4 Cells

4.1 Separation of T and B lymphocytes from human peripheral blood mononuclear cells using density perturbation methods

Patel, D., Rubbi, C.P. and Rickwood, D.
Clin. Chim. Acta, 240, 187-193 (1995)

The fractionation of sub-populations of peripheral blood mononuclear cells has become an essential routine procedure and some of the main fractionation methods used today are immunomagnetic separations. We describe a less expensive method for the separation of subpopulations of mononuclear cells using density perturbation, which uses the binding of antibody-coated dense polystyrene beads to increase the density of specific sub-populations of cells. By incubating a total mononuclear fraction from human peripheral blood together with antibody-coated beads, in a commercially-available lymphocyte separation medium (Nycoprep 1.077), a depletion of 94.9 ± 1.68% of the T cells could be obtained by this procedure; a depletion of 69.7 ± 1.78% of the B cells was also achieved. These results indicate the potential for the separation of different sub-populations of peripheral blood mononuclear cells on the basis of the immunological identity of the surface of cells using density perturbation methods involving antibody-coated dense polystyrene beads.

4.2 Optimization of conditions for specific binding of antibody-coated beads to cells

Patel, D.and Rickwood, D.
*J. Immunol. Methods, **184**, 71-80 (1995)*

It has previously been demonstrated that cells can bind antibody-coated beads: this effect can be used to enhance the fractionation of cells using magnetic fields or by centrifugation on isopycnic, isotonic density gradients. As a general rule, the higher the expression of surface antigens the more beads to bind to cells. However, we have also noted that other factors also affect the number of beads found bound to cells. Experiments have been carried out to determine what factors affect binding of antibody-coated beads to cells. The optimum conditions for binding of antibody-coated beads to MOLT-4 T cells were found to be, namely, a 20:1 bead to cell ratio in a 1 ml incubation volume, with continuous end-over-end mixing for 1 h at 25°C. Furthermore, the optimum centrifugation conditions at which the samples were separated on isopycnic, isotonic density gradients were determined as $220xg_{max}$ for 90 min, at 20°C. The results indicate the preferred conditions that are necessary to achieve optimum bead binding by cells and their subsequent fractionation. Different antibody-coated beads were examined including Dynabeads M-450, used as a known standard. In addition we describe, as a possible alternative to Dynabeads, dense polystyrene beads, for the separation of cells on the basis of the immunological identity of the surface of cells using density perturbation methods.

4.3 Use of iodixanol as a density gradient material for the isolation of motile, morphologically normal human sperm from semen.

Smith, T.T., Turner, D. and Whitford, W.
J. Andrology, Supplement 1996

Although highly desirable, the efficient isolation of motile, morphologically normal spermatozoa from semen has been limited due to the unavailability of an iso-osmotic gradient material with a density higher than human sperm. A new gradient material, iodixanol (60% w/v, **OptiPrep**, Nycomed), with its relatively high osmolality (260 mOsm) and a density (1.32 g/ml), may be suitable for this purpose. Iodixanol solutions, 1.154 g/ml and 1.054 g/ml (pH 7.3), were prepared using modified Human Tubal Fluid (m-HTF) medium. Osmolarity was adjusted to 300 mOsm with NaCl. Semen specimens (n=15) were obtained from 10 normozoospermic donors. Semen volume, sperm concentration (C), motility (%M), normal forms (%N; Tygerberg strict criteria), total motile (TM), and total normal (TN) sperm were determined. Semen was diluted 4:6.5 with OptiPrep and layered under the 1.154 and 1.054 g/ml solutions (3 ml each) in 4 tubes. The discontinuous gradient was centrifuged for 40 min at 1,500g during which time sperm rose to the 1.154/1.054 g/ml interface. The sperm were collected from the interface and diluted 1:5 in m-HTF, centrifuged for 15 min. at 500g and then resuspended in 1.0 ml of m-HTF. Final volume, C, %M, forward progressive velocity (V_f), TM, %N and TN were determined. To identify any possible cytotoxic effect of iodixanol, an aliquot of prepared sperm suspension was diluted to $10x10^6$/ml in 1 ml of m-HTF supplemented with 0.4% human serum albumin and incubated under mineral oil at 37°C in air for 24 h after which %M and V_f were determined again. Values are expressed ∀SE. Following centrifugation, a mean of 32.0 ∀ 3.8 $x10^6$ motile sperm were recovered from the 1.154/1.054 g/ml interface, representing 21

∀1.8% of the motile and 19.5 ∀ 2.5% of the morphologically normal sperm. Following 24 h of incubation at 37°C, %M and V_f had increased 18.1 ∀ 2.4% and 14.3 ∀ 1.7 μ/s respectively.

The results of this preliminary study show that iodixanol may be used to obtain motile, morphologically normal sperm for IUI, IVF and ICSI. The relatively small decline in %M and V_f at 24 h after recovery indicates iodixanol was not toxic to the sperm. Since the use of iodixanol as a gradient material appears promising, future studies will involve fine tuning the gradient densities to enhance the yield of motile, morphologically normal sperm.

4.4 Phenotypic and functional characterization of CD11c$^+$ dendritic cell population in mouse Peyer's patches

Ruedl, C., Rieser, C., Bock, G., Wick, G. and Wolf, H.
*Eur. J. Immunol., **26**; 1801-1806 (1996)*

The antigen-presenting cell system in the gastrointestinal tract, one of three main sites (skin and lung being the others) of primary antigen contact, is poorly understood. Our study focused on dendritic cells (DC) as possible candidates for antigen uptake, processing and presentation in mucosal inductive sites, such as Peyer's patches (PP). To investigate the morphology, immunophenotype and stimulatory activity of intestinal DC, a procedure was developed to obtain a cell population by using collagenase digestion of PP, density centrifugation and cell sorting on the basis of CD11c expression. The resultant low-density cell fraction consisted of a nonadherent cell population expressing different intensities of CD11c that could at least be characterized by typical DC morphology *(e.g.* abundant cytoplasma with veil-like cytoplasmatic dendrites, irregularly shaped nuclei, multivesicular and multilamellar bodies), constitutive levels of surface MHC class II, the presence of macrophage-specific markers, such as F4/80, Mac-I and Fc receptors, respectively, on subpopulations of CD11c$^+$ sorted cells and expression of adhesion and co-stimulatory receptors like ICAM-1 and CD44. The capability of this low-density CDl1c$^+$ fraction to stimulate T cell responses was demonstrated in primary allergenic mixed-lymphocyte reactions (MLR). Herein, we show that the freshly isolated CD11c$^+$ cells showed weak accessory function, but develop this capacity following short-term culture *in vitro* in the presence of granulocyte/macrophage colony-stimulating factor. Although the nature and functional capacity of the isolated CD11c$^+$ needs further clarification, these preliminary results describing phenotype and accessory function provide some evidence that these cells isolated from the PP may be immature forms of DC and play a crucial role as antigen-presenting cells with important implications for understanding the complex network regulating intestinal antigen uptake, processing and presentation.

4.5 Rejection of MHC Class II-transfected tumor cells requires induction of tumor-encoded B7-1 and/or B7-2 co-stimulatory molecules

Baskar, S et al.
*J. Immunol., **156**, 3821-3827 (1996)*

Many tumor cells that have been transfected with genes encoding B7 co-stimulatory molecules become effective cellular vaccines against wild-type tumor. The improved immunity is dependent on newly induced tumor-specific CD8$^+$ and/or CD4$^+$ T cells and presumably occurs because the B7 transfectants provide the requisite second signal for activation of T cells in conjunction with tumor cell-presented MHC class I/tumor peptide and/or MHC class II/tumor peptide complexes, respectively. Since B7 expression is such a potent enhancer of tumor immunity, and yet some tumors are immunogenic in the absence of B7 transfection, we have used class I$^+$ class II-transfected tumors to investigate whether co-stimulatory molecules are also involved in rejection of immunogenic, non-B7-transfected tumor. Blocking studies with B7 mAbs demonstrate that induction of tumor immunity in naive mice requires B7-1 and/or B7-2 expression, while experiments with tumor-primed mice indicate that once antitumor immunity is established, expression of B7 is not necessary. Flow cytometry analyses demonstrate that co-stimulatory molecules are expressed by the tumor cells via an in vivo induction process. Experiments with class II genes with truncated cytoplasmic tails indicate that the cytoplasmic region of the tumor-expressed class II heterodimer is involved in induction of B7. We therefore conclude that for this class I$^+$ class II-transfected tumor, generation of tumor immunity requires induction of tumor cell-encoded B7 molecules that are mediated by the cytoplasmic region of the transfected class II heterodimer.

4.6 Iodixanol as a density gradient medium for the isolation of motile spermatozoa.
Harrison, K.
*J. Assist. Reprod. and Gen., **14(7)**, 385-387 (1997)*

Purpose: The purpose of this study was to establish concentrations of **Iodixanol** which could be used in a similar manner to the widely used Mini-Percoll technique for the separation of motile sperm.
Methods: Various density gradient combinations of Iodixanol were compared for the isolation of motile spermatozoa from cryopreserved donor semen. The toxicity of Iodixanol was tested by its effect on the growth of two-cell mouse embryos and fresh sperm survival.
Results: The best sperm recovery (32%) came from the pellet of sperm passing through a discontinuous gradient of 25% over 40% OptiPrep after 20 min of centrifugation at 400g. There was no evidence of toxicity to mouse embryo growth or sperm survival.
Conclusions: Iodixanol (OptiPrep; Nycomed) provides a satisfactory alternative for the efficient separation of motile spermatozoa for ART procedures.

4.7 The use of iodixanol as a density gradient material for separating human sperm from semen.
Smith, T.T. , Byers, M., Kaftani, D. and Whitford, W.
*Arch. Androl., **38**, 223-230 (1997)*

Iodixanol, a new nonionic density gradient material with relatively low osmolality and high density, was evaluated to determine its suitability for the separation of human sperm from semen for their subsequent therapeutic use. Using a three-layer iodixanol gradient (1.17/1.15/1.05 g/ml), sperm were centrifuged at 1000g for 30 min and collected from the 1.05/1.15 interface. Using this method, a mean of 78% of the motile and 99% of the morphologically normal sperm originally present in the semen were recovered at the interface. There was no significant increase in the percentage of motile or morphologically normal sperm in the final preparation compared to the original semen. Sperm survived iodixanol density gradient centrifugation well, showing only modest declines in motility (18%) and velocity (35%) during a subsequent 24 h incubation period. Iodixanol provides a suitable, nontoxic alternative to Percoll for the preparation of human sperm for therapeutic use.

4.8 A quick, easy and inexpensive method for the isolation of human peripheral blood monocytes.
Graziani-Bowering, G.M., Graham, J. and Filion, L.G.
*J. Immunol. Methods, **207**, 157-168 (1997)*

A commercial monocyte isolation technique based on **OptiPrep** density-gradient medium was up-scaled with respect to sample and reagent volumes. The results of 7 isolations are reported in which the average purity ranged from 87.9 to 96.4%. In all but the initial isolation, monocytes were defined as $CD15^+$ dim $CD4^+$ dim as assessed by flow cytometric analysis; in the first isolation, monocytes were defined by the traditional $CD14^+$ $CD4^+$ dim combination. The mean yield (the number of isolated monocytes relative to the number present in the buffy coat) of all isolations was 26.1%, with the individual yields ranging from 10.8 to 41.4%. The mean number of isolated monocytes per experiment was $3.6x10^6$ monocytes for those isolations performed using 14 ml of buffy coat/OptiPrep mixture (n=4). The isolated cells were viable (>95%) and were not activated, according to HLA-DR expression. This technique is a convenient, fast (less than 2 h), relatively simple, and inexpensive alternative to traditional monocyte isolation techniques). The up-scaled version of this method also results in significantly higher numbers of monocytes per isolation than some traditional techniques. Furthermore this is the first literature report of the use of OptiPrep density gradient medium for the isolation of monocytes.

4.9 A new method for the purification of human motile spermatozoa applying density-gradient centrifugation; Polysucrose media compared to Percoll media.
Andersen, C.Y. and Grinsted, J.
*J. Assist. Reprod. and Gen., **14(10)**, 624-628 (1997)*

Purpose: A newly developed method for the isolation of human motile spermatozoa using density-gradient centrifugation was compared with the traditionally used Percoll technique.
Method: Sperm samples were divided into two equal aliquots, which were purified with either the traditionally performed Percoll technique or a new alternative based on polysucrose/ **OptiPrep** media. For each sample the isolation was performed during the same run of the centrifuge.
Results: The average recovery of progressively motile spermatozoa with the Polysucrose/ OptiPrep method

was significantly higher (48∀7%) than with the Percoll method (38∀6%) (n=18). The average percentage of motile spermatozoa and the motility score were similar in the purified preparations.
Conclusion: The new polysucrose/OptiPrep-based density-gradient centrifugation technique for the isolation of motile human spermatozoa is as good as the traditionally used Percoll method and may replace it in connection with assisted reproduction techniques.

4.10 The use of OptiPrep to prepare human sperm for the assisted reproductive technologies.
Kaftani, D., Byers, M. and Smith, T.T.
ASRM Abstracts 1997

Objectives: Concern regarding the safety of silica-based density gradient materials (e.g. Percoll) for human sperm processing has resulted in the need to find safe, cost-effective alternatives. The purpose of this study was to determine the suitability of **OptiPrep**, a non-toxic density gradient material, for the preparation of human sperm for subsequent therapeutic use.

Design: Two-layer discontinuous density gradients of OptiPrep and Percoll were compared for their ability to separate motile, morphologically normal sperm from semen. The functional status of processed sperm was evaluated by assessing percent motility (%M) and straight line velocity (m/s, VSL) at 0, 24 and 48 h post-isolation.

Material and Methods: Isotonic Optiprep gradients (35 and 17.5%) were prepared by diluting stock OptiPrep (60% iodixanol) with modified Human Tubal Fluid (mHTF). Likewise, Percoll gradients (90 and 45%) were prepared by diluting isotonic Percoll with mHTF. Two layer (1 ml each) discontinuous density gradients were prepared in 15 ml conical centrifuge tubes. Ten semen samples were obtained from 3 normozoospermic donors. Seminal sperm concentration C, %M and percent normal morphology (%N, strict criteria) were determined. Semen aliquots (0.5 ml) from the same ejaculate were layered over OptiPrep and Percoll gradients and centrifuged at 340 g for 20 min. Sperm pellets were washed once (OptiPrep) or twice (Percoll), resuspended in 0.5 ml of HTF and analyzed for C, %M and %N. The percent recovery of motile and morphologically normal sperm was calculated. At 0, 24 and 48 h, %M and VSL were determined using computer assisted sperm analysis. The percent decline over time in motility and VSL was determined. Data, expressed below as mean ± SEM, were compared using a paired *t*-test.

Results: There was no significant difference between OptiPrep and Percoll density gradients in the % recovery of motile sperm (106 ± 10.6 vs. 112.6 ± 6.7) or morphologically normal sperm (90.2 ± 9.5 vs. 87.9 ± 8.5). Likewise, there was no significant difference in the % decline in motility at 24 h (4.9 ± 2.5 vs. 5.1 ± 2.3) or 48 h (36.9 ± 6.5 vs. 33.0 ± 6.2), or the decline in VSL at 24 h (11.0 ± 5.7 vs. 8.6 ± 6.8) or 48 h (48.9 ± 0.7 vs. 45.2 ± 6.1).

Conclusion: These data indicate that OptiPrep provides a suitable, cost-effective, non-toxic alternative to silica-based density gradient materials for the isolation of motile, morphologically normal sperm for subsequent therapeutic use.

4.11 Selection of motile spermatozoa of normal morphology from bovine ejaculates by centrifugation in an iodixanol gradient.
Revell, S.G. et al
Liverpool John Moores University, "Control of Human Fertility", Seminar Report Nov. 1997

Bovine ejaculates vary in the quality and numbers of the sperm present and if the number of motile sperm in an ejaculate falls below a particular standard percentage, the ejaculate will be discarded. Although the percentage of motile sperm may be too low, the actual numbers can be very high, and it would be economically useful if the motile sperm could be separated from the non-motile and thus be used for Artificial Insemination (A.I.). A new centrifugation medium, **OptiPrep™**, offers a method for the separation of dead and deformed sperm from motile sperm of normal morphology. The method involves low-speed centrifugation of an ejaculate on a discontinuous density gradient which leaves the dead sperm as a pellet, live sperm of normal morphology at one interface of the gradient and deformed sperm, i.e. those with bent and twisted tails or with cytoplasmic droplets, rise to the top of the gradient. The band of motile sperm with normal morphology always contained at least 90% of sperm with normal morphologies. The pelleted sperm always have been shown to be at least 95% dead. After freezing and thawing sperm harvested from the "normal" band, recovered sperm were 75% live with excellent motility.

4.12 Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo
Brocker, T., Riedinger, M. and Karjalainen, K.
J. Exp. Med., ***185(3)****, 541-550 (1997)*

It is well established that lymphoid dendritic cells (DC) play an important role in the immune system. Beside their role as potent inducers of primary T cell responses, DC seem to play a crucial part as major histocompatibility complex (MHC) class II^+ "interdigitating cells" in the thymus during thymocyte development. Thymic DC have been implicated in tolerance induction and also by some authors in inducing major histocompatibility complex restriction of thymocytes. Most of our knowledge about thymic DC was obtained using highly invasive and manipulatory experimental protocols such as thymus reaggregation cultures, suspension cultures, thymus grafting, and bone marrow reconstitution experiments. The DC used in those studies had to go through extensive isolation procedures or were cultured with recombinant growth factors. Since the functions of DC after these in vitro manipulations have been reported to be not identical to those of DC in vivo, we intended to establish a system that would allow us to investigate DC function avoiding artificial interferences due to handling. Here we present a transgenic mouse model in which we targeted gene expression specifically to DC. Using the CD 11c promoter we expressed MHC class II I-E molecules specifically on DC of all tissues, but not on other cell types. We report that I-E expression on thymic DC is sufficient to negatively select I-E reactive $CD4^+$ T cells, and to a less complete extent, $CD8^+$ T cells. In contrast, if only DC expressed I-E in a class II-deficient background, positive selection of $CD4^+$ T cells could not be observed. Thus negative, but not positive, selection events can be induced by DC in vivo.

4.13 Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expression dendritic cells
Brocker, T
J. Exp. Med., ***186(8)****, 1223-1232 (1997)*

Thymic T cell development is controlled by T cell receptor (TCR)-major histocompatibility complex (MHC) interactions, whereas a further dependence of peripheral mature T cells on TCR-MHC contact has not been described so far. To study this question, CD4 T cell survival was surveyed in mice lacking MHC class II expression and in mice expressing MHC class II exclusively on dendritic cells. Since neither of these mice positively select CD4 T cells in the thymus, they were grafted with MHC class II-positive embryonic thymic tissue, which had been depleted of bone marrow derived cells. Although the thymus grafts in both hosts were repopulated with host origin thymocytes of identical phenotype and numbers, an accumulation of $CD4^+$ T cells in peripheral lymphoid organs could only be observed in mice expressing MHC class II on dendritic cells, but not in mice that were completely MHC class II deficient. As assessed by histology, the accumulating peripheral CD4 T cells were found to be in close contact with MHC class II^+ dendritic cells, suggesting that CD4 T cells need peripheral MHC class II expression for survival and that class II^+ dendritic cells might play an important role for the longevity of CD4 T cells.

4.14 Manual and automated methods for the determination of leukocyte counts at extreme low levels: comparative evaluation of the Nageotte chamber
Müller, T.H., Döscher, A., Schunter, F. and Scott, C.S.
Transfus. Sci., ***18(4)****, 505-515 (1997)*

Leukodepleted or leukocyte-poor blood products (fresh-frozen plasma, packed red cell and platelet concentrates in particular) are widely used in current clinical practice. However, because the monitoring of leukodepletion efficiency is generally carried out (if at all) using the labour-intensive and relatively inaccurate manual Nageotte chamber technique, it is clear that any increased demand for leukodepletion monitoring would be difficult, if not impossible, to meet. As the need to identify an automated alternative to the Nageotte technique is important, this study was undertaken to evaluate such a possibility. White blood cells were enumerated in a representative series of filtered and non-filtered human blood components by both microscopic counting in the Nageotte chamber, and with the Abbott CD3500 automated haematology analyser. For the Nageotte estimate, a single analysis was made in accordance with standard procedures, whereas the automated analysis was achieved by making six replicate counts and determining the mean of four replicates after excluding the highest and lowest estimates. To determine linearity limits of the manual and automated procedures, freshly isolated leukocytes were admixed with cell-free plasma-pheresis plasma. Reasonable reproducibility (mean CV 10% for cell counts exceeding 100 cells/microL) and good linearity ($r > 0.9$) were observed for CD3500 determinations in four separate

experiments. The manual and automated measurements also correlated well (r > 0.9) with no obvious inter-method bias for cell counts up to 40 cells/microL although there was some suggestion of lower absolute CD3500 counts in the range 40-130 cells/microL. For the comparative studies with filtered and non-filtered blood products, no significant method bias was seen with 70 individual red cell concentrates, but systematically higher CD3500 white blood cell counts were observed in the series of 68 platelet concentrates (probably due to the presence of platelet clumps). This study concludes that automation of white cell counts in blood products with the CD3500 analyser is feasible for quality control in the preparation of fresh-frozen plasma and red cell concentrates but is limited for the analysis of filtered platelet concentrates.

4.15 Endotoxin contamination of reagents used during isolation and purification of human pancreatic islets.

Linetsky, E., Inverardi, L., Kenyon, N.S., Alejandro, R. and Ricordi, C.
*Transplantation Proc., **30**, 345-346 (1998)*

A substantial number of islet allografts never function or lose function in the first several weeks after transplantation. This early allograft failure has been attributed to several mechanisms, such as increased imunogenicity of the islet preparation, acute allograft rejection, and recurrence of autoimmunity in recipients with insulin dependent diabetes mellitus. More recently, increasing attention has been directed also to microenvironmental factors at the site of the islet implantation (e.g. intrahepatic). A specific tissue injury, inflammation, and ischemia/reperfusion injury can affect the microenvironment at the transplant site, resulting in the activation of macrophages and other antigen-presenting cells. This can result not only in the local generation of cytokines and radicals known to be deleterious to islet cells, but also in the activation of allospecific immune response as well as β cell-specific autoimmunity. Results from experimental studies indicated that macrophages may play a determining role in the occurrence of early loss of transplanted islets. Endotoxins are well known inducers of macrophage activation and related cytokine and radical generation. The presence of endotoxins in the islet preparation could, by inducing macrophage activation, amplify an early inflammatory response. Endotoxins have been previously associated with failure of intrahepatic islet transplants, and it has always been important to identify reagents for human isolation that are endotoxin free. However, only recently new products have become available to fulfill this need. The aim of this study was to evaluate the endotoxin content of reagents commonly used in the isolation and purification of human pancreatic islets.

4.16 Porcine islet preservation during isolation in University of Wisconsin solution.

Van der Burg, M.P.M., Basir, I., Zwaan, R.P. and Bouwman, E.
*Transplantation Proc., **30**, 360-361 (1998)*

Substitution of the University of Wisconsin Solution (UWS) for the conventional Hanks= Balanced Salt Solution (HBSS) during collagenase digestion of the porcine pancreas has been reported to increase the yield of isolated porcine islets. Little is known, however, on the effect of the collagenase solution and different solutions during subsequent steps of the isolation and purification procedure on islet viability. Since the UWS probably also best preserves the islet tissue during the cold steps of the procedure, we compared the use of UWS for all steps of the isolation procedure and also during purification on our novel UWS-based **OptiPrep** gradient versus the use of HBSS for digestion and dispersion and similar OptiPrep purification by in parallel processing of two paired segments of the pancreatic body of market-age slaughterhouse pigs.

4.17 No porcine islet loss during density gradient purification in a novel iodixanol in University of Wisconsin solution.

Van der Burg, M.P.M., Basir, I. and Bouwman, E.
*Transplantation Proc., **30**, 362-363 (1998)*

The marked fragility and rapid dissociation and loss of (juvenile) porcine islets during the isolation and especially the purification process are considered a major barrier on the way to pig-to-human islet transplantation. We developed a novel simple density gradient of iodixanol **(OptiPrep)** in University of Wisconsin solution (UWS) that allows the purification of juvenile islets with no loss or fragmentation and an improved purity and viability as compared to conventional ficoll-sodium-diatrizoate (Histopaque, Sigma, St. Louis, Mo) gradients.

4.18 Markedly improved outcome of adult porcine islet isolation, purification, and culture using Liberase-P1 versus Collagenase-P, and a novel gradient of OptiPrep in University of Wisconsin solution.

Van der Burg, MPM, Zwaan, RP. and Bouwman, E.
*Horm. Metab. Res., **30**, A23 (1998)*

Isolated porcine islet yield, integrity, and viability is endangered by the use of crude enzyme mixtures for digestion, and the changing conditions generally introduced by the use of several different solutions during digestion and further isolation and purification steps. We, therefore, aimed at improving the preservation of pig islets by keeping the islets in the University of Wisconsin solution (UWS) both during isolation and the subsequent density purification in a novel iodixanol (**OptiPrep**, Nycomed Pharma) in UWS; and we compared a novel Liberase-P1 enzyme blend vs. collagenase-P for islet isolation by in parallel processing of two, paired, segments of the pancreas (whole organ weight 254∀15 gram) of large sows in 6 consecutive experiments. After 22 min warm- and 100 min cold ischemia, the segments were digested using 0.5-0.75 mg/ml Liberase for the one- and 2 mg/ml collagenase-P for the other segment, by the stationary single-endpoint method for 32 min at 36°C, and dispersed by shaking and sieving (400 μm) for –30 min in UWS on ice. The digest suspension was mixed with half the volume of a 30% w/v iodixanol in UWS (final density 1.10 g/ml, and osmolality 380 mOsm) and topped with a 1.090 g/ml OptiPrep-UWS, and plain UWS. After centrifugation at 500g and 4°C for 5 min, purified islets were obtained from the top layer, and cultured for 24 h in RPMI-1640 supplemented with 10% adult porcine serum. Islet yield was expressed in islet equivalents (IEQs; mass converted to the equivalent number of islets with an 150 μm diameter). Viability was assessed by fluorometry using acridine orange and propidium iodide. Islet yield (overall mean 2444±446 IEQs/g pancreas) and size (volume-average diameter 155±11 μm) did not differ after Liberase and collagenase-P digestion. After purification, comparison of the Liberase vs. collagenase-P isolated islets, demonstrated a similar >95% purity, but higher yields (2448±392 vs. 1319±224 IEQs; $P<.05$), larger islets (185±16 vs. 140±8; $P=.08$), and an improved viability (90±2 vs. 79±4%; $P<.05$) of the Liberase-isolated islets. Postculture recovery was 24±9% of the IEQs in the Liberase digest vs. 14±8% collagenase-P islets, $P<.05$). Viability was corroborated in vivo by postprandial normoglycemia (<200 mg%) from day- 1 after grafting of 2500 IEQs under the kidney capsule in streptozotocin diabetic nude mice. We conclude that islet integrity and viability are markedly improved by Liberase digestion, and preservation in UWS throughout isolation and purification in our novel OptiPrep-UWS gradient.

4.19 Efficacy of the novel iodixanol – UWS density gradient for human islet purification

Van der Burg, M.P.M., Ranuncoli, A., Molano, R., Kirlew, T., Ringers, J., Bouwman, E. and Ricordi, C.
*Acta Diabetol., **35**, 247 (1998)*

Consistent human islet isolation and purification success is hampered by the large variability of donor and procurement related factors. Substantial progress in purification has nevertheless been made over the last few years by simple changes in (pre-) purification solutions, suggesting that considerable scope still exists for further improvement in the density gradient purification of human islets. We recently developed a ~100% efficient (approx. complete islet recovery and purity) density gradient of iodixanol (**OptiPrep**) in University of Wisconsin solution (UWS) in the difficult pig model. This success prompted us to test this gradient during 5 consecutive human islet isolations. Pancreases distantly procured from multiorgan cadaveric donors (27-57 y) and cold preserved with UWS for approx. 12 h were digested with 1.4 mg/ml Liberase-HI in HBSS by the automated method. The digest was collected with cold RPMI, and a small sample of the digest was taken for these pilot experiments – the remainder was used in non-related experiments. Next, the tissue was incubated 60 min in UWS on ice. After taking an aliquot for assessment, half of the prep was loaded in a 1.086 – 1.075 –UWS gradient in 50 ml conical tubes, the other half was saved on ice for optional testing of other density layers. The 1.086 bottom was prepared by mixing 30 ml digest (in UWS) with 10 ml Working OptiPrep (WOP; an 1:1 mixture of OptiPrep and double-strength UWS). The 1.075 (or 1.070) barrier layer was prepared by mixing 5 ml WOP with 22.6 ml (or 28.3) UWS. After 5 min 500g centrifugation at 4°C tissue was collected from the top (UWS – 1.075 interface) and second layer. The 1.086-1.075-UWS gradient was successful (> 80% purity and recovery) during all 4 experiments. In the last experiment, purity was 35%, but again > 80% purity and recovery was obtained by using an 1.070 barrier with the other half of the digest prep. On average the digest loaded in the gradient contained 15287 ± 3712 IEQs and the volume average islet diameter was 238 ± 22 μm. After purification at the top 12766 ± 3129 IEQs were recovered (83 ± 2 % recovery), islet diameter was 204 ± 13 μm (NS) and purity was 89 ± 3 % Viability of the islets was corroborated histologically 1 to 3 weeks after transplantation under the kidney capsule in 3 nude mice. Thus, the consistent high efficacy of this simple

OptiPrep-UWS gradient under mild hyperosmotic conditions (approx. 360 mOsm) in this pilot, and other favourable characteristics such as a low endotoxin content, suggest that the gradient may become a new powerful tool for human islet purification.

4.20 Large scale isopycnic islet purification utilizing non-toxic, endotoxin-free media facilitates immediate single-donor pig islet allograft function.
Matsumoto, S, Zhang, H.J., Gilmore, T., van der Burg, M.P., Sutherland, D.E.R. and Hering, B.J.
Transplantation, ***66(8),*** S30 (1998)

Early graft function has been difficult to accomplish in clinical islet allotransplantation. It is becoming apparent that reagents used for islet preparation may stimulate the inflammatory response to islet grafts and may thereby interfere with early islet function and engraftment. The purpose of this study therefore was to develop islet purification density gradient media restricted to non-toxic, endotoxin-tested components and to subsequently test whether islets purified on the refined gradients immediately reverse diabetes in the relevant preclinical single-donor pig allograft model.

Density gradients were constructed based on **iodixanol** x-ray contrast media and UW solution which are both approved for clinical use. In the first set of experiments, the osmolality most suitable for islet separation was identified. The effect of four different osmolalities (320, 350, 375 and 400 mosm/kg) on the density profile of islet and acinar tissue was studied by means of continuous test gradients. In the second part of the study, iodixanol/UW gradients were applied for large scale continuous islet density gradient purification on a Cobe 2991 and the percentage recovery of islet equivalents (IE) in fractions with purities > 90% from iodixanol/UW was compared to Ficoll Na-diatrizoate gradients (previous standard). To test whether iodixanol/UW gradients interfere with immediate reversal of hyperglycemia posttransplant, 7134 ± 1830 purified IE/kg body weight were intraportally transplanted into five streptozotocin-diabetic pigs. The percentage of exocrine tissue contaminating 95% yield of islets were 0.45 ± 0.96%, 0.0 ± 0.0%, 0.45 ± 0.69%, and 0.80 ± 1.00% for 320, 350, 375 and 400 mosm/kg iodixanol/UW gradients, respectively. The percentage recovery of pure islet equivalents from 350 mosm/kg iodixanol/UW (n=6) gradients was significantly higher compared to the standard Ficoll Na-diatrizoate (n=4) gradient (86.2 ± 11.8% vs. 67.0 ± 9.3%, p=0.013). Four out of five consecutive diabetic single-donor pig islet allograft recipients became normoglycemic and insulin-independent within 24 hrs following transplantation.

The data indicate that single donor pig islet allografts purified on non-toxic, endotoxin-free gradients establish independence immediately. Osmolality adjustments should make iodixanol/UW gradients also suitable for human islet purification. Failure to achieve immediate insulin independence in the clinical setting would then point to non-technical obstacles.

4.21 Identification, culture, and characterization of pancreatic stellate cells in rat and humans
Bachem, M.G. et al
Gastroenterology, ***115****, 421-432 (1998)*

Until now, the basic matrix-producing cell type responsible for pancreas fibrosis has not been identified. In this report, retinoid-containing pancreatic stellate cells (PSCs) in rat and human pancreas are described, and morphological and biochemical similarities to hepatic stellate cells are shown. *Methods:* Electron and immunofluorescence microscopy (collagen types I and III, fibronectin, faminin, α-actin, and desmin) was performed using pancreatic tissue and cultured PSCs. Extracellular matrix synthesis was shown using quantitative immunoassay and Northern blot analysis. *Results:* PSCs are located in interlobular areas and in interacinar regions. Early primary cultured PSCs contain retinol and fatty acid retinyl-esters. Addition of retinol to passaged cells resulted in retinol uptake and esterification. During primary culture, the cells changed from a quiescent fat-storing phenotype to a highly synthetic myofibroblast-like cell expressing iso-α-smooth muscle actin (> 90%) and desmin (20%-40%) and showing strong positive staining with antibodies to collagen types I and III, fibronectin, and laminin. As determined on protein and messenger RNA level serum growth factors stimulated the synthesis of collagen type I and fibronectin. *Conclusions:* The identification of PSCs, particularly in fibrotic areas, and the similarities of these cells to hepatic stellate cells suggest that PSCs participate in the development of pancreas fibrosis.

4.22 Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir

Byrn, R.A. and Kiessling, A.A.
*J. Reprod. Immunol., **41**, 161-176 (1998)*

It is well established that HIV is found in semen, either as cell-free or cell associated virus, yet many questions remain about the source of the virus. A number of factors, including anatomic features of the male reproductive tract, the restricted access of the immune system to the germ cell compartment, and the results from sexually transmitted virus studies, suggest that the source of HIV in semen may be different from that in the peripheral blood. In this study, we examine the HIV in the infected cells of semen as indicators of the virus-producing reservoir. The frequency of HIV positive leukocytes in semen is compared to that of concurrent blood samples from eight donors and these values are found to be highly variable and frequently discordant. The protease gene sequences of HIV strains isolated from semen cells and blood cells were determined and phylogenetic analyses were performed which indicate the virus populations in the two sources are genetically distinct. In one patient receiving anti-HIV protease inhibitor therapy, gene sequences indicative of protease inhibitor resistance were found in the blood, but not the semen cell compartment. These results suggest that HIV in the semen and blood compartments are distinct, and further, may respond differently to antiviral therapy.

4.23 Residual MHC class II expression on mature dendritic cells and activated B cells in RFX5-deficient mice

Clausen, B.E. et al
*Immunity, **8**, 143-155 (1998)*

Patients with major histocompatibility complex class II (MHC-II) deficiency are known to carry mutations in either the RFX complex or the *trans*-activator CIITA. While the pivotal role of CIITA for MHC-II gene transcription is supported by the essential absence of MHC-II molecules in CIITA-deficient mice, we demonstrate here that $RFX5^{-/-}$ mice retain expression of MHC-II in thymic medulla, mature dendritic cells, and activated B cells. Nevertheless, $RFX5^{-/-}$ mice develop a severe immunodeficiency due to the lack of MHC-II in thymic cortex, failure of positive selection of $CD4^{+}$ T cells, and absence of MHC-II on resting B cells and resident or IFNγ-activated macrophages. This differential requirement for CIITA and RFX5 in subsets of antigen-presenting cells may be specific for the mouse: it may, however, also exist in humans without having been noticed so far.

4.24 Drug-metabolizing enzymes in rat liver myofibroblasts

Peterson, T.C. and Rowden, G.
Biochem. Pharmacol., 55, 703-708 (1998)

The myofibroblast is considered to be a key component in the pathogenesis of hepatic fibrosis. There is a need for therapeutic intervention in hepatic fibrosis, and, to date, the number of efficacious anti-fibrotic drugs is negligible. At best, the current therapeutic modalities reduce liver enzymes, an indicator of liver damage, but cannot reduce or prevent fibrosis. We have described the anti-fibrotic effect of pentoxifylline in an experimental model of hepatic fibrosis. Evidence suggests that, in addition to pentoxifylline itself, at least two of the metabolites of pentoxifylline are of therapeutic interest. We have reported that one of these metabolites (M-1) has a biological activity similar to that of its parent drug. The second metabolite (M-1R) has been reported to be more potent than the parent drug. Recent evidence suggests that inhibition of cytochrome P450 1A2 (CYP1A2) results in higher levels of pentoxifylline and M-1 and may be responsible for the production of the novel, potent metabolite (M-1R). We therefore investigated whether the myofibroblast, the cell with a crucial role in fibrosis, contains drug-metabolizing enzymes and thus may play a critical role in the anti-fibrotic actions of pentoxifylline. Our results showed that myofibroblasts contain aryl hydrocarbon hydroxylase activity, ethoxyresorufin O-deethylase activity, and methoxyresorufin O-demethylase activity. The results presented here also indicate that aryl hydrocarbon hydroxylase and methoxyresorufin O-demethylase activities can be increased by treatment of cells with dibenzanthracene, an inducer of CYP1A activities.

4.25 Spleen cells of non-obese diabetic mice fed with pig splenocytes display modified proliferation and reduced aggressiveness in vitro against pig islet cells

You, S., Gouin, E. and Saï, P.
*Diabetologia, **41**, 955-962 (1998)*

A new means of modifying xenogeneic reaction to pig islet cells, which involves pre-feeding with pig spleen cells, was investigated for the first time in the non-obese diabetic (NOD) mouse. Compared with controls, mice fed with pig spleen cells displayed much higher splenocyte proliferation in response to pig spleen and islet cells ($p < 0.0001$). This enhanced proliferation was specific for the species providing the fed cells. Positive relationships ($p < 0.01$) were found between increased splenocyte proliferation in response to pig spleen or islet cells and the number of cells per feeding or the number of daily feedings. Concomitantly, while co-incubation with splenocytes from control mice led to inhibition of both basal and stimulated insulin releases from pig islet cells ($p < 0.001$), this aggressiveness was abolished ($p < 0.001$) after co-culture with splenocytes from mice fed with pig spleen cells. The proliferative responses of splenocytes from fed or control mice to pig islet or spleen cells were abolished after removal of plastic-adherent cells, indicating that the major indirect pathway of T-cell activation was unchanged by pig spleen cell feeding. The main T-splenocyte subsets involved were restricted to MHC class II as they did not proliferate in the presence of monoclonal antibodies (mAbs) directed at I-A molecules. In mice fed with pig spleen cells, as well as in control mice, the blocking of CD4 + T cells with mAbs led to abolition of proliferation ($p < 0.002$), while the blocking of CD8 + led to a less marked effect. However, an increase in the blocking effect of anti-CD8 mAbs was noted in mice fed with pig spleen cells ($p < 0.02$). In control mice, the main splenocyte subset involved during proliferation in response to pig islet cells was Th1, since interferon <*gamma*> (IFN<*gamma*>) production increased significantly ($p < 0.01$) while that of interleukin-10 (IL-10) increased only slightly. The main change observed in mice fed with pig spleen cells was a marked increase in basal IL-10 production ($p < 0.01$) and the basal IL-10/IFN<*gamma*> ratio ($p < 0.001$). It seems likely that feeding with pig spleen cells shifted the Th1/Th2 balance towards a dominance of Th2-type class II-restricted CD4 + T cells, which may have been conducive to activating CD8 + suppressor T cells. In any event, oral administration of pig cells modified xenogeneic cellular response, which may have implications for xenografts of pig islets. In a more general sense, physiological feeding of cells from xenogeneic species would appear to have certain effects on the immune system.

4.26 Viability of fresh vs. cultured pig islets for transplant

Rijkelijkhuizen, J.K.R.A., Bouwman, E. and van der Burg, M.P.M.
*Cell Transplantation, **8(2)**, abstract 6 (1999)*

Probably because pig islets are difficult to isolate and culture, the impact of pre-transplant culture on graft viability has not been studied yet. We isolated islets from large sows (n=12) by Liberase digestion and **OptiPrep**-UWS purification. The yield of freshly prepared islets was 1924 ± 346 IEQs/g with a diameter of 162 ± 13 um, purity of 96 ± 2%, and viability of 82 ± 2% by acridine orange – propidium iodide (AOPI) staining. During culture at 37°C in RPMI + 10% porcine serum, at day 1 islet recovery was 21 ± 4%, size was 124 ± 6 um, and viability was 87 ± 2%; at 1 week recovery was 11 ± 2%, size was 113 ± 4 um, and viability was 90 ± 2%. Islet quality was further tested by grafting under the kidney capsule in STZ-diabetic nude mice of ~1500 (n=14) or 3200 (n=5) fresh islets as compared with ~1500 (n=18) or 800 (n=12) cultured islets. Only 1/14 recipients of 1500 fresh islets and 0/5 recipients of 3200 fresh islets became normoglycemic (< 10.7 mM non-fasting)). The other mice had a > 28 mM glycemia before being killed for severe diabetic complications at ~3 wk. Histology of the kidneys demonstrated substantial scarring and near-absence of islets. By contrast, 1500 cultured islets rendered 18/18 recipients normoglycemic within 2 days (1-wk cultured) or ~2 wk (1-day cultured) for > 100 days, with a mean glycemia of 5.5 mM. The 800 IEQs-dose was likewise successful in 5/8 recipients of 1-day and 4/4 recipients of 1-wk cultured islets. Histology of these grafts so far showed a substantial mass of well-preserved islets with little scarring at the graft's site. Thus, pre-transplant culture markedly improved the viability of the graft, and disintegration of part of the fresh islets may not only reduce the effective islet dose, but also hamper the engraftment of viable tissue, because of scarring and because the cellular debris probably will attract macrophages and induce the release of harmful cytokines.

4.27 OptiPrep for human islet purification.

Van der Burg, M.P.M., Ranuncoli, A., Molano, R., Kirlew, T., Ringers, J., Bouwman, E., Terpstra, O.T. and Ricordi, C.
*Cell Transplantation, **8(2)**, abstract 57 (1999)*

Considerable scope exists for improvement in the purification of human islets. We recently developed an approx. 100% efficient density gradient of iodixanol (**OptiPrep**) in University of Wisconsin solution (UWS) in the difficult pig model. This success prompted us to test this gradient during 5 consecutive human islet isolations. Pancreases distantly procured from multiorgan cadaveric donors (27-57 y) and cold preserved with UWS for ~12 h were digested with Liberase in HBSS by the automated method. The digest

was collected with cold RPMI, and a small sample of the digest was taken for these pilot experiments. Next, after pre-incubation for 60 min in UWS on ice, the tissue was bottom-loaded in a 1.086-1.075-1.070-UWS gradient in 50 ml conical tubes. The bottom was prepared by mixing 30 ml digest (in UWS) with 10 ml OptiUWS (an 1:1 mixture of OptiPrep and double-strength UWS). The 1.075 and 1.070 layers were prepared by mixing 5 ml OptiUWS with 22.6 and 28.3 ml UWS, resp. After 5 min 500g centrifugation at 4°C purified islets were collected from the top or two uppermost layers. The digest loaded in the gradients contained 15287 ± 3712 IEQs and the volume-average islet diameter was 238 ± 22 um. After purification at the top 12766 ± 3129 IEQs were recovered (83 ± 2% recovery), islet diameter was 204 ± 13 um (NS) and purity was 89 ± 3%. Viability of the islets was corroborated histologically 1 to 3 wk after transplantation under the kidney capsule in 3 nude mice. Thus, the consistent high efficacy of this simple OptiPrep-UWS gradient under mild hypertonic conditions (~360 mOsm) in this pilot, and other favorable characteristics such as low endotoxin content, suggest that the gradient may become a new powerful tool for human islet purification.

4.28 Adult pig islet recovery during Liberase isolation, OptiPrep purification and culture for transplantation in nude mice

Van der Burg, M.P.M., Rijkelijkhuizen, J.K.R.A., Zwaan, R.P. and Bouwman, E.
*Cell Transplantation, **8(2)**, abstract 58 (1999)*

We aimed at improving the yield of viable intact pig islets by keeping the islets in the University of Wisconsin solution (UWS) both during isolation and during purification in a novel gradient of **OptiPrep** (Nycomed) in UWS, and by testing the new Liberase-PI blend (0.5 mg/ml) head to head with collagenase-P (2 mg/ml) for digestion of two paired segments of the pancreas (WIT 22 min) of large sows (n=6). The segments were digested for ~ 30 min at 36°C, and dispersed by shaking and sieving in UWS on ice. For purification the bottom (density 1.10 g/ml, 380 mOsm) was prepared by mixing the digest 2:1 with OptiUWS (an 1:1 mixture of OptiPrep and double-strength UWS), layered with 1.090 g/ml OptiPrep-UWS, and topped with UWS. After centrifugation at 500 g and 4°C for 5 min, purified islets were obtained from the top. Islet yield (mean 2444 ± 446 IEQs/g pancreas) and size (volume-average diameter 155 ± 11 um) did not differ after Liberase and collagenase-P digestion. After purification, comparison of the Liberase vs. collagenase-P isolated islets demonstrated a similar > 95% purity, but higher yields (2448 ± 392 vs. 1319 ± 224 IEQs; $P<0.05$), larger islets (185 ± 16 vs. 140 ± 8 um, $P=0.08$), and superior viability (90 ± 2 vs. 79 ± 4%; $P<0.05$; acridine orange – propidium iodide staining) of the Liberase-isolated islets. Overnight culture IEQ recovery was 24 ± 9% for Liberase vs. 14 ± 8% for the collagenase-P islets ($P<0.05$). After grafting of cultured Liberase islets under the kidney capsule in STZ-diabetic nude mice normoglycemia was obtained in 5/8 recipients of 800 IEQs within 1 month and in 9/9 recipients of 1500 IEQs within ~ 2 wk. We conclude that islet preservation is markedly improved by Liberase digestion, and the use of UWS throughout isolation and purification in our novel OptiPrep-UWS gradient.

4.29 Autocontrolled, randomized comparison between a tri-layer density gradient (OptiPrep) and the migration-sedimentation-gravity method

Van den Bergh, M., Emiliani, S., Biramane, J., Vannin, A.A.S. and Englert, Y.
*Human Reprod. Suppl., **14**, **P211**, 246 (1999)*

Introduction: OptiPrep is a non-ionic, iodinated derivative from compounds initially developed for X-ray contrast which were by nature of their original purpose rigorously tested to ensure their safety. OptiPrep appears therefore to be a possible alternative for semen density gradient preparation.

Materials and methods: Each of 30 semen samples from men attending a classical in-vitro fertilization procedure was divided in two equal volumes. One part was prepared by the migration-sedimentation-gravity (MSG) technique and the other part by a tri-layer density gradient (50%-25%-12.5% v/v) OptiPrep (Op3). The oocytes were randomized to be inseminated either with the MSG either with the Op3 preparation of the same semen sample. The same concentration of spermatozoa was added to the two series. Initial volume, motility and concentration, as well as final volume, motility and concentration were recorded. Morphology according to strict criteria was assessed on both final preparations after Spermac® staining. Fertilization, cleavage and embryo quality obtained by both preparations was compared. Data were analyzed by Crosstabs and the Altman Bland (AB) method.

Results: Initial concentration varied between $25x10^6$/ml with a median of $100x10^6$/ml. Initial volume was between 1.2 and 22 ml with a median of 4.2 ml. The motility ranged between 12 and 76% with a median of

53%. The percentage recovery of initial motile spermatozoa used in each preparation was calculated. The recovery for the MSG method ranged between 0.5 and 68% with a median of 3.85% and for the Op3 from 0.17 to 24% with a median of 1.19%. The AB analysis resulted in a linear regression curve $P < 0.01$ with $R = 0.8$ for the recovery, indicating that the observed differences were significantly method dependent. The final concentration after preparation ranged from $0.85x10^6$/ml to $320x10^6$/ml with a median of $8.25x10^6$/ml for MSG and from $1.1x10^6$/ml to $250x10^6$/ml with a median of $7.5x10^6$/ml for Op3. The AB analysis resulted in linear regression curve $P< 0.01$ with $R = 0.65$ for final concentration, indicating that the observed differences were significantly method dependent. The percentage normal forms varied from 9 to 33% with a median of 15.5% for MSG and between 1 and 23% with a median of 10% for Op3. The AB analysis did not result in any correlation, indicating that the observed differences were not method dependent. The motility after preparation ranged between 50 and 100% with a median of 96% for MSG and from 3 to 97% with a median of 43% for Op3. The AB analysis resulted in a linear regression curve $P < 0.01$ with $R = -0.93$ for the final motility, indicating that the observed differences were significantly method dependent. From 163 oocytes inseminated with MSG-prepared semen, 65 (39%) fertilized, whereas 74 (40%) fertilized out of 188 inseminated with Op3-prepared semen (not significant). The embryo score based on a scale between 0 and 6 taking in account the cleavage speed at 45 h post insemination, ranged for both preparations between 0 and 5 with an identical median of 3, reflecting that there was no difference in embryo quality. Thirteen pregnancies were obtained in these 30 patients (= cycles) with two abortions. As the best embryos were selected for embryo transfer, embryos obtained by the two semen preparations were mixed in the transfer and no conclusion can be made concerning the implantation rates.

Conclusion: The differences in final percentage motile spermatozoa, in the final concentration and in the percentage recovery were method dependent. The difference in final percentage spermatozoa with normal morphology was not dependent on method. The results obtained in terms of fertilization and embryo cleavage and quality was not influenced by the method of preparation.

4.30 Immediate reversal of diabetes in primates following intraportal transplantation of porcine islets on a new histidine-lactobionate-iodixanol gradient

Matsumoto, S et al

*Transplantation, **67(7)**, abstract 856 (1999)*

Information on islet xenotransplantation in relevant preclinical models is limited. One of the first issues to be addressed is whether porcine islets transplanted into primates are subject to hyperacute rejection. Previous work by others has emphasized the importance of density gradient media. To assure that technical factors do not compromise interpretation of early graft function in this model we sought to refine density gradient media such that immediate function of purified and cultured islets is consistently achieved in diabetic nude mouse recipients. Recently we have shown large-scale isopycnic islet purification utilizing University of Wisconsin solution with iodixanol (UWI) facilitates immediate single-donor pig islet allograft function. However, histidine-lactobionate (HL) solution was shown to be superior to UW for cold storage of purified islets. Therefore, we developed new density gradient media based on HL and iodixanol (HLI). The purpose of this study is to compare HLI to UWI-gradient media and examine whether porcine islets purified with new designed gradient media reverse diabetes promptly in nude mice and rhesus monkeys.
Methods: Porcine islets were isolated by the automated method. Free islets were separated from non-islet tissue utilizing continuous UWI or HLI gradients on a Cobe 2991 cell separator. After a 48 hr culture period, islet viability was assessed by a trypan blue uptake test and functional integrity in vivo was evaluated by renal subcapsular transplantation of 2,000islet equivalents (IEQ) into diabetic nude mice. HLI purified and 48 hr cultured islets (20,000 IEQ/kg) were transplanted intraportally into streptozotocin-diabetic rhesus monkeys.
Results: The percent recovery and purity of HLI purified islets were significantly higher compared to UWI gradient ($100.0 \pm 5.8\%$ vs. $81.8 \pm 5.0\%$ $p<0.05$ and 93.0 ± 0.6 vs. $89.3 \pm 2.8\%$ $p<0.03$), respectively. Using HLI, culture recovery was significantly higher ($79.3 \pm 4.1\%$ and $57.3 \pm 2.5\%$, respectively ($p<0.01$)) and viability using trypan blue uptake test was also significantly higher ($99.4 \pm 0.1\%$ vs. $96.8 \pm 0.5\%$ respectively ($p<0.001$)). All nude mice recipients of HLI-purified islets became normoglycemic within one day ($n=8$) in contrast to recipients of UWI islets (2.3 ± 0.5 days, $p<0.02$). Diabetes was reversed in 5 of 6 rhesus monkeys within 24 hrs after transplantation of HLI islets.
Conclusion: HLI gradients recovered viable porcine islets perfectly. Islets purified by HLI reverse diabetes promptly both in nude mice and rhesus monkeys and seem therefore adequate to be used in ongoing studies designed to clarify the mechanism underlying islet xenograft rejection in primates.

4.31 Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse

AnjuΠre, F. et al
*Blood, **93(2)**, 590-598 (1999)*

Dendritic cells (DC) are highly efficient antigen-presenting cells (APC) that have an essential function in the development of immune responses against microbial pathogens and tumors. Although during the past few years our understanding of DC biology has remarkably increased, a precise characterization of the different DC subpopulations remains to be achieved with regard to their phenotype and lineage relationships. In this report, we have extensively studied the DC subpopulations present in the thymus, spleen, Peyer's patches, lymph nodes (LN) and skin of the mouse. Thymus DC and 60% spleen DC have a lymphoid DC phenotype, i.e., $CD8^+$ DEC-205^{high} Mac-1^{low}, whereas 40% spleen DC have a myeloid DC phenotype, i.e., $CD8^-$ DEC-205^{low} Mac-l^{high}. Both $CD8^+$ and $CD8^-$ DC are leukocyte function-associated antigen-1 (LFA-l)^{high} and highly adherent. Within Peyer's patches the majority of DC correspond to the $CD8^+$ DEC-205^{high} Mac-l^{low} lymphoid category. In the LN, together with $CD8^+$ and $CD8^-$ DC, an additional nonadherent $CD8^{int}$ LFA-l^{int} subpopulation with lymphoid DC characteristics is described. Finally, in the skin both epidermal Langerhans cells (LC) and dermal DC are $CD8^-$ DEC-205^{high} Mac-$1^{high,}$ and do not express LFA-1. Interestingly, LC migrations experiments indicate that LC underwent the upregulation of CD8 and LFA-1 upon migration to the LN, supporting the hypothesis that LC belong to the $CD8^+$ lymphoid lineage.

4.32 $CD8^+$ T cells mediate CD40-independent maturation of dendritic cells in vivo

Ruedl, C., Kopf, M. and Bachmann, M.F.
*J. Exp. Med., **189(12)**, 1875-1883 (1999)*

Induction of cytotoxic T lymphocyte (CTL) responses against minor histocompatibility antigens is dependent upon the presence of T cell help and requires the interaction of CD40 on dendritic cells (DCs) with CD40 ligand on activated T helper cells (Th). This study demonstrates that CD40 is neither involved in Th-dependent nor Th-independent antiviral CTL responses. Moreover, the data show that DC maturation occurs in vivo after viral infection in the absence of CD40 and Th. This maturation did not require viral infection of DCs but was mediated by peptide-specific $CD8^+$ T cells. Surprisingly, naive $CD8^+$ T cells were able to trigger DC maturation within 24 h after activation in vivo *and* in vitro. Moreover, peptide-activated $CD8^+$ T cells were able to induce maturation in trans, as DCs that failed to present the relevant antigen in vivo also underwent maturation. Upon isolation, the in vivo-stimulated DCs were able to convert a classically Th-dependent CTL response (anti-HY) into a Th-independent response in vitro. Thus, antiviral $CD8^+$ T cells are sufficient for the maturation of DCs in the absence of CD40.

4.33 Thrombopoietin-induced activation of the mitogen-activated protein kinase (MAPK) pathway in normal megakaryocytes: role in endomitosis

Rojnuckarin, P., Drachman, J.G. and Kaushansky, K.
*Blood, **94(4)**, 1273-1282 (1999)*

Thrombopoietin (TPO) plays a critical role in megakaryocyte proliferation and differentiation. Using various cultured cell lines, several recent studies have implicated the mitogen-activated protein kinase (MAPK) pathway in megakaryocyte differentiation. In the study reported here, we examined the role played by thrombopoietin-induced MAPK activity in a cytokine-dependent cell line (BAF3/Mpl) and in primary murine megakaryocytes. In both systems, extracellular signal regulated protein kinase (ERK) 1 and 2 MAPK phosphorylation was rapidly induced by TPO stimulation. To identify the Mpl domain responsible for MAPK activation, BAF3 cells expressing truncated forms of the Mpl receptor were studied. Phosphorylation of ERKs did not require elements of the cytoplasmic signaling domain distal to Box 2 and was not dependent on phosphorylation of the adapter protein Shc. ERK activation in murine megakaryocytes was maximal at 10 minutes and was markedly decreased over the subsequent 3 hours. Next, the physiologic consequences of MAPK inhibition were studied. Using the MAPK kinase (MEK) inhibitor, PD 98059, blockade of MAPK activity substantially reduced TPO-dependent proliferation in BAF3/Mpl cells and markedly decreased mean megakaryocyte ploidy in cultures. To exclude an indirect effect of MAPK inhibition on stromal cells in whole bone marrow, $CD41^+$ cells were selected and then cultured in TPO. The number of polyploid megakaryocytes derived from the CD41-selected cells was also significantly reduced by MEK inhibition, as was their geometric mean ploidy. These studies show an important role for MAPK in TPO-induced endomitosis and underscore the value of primary cells when studying the physiologic effects of signaling pathways.

4.34 A novel β_1 integrin-dependent mechanism of leukocyte adherence to apoptotic cells
Schwartz, B.R., Karsan, A., Bombeli, T. and Harlan, J.M.
*J. Immunol., **162**, 4842-4848 (1999)*

Adherence of leukocytes to cells undergoing apoptosis has been reported to be dependent on a variety of recognition pathways. These include $\alpha_v\beta_3$, (CD51/CD61, vitronectin receptor), CD36 (thrombospondin receptor), macrophage class A scavenger receptor, phosphatidylserine translocated to the outer leaflet of apoptotic cell membranes, and CD14 (LPS-binding protein). We investigated the mechanism by which leukocytes adhere to apoptotic endothelial cells (EC). Peripheral blood mononuclear leukocytes and U937 monocytic cells adhered to human or bovine aortic EC induced to undergo apoptosis by withdrawal of growth factors, treatment with the promiscuous protein kinase inhibitor staurosporine, with the protein synthesis inhibitor and protein kinase activator anisomycin, or with the combination of cycloheximide and TNFα. Expression of endothelial adherence molecules such as CD62E (E-selectin), CD54 (ICAM-1), and CD106 (VCAM-1) was not induced or increased by these treatments. A mAb to $\alpha_v\beta_3$, exogenous thrombospondin, or blockade of phosphatidylserine by annexin V did not inhibit leukocyte adherence. Further, leukocyte binding to apoptotic EC was completely blocked by treatment of leukocytes but not EC with mAb tβ_1, integrin. These results define a novel pathway for the recognition of apoptotic cells.

4.35 Regulation of low shear flow-induced HAEC VCAM-1 expression and monocyte adhesion
Mohan, S., Mohan, N., Valente, A.J. and Sprague, E.A.
*Am. J. Physiol., **276**, C1100-C1107 (1999)*

We recently reported that prolonged exposure of human aortic endothelial cells (HAEC) to low shear stress flow patterns is associated with a sustained increase in the activated form of the transcriptional regulator nuclear factor-κB (NF-κB). Here we investigate the hypothesis that low shear-induced activation of NF-κB is responsible for enhanced expression of vascular cell adhesion molecule (VCAM-1) resulting in augmented endothelial cell-monocyte (EC-Mn) adhesion and that this activation is dependent on intracellular oxidant activity. Before exposure to low shear (2 dyn/cm^2) for 6 h, HAEC were preincubated with or without the antioxidants pyrrolidine dithiocarbamate (PDTC) or N-acetyl-L-cysteine (NAC). PDTC strongly inhibited low shear-induced activation of NF-κB, expression of VCAM-1, and EC-Mn adhesion. Paradoxically, NAC exerted a positive effect on low shear-induced VCAM-1 expression and EC-Mn adhesion and only slightly downregulated NF-κB activation. However, cytokine-induced NF-κB activation and VCAM-1 expression are blocked by both PDTC and NAC. These data suggest that NF-κB plays a key role in low shear-induced VCAM-1 expression and that pathways mediating low shear- and cytokine-induced EC-Mn adhesion may be differentially regulated.

4.36 Lipopolysaccharide-activated macrophages stimulate the synthesis of collagen type 1 and C-fibronectin in cultured pancreatic stellate cells
Schmid-Kotsaas, A. Et al
*Am. J. Pathol., **155**, 1749-1758 (1999)*

We have recently identified and characterized pancreatic stellate cells (PSC) in rats and humans (Gastroenterology 1998, 15:421-435). PSC are suggested to represent the main cellular source of extracellular matrix in chronic pancreatitis. Now we describe a paracrine stimulatory loop between human macrophages and PSC (rat and human) that results in an increased extracellular matrix synthesis. Native and transiently acidified supernatants of cultured macrophages were added to cultured PSC in the presence of 0.1% fetal calf serum. Native supernatants of lipopolysaccharide-activated macrophages stimulated the synthesis of collagen type 1 1.38 ± 0.09-fold of control and c-fibronectin 1.89 ± 0.18-fold of control. Transiently acidified supernatants stimulated collagen type I and c-fibronectin 2.10 ± 0.2-fold and 2.80 ± 0.05-fold of control, respectively. Northern blot demonstrated an increased expression of the collagen-I-(α-l)-mRNA and fibronectin-mRNA in PSC 10 hours after addition of the acidified macrophage supernatants. Cell proliferation measured by bromodeoxyuridine incorporation was not influenced by the macrophage supernatants. Unstimulated macrophages released 1.97 pg TGFβ1/μg of DNA over 24 hours and lipopolysaccharide-activated macrophages released 6.61pg TGFβ/μg of DNA over 24 hours. These data together with the results that, in particular, transiently acidified macrophage supernatants increased matrix synthesis, identify TGFβ as the responsible mediator. In conclusion, our data demonstrate a paracrine stimulation of matrix synthesis of pancreatic stellate cells via TGFβ1 released by activated macrophages. We suggest that macrophages might play a pivotal role in the development of pancreas

fibrosis.

4.37 Synaptophysin: a novel marker for human and rat hepatic stellate cells
Cassiman, D. et al
*Am. J. Pathol., **155(6)**, 1831-1839 (1999)*

Synaptophysin is a protein involved in neurotransmitter exocytosis and is a neuroendocrine marker. We studied synaptophysin immunohistochemical expression in 35 human liver specimens (normal and different pathological conditions), in rat models of galactosamine hepatitis and carbon tetrachloride-induced cirrhosis, and in freshly isolated rat stellate cells. Synaptophysin reactivity was present in perisinusoidal stellate cells in both human and rat normal liver biopsies. The number of synaptophysin-reactive perisinusoidal cells increased in pathological conditions. Double staining for α-smooth muscle actin and synaptophysin, detected by confocal laser scanning microscopy, unequivocally demonstrated colocalization of both markers in lobular stellate cells. In addition, freshly isolated rat stellate cells expressed synaptophysin mRNA (detected by polymerase chain reaction) and protein. Finally, electron microscopy showed the presence of small electron translucent vesicles, comparable to the synaptophysin-reactive synaptic vesicles in neurons, in stellate cell projections. We conclude that synaptophysin is a novel marker for quiescent as well as activated hepatic stellate cells. Together with the stellate cell's expression of neural cell adhesion molecule, glial fibrillary acidic protein, and nestin, this finding raises questions about its embryonic origin and its differentiation. In addition, the presence of synaptic vesicles in stellate cell processes suggests a hitherto unknown mechanism of interaction with neighboring cells.

4.38 Persistent activation of nuclear factor - κB in cultured rat hepatic stellate cells involves the induction of potentially novel Rel-like factors and prolonged changes in the expression of IκB family proteins
Elsharkawy, A.M. et al
*Hepatology, **30**, 761-769 (1999)*

Rat hepatic stellate cells (HSC) cultured in serum-containing medium underwent a rapid (3-hour) classical induction of p5O:p65 and p65:p65 nuclear factor-κB (NFκ**B)** dimers. Subsequent culturing was associated with prolonged expression of active p50:p65 and persistent induction of a high-mobility NF-κB DNA binding complex consisting of potentially novel Rel-like protein(s). Formation of the latter complex was competed for by specific double-stranded oligonucleotides, was upregulated by treatment of HSCs with tumor necrosis factor α (TNF-α), and was maintained at basal levels of expression by a soluble HSC-derived factor. An NF-κB-responsive CAT reporter gene was highly active in early cultured HSCs but was also trans-activated at a lower but significant level in longer-term cultured cells and could be completely suppressed by expression of dominant negative IκB-α. Physiological significance of the lower persistent NF-κB activities was also demonstrated by the ability of long-term cultured HSCs to support the activity of the NF-κB-dependent human intercellular adhesion molecule-1 (ICAM-1) promoter. Freshly isolated HSCs expressed high levels of IκB-α and IκB-β. Culture activation was accompanied by a long-term reduction in levels of IκB-α with no detectable expression in the nuclear fraction of cells, under these conditions p50:p65 was detected in the nucleus. IκB-β expression was transiently reduced and, upon replenishment, was associated with appearance of a lower-mobility IκB-β antibody-reactive species. Bcl3 expression was absent in freshly isolated HSC but was induced during culturing and became a persistent feature of the activated HSC. Inhibition of NF-κB DNA binding activity by gliotoxin was associated with increased numbers of apoptotic cells. We suggest that activation of NF-κB in cultured HSC is required for expression of specific genes associated with the activated phenotype such as ICAM-1 and may be antiapoptotic for rat HSCs.

4.39 Dynamic regulation of expression and phosphorylation of Tau by fibroblast growth factor-2 in neural progenitor cells from adult rat hippocampus
Tatebayashi, Y., Iqbal, K. and Grundke-Iqbal, I.
*J. Neurosci., **19(13),** 5245-5254 (1999)*

The nature of the extracellular signals that regulate the expression and the phosphorylation of the microtubule-associated protein tau, which is aberrantly hyperphosphorylated in Alzheimer disease and other adult-onset neurodegenerative diseases, is not known. We have found that neural progenitor cells from adult rat hippocampus express adult isoforms of tau and that the expression and the phosphorylation of tau are regulated by fibroblast growth factor-2 (FGF-2). Astrocytes that are differentiated from these cells by stimulation with ciliary neurotrophic factor express phosphorylated tau similarly when cultured in

the presence of FGF-2. In fetal progenitor cells that express only the fetal tau isotope expression, but not the phosphorylation, of this protein is regulated by FGF-2 in cultures of higher passages. The FGF-2-mediated tau hyper-phosphorylation is inhibited by lithium an inhibitor of glycogen synthase kinase-3 (GSK-3), but not by inhibitors of mitogen-activated protein kinase or the cyclin-dependent kinases. Furthermore, both GSK-3 activity and the phosphorylation of tau increase when the concentration of FGF-2 is increased up to 40 ng/ml. These results demonstrate that proliferating adult rat hippocampal progenitor cells express adult isoforms of tau stably and that FGF-2 upregulates the expression and, by upregulating GSK-3 activity, the phosphorylation of tau.

4.40 Effect of the nonpeptide neurotrophic compound SR57746A on the phenotypic survival of purified mouse motoneurons.
Duong, F.H.T., Warter, J.M., Poindron, P. and Passilly, P.
Br. J. Pharmacol., ***128***, *1385-1392 (1999)*

1 Neurotrophic factors have been used for the treatment of several neurodegenerative diseases. However, their use is limited by their inability to cross the blood-brain barrier, their short half-life and their side effects. SR 57746A is a new orally active compound that exhibits *in vivo* and *in vitro* neurotrophic effects in several experimental models.
2 We show here that SR 57746A (1μM) increases the phenotypic survival of embryonic purified mouse motoneurons *in vitro* to the same extent as brain-derived neurotrophic factor (100 ng ml^{-1}) and increases the outgrowth and number of their neurites. It acts in a dose-dependent manner up to 1 μM which is the optimal concentration. Above this concentration, its neurotrophic effect decreases.
3 Genistein (10 μM), a protein tyrosine kinase inhibitor, also increases the phenotypic survival and differentiation of mouse motoneurons. It does not act in a synergistic or additive manner with SR 57746A. However, at concentrations equal or superior to 25 μM, it decreases the survival of motoneurons. This suggests that the neurotrophic effect of genistein is due to a favourable alteration of equilibrium between phosphorylated and dephosphorylated states of proteins involved in survival and differentiation of motoneurons.
4 Like genistein. SR 57746A should be used at a critical concentration (1 μM) to exert its optimal effects. Since SR 57746A does not act synergistically with genistein. It is likely that its mechanism of action involves a pathway similar to that affected by this tyrosine kinase inhibitor.
5 At the present time, SR 57746A is the only orally active compound and the only synthetic compound shown to be active on motoneurons *in vitro*. It should thus be considered as a good candidate for the treatment of motoneuron diseases.

4.41 Activation and expression of ERK, JNK, and p38 MAP-kinases in isolated islets of Langerhans: implications for cultured islet survival
Paraskevas, S. et al
FEBS Letters, ***455***, *203-208 (1999)*

Isolation and purification of islet cells exposes them to ischemic, osmotic and mechanical stress. The objective of this study was to determine the roles of MAP-kinases in islets immediately following isolation. During the first 48 h, activity of JNK1 and JNK2 declined markedly. Activity of p38 increased steadily with time in culture while extracellular signal regulated kinase (ERK) activity declined dramatically within 24 h post-isolation. High p38 activation relative to ERK activation immediately following isolation correlated with a decrease in islet survival after 36 h in culture. Absence and/or transiency of ERK signaling in conjunction with sustained activation of p38 pathway could be an important regulator of cell death in islets during and following their isolation by commonly employed procedures.

4.42 Comparison of two colloidal silica-based sperm separation media with a non-silica-based medium
Makkar, G., Ng, H-Y-. Yeung, S-B. and Ho, P-C.
Fert. Steril., ***72(5)***, *796-802 (1999)*

Objective: To study and compare the effects of three sperm separation media, two silica-based (Percoll; Pharmacia Biotech AB, Uppsala, Sweden, and Isolate; Irvine Scientific, Santa Ana, CA) and one non–silica-based (Ixaprep; Medicult, Copenhagen, Denmark), on the recovery of progressive motile sperm, the percentage of sperm with normal morphology, various sperm motion characteristics determined by computer-aided sperm analysis, and the percentage of acrosome-reacted sperm.
Design: Prospective study.
Setting: A university-based assisted reproductive technology center.

Patient(s): Male partners of couples attending our infertility clinic.
Intervention(s): None.
Main Outcome Measure(s): Various semen parameters.
Result(s): Both Isolate and Ixaprep resulted in enhanced recovery of motile spermatozoa compared with Percoll. The percentage of sperm with forward progressive motility, the percentage of sperm with normal morphology, and various sperm motion characteristics were similar after the use of Percoll and Isolate and were significantly better than after the use of Ixaprep. The same percentage of acrosome-reacted spermatozoa was observed with all three media. Similar results were observed in both normal and subnormal semen samples.
Conclusion(s): The use of Isolate and Ixaprep resulted in better recovery of motile spermatozoa. Other semen parameters were similar with the use of Isolate and Percoll, whereas the use of Ixaprep was associated with lower sperm velocities and fewer morphologically normal spermatozoa.

4.43 Intensity and mechanisms of in vitro xenorecognition of adult pig pancreatic islet cells by CD_4^+ and CD_8^+ lymphocytes from type I diabetic or healthy subjects
Lalain, S., Chaillous, L., Gouin, E. and Saï, P.
Diabetologia, 42, 330-335 (1999)

The intensity and mechanisms of cell-mediated rejection of pig islet cells were studied in 49 Type I diabetic and 34 healthy subjects. Human peripheral mononuclear cells proliferated strongly in response to pig islet cells ($p < 0.001$), though with notable interindividual variations (stimulation index 2 to 215). The variance of stimulation index was higher in diabetic than healthy subjects ($p < 0.0001$). The response to islet cells was stronger ($p < 0.01$) than that to pig splenocytes. Proliferation in response to islet cells was strongly decreased ($p < 0.01$) when CD_4^+ T cells were blocked with monoclonal antibodies, whereas the blocking of CD_8^+ cells or NK cells gave less pronounced effects. The response to islet cells was decreased ($p < 0.01$), but not abolished, after antigen-presenting cells were removed. Purified CD_4^+ cells alone did not proliferate in response to islet cells but recovered their proliferative ability when mixed with antigen-presenting cells, whereas CD_8^+ cells alone proliferated in the presence of interleukin-2 in response to islet cells. Proliferation was blocked ($p < 0.01$) by anti-DR monoclonal antibodies. During proliferation in response to islet cells, interleukin-10 increased 43-fold ($p < 0.01$) but interferon-<*gamma*> increased only slightly. No statistical differences were detected between diabetic and control subjects with respect to lymphocyte subsets and the recognition mechanisms or to interferon-<*gamma*> / interleukin-10 production in response to islet cells. These results provide the first detailed information on human cell-mediated xenoreaction to pig islet cells. This situation involves a dominant CD_4 class II-restricted Th2 response, with an indirect recognition pathway, as well as a CD_8 T-cell response resulting from direct recognition. This strong reaction constitutes a serious obstacle which may vary in degree among subjects.

4.44 Increased levels of soluble Fcγ receptor III in gingival fluid from periodontal lesions
Yuan, Z-N, Tolo, K., Schenck, K. and Helgeland, K.
*Oral Microbiol. Immunol., **14**, 172-175 (1999)*

Enzyme-linked immunosorbent assay was used for determination of the concentration of soluble Fcy receptor III (FcγRIII) in 40 samples of gingival fluid obtained from periodontal pockets in 30 patients with periodontitis. The assay was based on a monoclonal immobilized antibody binding FcγRIII and a poly-clonal FcγIII rabbit antibody for its quantification. The results indicate a substantially increased concentration of soluble FcγRIII in gingival fluid as compared to the serum level. This increased concentration of soluble FcγRIII may interfere with phagocytosis and immune homeostasis in the periodontal lesions.

4.45 Calcium and ATP regulation of ion transport in larval frog skin
Cox, T.C.
*J. Comp. Physiol. B **169**, 344-350 (1999)*

Ion transport measured as short circuit cur rent (Isc) across the skin of larva! frogs is activated by amiloride, acetylcholine, and ATP. In many epithelia, ATP stimulation of Isc involves an increase in intracellular calcium. To define the role of changes in intracellular calcium in ATP stimulation of Isc in larval frog skin, epithelial cells were loaded with calcium by adding 5μM ionomycin to a 2 mM calcium apical Ringer's solution. Calcium loading had no observable effect on baseline Isc or on stimulation by ATP. Minimizing changes in intracellular calcium by loading the cell with the calcium chelator BAPTA also had no measurable effect on ATP stimulation of Isc. When the apical side was bathed with Ca^{2+} -free

Ringer's solution, ionomycin increased Isc up to 15μA. This increase was partially blocked by 2 mM Ca^{2+}, 2 mM Mg^{2+}, and 10μM W-7. Other experiments showed that baseline-stimulated and ATP-stimulated Isc were always larger in 2 mM Mg^{2+} Ringer's compared to 2 mM Ca^{2+}. In dissociated cells bathed in 2 mM Ca^{2+} Ringer's, ATP had no effect on intracellular calcium as measured by Fluo-LR fluorescence changes. In conclusion, ATP apparently stimulates Isc without concomitant changes in intracellular calcium. This is consistent with a directly ligand-gated receptor at the apical membrane with P2X-like characteristics.

4.46 Circulating vascular endothelial growth factor is not increased during relapses of steroid-sensitive nephritic syndrome

Webb, N.J.A. et al
*Kidney Int., **55**, 1063-1071 (1999)*

Background. An uncharacterized circulating factor that increases vascular permeability has previously been described in childhood steroid-sensitive nephrotic syndrome (SSNS). The aim of this study was to determine whether this factor is vascular endothelial growth factor (VEGF), the recently described endothelial cell mitogen and enhancer of vascular permeability.
Methods. Plasma and urine VEGF levels were measured in children with SSNS in both relapse and remission and in normal age- and sex-matched controls. Semiquantitative reverse transcriptase-polymerase chain reaction studies investigating VEGF mRNA expression were performed on peripheral blood mononuclear cells isolated from children with SSNS in relapse and controls. In two experimental models (one-hour and three-day follow-up postinfusion), Sprague-Dawley rats were intravenously administered 50 μg rVEGF to determine whether this induced either proteinuria or glomerular histologic change.
Results. Plasma VEGF levels and urine VEGF/creatinine ratios were not elevated in SSNS relapse compared with remission and control samples. Peripheral blood mononuclear cell VEGF mRNA expression was no different in SSNS patients compared with controls. The administration of VEGF to rats induced an acute reversible fall in systemic blood pressure but did not result in the development of either proteinuria or glomerular histologic change.
Conclusion, Increased circulating VEGF levels are not responsible for the proteinuria observed during relapses of SSNS. Further studies are warranted to investigate intrarenal VEGF expression.

4.47 Functional and phenotypic analysis of thymic B cells: role in the induction of T cell negative selection

Ferrero, I., Anjuere, F., Martin, P., Martinez del Hoyo, G., Lopez Fraga, M., Wright, N., Varona, R., Marquez, G. and Ardavin, C.
*Eur. J. Immunol., **29(5)**, 1598-1609 (1999)*

The phenotype of mouse thymic B cells and their capacity to induce T cell negative selection in vitro were analyzed. Thymic B cells expressed B cell markers such as IgM, Fcϒ receptor, CD44, heat-stable antigen, LFA-1 and CD40. In addition, they were positive for the activation molecule CD69 and displayed high levels of B7-2. Although thymic B cells expressed CD5 on their surface, no CD5-specific mRNA was detected. Moreover, thymic B cells induced a stronger deletion of TCR-transgenic (TG) thymocytes than splenic B cells, which had low CD69 and B7-2 levels. Interestingly, CD40-activated splenic B cells up-regulated CD69 and B7-2 and acquired a capacity to induce T cell deletion comparable to that of thymic B cells. Moreover, thymic B cells from CD40-deficient mice displayed lower CD69 and B7-2 levels than control thymic B cells, and lower capacity to induce the deletion of TCR TG thymocytes. These results support the hypothesis that CD40-mediated activation of thymic B cells determines a high efficiency of antigen presentation, suggesting that within the thymus B cells may play an important role in the elimination of autoreactive thymocytes.

4.48 Fractionation of differentiating cells using density perturbation

Bildirici, L. and Rickwood, D.
*J. Immunol. Methods, **240 (1-2)**, 93-99 (2000)*

This paper describes the development of a new method for the fractionation of purified subpopulations of partially differentiated cells on continuous isopycnic gradients, using a density perturbation method based on the ability of cells to bind dense antibody-coated beads. Until now none of the available fractionation techniques, such as magnetic cell fractionation has been efficient for separating subpopulations of partially differentiated cells. The fractionation experiments described in this report used promyeolytic HL-60 and DMSO-induced granulocytic HL-60 cells as a model system. Populations of cells, modified by the binding of dense beads were fractionated on isotonic, isopycnic OptiPrep gradients by centrifugation at 220xg for

90 min at 20 degrees C. Examination of the different gradient fractions showed that, as cells bind increasing numbers of beads, they are found in the denser regions of the isopycnic gradients. Indirect immunofluorescence was combined with flow cytometric techniques to characterize the fractionation of partially differentiated cells. Flow cytometric results confirmed that as antigenic determinants appear on the surface at higher levels of expression, the number of beads binding to each cell increased. Furthermore, after fractionation, when the bead-bound and non-bead-bound cells were cultured in the presence of DMSO, those cells that had bound more beads targeted to differentiated cells were found to achieve terminal differentiation faster than those cells that had not been associated with any beads.

4.49 Stromal cell-derived factor-1 (SDF-1) acts together with thrombopoietin to enhance the development of megakaryocytic progenitor cells (CFU-MK)
Hodohara, K., Fujii, N., Yamamoto, N. and Kaushansky, K
*Blood, **95(3)**, 769-775 (2000)*

Stromal cell-derived factor-I (SDF-1) is a CXC chemokine that acts as a stimulator of pre-B lymphocyte cell growth and as a chemoattractant for T cells, monocytes, and hematopoietic stem cells. More recent studies also suggest that megakaryocytes migrate in response to SDF-1. Because genetic elimination of SDF-1 or its receptor lead to marrow aplasia, we investigated the effect of SDF-1 on megakaryocyte progenitors (colony-forming units-megakaryocyte [CFU-MK]). We report that SDF-1 augments the growth of CFU-MK from whole murine bone marrow cells when combined with thrombopoietin (TPO). The addition of SDF-1 to interleukin-3 (IL-3) or stem cell factor (SCF) had no effect. Specific antagonists for CXCR4 (the sole receptor for SDF-1), T22, and 1-9 (P2G) SDF-1 reduced megakaryocyte colony growth induced by TPO alone, suggesting that many culture systems contain endogenous levels of the chemokine that contributes to the TPO effect**.** To examine whether SDF-1 has direct effects on CFU-MK, we developed a new protocol to purify megakaryocyte progenitors. CFU-MK were highly enriched in $CD41^{high}$ $c\text{-}kit^{high}$ cells generated from lineage-depleted TPO-primed marrow cells. Because the growth-promoting effects of SDF-1 were also observed when highly purified populations of CFU-MK were tested in serum-free cultures, these results suggest that SDF-1 directly promotes the proliferation of megakaryocytic progenitors in the presence of TPO, and in this way contributes to the favorable effects of the bone marrow microenvironment on megakaryocyte development.

4.50 Tumor necrosis factor alpha and interleukin 1β up-regulate gastric mucosal Fas antigen expression in *Helicobacter pylori* infection
Houghton, J., Macera-Bloch, L.S., Harrison, L., Kim, K.H. and Korah, R.M.
*Infection and Immunity, **68(3)**, 1189-1195 (2000)*

Fas-mediated gastric mucosal apoptosis is gaining attention as a cause of tissue damage due to *Helicobacter pylori* infection. We explored the effects of *H. pylori* directly, and the effects of the inflammatory environment established subsequent to *H. pylori* infection, on Fas-mediated apoptosis in a nontransformed gastric mucosal cell line (RGM-1). Exposure to *H. pylori*-activated peripheral blood mononuclear cells (PBMCs), but not *H. pylori* itself, induced Fas antigen (Fas Ag) expression, indicating a Fas-regulatory role for inflammatory cytokines in this system. Of various inflammatory cytokines tested, only interleukin lβ and tumor necrosis factor alpha induced Fas Ag expression, and removal of either of these from the conditioned medium abrogated the response. When exposed to Fas ligand, RGM- 1 cells treated with PBMC-conditioned medium underwent massive and rapid cell death, interestingly, with a minimal effect on total cell numbers early on. Cell cycle analysis revealed a substantial increase in S phase cells among cells exposed to Fas ligand, suggesting an increase in their proliferative response. Taken together, these data indicate that the immune environment secondary to *H. pylori* infection plays a critical role in priming gastric mucosal cells to undergo apoptosis or to proliferate based upon their Fas Ag status.

4.51 Upstream tissue inhibitor of metalloproteinases-1 (TIMP-1) element-1, a novel and essential regulatory DNA motif in the human TIMP-1 gene promoter, directly interacts with a 30-kDa nuclear protein
Trim, J.E. et al
*J. Biol. Chem., **275(9)**, 6657-6663 (2000)*

Elevated expression of the tissue inhibitor of metalloproteinases-1 (TIMP-1) protein and mRNA has been reported in human diseases including cancers and tissue fibrosis. Regulation of TIMP-1 gene expression is mainly mediated at the level of gene transcription and involves the activation of several well-known transcription factors including those belonging to the AP-1, STAT, and Pea3/Ets families. In the current

study, we have used DNase-1 footprinting to identify a new regulatory element (5'-TGTGGTTTCCG-3') present in the human TIMP-1 gene promoter. Mutagenesis and transfection studies in culture-activated rat hepatic stellate cells and the human Jurkat T cell line demonstrated that the new element named upstream TIMP-1 element-1 (UTE-1) is essential for transcriptional activity of the human TIMP-1 promoter. Electrophoretic mobility shift assay studies revealed that UTE-1 can form protein-DNA complexes of distinct mobilities with nuclear extracts from a variety of mammalian cell types and showed that induction of a high mobility UTE-1 complex is associated with culture activation of freshly isolated rat hepatic stellate cells. A combination of UV-cross-linking and Southwestern blotting techniques demonstrated that UTE-1 directly interacts with a 30-kDa nuclear protein that appears to be present in all cell types tested. We conclude that UTE-1 is a novel regulatory element that in combination with its cellular binding proteins may be an important component of the mechanisms controlling TIMP-1 expression in normal and pathological states.

4.52 Notch1 deficiency dissociates the intrathymic development of dendritic cells and T cells
Radtke, F. et al
J. Exp. Med., ***191(7)****, 1085-1093 (2000)*

Thymic dendritic cells (DCs) form a discrete subset of bone marrow (BM)-derived cells, the function of which is to mediate negative selection of autoreactive thymocytes. The developmental origin of thymic DCs remains controversial. Although cell transfer studies support a model in which T cells and thymic DCs develop from the same intrathymic pluripotential precursor, it remains possible that these two types of cells develop from independent intrathymic precursors. Notch proteins are cell surface receptors involved in the regulation of cell fate specification. We have recently reported that T cell development in inducible Notchl-deficient mice is severely impaired at an early stage, before the expression of T cell lineage markers. To investigate whether development of thymic DCs also depends on Notchl, we have constructed mixed BM chimeric mice. We report here that thymic DC development from Notchl$^{-/-}$ BM precursors is absolutely normal (in terms of absolute number and phenotype) in this competitive situation, despite the absence of Notchl$^{-/-}$T cells. Furthermore, we find that peripheral DCs and Langerhans cells are also not affected by Notchl deficiency. Our results demonstrate that the development of DCs is totally independent of Notchl function, and strongly suggest a dissociation between intrathymic T cell and DC precursors.

4.53 Phosphorylation of paxillin via the ERK mitogen-activated protein kinase cascades in EL4 thymoma cells
Ku, H. and Meier, K.E.
J. Biol. Chem., ***275(15)****, 11333-11340 (2000)*

Intracellular signals can regulate cell adhesion via several mechanisms in a process referred to as "inside-out" signaling. In phorbol ester-sensitive EL4 thymoma cells, phorbol-12-myristate 13-acetate (PMA) induces activation of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinases and promotes cell adhesion. In this study, clonal EL4 cell lines with varying abilities to activate ERKs in response to PMA were used to examine signaling events occurring downstream of ERK activation. Paxillin, a multifunctional docking protein involved in cell adhesion, was phosphorylated on serine/ threonine residues in response to PMA treatment. This response was correlated with the extent and time course of ERK activation. PMA-induced phosphorylation of paxillin was inhibited by compounds that block the ERK activation pathway in EL4 cells, primary murine thymocytes, and primary murine splenocytes. Paxillin was phosphorylated in vitro by purified active ERK2. Two-dimensional electro-phoresis revealed that PMA treatment generated a complex pattern of phosphorylated paxillin species in intact cells, some of which were generated by ERK-mediated phosphorylation in *vitro*. An ERK pathway inhibitor interfered with PMA induced adhesion of sensitive EL4 cells to substrate. These findings describe a novel inside-out signaling pathway by which the ERK cascade may regulate events involved in adhesion.

4.54 Cooperation among Stat1, glucocorticoid receptor, and PU.1 in transcriptional activation of the high-affinity Fcγ receptor I in monocytes
Aittomaki, S. et al.
J. Immunol., ***164****, 5689-5697 (2000)*

IFN-γ and glucocorticoids regulate inflammatory and immune responses through Stat1 and glucocorticoid receptor (GR) transcription factors, respectively. The biological responses to these polypeptides are determined by integration of various signaling pathways in a cell-type and promoter-dependent manner. In this study we have characterized the molecular basis for the functional cooperation between IFN-γ and

dexamethasone (Dex) in the induction of the high-affinity Fcγ receptor I (FcγRI) in monocytes. Dex did not affect IFN-γ induced Statl DNA binding activity or induce novel DNA-binding complexes to the FCγRI promoter. By using cell systems lacking functional GR or Stat1, we showed that GR stimulated Statl-dependent transcription in a ligand-dependent manner, while Stat1 did not influence GR-dependent transcription. The cooperation required phosphorylation of Tyr^{701}, DNA binding, and the *trans*-activation domain of Stat1, but did not involve Ser^{727} phosphorylation of Stat1 or physical interaction between GR and Statl. The costimulatory effect of Dex was not dependent on a consensus glucocorticoid response element in the Stat1-responsive promoters, but required the DNA-binding and *trans*-activation functions of GR and Dex-induced protein synthesis. GR activated the natural FcγRI promoter construct, and this response required both Statl and the Ets family transcription factor PU.I. Previously, physical association between GR and Stat5 has been shown to enhance Stat5-dependent and suppress GR-dependent transcription. The results shown here demonstrate a distinct, indirect mechanism of cross-modulation between cytokine and steroid receptor signaling that integrates Statl and GR pathways with cell type-specific PU.1 transcription factor in the regulation of FcγRI gene transcription.

4.55 Rebound from nitric oxide inhibition triggers enhanced monocyte activation and chemotaxis
Magazine, H.I., Chang, J., Goumon, Y. and Stefano, G.B.
*J. Immunol., **165**, 102-107 (2000)*

Exposure of human peripheral blood monocytes to the NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) resulted in a rapid shift in cellular conformation of spontaneously activated cells from amoeboid to round. The population of activated cells, ~7.1 ± 1.2%, was reduced 7-fold to 1.1 ± 0.4% following 0.5 h exposure to SNAP. Observation of monocytes for 6 h demonstrated a gradual release from NO inhibition initiating at 2.5 h following SNAP treatment and a period of hyperactivity that was maximal at ~5 h following SNAP exposure. During the rebound from the NO inhibition phase, there was a significant increase in the population of activated monocytes and an increased responsiveness to chemotactic agents such as IL-1, IL-8, and fMLP relative to that of cells treated with the chemotactic agents alone. Conformational changes induced by SNAP were associated with a reduction in F-actin and loss of filopodial extension. The loss and recovery of F-actin staining paralleled changes in cell activity, suggesting that NO may alter cellular activity by modulation of cytoskeletal actin. These data taken together suggest that inhibition of monocyte activity by NO results in an excitatory phase observed subsequent to release from NO inhibition and increased sensitivity to chemotactic agents. We propose that this rebound from NO inhibition may provide increased immunosurveillance to rectify immunological problems that have been encountered during the period of inhibition.

4.56 Sterol 27-hydroxylase acts on 7-ketocholesterol in human atherosclerotic lesions and macrophages in culture
Brown, A.J., Watts, G.F., Burnett, J.R., Dean, R.T. and Jessup, W
*J. Biol. Chem., **275(36)**, 27627-27633 (2000)*

27-Hydroxycholesterol (27OH) is the major oxysterol in human atherosclerotic lesions, followed by 7-ketocholesterol (7K). Whereas 7K probably originates nonenzymically, 27OH arises by the action of sterol 27-hydroxylase, a cytochrome P450 enzyme expressed at particularly high levels in the macrophage and proposed to represent an important pathway by which macrophages eliminate excess cholesterol. We hypothesized and here show that 27-hydroxylated 7-ketocholesterol (27OH-7K) is present in human lesions, probably generated by the action of sterol 27-hydroxylase on 7K. Moreover, [^{3}H]27OH-7K was produced by human monocyte-derived macrophages (HMDMs) supplied with [^{3}H]7K but not in HMDMs from a patient with cerebrotendinous xanthomatosis (CTX) shown to have a splice-junction mutation of sterol 27-hydroxylase. Whereas [^{3}H]27OH-7K was predominantly secreted into the medium, [^{3}H]-27OH formed from [^{3}H]-cholesterol was mostly cell-associated. The majority of supplied [^{3}H]7K was metabolized beyond 27OH-7K to aqueous-soluble products (apparently bile acids derived from the sterol 27-hydroxylase pathway). Metabolism to aqueous-soluble products was ablated by a sterol 27-hydroxylase inhibitor and absent in CTX cells. Sterol 27-hydroxylase therefore appears to represent an important pathway by which macrophages eliminate not only cholesterol but also oxysterols such as 7K. The fact that 7K (and cholesterol) still accumulates in lesions and foam cells indicates that this pathway may be perturbed in atherosclerosis and affords a new opportunity for the development of therapeutic strategies to regress atherosclerotic lesions.

4.57 Differential infectivity and division of *Toxoplasma gondii* in human peripheral blood leukocytes
Channon, J.Y., Seguin, R.M. and Kasper, L.

*Infection and Immunity, **68(8)**, 4822-4826 (2000)*

When tachyzoites were incubated with human peripheral blood leukocytes in vitro, more monocytes and dendritic cells than neutrophils were infected. Although tachyzoites were able to divide in each of these cell types, monocytes and dendritic cells were more permissive to rapid tachyzoite division than neutrophils or lymphocytes.

4.58 Langerhans cells develop from a lymphoid-committed precursor

AnjuΓre, F., Martinez del Hoyo, G., Martin, P. and Ardavin, C
*Blood, **96**, 1633-1637 (2000)*

Langerhans cells (LCs) are specialized dendritic cells (DCs) strategically located in stratified epithelia, such as those of the skin, oral cavity, pharynx, esophagus, upper airways, urethra, and female reproductive tract, which are exposed to a wide variety of microbial pathogens. LCs play an essential role in the induction of T-lymphocyte responses against viruses, bacteria, and parasites that gain access to those epithelial surfaces, due to their high antigen capture and processing potential and their capacity to present antigen peptides to T cells on migration to the lymph nodes. Although LCs have been classically considered of myeloid origin, recent reports which demonstrate that existence of lymphoid DCs derived from multipotent lymphoid precursors devoid of myeloid differentiation potential, raise the question of the lymphoid or myeloid origin of LCs. The present study shows that mouse lymphoid-committed CD^{low} precursors, with the capacity to generate T cells, B cells, $CD8^+$ lymphoid DCs, and natural killer cells, also generate epidermal LCs on intravenous transfer, supporting the view that LC belong to the lymphoid lineage.

4.59 Interferon-α directly represses megakaryopoiesis by inhibiting thrombopoietin- induced signaling through induction of SOCS-1

Wang, Q., Miyakawa, Y., Fox, N. and Kaushansky, K.
*Blood, **96**, 2093-2099 (2000)*

Interferon (IFN)-α has proven useful for treating several clinical conditions, including chronic viral hepatitis and chronic myeloproliferative and lymphoproliferative disorders. In addition to its well-known antiviral effects, the cytokine exerts antiproliferative effects on many cell types, helping to explain its therapeutic usefulness in these latter conditions. However, this same property accounts for several undesirable effects, including thrombocytopenia, which can interfere with the successful clinical application of IFN-α. Unfortunately, the mechanisms responsible for the myelosuppressive effects of the cytokine are incompletely understood. The effects of IFN-α on megakaryocyte (MK) development were studied. Using several marrow cell purification techniques and quantitative culture methods, it was found that IFN-α directly inhibits thrombopoietin (TPO)-induced MK growth. Previous studies indicated that Janus kinase (JAK) and its substrates mediate the effects of TPO on cellular proliferation and survival. It was found that IFN-α directly suppresses TPO-induced phosphorylation of the JAK2 substrates c-Mpl and STAT 5 in a TPO-dependent hematopoietic cell line and of Mpl and STAT3 in primary murine MK. Moreover, IFN-α induces SOCS-1 production in these cells, which has been shown to inhibit TPO-induced cell growth. Because SOCS protein expression is induced by many cytokines and has been reported to extinguish signaling from several hematopoietic cytokine receptors, these results identify a molecular mechanism responsible for cytokine receptor cross-talk.

4.60 Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion

Belcher, J.D., Marker, P.H., Weber, J.P., Hebbel, R.P. and Vercellotti, G.M.
*Blood, **96**, 2451-2459 (2000)*

Sickle cell anemia is characterized by painful vaso-occlusive crises. It is hypothesized that monocytes are activated in sickle cell disease and can enhance vaso-occlusion by activating endothelium. To test this hypothesis, human umbilical vain endothelial cells (HUVEC) and human microvascular endothelial cells (MVEC) with sickle and normal mononuclear leukocytes were incubated, and endothelial activation was measured. Endothelial cells incubated with sickle mononuclear leukocytes were more activated than those incubated with normal mononuclear leukocytes, as judged by the increased endothelial expression of adhesion molecules and tissue factor and the adhesion of polymorphonuclear leukocytes (PMNL). Monocytes, not lymphocytes or platelets, were the mononuclear cells responsible for activating endothelial cells. Sickle monocytes triggered endothelial nuclear factor-kappa B (NF-κB) nuclear translocation. Cell-

to-cell contact of monocytes and endothelium enhanced, but was not required for, activation. Antibodies to tumor necrosis factor-alpha (TNF-α) and interleukin-l-beta (IL-1β) blocked activation of the endothelium by monocytes. Peripheral blood monocytes from patients with sickle cell disease had 34% more IL-1β *(P* = .002) and 139% more TNF-α (*P* = .002) per cell than normal monocytes. Sixty percent of sickle monocytes expressed the adhesion molecule ligand CD11b on their surfaces compared with only 20% of normal monocytes *(P* = .002). Serum C-reactive protein, a marker of systemic inflammation, was increased 12-fold in sickle serum than in normal serum (*P* = .003). These results demonstrate that sickle monocytes are activated and can, in turn, activate endothelial cells. It is speculated that vascular inflammation, marked by activated monocytes and endothelium, plays a significant role in the pathophysiology of vaso-occlusion in sickle cell anemia.

4.61 Concept of lymphoid versus myeloid dendritic cell lineages revisited: both $CD8\alpha^-$ and $CD8\alpha^+$ dendritic cells are generated from $CD4^{low}$ lymphoid-committed precursors
Martin, P. et al
*Blood, **96**, 2511-2519 (2000)*

Two dendritic cell (DC) subsets have been identified in the murine system on the basis of their differential CD8α expression. $CD\alpha^+$ DCs and $CD8\alpha^-$ DCs are considered as lymphoid- and myeloid-derived, respectively, because $CD8\alpha^+$ but not $CD8\alpha^-$ splenic DCs were generated from lymphoid $CD4^{low}$ precursors, devoid of myeloid reconstitution potential. Although $CD8\alpha^-$ DCs were first described as negative for CD4, our results demonstrate that approximately 70% of them are $CD4^+$. Besides $CD4^-$ $CD8\alpha^-$ and $CD4^+$ $CD8\alpha^-$ DCs displayed a similar phenotype and T-call stimulatory potential in mixed lymphocyte reaction (MLR), although among $CD8\alpha^-$ DCs, the $CD4^+$ subset appears to have a higher endocytic capacity. Finally, experiments of DC reconstitution after irradiation in which, in contrast to previous studies, donor-type DCs were analyzed without depleting $CD4^+$ cells, revealed that both $CD8\alpha^+$ DCs and $CD8\alpha^-$ were generated after transfer of $CD4^{low}$ precursors. These data suggest that both $CD8\alpha^+$ and $CD8\alpha^-$ DCs derive from a common precursor and, hence, do not support the concept of the $CD8\alpha^+$ lymphoid-derived and $CD8\alpha^-$ myeloid-derived DC lineages. However, because this hypothesis has to be confirmed at the clonal level, it remains possible that $CD8\alpha^-$ DCs arise from a myeloid precursor within the $CD4^{low}$ precursor population or, alternatively, that both $CD8\alpha^+$ and $CD8\alpha^-$ DCs derive from an independent nonlymphoid, nonmyeloid DC precursor. In conclusion, although we favor the hypothesis that both $CD8\alpha^+$ and $CD8\alpha^-$ DCs derive from a lymphoid-committed precursor, a precise study of the differentiation process of $CD8\alpha^+$ and $CD8\alpha^-$ DCs is required to define conclusively their origin.

4.62 Protective immunity in macaques vaccinated with a modified vaccinia virus Ankara-based measles virus vaccine in the presence of passively acquired antibodies
Stittelaar, K. et al
*J. Virol., **74(9)**, 4236-4243 (2000)*

Recombinant modified vaccinia virus Ankara (MVA), encoding the measles virus (MV fusion (F) and hemagglutinin (H) (MVA-FH) glycoproteins, was evaluated in an MV vaccination-challenge model with macaques. Animals were vaccinated twice in the absence or presence of passively transferred MV-neutralizing macaque antibodies and challenged 1 year later intratracheally with wild-type MV. After the second vaccination with MVA-FH, all the animals developed MV-neutralizing antibodies and MV-specific T-cell responses. Although MVA-FH was slightly less effective in inducing MV-neutralizing antibodies in the absence of passively transferred antibodies than the currently used live attenuated vaccine, it proved to be more effective in the presence of such antibodies. All vaccinated animals were effectively protected from the challenge infection. These data suggest that MVA-FH should be further tested as an alternative to the current vaccine for infants with maternally acquired MV-neutralizing antibodies and for adults with waning vaccine-induced immunity.

4.63 Anatomical origin of dendritic cells determines their life span in peripheral lymph nodes
Ruedl, C., Koebel, P., Bachmann, M., Hess, M. And Karjalainen, K.
*J. Immunol., **165,** 4910-4916 (2000)*

Dendritic cells (DCs) exhibit considerable heterogeneity in their anatomical location, surface phenotype, and functional properties. In this study, we demonstrate that peripheral lymph nodes contain at least four major, functionally separable, and independently derived, DC subsets, which can be clearly demarcated by their CD11c, CD40, and CD8 expression pattern. Surprisingly, all DCs derived directly from the bone

marrow, the myeloid- and the lymphoid-related subsets, turned over fast with $t_{1/2}$ of a couple of days. In contrast, DCs exported from the skin, both dermal and epidermal, accumulated 3- to 4-fold slower, turnover that is dramatically increased by cutaneous inflammation.

4.64 Enteric infection acts as an adjuvant for the response to a model food antigen
Shi, H.N., Liu, Y. and Nagler-Anderson, C.
*J. Immunol., **165**, 6174-6182 (2000)*

Oral administration of soluble protein Ags typically induces Ag-specific systemic nonresponsiveness. However, we have found that feeding a model food protein, OVA, to helminth-infected mice primes for a systemic OVA-specific Th2 response. In this report we show that, in addition to creating a Th2-priming cytokine environment, helminth infection up-regulates costimulatory molecule expression on mucosal, but not peripheral, APCs. To examine the consequences of mucosal infection for the T cell response to orally administered Ag, we adoptively transferred, OVA-specific, T cells into normal mice. We found that helminth infection enhances the expansion and survival of transgenic T cells induced by Ag feeding. Transfer of 5,6-carboxyfluorescein diacetate succinimidyl ester-labeled donor cells showed that T cell proliferation in response to Ag feeding takes place primarily in the mesenteric lymph nodes. Upon subsequent peripheral exposure to Ag in adjuvant, the proliferative capacity of the transferred transgenic T cells was reduced in noninfected mice that had been fed OVA. Helminth infection abrogated this reduction in proliferative capacity. Our data suggests that enteric infection can act as an adjuvant for the response to dietary Ags and has implications for allergic responses to food and the efficacy of oral vaccination.

4.65 Successful suppression of the early rejection of pig islets in monkeys
Rijkelijkhuizen, JK. et al
*Cell Transplant., **9(6)**, 909-912 (2000)*

Primary nonfunction (PNF) is seen very frequently after xenogeneic transplantation of islets of Langerhans. In a pig-to-rat model we recently observed that no PNF occurs when the islets are kept in culture at 37 degrees C for 1-2 weeks prior to transplantation. In order to investigate the rejection mechanisms in a preclinical model, we transplanted cultured porcine islets under the capsule of both kidneys in four cynomolgous monkeys. Islets were isolated from adult sows by means of digestion with Liberase in University of Wisconsin solution (UWS). The digest was purified by a density gradient of OptiPrep in UWS. Highly purified (>95%) islets were cultured 1-2 weeks in RPMI. All monkeys showed significant titers of preformed anti-pig antibodies. The immunosuppression of the monkeys consisted of cyclophosphamide (Cy) (2 days), cyclosporin A (CsA), and prednisolone. Anticipating a fast rejection we carried out nephrectomies at different time points within 2 weeks after transplantation. Following unilateral nephrectomy, well-preserved islets with no signs of rejection were observed between 3 and 7 days posttransplant. Later, between days 11 and 15 posttransplant, histology in the first three animals demonstrated no islets. In the fourth monkey histology on day 11 showed islets with excellent morphology and some small focal infiltrates. The highest CsA blood levels (around 1000 ng/ml) were found in animals with the best graft survival. We conclude that cultured porcine islets can be grafted without hyperacute rejection in monkeys with preformed anti-pig antibodies. In the presence of high levels of CsA only marginal signs of a cellular immune response were observed 11 days after transplantation.

4.66 The LIM and SH3 domain-containing protein, lasp-1, may link the cAMP signaling pathway with dynamic membrane restructuring activities in ion transporting epithelia
Chew, C.S., Parente, J.A., Chen, X., Chaponnier, C. and Cameron, R.S.
*J. Cell Science, **113(11)**, 2035-2045 (2000)*

Lasp-1 is a unique LIM and src homology 3 (SH3) domain containing protein that was initially identified as a 40 kDa cAMP-dependent phosphoprotein in the HCI-secreting gastric parietal cell. Because cAMP is a potent stimulator of parietal cell acid secretion, we have hypothesized that changes in lasp1 phosphorylation might be involved in the regulation of ion transport-related activities, perhaps by modulating interactions among cytoskeletal and/or vesicle-associated proteins. In this study, we demonstrate that the cAMP dependent acid secretary agonist, histamine, induces a rapid, sustained rise in parietal cell lasp-1 phosphorylation and this increase in phosphorylation is closely correlated with the acid secretary response. In addition, elevation of intracellular cAMP concentrations appear to induce a partial redistribution of lasp-1 from the cell cortex, where it predominates along with the γ-isoform of actin in unstimulated cells, to the β-actin enriched, apically-directed intracellular canalicular region, which is the site of active proton transport in the parietal cell. Additional studies demonstrate that although lasp-1

mRNA and protein are expressed in a wide range of tissues, the expression is specific for certain actin-rich cell types present within these tissues. For example, gastric chief cells, which contain relatively little F-actin and secrete the enzyme, pepsinogen, by regulated exocytosis, do not appear to express lasp- 1. Similarly, lasp- 1 was not detected in pancreatic acinar cells, which secrete enzymes by similar mechanisms and also contain relatively low levels of F-actin. Lasp- 1 also was not detectable in proximal tubules in the kidney, in gastrointestinal smooth muscle, heart or skeletal muscle. In contrast, expression was prominent in the cortical regions of ion-transporting duct cells in the pancreas and in the salivary parotid gland as well as in certain F-actin-rich cells in the distal tubule/collecting duct. Interestingly, moderate levels of expression were also detected in podocytes present in renal glomeruli and in vascular endothelium. In primary cultures of gastric fibroblasts, lasp-1 was present mainly within the tips of lamellipodia and at the leading edges of membrane ruffles. Taken together these results support the hypothesis that the lasp-1 plays an important role in the regulation of dynamic actin-based, cytoskeletal activities. Agonist-dependent changes in lasp-1 phosphorylation may also serve to regulate actin-associated ion transport activities, not only in the parietal cell but also in certain other F-actin-rich secretory epithelial cell types.

4.67 SpC3, the complement homologue from the purple sea urchin, *Strongylocentrotus purpuratus*, is expressed in two subpopulations of the phagocytic coelomocytes
Gross, P.S., Clow, L.A., Courtney Smith, L.
*Immunogenetics, **51**, 1034-1044 (2000)*

The lower deuterostomes, including the echinoderms, possess an innate immune system that includes a subsystem with similarities to the vertebrate complement system. A homologue of the central component of this system, C3, has recently been identified in the purple sea urchin, *Strongylocentrotus purpuratus*, and is called SpC3. We determined previously that coelomocytes specifically express the SpC3 gene (*Sp064*); however, the sea urchin has at least four different types of coelomocytes: amoeboid phagocytes, red spherule cells, colorless spherule cells, and vibratile cells. To determine which of these subpopulations expresses *Sp064* and produces SpC3, coelomocytes were separated by discontinuous gradient density centrifugation. Relatively homogenous fractions were obtained consisting of the four major cell types in addition to two types of amoeboid phagocytes with different densities and distinct morphologies. Analysis of proteins from separated cell subpopulations by Western blot and analysis of gene expression by RT-PCR revealed that phagocytes express the gene and contain the protein. Immunolocalization showed that $SpC3^{+}$ phagocytes are present as subsets of both the low- and high-density subpopulations of phagocytes; however, the subcellular localization of SpC3 is different in these two subpopulations.

4.68 Treatment of rat pancreatic islets with reactive PEG
Panza, J.L. et al
*Biomaterials, **21**, 1155-1164 (2000)*

Covalent attachment of polymers to cells and tissues could be used to solve a variety of problems associated with cellular therapies. Insulin-dependent diabetes mellitus is a disease resulting from the autoimmune destruction of the beta cells of the islets of Langerhans in the pancreas. Transplantation of islets into diabetic patients would be an attractive form of treatment, provided that the islets could be protected from the host's immune system in order to prevent graft rejection. If reaction of polyethylene glycol (PEG) segments with the islet surface did not damage function, the immunogenicity and cell binding characteristics of the islet could be altered. To determine if this process damages islets, rat islets have been isolated and treated with protein-reactive PEG-isocyanate (MW 5000) under mild reaction conditions. An assessment of cell viability using a colorimetric mitochondrial activity assay showed that treatment of the islets with PEG-isocyanate did not reduce cell viability. Insulin release in response to secretagogue challenge was used to evaluate islet function after treatment with the polymer. The insulin response of the PEG-treated islets was not significantly different than untreated islets in a static incubation secretagogue challenge. In addition, PEG-isocyanate-treated islets responded in the same manner as untreated islets in a glucose perifusion assay. Finally, the presence of PEG on the surface of the islets after treatment with the amine-reactive N-hydroxysuccinimide-PEG-biotin (not PEG-isocyanate) was confirmed by indirect fluorescence staining. These results demonstrate the feasibility of treating pancreatic islets with reactive polymeric segments and provide the foundation for further investigation of this novel means of potential immunoisolation.

4.69 Multiple drug resistance mutations in human immunodeficiency virus in semen but not blood of a man on antiretroviral therapy

Eyre, R.C., Zheng, G. And Kiessling, A.A.
*Urology, **55**, 591xvii-591xx (2000)*

The concept that the male reproductive tract harbors isolated reservoirs of human immunodeficiency virus (HIV) infection has now been widely accepted. The significance of semen viral burden to sexual transmission of HIV is obvious; however, its contribution to disease progression is unknown. We report a case study that demonstrates the emergence of resistance-conferring mutations to antiviral therapy in infected seminal leukocytes from a man with asymptomatic prostatitis associated with leukospermia. This finding demonstrates the potential importance of male reproductive tract organs to the development of therapy resistance in HIV-infected men.

4.70 Investigation of the suitability of a new purification medium in comparison with Percoll to separate bone marrow

Ruijs, W.P.M., Preijers, F. and Schattenberg, A.
ABS2000, Poster presentation (2000)

Percoll is commonly used to enrich mononuclear cells from bone marrow (BM) prior to Counterflow Centrifugation Elutriation. Since Percoll contains high levels of endotoxins and regulations are increasingly imposing us to use pharmaceutical approved media, we were forced to search for alternatives. Recently Medi-Cult (Denmark) formulated for us a new clinical grade Purification Medium (PM). In contrast to the silica-based Percoll, PM is based on a specific molecular fraction of polysuccrose and iodixanol. We compared Percoll and PM in floatation gradients in order to establish its separation capacity on bone marrow cells. Therefore 20 ml of buffer was pumped in a 250 ml centrifugation bottle followed by 80 ml of 1.073 g/ml Percoll or PM, respectively. After plasma extraction BM was mixed with 90% Percoll or PM, respectively, to obtain a density of 1.085 g/ml. The mixture was pumped beneath the 1.073 g/ml layer and the 250 ml bottles were centrifuged. Different gradient fractions were collected and cell numbers were counted, analyzed in flow cytometry and clonogenic assays (CFU-GM, BFU-E and GFU-GEMM). The differences in numbers of CD34+ cells, T cells (CD4 and CD8), B cells, NK cells (CD3-/CD56), monocytes and granulocytes (CD66e) were determined as well as the number of dead cells (7AAD) in all these populations. Most CD34 cells, lymphocytes and monocytes were found in the 1.073 interfase layers whereas granulocytes were located in the 1.085 interphase layers. Erythrocytes were found in the bottom layers. Although no significant differences were found in the separation profiles of nucleated cells, the best separation of erythrocytes was found with PM.
We conclude that the new Purification Medium can be used as a good alternative for the current gradient separation media in order to isolate mononuclear cells from bone marrow.

4.71 Expression of the HML-1 epitope on human monocytes is independent of αE integrin mRNA

Miller, L.A., Li, C and Hyde, D.M.
*Inflammation, **24(3)**, 195-205 (2000)*

Interferon gamma (IFN γ)-mediated activation of the myelomonocytic cell line HL-60 and peripheral blood monocytes will induce expression of an epitope for the monoclonal antibody which recognizes human αEβ7 integrin, HML-1. Here, we found that the anti-human αEβ7 monoclonal antibody Ber-ACT8 did not recognize IFN γ-stimulated, HML-1 positive HL-60 cells. Culture of blood monocytes with IFN γ also induced expression of HML-1 but not Ber-ACT8 epitopes. Moreover, migration of monocytes across a monolayer of human airway epithelial cells rapidly induced expression of HML-1, with no detectable Ber-ACT8 staining. Using two different sets of primers specific for the human αE integrin gene, we were unable to detect αE mRNA within HL-60 cells or isolated monocytes by reverse transcriptase polymerase chain reaction methods. We conclude that reactivity of the HML-1 antibody for HL-60 cells and monocytes does not correspond with the expression of the αE integrin subunit, and instead detects a marker for cellular activation.

4.72 Monocyte chemotactic protein-1 stimulates the killing of Leishmania major by human monocytes, acts synergistically with IFN-ϒ and is antagonized by IL-4

Ritter, U. and Moll, H.
*Eur. J. Immunol., **30(11)**, 3111-3120 (2000)*

We recently demonstrated that monocyte chemotactic protein-1 (MCP-1) is strongly expressed in lesions of patients with self-healing localized cutaneous leishmaniasis (LCL) whereas it is scarce in those of chronic diffuse cutaneous leishmaniasis (DCL). This finding indicated that MCP-1 may contribute to the healing process. In the present study, we analyzed the capacity of MCP-1 to trigger leishmanicidal activities. The results show that MCP-1 directly stimulates the elimination of intracellular *Leishmania* parasites by human monocytes, a potential that correlates with the induction of reactive oxygen intermediates. Release of NO was not detected. To understand the cross-talk between the chemokine and T cell-associated cytokines, we studied the influence of the Th1 cytokine IFN-ϒ and the Th2 cytokine IL-4 on MCP-1-mediated activation of human monocytes. The data demonstrate that IFN-ϒ and MCP-1 synergistically activate monocytes to clear intracellular parasites, whereas IL-4 abrogates the effect of MCP-1. Furthermore, IL-4 inhibits MCP-1 expression by infected monocytes, a finding that may explain the lack of MCP-1 in chronic lesions. The data suggest a novel model for macrophage activation in cutaneous leishmaniasis. In lesions of LCL, the synergistic action of MCP-1 and IFN-ϒ may stimulate the killing of parasites by macrophages and promote healing, whereas the presence of IL-4 in DCL lesions may favor the suppression of MCP-1 and, together with the lack of IFN-ϒ, the progression of disease.

4.73 Maturation of vulnerability to excitotoxicity: intracellular mechanisms in cultured postnatal hippocampal neurons

Marks, J.D., Bindokas, V.P. and Zhang, X-M.
*Develop. Brain Res., **124**, 101-116 (2001)*

Neuronal vulnerability to excitotoxicity changes dramatically during postnatal maturation. To study the intracellular mechanisms by which maturation alters vulnerability in single neurons, we developed techniques to maintain hippocampal neurons from postnatal rats in vitro. After establishing their neuronal phenotype with immunohistochemistry and electrophysiology, we determined that these neurons exhibit developmentally regulated vulnerability to excitotoxicity. At 5 days in vitro, NMDA-induced cell death at 23 h increased from 3.6% in 3-day-old rats to > 90% in rats older than 21 days. Time-laps imaging of neuronal morphology following NMDA demonstrated differences in peak NMDA-induced [Ca^{2+}] increases between neurons from younger and older animals. However, neurons from older animals were uniformly distinguished from those from younger animals by their subsequent loss of [Ca^{2+}]$_i$ homeostasis. Because of the role of mitochondrial Ca^{2+} buffering in [Ca^{2+}]$_i$ homeostasis, we measured NMDA-induced changes in mitochondrial membrane potential ($\Delta\Psi$) as a function of postnatal age. NMDA markedly dissipated $\Delta\Psi$ in neurons from mature rats, but minimally in those from younger rats. These data demonstrate that, in cultures of postnatal hippocampal neurons (a) vulnerability to excitotoxicity increases as a function of the postnatal age of the animal from which they were harvested, and (b) developmental regulation of vulnerability to NMDA occurs at the level of the mitochondria.

4.74 Is islet transplantation a realistic therapy for the treatment of type 1 diabetes in the near future?

Stevens, R.B., Matsumoto, S. and Marsh, C.
*Clinical Diabetes, **19(2)**, 51-60 (2001)*

Shapiro and colleagues recently reported a 100% cure rate for type 1 diabetes with their “Edmonton protocol” for islet transplantation. This unprecedented success has caused a groundswell of enthusiasm and an unparalleled effort to replicate their experience. It has also raised questions about the clinical reality of this therapy and sparked a dialog about which patients should benefit from receiving this scarce allocated resource. This article reviews the factors contributing to the Edmonton success and obstacles to immediate and long-term expansion of islet transplantation. The authors argue that use of the two-layered method of pancreas preservation will enable the Edmonton protocol to cure diabetes from single and marginal cadaveric donors. A concerted effort will be required to expedite routing of pancreases to islet processing centers and transplant programs. The long-term success and expansion of islet transplantation will depend on not only safer forms of immunosuppression, but also new sources of islet tissue.

4.75 In vivo-matured Langerhans cells continue to take up and process native proteins unlike in vitro-matured counterparts

Ruedl, C., Koebel, P. and Karjalainen, K.
J. Immunol., ***166(12)***, *7178-7182 (2001)*

We have been able to identify the cell subset derived from Langerhans cells in the total dendritic cell population of the peripheral lymph node and hence to follow their trafficking under normal physiological conditions as well as upon skin irritation. As expected, the rapid mobilization of Langerhans cells triggered by inflammatory signals into the draining lymph node correlated with an up-regulation of costimulatory molecules and with an enhanced immunostimulatory capacity. Surprisingly, however, these cells, instead of shutting down, maintain the capacity to capture and process protein. Ags during the couple of days they stay alive in stark contrasts to in vitro-matured dendritic cells.

4.76 The effect of leukocyte-reduction method on the amount of human cytomegalovirus in blood products: a comparison of apheresis and filtration methods

Dumont, L.J. et al
Blood, ***97(11)***, *3640-3647 (2001)*

This study examined the effectiveness of 3 leukocyte-reduction (LR) methods in depleting the residual level of cytomegalovirus (CMV) in blood products measured by quantitative polymerase chain reaction (QA-PCR). At 2 locations over 3 allergy seasons, apheresis platelets and whole blood were collected from 52 healthy CMV seropositive subjects having an elevated titer of CMV DNA (median = 2400 genome equivalents [GE]/mL) resulting in 32 evaluable LR apheresis platelets, 31 filtered platelets from whole blood, and 31 filtered red blood cells (RBCs) from whole blood. Leukoreduction by apheresis and filtration resulted in substantial reduction of detectable CMV DNA levels with 99.9% of the LR products expected to have less than 500 GE/mL of CMV DNA. No difference was found between methods (P = .52). CMV genomic leukocyte subset localization was determined by QA-PCR of fluorescence-activated cell sorter (FACS)-sorted peripheral blood from 20 seropositive subjects (n = 10 > 100 GE/mL, n = 10 QA-PCR negative). CMV was detected in monocyte (13 of 20) and granulocyte (3 of 20) fractions. Presence of competent virus in QA-PCR positive (> 100 GE/mL) peripheral blood samples was verified with 4 of 19 subjects positive in shell vial assay, and 8 of 18 positive for CMV gene products (messenger RNA). We observed a seasonal DNAemia variation in seropositive subjects. CMV seropositive subjects (n = 45) entered into longitudinal monitoring in March/April 1999 were QA-PCR negative at baseline. Subjects converted to a positive QA-PCR coincident with increased seasonal allergen levels (Norfolk 15 of 18 evaluable in 43.4 ± 9.48 days. Denver, 16 of 23 evaluable in 96 ± 26.3 days). These data demonstrate effective reduction of CMV load by LR during periods of DNAemia in CMV seropositive subjects.

4.77 JunD regulates transcription of the tissue inhibitor of metalloproteinases-1 and interleukin-6 genes in activated hepatic stellate cells.

Smart, D. et al
J. Biol. Chem., ***276(26)***, *24414-24421 (2001)*

Activation of hepatic stellate cells to a myofibroblast-like phenotype is the pivotal event in hepatic wound healing and fibrosis. Rat hepatic stellate cells activated in vitro express JunD, and FosB as the predominant AP-1 DNA binding proteins and all three associate with an AP-1 sequence that is essential for activity of the tissue inhibitor of metalloproteinases-1 (TIMP-1) promoter. In this study we used expression vectors for wild type, dominant negative and forced homodimeric (Jun/eb1 chimeric factors) forms of JunD and other Fos and Jun proteins to determine the requirement for JunD in the transcriptional regulation of the TIMP-1 and interleukin-6 (IL-6) genes. JunD activity was required for TIMP-1 gene promoter activity, while over-expression of Fra2 or FosB caused a repression of promoter activity. The ability of homodimeric JunD/eb1 to elevate TIMP-1 promoter activity supports a role for JunD homodimers as the major AP1 dependant trans-activators of the TIMP-1 gene. IL-6 promoter activity was induced with activation of hepatic stellate cells and also required JunD activity, however, expression of JunD/eb1 homodimers resulted in transcriptional repression. Mutagenesis of the IL-6 promoter showed that an AP-1 DNA binding site previously reported to be an activator of transcription in fibroblasts functions as a suppressor of promoter activity in hepatic stellate cells. We conclude that JunD activates IL-6 gene transcription as a heterodimer and operates at an alternative DNA binding site in the promoter. The relevance of these findings to events occurring in the injured liver was addressed by showing that AP-1 DNA binding complexes are induced during HSC activation and contain JunD as the predominant Jun family protein. JunD is therefore an important transcriptional regulator of genes responsive to Jun homo-

and heterodimers in activated hepatic stellate cells.

4.78 Phosphatidylinositol 3-kinase is necessary but not sufficient for thrombopoietin- induced proliferation in engineered Mpl-bearing cell lines as well as in primary megakaryocytic progenitors
Geddis, A.E., Fox, N.E. and Kaushansky, K.
J. Biol. Chem. ***276(37)****, 34473-34479 (2001)*

Thrombopoietin and its receptor (Mpl) support survival and proliferation in megakaryocyte progenitors and in BaF3 cells engineered to stably express Mpl (BaF3/Mpl) (1). The binding of thrombopoietin to Mpl activates multiple kinase pathways, including the Jak/STAT, Ras/Raf/MAPK, and phosphatidylinositol 3-kinase, but it is not clear how these kinases promote cell cycling. Here we show that thrombopoietin induces phosphatidylinositol 3-kinase and that phosphatidylinositol 3-kinase is required for thrombopoietin-induced cell cycling in BaF33/Mpl cells and in primary megakaryocyte progenitors. Treatment of BaF3/Mpl cells and megakaryocytes with the phosphatidylinositol 3-kinase inhibitor LY294002 inhibited mitotic and endomitotic cell cycling. BaF3/Mpl cells treated with thrombopoietin and LY294002 showed both a G1 and a G2 cell cycle block. Expression of constitutively active Akt in BaF3/Mpl cells restored the ability of thrombopoietin to promote cell cycling in the presence of LY294002. Constitutively active Akt was not sufficient to drive proliferation of BaF3/Mpl cells in the absence of thrombopoietin. We conclude that in BaF3/Mpl cells and megakaryocyte progenitors, thrombopoietin-induced phosphatidylinositol 3-kinase activity is necessary but not sufficient for thrombopoietin-induced cell cycle progression. Phosphatidylinositol 3-kinase activity is likely involved in regulating the G1/S transition.

4.79 Human antibodies isolated from plasma by affinity chromatography increase the coxsackievirus B4-induced synthesis of interferon-α by human peripheral blood mononuclear cells *in vitro*
Chehadeh, W., Bouzidi, A., Alm, G., Wattr9, P.and Hober, D.
J. Gen. Virol., ***82****, 1899-1907 (2001)*

Coxsackievirus B4 (CVB4) can be found in circulating blood of patients; however, the interaction of CVB4 with peripheral blood mononuclear cells (PBMCs) is poorly understood. CVB4 induced low levels of IFN-α synthesis in PBMCs from healthy donors. In contrast, preincubation of infectious CVB4 with plasma from these donors containing anti-CVB4 antibodies strongly enhanced the synthesis of IFN-α. IgG obtained from plasma by chromatography formed immune complexes with CVB4 and increased significantly the CVB4-induced production of IFN-α by PBMCs. These antibodies did not have a neutralizing effect on CVB4 infection of Hep-2 cells. The role of CVB and adenovirus receptor (CAR), FcγRII and FcγIII in the increased synthesis of IFN-α induced by CVB4 preincubated with IgG was shown by inhibition with specific antibodies. The major interferon-α-producing cells in response to CVB4-IgG complexes were CD14 cells and monocyte-enriched PBMCs. With the latter, detection of IFN-α by immunostaining was positive whereas in monocyte-depleted PBMCs it was not. This study shows that CVB4-induced synthesis of IFN-α by PBMCs can be enhanced by an antibody-dependent mechanism through interactions between the virus, non-neutralizing antivirus antibodies, FcγII and III and CAR.

4.80 Neurohormonal regulation of secretion from isolated rat stomach ECL cells: a critical reappraisal
Lindstr m, E. and Håkanson, R.
Regulatory Peptides, ***97****, 169-180 (2001)*

ECL cells are endocrine/paracrine cells in the oxyntic mucosa. They produce, store and secrete histamine and chromogranin A-derived peptides such as pancreastatin. The regulation of ECL-cell secretion has been studied by several groups using purified ECL cells, isolated from rat stomachs. Reports from different laboratories often disagree. The purpose of the present study was to re-evaluate the discrepancies by studying histamine (or pancreastatin) secretion from standardized preparations of pure, well-functioning ECL cells. Cells from rat oxyntic mucosa were dispersed by pronase digestion, purified by repeated counter-flow elutriation and subjected to density gradient centrifugation. The final preparation consisted of more than 90% ECL cells (verified by histamine and/or histidine decarboxylase immunochemistry). They were maintained in primary culture for 48 h before they were exposed to candidate stimulants and inhibitors for 30 min after which the medium was collected for determination of mobilized histamine (or pancreastatin). Gastrin-17 and sulphated cholecystokinin octapeptide (CCK-8s) raised histamine secretion 4-fold, the EC_{50} for both peptides being around 100 pM. The neuropeptide pituitary adenylate cyclase activating peptide (PACAP-27) (5-fold increase) and the related neuropeptides vasoactive intestinal

peptide (VIP) and peptide histidine isoleucine (PHI) (3-fold increase) mobilized histamine with similar potency (EC_{50} ranging from 80 to 140 pM). Adrenaline, isoprenaline and terbutaline stimulated secretion by activating β_2 - receptor subtype, while acetylcholine and carbachol were without effect. Secretion experiments were invariably run in parallel with a gastrin standard curve. Somastatin, prostaglandin E_2 (PGE_2) and the PGE_1 congener misoprostol inhibited PACAP- and gastrin-stimulated secretion by more than 90%, with IC_{50} values ranging from 90-720 (somatostatin) to 40-200 (misoprostol) pM. The neuropeptide galanin inhibited secretion by 60-70% with a potency similar to that of somatostatin. Proposed inhibitors such as peptide YY, neuropeptide Y and the cytokines interleukinβ1- and tumor necrosis factorα induced at best a moderate inhibition of gastrin- or PACAP-stimulated secretion at high concentration, while calcitonin gene-related peptide, pancreatic polypeptide and histamine itself were without effect. Inhibition of gastrin- or PACAP-stimulated secretion was routinely compared to a somatostatin standard curve. In conclusion, gastrin, PACAP, VIP/PHI and adrenaline stimulated secretion. Somatostatin and PGE_2 were powerful inhibitors of both gastrin- and PACAP-stimulated secretion; although equally potent, galanin was less effective that somatostatin and PGE_2.

4.81 An investigation into the suitability of silica beads for cell separations based on density perturbation
Bildirici, L. and Rickwood, D.
*J. Immunol. Methods, **252**, 57-62 (2001)*

This study has investigated possible alternative types of beads for fractionating cells on the basis of density perturbation. It is well known that uniform magnetic beads can be extremely important tools for separating cells by both magnetic separation techniques and density perturbation. However, because of the inherent expense associated with the use of magnetic beads, it was decided to study the possible use of inexpensive silica beads for density perturbation in terms of their attachment and modification of density of cells and to compare them with uniform Dynabeads. Silica beads were analyzed to determine their size and effect on the density of cells. Differentiated HL60 cells were used as model system. As differentiation occurs, different levels of antigens are expressed on the cell surface and this results in different numbers of beads binding to cells. DMSO-differentiated HL60 cells were mixed with anti-CD11b-coated beads at a ratio of 20:1 (beads/cell), and gentle mixing was carried out at 20°C on the end-over-end mixer. The binding of antibody-coated silica beads and Dynabeads to partially differentiated HL60 cells were compared.
The conclusions reached on the basis of these experiments are that antibody-coated silica beads (Ab-coated silica) can be used as alternative beads for some cell fractionations. However, compared with Dynabeads, there are more beads that are only transiently associated with cells, possibly indicating that higher levels of detachment of beads from cells occur when silica beads are used. In addition, silica beads are usually heterogeneous in size and this would make it difficult to use these beads for the isolation of purified subpopulations of differentiated cells.

4.82 Culture and regeneration of human neurons after brain surgery
Brewer, G.J. et al
*J, Neurosci Meth., **107**, 15-23 (2001)*

Cortical human brain tissue was obtained from 11 craniotomies for intractable epilepsy or tumor resection. Neuregen transport medium preserved viability at 4^0C during transfer to the culture laboratory. Cells were isolated and cultured by methods previously developed for adult rat neurons (Brewer G.J. Isolation and culture of adult rat hippocampal neurons. J. Neurosci. Meth. 1997: 71: 143-155). In about 40% of the cases, cultures regenerated with a majority of neuron-like cells that stained for neurofilament and not GFAP. After 3 weeks of culture from a 70-year-old meningioma case, synapse-like structures were revealed by electron microscopy. Trophic support from basic human recombinant fibroblast growth was synergistically improved with the steroid hormone dehydroepiandrosterone 3-sulfate. Another 40% of the cases resulted in cultures that were predominantly GFAP positive astroglia. The remaining 20% of the cases did not regenerate cells with neuron-like or glial processes. Three postmortem cases did not regenerate neurites. These methods may aid development of human culture models of epilepsy as well as human pharmacology, toxicology and development of improved methods for brain grafts.

4.83 In vitro culture and synchronous release of *Sarcocystis neurona* merozoites from host cells
Ellison, S.P., Greiner, E. and Dame, J.B.
*Vet. Parasitol., **95**, 251-261 (2001)*

The growth of *Sarcocystic neurona*, isolate UCD1, in continuous culture was examined in 10 cell lines to identify growth conditions and methods for the preparation of parasites free of gross host cell

contamination for molecular studies. The unpredictable, slow release of merozoites in most cell lines prompted development of a method to synchronously release the parasites from infected host cells. The calcium ionophore A23187 at a concentration of 1 μM was found to release intracellular merozoites with a 40 min treatment at 37°C. The release of merozoites en masse from attached host cells allowed for the rapid collection of relatively pure parasites from the culture supernatant. This release of merozoites occurred in five different host cell lines. The ionophore-released parasites were highly infectious for host cells and appeared to be morphologically identical to naturally released merozoites, except that the treated merozoites had an increased number of micronemes when examined by electron microscopy. The ionophore did not enhance the release of sporozoites from sporocysts, but freezing in the presence of 5% DMSO released sporozoites that were infectious to bovine monocytes in *in vitro* culture.

4.84 ***In vitro*** **co-incubation of pig islet cells with xenogeneic human blood mononuclear cells causes loss of insulin release during perifusion: involvement of non-T-cell- and T-cell-mediated mechanism**
Lalain, S., Cl9menceau, B., Gouin, E. and Sai, P.
Human Immunol., ***62****, 607-614 (2001)*

Because the different steps of the human cellular immune rejection of pig islets are still poorly understood, our previous work concerned the intensity and mechanisms of the proliferation of human peripheral mononuclear cells (PBMC) to adult pig islet cells (PIC). As lymphocyte proliferation is not indicative of alteration of PIC, the present *in vitro* study evaluated cell-mediated immune effectors possible involved in impairment of adult PIC. A test was thus developed, based on perfusion analysis of the alteration of insulin release from PIC incubated with different human cells. Compared to PIC incubated alone or with autologous pig splenocytes, seven-day co-incubation with whole human peripheral blood mononuclear cells (PBMC) (n= 18) led to almost complete abolition of basal and stimulated insulin releases ($p < 0.0001$). This effect could not be reversed by extensive sequential washes before perifusion of PIC, and the number of PIC was decreased by 78% after seven-day co-incubation with PBMC. PBMC are a complex mixture of cells involved in different xenogeneic mechanisms, and two components of this PIC impairment were then detected separately. First, the effect of PBMC against PIC was decreased ($p< 0.0001$) after removal of either MHC class II+ or CD14+ cells from PBMC. On the contrary, decreasing effect ($p < 0.001$) on insulin secretion was observed when only plastic-adherent or CD14+ cells were co-incubated with PIC. Additionally, alteration of insulin release from PIC cultured with PBMC or plastic-adherent cells was abolished dose-dependently ($p < 0.0001$ and $p < 0.04$, respectively) by gadolinium chloride (which inhibits macrophages), but not modified by cyclosporin A or mycophenolate mofetil which did not alter insulin release from PIC but blocked the proliferation of PBMC against PIC. A second mechanism was also detected, since co-incubation of PIC with purified human T-cells remixed with antigen-presenting cells led to a decrease ($p < 0.0001$) of insulin release. This model based on the alteration of dynamic basal and stimulated insulin secretion provides detailed account of in vitro human cell-mediated impairment of PIC. It shows that the xenogeneic effect of whole mononuclear cells were strong and rapid. A crucial role was played by MHC class II+, CD14+, and plastic-adherent cells. Two mechanisms appear to be responsible for the role of these cells: 1) early direct effect, potentially involved in vivo in primary nonfunction of islets aggressed by monocytes/macrophages; and 2) the presentation of PIC xenoantigens leading to impairment by T lymphocytes, which may be involved in *in vivo* specific cellular rejection.

4.85 **Isolated rat stomach ECL cells generate prostaglandin E_2 in response to interleukin-1β, tumor necrosis factor-α and bradykinin**
Lindström, E., Lerner, U.H. and Håkanson, R.
Eur. J. Pharmacol., ***416****, 255-263 (2001)*

The ECL cells control parietal cells by releasing histamine in their immediate vicinity. Gastrin and pituitary adenylate-cyclase-activating peptide (PACAP) stimulate histamine secretion from isolated ECL cells, while somatostatin and galanin inhibit stimulated secretion. Prostaglandin E_2 and related prostaglandins likewise suppress ECL-cell histamine secretion. Conceivably, that is how they inhibit acid secretion. In the present study, we examined if prostaglandin E_2 can be generated by isolated ECL cells. Rat stomach ECL cells were purified (> 90% purity) by counterflow elutriation and gradient centrifugation and cultured for 48 h. ECL cell stimulants (gastrin and PACAP) and inflammatory agents (interleukin-1β, tumor necrosis factor-α and bradykinin) were tested for their ability to induce prostaglandin E_2 accumulation (24-h incubation), measured by radioimmunoassay. Gastrin and PACAP did not affect prostaglandin E_2 accumulation but interleukin-1β (300 pg/ml), tumor necrosis factor-α (10 ng/ml) and bradykinin (1 μM) induced a 2-fold to 3-fold increase in the amount of prostaglandin E_2 accumulated. While the combination of interleukin-1β and bradykinin induced a 9-fold increase, the combination

interleukin-1β + tumor necrosis factor-α and bradykinin + tumor necrosis factor-α induced additive effects only. The combination of interleukin-1β + tumor necrosis factor-α + bradykinin did not induce a greater effect than interleukin-1β + bradykinin. The effect of interleukin-1β + bradykinin was abolished by adding 10 nM hydrocortisone (suppressing phospholipase A_2 and cyclooxygenase) or 1 μM indomethacin (inhibiting cyclooxygenase). Incubating ECL cells in the presence of interleukin-1β + bradykinin for 24 h reduced their ability to secrete histamine in response to gastrin. The inhibitory effect was reversed by 1 μM indomethacin. Also, increasing the concentration of hydrocortisone in the medium resulted in an enhanced gastrin-stimulated histamine secretion. Hence, the previously described acid-inhibiting effect of inflammatory agents may be explained by inhibition of ECL-cell histamine mobilization, consequent to enhanced formation of prostaglandin E_2 by cells in the oxyntic mucosa, including the ECL cells themselves.

4.86 Neuregulins increase α7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus
Liu, Y., Ford, B., Mann, M.A. and Fischbach, G.D.
J. Neurosci., ***21***, *5660-5669 (2001)*

Neuregulins are highly expressed in the CNS, especially in the cholinergic neurons. We have examined the effect of neuregulin on nicotinic acetylcholine receptors (nAChRs) in neurons dissociated from the rat hippocampus. Rapid application of acetylcholine (ACh) induced a rapidly rising and decaying inward current in some of the neurons, which was completely blocked by methyllycaconitine, a specific antagonist of the α7 subunit of the nAChR. When the cells were treated with 5 nM neuregulin (NRG1-β1) for 2-4 d, a twofold increase in amplitude of the peak ACh-induced current was observed, and there was a comparable increase in ^{125}I-α-bungarotoxin binding. The fast ACh-induced peak current was prominent in large neurons that also contained GABA immunoreactivity. These presumptive GABAergic neurons constituted ~ 10% of neurons present in 7-to-9-d-old cultures. In addition to the large inward peak current, ACh also evoked transmitter release from presynaptic nerve terminals. Pharmacologic experiments indicated that the shower of PSCs was mediated by glutamate, with a small minority caused by the action of GABA. Chronic exposure to NRG1-β1 increased the amplitude of ACh-evoked PSCs but not the minimum "quantal" PSC. NRG1-β1 also increased the percentage of neurons that exhibited ACh-evoked PSCs.

4.87 The peroxisome proliferator-activated receptor delta promotes lipid accumulation in human macrophages
Vosper, H et al
J. Biol. Chem. ***276***, *44258-44265 (2001)*

The Peroxisome Proliferator Activated Receptors (PPARs) are a family of fatty acid-activated transcription factors which control lipid homeostasis and cellular differentiation. PPARα (NR1C1) controls lipid oxidation and clearance in hepatocytes and PPARγ (NR1C3) promotes preadipocyte differentiation and lipogenesis. Drugs that activate PPARα are effective in lowering plasma levels of lipids and have been used in the management of hyperlipidaemia. PPARγ agonists increase insulin sensitivity and are used in the management of type 2 diabetes. In contrast, there are no marketed drugs that selectively target PPARδ (NR1C2) and the physiological roles of PPARδ are unclear. In this report we demonstrate that the expression of PPARδ is increased during the differentiation of human macrophages in vitro. In addition, a highly selective agonist of PPARδ (compound F) promotes lipid accumulation in primary human macrophages and in macrophages derived from the human monocytic cell line, THP-1. Compound F increases the expression of genes involved in lipid uptake and storage such as the class A and B scavenger receptors (SRA, CD36) and adipophilin. PPARδ activation also represses key genes involved in lipid metabolism and efflux i.e. cholesterol 27-hydroxylase and apolipoprotein E. We have generated THP-1 sub-lines that overexpress PPARδ and have confirmed that PPARδ is a powerful promoter of macrophage lipid accumulation. These data suggest that PPARδ may play a role in the pathology of diseases associated with lipid-filled macrophages, such as atherosclerosis, arthritis, and neurodegeneration.

4.88 Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene
Waldburger, J-M., Suter, T., Fontana, A., Acha-Orbea, H. and Reith, W.
J. Exp. Med., ***194(4)***, *393-406 (2001)*

MHC class II (MHCII) molecules play a pivotal role in the induction and regulation of immune responses. The transcriptional coactivator class II transactivator (CIITA) controls MHCII expression. The CIITA gene

is regulated by three independent promoters (pI, pIII, pIV). We have generated pIV knockout mice. These mice exhibit selective abrogation of interferon (IFN)-γ-induced MHCII expression on a wide variety of non-bone-marrow-derived cells, including endothelia, epithelia, astrocytes, and fibroblasts. Constitutive MHCII expression on cortical thymic epithelial cells, and thus positive selection of CD4+ T cells, is also abolished. In contrast, constitutive and inducible MHCII expression is unaffected on professional antigen-presenting cells, including B cells, dendritic cells, and IFN-γ-activated cells of the macrophage lineage. PIV-/- mice thus allowed precise definition of CTIITA pIV usage in vivo. Moreover, they represent a unique animal model for studying the significance and contribution of MHCII-mediated antigen presentation by nonprofessional antigen-presenting cells in health and disease.

4.89 Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression
Landmann, S. et al
J. Exp. Med., ***194(4)***, *379-391 (2001)*

Cell surface expression of major histocompatibility complex class II (MHCII) molecules is increased during the maturation of dendritic cells (DCs). This enhances their ability to present antigen and activate naive $CD4^+$ T cells. In contrast to increased cell surface MHCII expression, de novo biosynthesis of MHCII mRNA is turned off during DC maturation. We show here that this is due to a remarkably rapid reduction in the synthesis of class II transactivator (CIITA) mRNA and protein. This reduction in CIITA expression occurs in human monocyte-derived DCs and mouse bone marrow-derived DCs, and is triggered by a variety of different maturation stimuli, including lipopolysaccharide, tumor necrosis factor α, CD40 ligand, interferon α, and infection with *Salmonella typhimurium* or Sendai virus. It is also observed in vivo in splenic DCs in acute myelin oligodendrocytes glycoprotein induced experimental autoimmune encephalitis. The arrest in CIITA expression is the result of a transcriptional inactivation of the *MHC2TA* gene. This is mediated by a global repression mechanism implicating histone deacylation over a large domain spanning the entire *MHC2TA* regulatory region.

4.90 Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis
Flugel, A. et al
Immunity, ***14***, *547-560 (2001)*

Homing behavior and function of autoimmune $CD4^+$ T cells in vivo was analyzed before and during EAE, using MBP-specific T cells retrovirally engineered to express the gene of green fluorescent protein. The cells migrate from parathymic lymph nodes to blood and to the spleen. Preceding disease onset, large numbers of effector cells invade the CNS, with only negligible numbers left in the periphery. In early EAE, most (>90%) infiltrating $CD4^+$ cells were effector cells. Migratory effector cells downregulate activation markers (CD25, OX-40) but upregulate several chemokine receptors and absorb MHC class II on their membranes. Within the CNS, the effector cells are reactivated, with upregulated proinflammatory cytokines and downmodulated T cell receptor-associated structures, presumably reflecting autoantigen recognition in situ.

4.91 Characterization of virus-mediated inhibition of mixed chimerism and allospecific tolerance
Williams, M.A. et al
J. Immunol., ***167***, *4987-4995 (2001)*

Simultaneous blockade of the CD28 and CD40 T cell costimulatory pathways has been shown to effectively promote skin allograft survival in mice. Furthermore, blockade of one or both of these pathways has played a central role in the development of strategies to induce mixed hematopoietic chimerism and allospecific tolerance. It has recently been observed that the beneficial effects of CD40 blockade and donor splenocytes in prolonging skin graft survival can be abrogated by some viral infection, including lymphocytic choriomeningitis virus (LCMV). In this study, we shown that LCMV infection prevents allograft survival following CD28/CD40 combined blockade. We further show that LCMV prevents the induction of allospecific tolerance mixed hematopoietic chimerism, while delay of infection for 3-4 wk posttransplant has no effect on tolerance induction. Because of reports of anti-$H2^d$ activity following LCMV infection, we assayed the ability of LCMV-specific T cells to respond to alloantigen at a single cell level. Although we confirm that LCMV infection induces the generation of alloreactive cells, we also demonstrate that LCMV-specific T cells do not divide in response to alloantigen. The alloresponse suppressed by costimulation blockade is restored by LCMV infection and correlates with increased

dendritic cell maturation. We hypothesize that the costimulation blockade-resistant rejection mediated by LCMV could be partly attributable to the up-regulation of alternative co-stimulatory pathways subsequent to LCMV-induced dendritic cell maturation.

4.92 Regulation of E-box DNA binding during in vivo and in vitro activation of rat and human hepatic stellate cells

Vincent, K.J. et al
*Gut, **49**, 713-719 (2001)*

Background – Activation of hepatic stellate cells (HSCs) to a myofibroblastic phenotype is a key event in liver fibrosis. Identification of transcription factors with activities that are modulated during HSC activation will improve our understanding of the molecular events controlling HSC activation.
Aims – To determine if changes in E-box DNA binding activity occur during in vitro and in vivo activation of rat and human HSCs and to investigate mechanisms underlying any observed changes.
Methods – Nuclear extracts were prepared from rat HSCs isolated and cultured from normal and carbon tetrachloride injured rat livers and from HSCs isolated from human liver. EMSA analysis of E-box DNA binding activity was performed on nuclear extracts to determine changes during HSC activation. Western and northern blot analysis of MyoD and Id1 basic helix-loop-helix (bHLH) protein was performed to confirm expression in HSC.
Results – HSC activation was associated with inducible expression of two low mobility E-box binding complexes that were immunoreactive with an anti-MyoD antibody. MyoD and mRNA expression was found at similar levels in freshly isolated and activated HSCs. Activation of rat HSCs was accompanied by reduced expression of the inhibitory bHLH protein Id1.
Conclusions – In vitro and in vivo activation of rat and human HSCs is accompanied by induction of MyoD binding to E-box DNA sequences which appears to be mechanistically associated with elevated MyoD protein expression and reduced expression of the inhibitory Id1 protein. Clarification of the role of MyoD and Id1 proteins in HSC activation and liver fibrogenesis is now required.

4.93 Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors

Cassiman, D., Denef, C., Desmet, V.J. and Roskams, T.
*Hepatology, **33**, 148-158 (2001)*

The expression of neurotrophins and neurotrophin receptors in non-neural tissue is related to tissue remodeling, differentiation, proliferation and migration of target cells. The literature yields contradictory results on neurotrophin and neurotrophin receptor expression in the liver. We show immunoreactivity to antibodies to nerve growth factor (NGF), brain-derived neurotrophin (BDNF), neurotrophin 3 (NT-3), neurotrophin 4/5 (NT-4/5), the low-affinity nerve growth factor receptor p75 and the high-affinity tyrosine kinase receptors (Trk) B and C in hepatic stellate cells and weak reactivity for BDNF, NT-3, and NT-4/5 in hepatocytes, in cryosections of human and rat liver, in normal and varying pathologic conditions. Immunoreactivity is unequivocally localized to hepatic stellate cells by double staining with α-smooth muscle actin (α-SMA) and desmin, studied by confocal laser scanning microscopy. Finally, the presence of mRNA transcripts for the different neurotrophins and neurotrophin receptors, with the exception of Trk-B, is shown by reverse transcription polymerase chain reaction (RT-PCR) on RNA extracted from freshly isolated rat hepatic stellate cells, compared with hepatocyte RNA. Hepatocyte RNA was found to contain BDNF, NT-3, NT-4/5 mRNA (which is compatible with the immunohistochemical findings) and Trk-A mRNA. In conclusion, hepatic stellate cells are a source of several neurotrophins in the liver and they express neurotrophin receptors. These findings correspond with the known involvement of hepatic stellate cells in tissue remodeling, their production of extracellular matrix components and their proliferation in acute necrotizing liver pathology. In analogy with findings in other organs and systems, neurotrophins are hypothesized to play a role in the pathophysiology of liver disease.

4.94 Differential regulation of transendothelial migration of THP-1 cells by ICAM-1/LFA-1 and VCAM-1/VLA-4

Ronald, J.A., Ionescu, C.V., Rogers, K.A. and Sandig, M.
*J. Leukoc. Biol., **70**, 601-609 (2001)*

The adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expressed in atherogenic lesions are thought to regulate monocyte diapedesis. To better understand their specific roles we used function-blocking antibodies and examined in a culture model the morphology, motility, and diapedesis of THP-1 cells interacting with human coronary artery endothelial

cells. The number of motile THP-1 cells was reduced only when VCAM-1 or both ICAM-1 and VCAM-1 were blocked. Blockade of ICAM-1 and VCAM-1, either separately or together, reduced to the same degree the distance that THP-1 cells traveled. Diapedesis was reduced only during the simultaneous blockade of both adhesion molecules. Blockade of either ICAM-1 or VCAM-1 inhibited pseudopodia formation, but ICAM-1 blockade induced the formation of filopodial. We suggest that the interactions of endothelial ICAM-1 and VCAM-1 with their ligands differentially regulate distinct steps of diapedesis by modulating the ratio of active and inactive forms of small GTPases such as Rho, Rac, and Cdc42.

4.95 Effect of 6-hydroxydopamine on host resistance against *Listeria monocytogenes* infection
Miura, T et al
*Infection and Immunity, **69(12)**, 7234-7241 (2001)*

Recent studies have shown that immunocompetent cells bear receptors of neuropeptides and neurotransmitters and that these ligands play roles in the immune response. In this study, the role of the sympathetic nervous system in host resistance against *Listeria monocytogenes* infection was investigated in mice pretreated with 6-hydroxydopamine (6-OHDA), which destroys sympathetic nerve termini. The norepinephrine contents of the plasma and spleens were significantly lower in 6-OHDA-treated mice than in vehicle-treated mice. The 50% lethal dose of *L. monocytogenes* was about 20 times higher for 6-OHDA-treated mice than for vehicle-treated mice. Chemical sympathectomy by 6-OHDA upregulated interleukin-12 (IL-12) and tumor necrosis factor-alpha (TNF-α) production in enriched dendritic cell cultures and gamma interferon (IFNγ) and TNF-α production in spleen cell cultures, whereas chemical sympathectomy had no apparent effect on phagocytic activities, listericidal activities, and nitric oxide production in peritoneal exudates cells and splenic macrophages. Augmentation of host resistance against *L. monocytogenes* infection by 6-OHDA was abrogated in IFN-$\gamma^{-/-}$ or TNF-$\alpha^{-/-}$ mice suggesting that upregulation of IFNγ, IL-12, and TNF-α production may be involved in 6-OHDA-mediated augmentation of antilisterial resistance. Furthermore, adoptive transfer of spleen cells immune to *L. monocytogenes* from 6-OHDA-treated mice resulted in untreated naive recipients that had a high level of resistance against *L. monocytogenes* infection. These results suggest that the sympathetic nervous system may modulate host resistance against *L. monocytogenes* infection through regulation of production of IFN-γ, IL-12, and TNF-α, which are critical in antilisterial resistance.

4.96 Differential regulation of calcitonin secretion in normal and neoplastic pulmonary neuroendocrine cells in vitro
Pu, F.R., Manning, F.C.R., Brannigan, A.E. and Crosby, S.R.
*Exp. Lung Res., **27**, 689-703 (2001)*

Within the mammalian lung, cells with a neuroendocrine phenotype are few in number and are sparsely distributed. In contrast, neuroendocrine neoplasms represent a major group of lung cancers. The aim of this study was to develop a model of mammalian PNECs and to compare glucocorticoid regulation of calcitonin secretion in normal and neoplastic cells with neuroendocrine differentiation. Cell cultures of PNECs were initiated after the disaggregation of neonatal hamster lungs with 0.1% collagenase and fractionation of the resultant cell suspension on a gradient of **iodixanol** (1.320 g/ml). Cell fractions enriched in PNECs were identified by positive staining for 5-hydroxytryptamine and the presence of calcitonin. Calcitonin secretion was investigated after exposure to hydrocortisone (0 to 1000 nM). A dose-dependent inhibition of calcitonin secretion was seen after 7 days between 10 nM (55% of control), and 1000 nM (29%) hydrocortisone. Cell cultures grown in the presence of hydrocortisone also contained significantly fewer PNECs between 10 nM (90% of control), and 1000 nM (45%). Human bronchial carcinoid cells (NCIH727) cultured under identical conditions showed a similar inhibition of calcitonin secretion between 10 nM (53%) and 1000 nM (52%), although at these concentrations, no reduction in cell number was seen. In contrast, 2 human small cell lung cancer cell lines (DMS-79 and COR-L24 cells) showed no dose-dependent inhibition of calcitonin secretion and no effect on cell proliferation in response to hydrocortisone. These results show that enriched cultures of mammalian PNECs can be used to investigate functional aspects of their biology, including peptide secretion in response to potential regulators. Furthermore, calcitonin secretion is inhibited in normal PNECs and bronchial carcinoid cells at physiological concentrations of glucocorticoids, but this feature appears not to be present in the 2 more invasive neuroendocrine neoplasms (small cell lung cancer cells) investigated in this study.

4.97 A population of PC12 cells that is initiating apoptosis can be rescued by nerve growth factor
Francois, F., Godinho, M.J., Dragunow, M. and Grimes, M.L.
*Mol. Cell. Neurosci., **18**, 347-362 (2001)*

Programmed cell death, or apoptosis, occurs asynchronously in neuronal cells. To overcome this asynchrony, rat pheochromocytoma (PC12) cells were separated at different stages of apoptosis on the basis of cell density. Live cells that exhibited no apoptotic features floated to the top of density gradients. The most dense cells showed extensive loss of cytochrome C from mitochondria, caspase activation, chromatin condensation, and DNA fragmentation. These cells were committed to apoptosis and could not be rescued by reculturing in with nerve growth factor (NGF). Cells of intermediate density displayed no DNA fragmentation, but had begun to show cytochrome C loss, caspase activation, and chromatin condensation. This population displayed upregulation of the prodeath factor, c-Jun, and downregulation of prosurvival kinase, Akt. Importantly, apoptosis was reversible by NGF in this population. These studies suggest that increased cell density correlates with an initial step in the apoptosis mechanism that precedes irreversible commitment to suicide.

4.98 Age-related differences in NMDA responses in cultured rat hippocampal neurons
Cady, C., Evans, M.S. and Brewer, G.J.
Brain Res., ***921***, *1-11 (2001)*

The N-methyl-D-aspartate receptor (NMDAR) is expressed in the cerebral cortex and hippocampus and is important in learning and memory. NMDARs are influenced by aging and implicated in neurodegenerative disorders. We investigated age-related differences in NMDAR ionic currents and intracellular calcium in embryonic (E18), middle-age (9-10 months) and old (26 months) rat hippocampal neurons cultured in serum-free medium for 7-12 days. Responses to 200 uM NMDA with 50 uM glycine were measured using whole cell voltage clamp and fura-2 fluorescence. Embryonic neurons exhibited significantly larger and faster NMDA responses than adults. Old rats had 1.5 fold greater normalized NMDA peak current compared to middle-age rats, while intracellular calcium rose 1.3 fold higher. Differences in regression slopes generated from the integral of NMDA current versus normalized NMDA current indicate age-related differences are not exclusively due to changes in receptor density but likely influenced by changes in receptor function. Corresponding age-related measures of intracellular calcium by fura-2 fluorescence in response to NMDA showed a strong correlation with peak current ($r_2 = 0.996$). Our data support the hypothesis that NMDAR responsiveness is altered during aging with an enhanced NMDA peak current in both old and embryonic neurons compared to middle-age neurons.

4.99 Highly efficient isolation of porcine islets of Langerhans for xenotransplantation: numbers, purity, yield and in vitro function
Krickhahn, M., Meyer, T., Buchler, C., Thiede, A. and Ulrichs, K.
Ann. Transplant., ***6(3)***, *48-54 (2001)*

Xenogeneic transplantation of porcine islets of Langerhans is regarded as a future treatment for diabetes mellitus. Despite considerable biotechnological progress, however, it is still very difficult and often unreliable to isolate sufficient numbers of highly purified, intact islets from the porcine pancreas with good in vitro function.
OBJECTIVE: Of this study was to describe an efficient and reliable method to isolate sufficient numbers of highly purified islets of Langerhans with good in vitro function from adult as well as from young hybrid pigs.
METHODS: Islets were isolated from the pancreas of young (4-6 months) hybrid pigs and old (2-3 years) retired breeders using Liberase PI and digestion-filtration. Average islet size was detected by dithizone staining of tissue sections prior to isolation; only organs with an average islet size > or = 200 micron were used. Density gradient purification with **OptiPrep** was performed in a COBE 2991 cell processor. Viability was investigated using fluorescence staining. Perifusion studies were carried out to assess in vitro function of isolated islets.
RESULTS: Islets were successfully isolated from young hybrid pigs (3,671 ± 598 IEQ/g) and old retired breeders (5,182 ± 545 IEQ/g). After purification islet purity was 92% for retired breeders and 87% for young hybrid pigs. Yield after purification was still not satisfactory: 64% for retired breeders (3,209 ± 444 IEQ/g) and 44% for young hybrid pigs (1,669 ± 386 IEQ/g). Viability of isolated islets was 80-95%. Perifusion studies of porcine islets showed sufficient insulin release upon glucose challenge; however, the level of insulin release depended on the density of islets within the perifusion chamber. Low temperature culture (24°C) prior to perifusion studies had no detrimental effect on insulin release. Long-term culture over 11 days was followed by a dramatic loss of islet function.
CONCLUSION: If xenograft rejection can be overcome and the risk of xenosis can be minimised, sufficient numbers of purified porcine islets with good in vitro function can be isolated to serve as a

potential source for islet transplantation in diabetic patients.

4.100 Septic shock and acute lung injury in rabbits with peritonitis

Matute-Bello, G. et al

Am. J. Respir. Crit. Care Med., ***163***, *234-243 (2001)*

The major goal of this study was to investigate the mechanisms that link the host response to a local infection in the peritoneal cavity with the development of sepsis and lung injury. Rabbits were infected by intraperitoneal inoculation of fibrin clots containing *E.coli* cells at 10^8, 10^9, or 10^{10} cfu/clot. Physiologic, bacteriologic, and inflammatory responses were monitored, and the lungs were examined postmortem. At a dose of 10^8 cfu/clot the animals had resolving infection, and a dose of 10^9 cfu/clot resulted in persistent infection at 24h, with minimal systemic manifestations. In contrast, inoculation at 10^{10} cfu/clot resulted in rapidly lethal local infection, with septic shock and lung injury. The onset of septic shock was associated with a paradoxical lack of identifiable polymorphonuclear leukocytes (PMN; neutrophils) in the peritoneal cavity. The absence of PMN in the peritoneum was due in part to lysis of intraperitoneal PMN, because the peritoneal fluids contained free myeloperoxidase and induced rapid death of normal rabbit PMN in vitro. Although most animals became bacteremic, only those with a severe systemic inflammation response developed lung injury. These data show that control of an infection in the first compartment in which bacteria enter the host is a critical determinant of the systemic response. Above a threshold dose of bacteria, failure of the local neutrophil response is a key mechanism associated with deleterious systemic responses. Bacteremia alone is not sufficient to cause lung injury. Lung injury occurs only in the setting of a severe systemic inflammatory response and an inadequate leukocyte response at the primary site of infection.

4.101 Gravity sensing in moss protonemata

Sack, F.D., Schwuchow, J.M., Wagner, T. and Kern, V.
Adv. Space Res., ***27(5)***, *871-876 (2001)*

Moss protonemata are a valuable system for studying gravitropism because both sensing and upward curvature (oriented tip growth) take place in the same cell. We review existing evidence, especially for *Ceratodon purpureus*, that addresses whether the mass that functions in sensing is that of amyloplasts that sediment. Recent experiments show that gravitropism can take place in media that are denser than the apical cell. This indicates that gravity sensing relies on an intracellular mass rather than that of the entire cell and provides further support for the starch-statolith hypothesis of sensing. Possible mechanisms for how amyloplast mass functions in sensing and transduction are discussed.

4.102 Origin and differentiation of dendritic cells

Ardivin, C. et al.
TRENDS Immunol., ***22(12)***, *691-700 (2001)*

Despite extensive, recent research on the development of dendritic cells (DCs), their origin is a controversial issue in immunology, with important implications regarding their use in cancer immunotherapy Although, under defined experimental conditions, DCs can be generated from myeloid or lymphoid precursors, the differentiation pathways that generate DCs *in vivo* remain unknown largely. Indeed, experimental results suggest that the *in vivo* differentiation of a particular DC subpopulation could be unrelated to its possible experimental generation. Nevertheless, the analysis of DC differentiation by *in* vivo and *in vitro* experimental systems could provide important insights into the control of the physiological development of DCs and constitutes the basis of a model of common DC differentiation that we propose.

4.103 Preptin derived from proinsulin growth factor II (preIGF-II) is secreted from pancreatic islet β-cells and enhances insulin secretion

Buchanan, C.M., Phillips, A.R.J. and Cooper, G.J.S.
Biochem. J., ***360***, *431-439 (2001)*

Pancreatic islet β-cells secrete the hormones insulin, amylin and pancreastatin. To search for further β-cell hormones, we purified peptides from secretory granules isolated from cultured murine βTC6-F7 β-cells. We identified a 34-amino-acid peptide (3948 Da), corresponding to Asp^{69} –Leu^{102} of the proinsulin-like growth factor II E-peptide, which we have termed ‘preptin’. Preptin is present in islet β-cells and undergoes glucose-mediated co-secretion with insulin. Synthetic preptin increases insulin secretion from glucose-stimulated βTC6-F7 cells in a concentration-dependent and saturable manner. Preptin infusion into the isolated, perfused rat pancreas increases the second phase of glucose-mediated insulin secretion by 30% while antipreptin immunoglobulin infusion decreases the first and second phases of insulin secretion by 29 and 26% respectively. These findings suggest that preptin is a physiological amplifier of glucose-mediated insulin secretion.

4.104 Low-speed isopycnic islet separation is effeictive and yields islets with superior quantity and quality
Sageshima, S.S. et al
IPITA 2001 abstracts P3-06 (2001)

Background: The inconsistency of islet processing remains the major obstacle to the application of islet transplantation. This study addresses the impact of centrifugal force (CF) applied during isopycnic islet separation. Method: Islets were purified in an alternate fashion using low (100G) and standard (800 G) CF on a Cobe2991 (n=18). Continuous iodixanol gradients with low viscosity were used in both groups. The low and standard CF-purified islet were compared with respect to islet enumeration, purity, islet equivalent/islet count (IE/IC), and glucose-stimulated insulin release in vitro. The posttransplant function of low CF-purified islets was evaluated in diabetic pigs using the small bowel intramuscular implantation site.
Results: The %recovery and purity of low CF-purified islets were significantly higher compared to islets separated at standard CF (118 vs. 100%, 90.3 vs. 88.8%). The insulin secretory response to glucose was higher for islets separated at low vs. standard CF (2.2 vs. 1.2). At standard CF, the IE/IC after purification was significantly lower compared to that before purification (0.73 vs. 0.84), which is suggestive of islet fragmentation. In contrast, there was no significant difference in IE/IC before and after islet separation at low CF (0.73 vs. 0.73). Low CF-purified islets consistently reversed diabetes in the pig allotransplant model with a follow-up greater than 30days in all immunosuppressed pigs.
Conclusions: Low CF-purified islet separation yields islets with superior results compared to standard method with respect to both islet recovery and quality. Low CF islet separation represents an improvement and warrants evaluation for human islet separation.

4.105 Human islet cell transplantation – future prospects
White, S.A., James, R.F.L., Swift, S.M., Kimber, R.M. and Nicholson, M.L.
*Diabetic Med., **18**, 78-103 (2001)*

Background Islet transplantation has the potential to cure diabetes mellitus. Nevertheless despite successful reversal of diabetes in many small animal models, the clinical situation has been far more challenging. The aim of this review is to discuss why insulin-independence after islet allotransplantation has been so difficult to achieve.

Methods A literature review was undertaken using Medline from 1975 to July 2000. Results reported to the International Islet Transplant Registry (ITR) up to December 1998 were also analysed.

Results Up to December 1998, 405 islet allotransplants have been reported the ITR. Of those accurately documented between 1990 and 1998 *(n* = 267) only 12% have achieved insulin-independence (greater than 7 days). However with refined pen-transplant protocols insulin independence at 1 year can reach 20%.

Conclusions There are many factors which can explain the failure of achieving insulin-independence after islet allotransplantation. These include the use of diabetogenic immunosuppressive agents to abrogate both islet allo-immunity and auto-immunity, the critical islet mass to achieve insulin-independence and the detrimental effects of transplanting islets in an ectopic site. However recent evidence most notably from the Edmonton group demonstrates that islet allotransplantation still has great potential to become an established treatment option for diabetic patients.

4.106 Innate IFN-γ production in cattle in response to MPP14, a secreted protein from *Mycobacterium avium* subsp. *paratuberculosis*
Olsen, I. and Storset, A.K.
Scand. J. Immunol., ***54***, *305-313 (2001)*

Calves experimentally infected with *Mycobacterium avium* subsp. *paratuberculosis* and uninfected calves were tested for interferon(1FN)-γ production after stimulation with purified protein derivative from *M. avium* subsp. *paratuberculosis* (PPDp) or a secreted 14 kDa protein (MPP14) specific for the *M. avium-intracellularescrofulaceum* (MAIS) complex. Several calves in both groups responded strongly up to about *5* months to both antigens. Two uninfected calves responded repeatedly, but not always, to MPPI4 and PPDp throughout the study. The responses in the uninfected animals seemed to be independent of cell contact between the antigen presenting cells (APC) and the responding population. The supernatant from adherent cells stimulated with MPPI4 induced similar levels of IFN-γ production in $CDl4^+$/B-ce11 depleted peripheral blood mononuclear cells (PBMC) as when the antigen was used directly on PBMC. In contrast, APCiT-cell contact was necessary to induce the IFN-γ production in infected animals, suggesting that both innate and adaptive IFN-γ production in response to MPP14 could occur. $CD8^+$ cells contributed to some of the 1FN-γ production in response to MPP14, but the rest could not be explained, while $CD4^+$ cells were responsible for the adaptive response to PPDp. This study showed that secreted proteins could induce innate IFN-γ production that interferes with diagnostic testing using the IFN-γ-test.

4.107 A microfluidic device for measuring cellular membrane potential
Farinas, J., Chow, A.W. and Wada, H.G.
Anal. Biochem., ***295***, *138-142 (2001)*

Recent developments in microfluidics have enabled the design of a lab-on-a-chip system capable of measuring cellular membrane potential. The chip accesses liquid samples sequentially by sipping from a micro-plate through a capillary, mixes the samples with cells flowing through a microchannel, contacts the cells with potential-sensitive dyes, and reads out cellular responses using fluorescence detection. The rate of cellular uptake of membrane-permeable, ionic fluorophores by THP-1 cells was found to depend strongly on membrane potential. The ratio of the fluorescence of the anionic dye $DiBAC_4(3)$ and the cationic dye Syto 62 taken up by cells was found to double for every 33 mV change in membrane potential. The utility of this approach was demonstrated by assaying ion channel activity in human T lymphocytes. Because of the high sensitivity, low cellular and reagent consumption, and high data quality obtained with the microfluidic device, the lab-on-a-chip system should be widely applicable in high-throughput screening and functional genomics studies.

4.108 Co-incubation of pig-islet cells with spleen cells from non-obese mice causes decreased insulin release by non-T-cell- and T-cell-mediated mechanisms

You, S., Rivereau, A-S., Gouin, E. and Sai, P.

Clin. Exptl. Immunol., ***125***, *25-31 (2001)*

In vitro studies were conducted in the non-obese diabetic (NOD) mouse, prone to Type 1 autoimmune diabetes, to investigate the mechanisms involved in cell-mediated rejection of pig islet xenografts. Our previous work concerning the mechanisms of proliferation of xenogeneic lymphocytes to pig islet cells (PIC) was not indicative of PIC impairment. Consequently, a test was developed based on perfusion analysis of the alteration of basal and stimulated insulin release from adult PIC incubated with mouse splenocytes or subsets. Compared with PIC incubation alone or with syngeneic pig splenocytes, co--incubation with mouse whole spleen cells resulted in a decrease of basal and stimulated insulin release ($P < 0.001$). Two components of this alteration were detected separately: PIC impairment was decreased ($P < 0.01$) after removal of plastic-adherent cells from spleen cells, but maintained ($P < 0.01$) when plastic-adherent cells alone were co-incubated with PIC. The increase of murine interleukin-lβ when mouse plastic-adherent spleen cells were cultured with PIC ($P < 0.04$) was indicative of macrophage activation. Soluble factors produced during co-incubation of mouse splenocytes or plastic-adherent cells with PIC were involved in the impairment process, since supernatant fluids collected during previous PIC—mouse cell co-incubations directly altered ($P < 0.01$) insulin release from PIC. Moreover, impairment of PIC by mouse spleen cells was abolished ($P < 0.01$) by gadolinium chloride (which inhibits macrophages), but not by cyclosporin A. Another mechanism was apparent, since co-incubation of PIC with purified mouse T cells or $CD4^+$ T cells, re-mixed with antigen-presenting cells, led to a decrease ($P < 0.01$) of insulin release. This model, based on the alteration of dynamic basal and stimulated insulin release, is indicative of *in vitro* cell-mediated alteration of PIC in the NOD mouse, The effect of whole spleen cells was rapid, and a crucial role was played by plastic-adherent cells. Two mechanisms were responsible for the behaviour of these cells: an early direct effect (at least in part via soluble products): and the indirect presentation of PIC xenoantigens (leading to impairment by $CD4^+$ T lymphocytes).

4.109 Efficient cryopreservation of dendritic cells transfected with cDNA of a tumour antigen for clinical application

Pecher, G., Schirrmann, T., Kaiser, L. and Schenk, J.A.

Biotechnol. Appl. Biochem., ***34***, *161-166 (2001)*

Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system and are currently being investigated in clinical applications as cancer vaccines. An efficient cryopreservation method would greatly contribute to their use in clinical trials. We have established a method for freezing of DCs derived from peripheral blood mononuclear cells using the plasma expander Gelifundol®. This enabled us to reduce the concentration of the toxic DMSO to 5%. The method could be performed without the addition of fetal calf serum or any other serum. After freezing, the viability of the DCs was 90%. The cells exhibited all the phenotypic characteristics ($CD11c^+$, $HLA\text{-}DR^+$, $CD8O^+$, $CD83^+$, $CD86^+$) of DCs, as tested by flow cytometry. Cells transfected with cDNA for the tumour antigen mucin expressed this protein on their surfaces in the same manner as before freezing. The stimulating capacity of a mixed lymphocyte culture was also preserved. These findings offer an efficient method for the cryopreservation of DCs for use in clinical trials.

4.110 Improved in vivo pancreatic iselt function after prolonged in vitro islet culture
Gaber, A. O. et al
Transplant., ***72***, *1730-1736 (2001)*

Background. Difficulties with recovering and preserving pancreatic islets have hampered progress in islet transplantation. In previous in vitro studies, our laboratory successfully demonstrated that using serum-free medium for prolonged pancreatic Islet culture allows post-culture recovery ratios greater than those obtained with standard media with sustained In vitro Islet function. The goal of this study was to determine whether culturing of islets in a modified serum-free medium (MSFM) would sustain function in vivo.
Methods. Islets were isolated from pancreata procured from 12 cadaveric organ donors and cultured in the M-SFM for up to 2 months, cryopreserved at -70°C within 1-3 days of isolation for 2 months, or placed in short-term culture (3-5 days) before their transplantation under the kidney capsule of non-obese diabetic-severe combined immunodeficient mice (n=4-7 per group/time point). In vivo islet function was assessed by measuring the production of human insulin and C-peptide over a period of 3-15 months.
Results. After extended culture of Islets in M-SFM for 1 or 2 months, transplanted islets maintained their viability, and in some instances in vivo function improved when compared with short-term cultured islets transplanted from the same preparation ($P<0.01$). Improvement was particularly evident for islets cultured for 1 month. Furthermore, when compared with cryopreserved preparations, early function (postoperative day 7) of Islets from 1-month culture preparations was statistically better ($P<0.05$). Prolonged culture in M-SFM bad no significant impact on long-term function, inasmuch as cultured islets functioned for more than 120 days.
Conclusion. These data demonstrate that prolonged islet culture in M-SFM sustained viability and function, and in some instances had a positive effect on in vivo islet function, particularly in the 1-month cultures. No negative effect on long-term in vivo function was demonstrated in this study. Confirmation in clinical models utilizing extended (1-2 months) islet culture in M-SFM could significantly enhance islet transplantation by allowing the identification of best matched recipients, pre-transplantation recipient conditioning, and possible pre-transplantation islet modifications to promote engraftment and prolonged graft function.

4.111 In vitro recognition and impairment of pig islet cells by baboon immune cells
Lalain, S., Gianello, P., Gouin, E. and Sai, P.
Transplant., ***72***, *1541-1548 (2001)*

Background. Grafting pig islets into patients with type 1 diabetes requires control of the strong cellular - xenogeneic rejection. This in vitro study compared the cellular reaction of baboons and humans to pig Islet cells (PICs) to confirm the validity of using these animals for further in vivo preclinical trials.
Methods. Baboon or human peripheral blood mono-nuclear cells (PBMCs) or subsets were co-incubated with PICs from specific pathogen-free adult pigs for 7 days to determine the mechanisms and intensity of PBMC proliferation. Interleukin (IL) 10 and interferon (IFN) γ secretion were assessed by enzyme-linked immunosorbent assay. Because proliferation was not indicative of aggression, a test based on perifusion analysis of the alteration of basal and stimulated insulin releases from PIC incubated with different baboon and human cells was developed.
Results. Baboon PBMCs strongly proliferated in response to PICs (stimulation Index [SI]=24.8±6.9 [n=8] vs. 23.9±3.4 [n=34] for human PBMCs), showing considerable variation in intensity among animals ($2.3<SI<63$) and humans ($1.8<SI<97$). PBMC proliferation was inhibited in baboons and humans by anti-CD4 (% inhibition of SI: 71±10% and 75±7%, respectively) and anti-DR (75±35% and 80±6%) monoclonal antibodies (MoAbs) or by depletion of MHC class II+ cells (99±1% and 90±6%).Blocking by anti-CD8 or anti-CD16 MoAbs was weaker and variable among both animals and humans. IL-l0 production by baboon and human PBMCS in response to PICs increased more than IFN-γ production after 2 days of co-culture, but the IL-10/IFN-γ ratio was inverted after 5 days of co-culture. After 7 days (and even after only 2days) of co-culture with baboon (n=8) or human (n=18) PBMCs, basal and glucose-stimulated insulin secretions from PICs were almost completely abolished ($P<0.0001$). The drop in insulin release could have mainly resulted from lysis of PICs, because the number of PICs decreased by 78% after 7 days of co-incubation with PBMCs. A decrease of insulin release by PBMCs was reproduced with plastic-adherent cells and was abolished by depletion of MHC class II+ cells or by addition of 100 μg/ml gadolinium (which inhibits macrophages), but not by cyclosporine. In baboons, as in humans, insulin release was also decreased after co-culture of PICs with enriched T lymphocytes remixed with antigen-presenting cells (APCs).
Conclusions. This study provides the first data on in vitro comparison of baboon and human cell-mediated recognition and impairment of PICs. Proliferation of PBMCs against PICs involves mainly CD4 T cells,

with indirect recognition mediated by baboon or human MHC class II+ APCs. The Th2/Th1 profile of cytokines secreted in response to PICs was similar in baboon and human PBMCs. The model based on alteration of insulin secretion indicates that PIC impairment by whole mononuclear cells was strong and rapid and that a crucial role was played by MHC class II+ and plastic-adherent cells. Two mechanisms appear to be responsible for the role of these cells: (1) early and strong direct effect, which is potentially involved in vivo in primary non-function of islets aggressed by monocytes and macrophages; and (2) presentation of PIC xenoantigens, which leads to impairment by T lymphocytes possibly involved in in vivo-specific cellular rejection. The mechanisms and intensity of baboon cellular reactions to PICs in vitro were similar to those observed in humans, which suggests that the baboon is a suitable model for the study of cellular mechanisms during preclinical trials of pig islet xenografts.

4.112 Long-term follow-up failed to detect in vitro transmission of full-length porcine endogenous retroviruses from specific pathogen-free pig islets to human cells
Clemenceau, B., Jegou, D., Martignat, L. and Sai, P.
*Diabetologia, **44**, 2044-2055 (2001)*

Aims/hypothesis: Islets from specific pathogen-free (SPF) pigs could prevent the transmission of conventional zoonosis, but not endogenous retroviruses (PERV), from pigs to diabetic patients. We previously reported that the pancreas showed the lowest expression of PERV mRNA among pig tissues intended for grafting. This study aimed to determine whether PERV from pig islets infect human cells during co-incubation. Methods: Human cells (including highly PERV-sensitive 293 cells) were incubated with SPF pig islet cells under conditions designed to increase contact (a high islet to human cell ratio, extended period of co-culture, and repeated contacts). PK15 and G2 retrovirus-producing pig cells were used in place of islet cells as "positive infection controls". Infection of human cells was monitored on cellular extracts and supernatants by PCR or long PCR, and RT-PCR or long RT-PCR, to detect PERV DNA and mRNA, respectively. Reverse-transcriptase activity was monitored by PERT. Results: Despite the presence of all PERV sequences in pig islet cells, including full-length inserts, no DNA or RNA for gag, pol, and the 3 env sub-types were detected in any human cell line or blood mononuclear cells incubated with pig islet cells, during an 18-week follow-up period. No PERV sequences or RT activity were detected in supernatants. PERV signals were negative even when the pig islet to human cell ratio was increased to 100:1, the time of co-culture was extended to 5 days and two sequential co-incubations were done. By contrast, all PERV DNA and mRNA were detected in all human cells co-incubated with PK15 or G2 cells. Depending on human cell types, productive or non-productive infections were obtained: full-length PERV RNA and RT activity in supernatants were detected or not; and PERV sequences to previously unexposed human cells by PERV-infected human cells were transmitted or not. Some human cells were not productively infected by PK15 cells but became productively infected after co-incubation with PERV-infected 293 cells. Conclusion/interpretation: SPF pig islet cells, even with PERV inserts and transcripts, have very little probability of transmitting PERV to human cells during co-incubation. The sensitivity of human cells to stable and productive infection by PERV depends on the cell type. Human adaptation of PERV was observed.

4.113 Safety of modified vaccinia virus Ankara (MVA) in immune-suppressed macaaaques
Stittelaar, K.J. et al
*Vaccine, **19**, 3700-3709 (2001)*

Modified vaccinia virus Ankara (MVA)-based recombinant viruses have been shown to be potent vaccine candidates for several infectious and neoplastic diseases. Since a major application of these live, replication-deficient vectors would be their use in immunocompromised or potentially immunocompromised individuals, a preclinical safety study was carried out. Macaques were inoculated with high doses of MVA (10^9) via various routes, after immune-suppression by total-body irradiation, anti-thymocyte globulin treatment, or measles virus (MV) infection. No clinical, haematological or pathological abnormalities related to MVA inoculation were observed during a 13-day follow-up period. The presence of MVA genomes was demonstrated by nested PCR during the course of the experiment in all macaques, but from none of these animals replication competent MVA could be reisolated. These data suggest that MVA can safely be used as a basis for recombinant human vaccines, and that it is also safe for use in immunocompromised individuals.

Gastrin and the neuropeptide PACAP evoke secretion from rat stomach histamine-containing (ECL) cells by stimulating influx of Ca^{2+} through different Ca^{2+} channels
Lindström, E., Eliasson, L., Björkqvist, M. and Håkonson, R.

J. Physiol., ***535(3)***, *663-677 (2001)*

Gastrin and PACAP stimulate secretion of histamine and pancreastatin from isolated rat stomach ECL cells. We have examined whether or not secretion depends on the free cytosolic Ca2+ concentration ([Ca2+]i) and the pathways by which gastrin and PACAP elevate [Ca2+]i. Secretion was monitored by radioimmunoassay of pancreastatin and changes in [Ca2+]i by video imaging. The patch clamp technique was used to record whole-cell currents and membrane capacitance (reflecting exocytosis).
In the presence of 2 mM extracellular Ca^{2+}, gastrin and PACAP induced secretion and raised $[Ca^{2+}]_i$. Without extracellular Ca^{2+} (or in the presence of La^{3+}) no secretion occurred. The extracellular Ca^{2+} concentration required to stimulate secretion was 10 times higher for gastrin than for PACAP. Depletion of intracellular Ca^{2+} pools by thapsigargin had no effect on the capacity of gastrin and PACAP to stimulate secretion.
Gastrin-evoked secretion was inhibited 60-80 % by L-type channel blockers and 40 % by the N-type channel blocker ω-conotoxin GVIA. Combining L-type and N-type channel blockers did not result in greater inhibition than L-type channel blockers alone. Whole-cell patch clamp measurements confirmed that the ECL cells are equipped with voltage-dependent inward Ca^{2+} currents. A 500 ms depolarising pulse from -60 mV to +10 mV which maximally opened these channels resulted in an increase in membrane capacitance of 100 fF reflecting exocytosis of secretory vesicles.
PACAP-evoked secretion was reduced 40 % by L-type channel blockers but was not influenced by inhibition of N-type channels. SKF 96365, a blocker of both L-type and receptor-operated Ca^{2+} channels, inhibited PACAP-evoked secretion by 85 %. Combining L-type channel blockade with SKF 96365 abolished PACAP-evoked secretion.
The results indicate that gastrin- and PACAP-evoked secretion depends on Ca^{2+} entry and not on mobilisation of intracellular Ca^{2+}. While gastrin stimulates secretion via voltage-dependent L-type and N-type Ca^{2+} channels, PACAP acts via L-type and receptor-operated Ca^{2+} channels.

Serum-Free Media for Neural Cell Cultures
Price, P.J. and Brewer, G.J.
Protocols for Neural Cell Culture, Ed. Fedoroff, s. and Richardson, A., Humana Press 255-264 (2001)

The ability to grow primary neurons under serum-free conditions is facilitating better control in studies of neuronal development, mechanisms of neuronal signaling, electrophysiology, pharmacology, plasticity, in vitro growth requirements, gene expression, and neurotoxicity. Some of the new commercially available media combinations allow for the growth of sparse populations of neurons, which in turn allow for the study of individual neurons and synapses. This has not been possible using serum-supplemented media without a feeder layer of glial cells. In serum-supplemented media, glial cells continue to multiply, necessitating the use of cytotoxic mitotic inhibitors (*Wallace and Johnson, 1989*). Serum also contains unknown and variable levels of growth factors, hormones, vitamins, and proteins.

4.114 Kinetics of endomitosis in primary murine megakaryocytes
Carow, C.E., Fox, N.E. and Kaushansky, K.
J. Cell. Physiol., ***188(3)***, *291-303 (2001)*

Megakaryocytes (MKs) develop from diploid progenitor cells via successive rounds of DNA synthesis in the absence of cell division, a process termed endomitosis (EnM). While the mechanism underlying EnM is not known, studies in yeast and leukemic cell lines have suggested that it may be due to reduced levels of cyclin B1 or cdc2, leading to a decrease in mitotic kinase activity. Using flow cytometry to study EnM highly purified marrow-derived MK precursors, we found that: (1) on average, 36% of 8N-32N MKs expressed abundant cyclin B during G2/M. The percentage of cells in G2/M decreased in >64N MKs, suggesting the limit of EnM, (2) the level of cyclin B per G2/M MK increased linearly with ploidy, (3) cyclin B expression oscillated normally in polyploid MKs, (4) MPM-2, a phosphoepitope created by the action of mitotic kinases and specific to M-phase cells, was expressed in a significant fraction of polyploid MKs, and (5) there was an apparent increase of cyclin B in G1-phase in polyploid MKs. This study provides the first qualitative kinetic data regarding the cell cycle status of MKs within individual ploidy classes. It also demonstrates the feasibility of using anti-cyclin B antibody and flow cytometry to resolve G1 from G2/M populations in polyploid MKs. Finally, these findings establish that neither a relative nor absolute deficiency of mitotic kinase components is responsible for EnM, suggesting that the departure from normal cell division kinetics seen in polyploid MKs is likely due to alterations in other cell cycle regulators.

4.115 Temperature and time interval for culture of postmortem neurons from adult rat cortex
Viel, J.J., McManus, D.Q., Cady, C., Evans, M.S. and Brewer, G.J.
*J. Neurosci. Res., **64(4)**, 311-321 (2001)*

For a model of neurological disease and ischemia, we extended recent work to culture adult postmortem rat brain neurons. Frontal cortex sections were removed from adult rats immediately following sacrifice and at different postmortem intervals and with the brain at either 22°C or 4°C. Brain could be stored four times longer at 4°C between sacrifice and neuronal disaggregation to achieve the same 20% recovery of live cells from those plated compared to 22°C. Each milligram of rat frontal cortex was estimated by the optical disector method to contain 160,000 neurons. When cells were isolated as rapidly as possible, 9% of the neurons originally present in the brain were viable. Various postmortem intervals from 2 to 24 hr resulted in a reduction from 6% to 3% of the cells originally present. After 5 days in culture, viable neurons were 23–42% of those isolated. Neuron-like cells that survived represented 40–75% of the viable cells, or 0.5–2.75% of those originally estimated to be present in the brain. Electrophysiology experiments show that cells isolated 0 and 24 hr postmortem had neuronal electrical properties, including an average resting membrane potential of –48 mV, voltage-sensitive currents, and action potentials. Neuron-like cells were immunoreactive for neuron-specific enolase, neurofilament 200, glutamate, MAP2, and tau after 2 weeks in culture. These experiments show that neuron-like cells can be reliably cultured from adult rat cortex up to 6 hr postmortem when stored at 22°C and up to 24 hr postmortem when stored at 4°C. These findings should encourage donation of human postmortem brain neurons for studies on ischemia, adult pharmacology, and neurological disease.

4.116 Isolation of Mouse Thymic Dendritic Cells
Anjuere, F. and Ardavin, C.
*Methods in Mol. Med., **64**, 23-28 (2001)*

No abstract available

4.117 Fractalkine is expressed by smooth muscle cells in response to IFN-γ and TFN-α and is modulated by metalloproteinase activity
Ludwig, A., Berkhout, T., Moores, K., Groot, P. and Chapman G.
*J. Immunol., **168**, 604-612 (2002)*

Fractalkine/CX3C-chemokine ligand1 is expressed as a membrane-spanning adhesion molecule that can be cleaved from the cell surface to produce a soluble chemoattractant. Within the vasculature, fractalkine is known to be generated by endothelial cells, but to date there are no reports describing its expression by smooth muscle cells (SMC). IN this study we demonstrate that IFNγ and TNF-α, but not IL-1β, cooperate synergistically to induce fractalkine mRNA and protein expression in cultured aortic SMC. We also report the release of functional, soluble fractalkine from the membranes of stimulated SMC. This release is inhibited by the zinc metalloproteinase inhibitor batimastat, resulting in the accumulation of membrane-associated fractalkine on the SMC surface. Therefore, an SMC-derived metalloproteinase activity is involved in fractalkine shedding. While soluble fractalkine present in SMC-conditioned medium is capable of inducing calcium transients in cells expressing the fractalkine receptor (CX3CR1), blocking experiments using neutralizing Abs reveal that it can be inactivated without affecting the chemotactic activity of SMC-conditioned media on monocytes. However, membrane-bound fractalkine plays a major role in promoting adhesion of monocytic cells to activated SMC. This fractalkine-mediated adhesion is further enhanced in the presence of batimastat, indicating that shedding of fractalkine from the cell surface down-regulates the adhesive properties of SMC. Hence, during vascular inflammation, the synergistic induction of fractalkine by IFN-γ and TNF-α together with its metalloproteinase-mediated cleavage may finely control the recruitment of monocytes to SMC within the blood vessel wall.

4.118 $CD8\alpha^+$ dendritic cells originate from $CD8\alpha^-$ dendritic cell subset by a maturation process involving CD8α, DEC-205, and CD24 up-regulation
Del Hoyo, G.M., Martin, P., Arias, F.C., Marin, A.R. and Ardavin, C.
*Blood, **99(3)**, 999-1004 (2002)*

$CD8\alpha^+$ and $CD8\alpha^-$ dendritic cells (DCs) have been considered as an independent DC subpopulation both ontogenetically and functionally during recent years. However, it has been demonstrated that both DC subsets can be generated from a single precursor population, supporting the concept that they do not represent separate DC lineages. By using highly purified splenic $CD8\alpha^-$ DCs, which were injected

intravenously and traced by means of an Ly5.1/Ly5.2 transfer system, this study shows that CD8α$^-$ DCs acquired the phenotypic characteristics of CD8α$^+$ DCs by a differentiation process involving CD8α, DEC-205, and CD24 up-regulation, paralleled by the down-regulation of CD11b, F4/80, and CD4. These data demonstrate that CD8α$^+$ DCs derive from CD8α$^-$ DCs, and strongly support that CD8α$^-$ and CD8α$^+$ represent different maturation or differentiation stages of the same DC population. Therefore, CD8α$^+$ DCs would represent the last stage of DC differentiation, playing an essential role in the induction of T-cell responses, due to their antigen-presenting potential, cross-priming ability, and capacity to secrete large amounts of key cytokines such as interferon γ and interleukin-12.

4.119 Characterization of mitotic neurons derived from adult rat hypothalamus and brain stem
Evans, J. et al
*J. Neurophysiol., **87**, 1076-1085 (2002)*

Embryonic or neonatal rat neurons retain plasticity and are readily grown in tissue culture, but neurons of the adult brain were thought to be terminally differentiated and therefore difficult to culture. Recent studies, however, suggest that it may be possible to culture differentiated neurons from the hippocampus of adult rats. We modified these procedures to grow differentiated neurons from adult rat hypothalamus and brain stem. At day 7 in tissue culture and beyond, the predominant cell types in hypothalamic and brain stem cultures had a stellate morphology and could be subdivided into two distinct groups, one of which stained with antibodies to the immature neuron marker α-internexin, while the other stained with the astrocytes neuron marker GFAP. The α-internexin positive cells were mitotic and grew to form a characteristic two-dimensional cellular network. These α-internexin positive cells coimmunostained for the neuronal markers MAP2, type III β-tubulin, and tau, and also bound tetanus toxin, but were negative for the oligodendrocytes marker Ga1C and also for the neurofilament triplet proteins NF-L, NF-M, and NF-H, markers of more mature neurons. Patch-clamp analysis of these α-internexin positive cells revealed small Ca^{2+} currents with a peak current of –0.5 ± 0.1 pA/pF at a membrane potential of –20 mV (n = 5) and half-maximal activation at –30 mV (n= 5). Na^+ currents with a peak current density of –154.5 ± 49.8 pA/pF at a membrane potential of –15 mV (n= 5) were also present. We also show that these cells can be frozen and regrown in tissue culture and that they can be efficiently infected by viral vectors. These cells therefore have the immunological and electrophysiological properties of immature mitotic neurons and should be useful in a variety of future studies of neuronal differentiation and function.

4.120 Dramatic increase in lymph node dendritic cell number during infection by the mouse mammary tumor virus occurs by a CD62L-dependent blood-borne DC recruitment
Martin, P. et al
*Blood, **99(4)**, 1282-1288 (2002)*

Despite the information dealing with the differential phenotype and function of the main mouse dendritic cell (DC) subpopulations, namely, CD8α$^-$ and CD8α$^+$ DCs, their origin and involvement in antiviral immune responses in vivo are still largely unknown. To address these issues, this study used the changes occurring in DC subpopulations during the experimental infection by the Swiss (SW) strain of the mouse mammary tumor virus (MMTV). MMTV(SW) induced an 18-fold increase in lymph node DCs, which can be blocked by anti-CD62L treatment, concomitant with the presence of high numbers of DCs in the outer cortex, in close association with high endothelial venules. These data suggest that the DC increase caused by MMTV(SW) infection results from the recruitment of blood-borne DCs via high endothelial venules, by a CD62L-dependent mechanism. In addition, skin sensitization assays indicate that MMTV(SW) infection inhibits epidermal Langerhans cell migration to the draining lymph node. Moreover, data on the kinetics of MMTV(SW)-induced expansion of the different DC subsets support the hypothesis that CD8$^-$ and CD8$^+$ DCs represent different maturation stages of the same DC population, rather than myeloid-and lymphoid-derived DCs, respectively, as previously proposed. Finally, the fact that DCs were infected by MMTV(SW) suggests their participation in the early phases of infection.

4.121 Alveolar epithelial ion and fluid transport Na transport proteins are expressed by rat alveolar epithelial type I cells
Borok, Z. et al
Am. J. Physiol. Lung Mol. Physiol., ***282****, L599-608 (2002)*

Despite a presumptive role for type I (AT1) cells in alveolar epithelial transport, specific Na transporters have not previously been localized to these cells. To evaluate expression of Na transporters in AT1 cells, double labeling immunofluorescence microscopy was utilized in whole lung and in cytocentrifuged preparations of partially purified alveolar epithelial cells (AEC). Expression of Na pump subunit isoforms and the α-subunit of the rat (r) epithelial Na channel (α-EnaC) was evaluated in isolated AT1 cells identified by their immunoreactivity with AT1 cell-specific antibody markers (VIIIB2 and/or anti-aquaporin-5) and lack of reactivity with antibodies specific for AT22 cells (anti-**surfactant** protein A) or leukocytes (anti-leukocyte common antigen). Expression of the Na pump α_1-subunit in AEC was assessed in situ. Na pump subunit isoform and α-rENaC expression was also evaluated by RT-PCR in highly purified (~ 95%) AT1 cell preparations. Labeling of isolated AT1 cells with anti-α_1 and anti-β_1 Na pump subunit and anti-α-rENaC antibodies was detected, while reactivity with anti-α_2 Na pump subunit antibody was absent. AT1 cells in situ were reactive with anti-α_1 Na pump subunit antibody. Na pump α_1 – and β_1 – (but not α_2-) subunits and α-rENaC were detected in highly purified AT1 cells by RT-PCR. These data demonstrate that AT1 cells express Na pump and Na channel proteins, supporting a role for AT1 cells in active transalveolar epithelial Na transport.

4.122 Apolipoprotein E4 inhibits, and apolipoprotein E3 promotes neurite outgrowth in cultured adult mouse cortical neurons through the low-density lipoprotein receptor-related protein
Nathan, B. et al
Brain Res., ***928****, 96-105 (2002)*

The apolipoprotein E4 (apoE4) genotype is a major risk factor for Alzheimer's disease (AD); however, the mechanism is unknown. We previously demonstrated that apoE isoforms differentially modulated neurite outgrowth in embryonic neurons and in neuronal cell lines. ApoE3 increased neurite outgrowth whereas apoE4 decreased outgrowth, suggesting that apoE4 may directly affect neurons in the brain. In the present study we examined the effects of apoE on neurite outgrowth from cultured adult mouse cortical neurons to examine if adult neurons respond the same way that embryonic cells do. The results from this study demonstrated that (1) cortical neurons derived from adult apoE-gene knockout (apoE KO) mice have significantly shorter neurites than neurons from adult wild-type (WT) mice; (2) incubation of cortical neurons from adult apoE KO mice with human apoE3 increased neurite outgrowth, whereas human apoE4 decreased outgrowth in a dose-dependent fashion; (3) the isoform specific effects were abolished by incubation of the neurons with either receptor associated protein (RAP) or lactoferrin, both of which block the interaction of apoE-containing lipoproteins with the low-density lipoprotein receptor-related protein (LRP). These data suggest a potential mechanism whereby apoE4 may play a role in regenerative failure and accelerate the development of AD.

4.123 Expression of Notch ligands, Jagged1, and Delta1 in antigen presenting cells in mice
Yamaguchi, E. et al
Immunol. Lett., ***81****, 59-64 (2002)*

Notch1 is indispensable for T cell development. It is anticipated that Notch1 and other Notch receptors expressed on the surface of thymic T cell precursors are activated by ligands present on environmental cells, including antigen presenting cells (APCs), and involved in positive and negative selections. Notch receptors on peripheral T cells may also be activated by ligands on APCs. Here, we examined the expression pattern of three Notch ligands, Jagged1, 2 and Delta1 in APCs by an immunofluorescence cell staining method and a reverse transcriptase-polymerase chain reaction (RT-PCR) method. Peritoneal macrophages were strongly positive for Jagged1 staining. In contrast, macrophages separated from spleen and dendritic cells (DCs) separated from spleen and thymus showed positive staining for all the three ligands at a similar intensity. An analysis by RT-PCR revealed that peritoneal and splenic macrophages and splenic and thymic DCs, show a distinct pattern in Notch ligand expression. These findings may represent that expression of various Notch ligands in APCs has a physiological relevance in each organ.

4.124 Increased production of nitric oxide stimulated by interferon-γ from peripheral blood monocytes in patients with complex regional pain syndrome
Hartrick, C.T.
Neurosci. Lett., ***323***, *75-77 (2002)*

This study examines immediate nitric oxide (NO) release from monocytes following interleukin-1beta (IL-1beta), interferon-gamma (IFN-gamma), and tumor necrosis factor-alpha (TNF-alpha) challenge in patients with complex regional pain syndrome (CRPS). Study patients exhibited the following: (1), mechanical allodynia; (2), evidence of either vasomotor or sudomotor disturbance; and (3), concordant painful allodynia documented with quantitative sensory testing that was temporarily abolished with sympathetic block. Ten subjects (CRPS, N=5; control, N=5) were enrolled. Peripheral blood monocytes were challenged with 100 μl of IL-1β (1 ng), IFN-γ (1 ng), TNF-α (0.01 ng), and normal saline (NS) and the resultant immediate NO release measured. Subjects with CRPS exhibited a statistically significant increase in NO release in response to IFN-γ (P<0.012) compared with controls. The NO responses to IFN-γ in excess of NS (P<0.025) and as the ratio IFN-γ/NS (P<0.022) were also significantly increased.

4.125 Tissue-specific mechanisms control the retention of IL-8 in lungs and skin
Frevert, C.W. et al
J. Immunol., ***168***, *3550-3556 (2002)*

Chemokines are a group of structurally related peptides that promote the directed migration of leukocytes in tissue. Mechanisms controlling the retention of chemokines in tissue are not well understood. In this study we present evidence that two different mechanisms control the persistence of the CXC chemokine, IL-8, in lungs and skin. ^{125}I-labeled IL-8 was injected into the airspaces of the lungs and the dermis of the skin and the amount of ^{125}I-labeled IL-8 that remained at specified times was measured by scintillation counting. The ^{125}I-labeled IL-8 was cleared much more rapidly from skin than lungs, as only 2% of the ^{125}I-labeled IL-8 remained in skin at 4 h whereas 50% of the ^{125}I-labeled IL-8 remained in lungs at 4 h. Studies in neutropenic rabbits showed that neutrophils shortened the retention of ^{125}I-labeled IL-8 in skin but not lungs. A monomeric form of IL-8, *N*-methyl-leucine 25 IL-8, was not retained as long in lungs as recombinant human IL-8, indicating that dimerization of IL-8 is a mechanism that increases the local concentration and prolongs the retention of ^{125}I-labeled IL-8 in lungs. These observations show that the mechanisms that control the retention of IL-8 in tissue include neutrophil migration and dimerization, and that the importance of these varies in different tissues.

4.126 Expression and function of 4-1BB and 4-1BB ligand on murine dendritic cells
Futagawa, T. et al
Int. Immunol., ***14(3)***, *275-286 (2002)*

4-1BB (CDw137) and its ligand (4-1BBL) have been implicated in cellular immune responses. To further characterize the expression and function of 4-1BBL, we newly generated an anti-mouse 4-1BBL mAb (TKS-1), which can inhibit the interaction of 4-1BBL with 4-1BB. Flow cytometric analyses using TKS-1 and an anti-mouse 4-1BB mAb indicated that 4-1BB was inducible on both $CD4^+$ and $CD8^+$ splenic T cells by stimulation with immobilized anti-CD3 mAb, but 4-1BBL was not expressed on resting or activated T cells. 4-1BBL expression was inducible on splenic B cells by stimulation with anti-IgM antibody plus anti-CD40 mAb, on peritoneal macrophages by stimulation with lipopolysaccharide (LPS) and on splenic dendritic cells (DC) by stimulation with anti-CD40 mAb or LPS. Interestingly, splenic DC expressed 4-1BB constitutively, which was down-regulated by anti-CD40 stimulation. Co-culture of splenic DC with 4-1BBL-transfected cells or 4-1BBL-expressing tumor cell lines led to cytokine (IL-6 and IL-12) production and co-stimulatory molecule up-regulation by splenic DC, indicating that 4-1BBL can directly activate DC. Moreover, IL-12 production by anti-CD40-stimulated DC was partially inhibited by TKS-1. These results suggest that 4-1BB expressed on DC may be involved in DC activation through DC–tumor interaction and DC–DC interaction.

4.127 CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells
Homann, D. et al
*Immunity, **16**, 403-415 (2002)*

Systemic treatment with antibody to CD40 ligand (aCD40L) can prevent autoimmunity and transplant rejection in several animal models and is currently under evaluation in clinical trials. While it is known that aCD40L administration inhibits expansion and effector functions of aggressive T cells, it is still unclear whether additional regulatory mechanisms are operative. Here we demonstrate that a single episode of CD40L blockade during development of the autoaggressive immune response completely prevented autoimmune disease in the RIP-LCMV mouse model for virally induced type 1 diabetes. Interestingly, protection could be transferred by a highly potent, bitypic cell population sharing phenotypic and functional properties of both natural killer (NK) and dendritic cells (DC). Furthermore, protection of prediabetic recipients was autoantigen specific and did not result in generalized immunosuppression. The origin, function, and therapeutic potential of these bitypic NK/DC regulatory cells are discussed.

4.128 Differentiated HL-60 cells are a valid model system for the analysis of human neutrophil migration and chemotaxis
Hauert, A.B., Martinelli, S., Marone, C. And Niggli, V.
*Int. J. Biochem. Cell Biol., **34**, 838-854 (2002)*

We have carried out a detailed comparison of the motile properties of differentiated HL-60 cells and human peripheral blood neutrophils. We compared the effects of chemotactic stimuli and of inhibitors of signaling proteins on morphology, chemokinesis and chemotaxis of neutrophils and differentiated HL-60 cells using videomicroscopy and a filter assay for chemotaxis. We also assessed expression of signaling and cytoskeletal proteins using Western blotting.

Chemotactic peptide induced a front-tail polarity in HL-60 cells comparable to that of neutrophils. Chemokinetic and chemotactic responses to chemotactic peptide were also very similar for both cell types, concerning mean speed of migration, the fraction of migrated cells and the concentration of stimulus optimal for activation. The cytokine interleukin-8 was in contrast clearly less effective in activating motile responses of differentiated HL-60 cells as compared to neutrophils.

An important functional role of Rho-activated kinases and phosphatidylinositol 3-kinase in motile responses of HL-60 cells, consistent with their upregulation during differentiation, could be confirmed using inhibitors with specificity for the corresponding enzymes. The only difference observed here between HL-60 cells and neutrophils concerned the differential effects of a protein kinase C inhibitor.

In summary, the results presented here show that differentiated HL-60 cells, stimulated with chemotactic peptide, are a valid model system to study molecular mechanisms of neutrophil emigration.

4.129 Expression of transforming growth factor-β1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis
Wai-Tsing Shek, F. et al
*Am. J. Pathol., **160(5)**,1787-1798 (2002)*

Pancreatic stellate cells mediate fibrosis in chronic pancreatitis. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs)-1 and -2 are crucial modulators of fibrosis. Transforming growth factor-β (TGF-β) is a key regulator of extracellular matrix production and myofibroblast proliferation. We have examined MMP and TIMP synthesis by transformed cultured pancreatic stellate cells and their regulation by TGF-β1. By Northern analysis they expressed mRNAs for procollagen 1, TIMP-1, TIMP-2, and MMP-2. Expression of membrane type-1 MMP was confirmed by Western blotting. By immunohistochemistry these enzymes localized to fibrotic areas in human chronic pancreatitis. Active TGF-β1 constitutes 2 to 5% of total TGF-β1 secreted by pancreatic stellate cells; they express TGF-β receptors I and II. Exogenous TGF-β1 (10 ng/ml) significantly increased procollagen-1 mRNA by 69% and collagen protein synthesis by 34%. Similarly TGF-β1 at 0.1, 1, and 10 ng/ml significantly reduced cellular proliferation rate by 37%, 44%, and 44%, respectively, whereas pan-TGF-β-neutralizing antibody increased proliferation by 40%. TGF-β1 (10 ng/ml) down-regulated MMP-9 by 54% and MMP-3 by 34% whereas TGF-β1-neutralizing antibody increased MMP-9 expression by 39%. Pancreatic stellate cells express both mediators of matrix remodeling and the regulatory cytokine TGF-β1 that, by autocrine inhibition of MMP-3 and MMP-9, may enhance fibrogenesis by reducing collagen degradation.

4.130 Expression of the vesicular inhibitory amino acid transporter in pancreatic islet cells
Chessler, S.D., Simonson, W.T., Sweet, I.R. and Hammerle, L.P.
*Diabetes, **51**, 1763-1771 (2002)*

γ-Aminobutyric acid (GABA) is stored in microvesicles in pancreatic islet cells. Because GAD65 and GAD67, which catalyze the formation of GABA, are cytoplasmic, the existence of an islet vesicular GABA transporter has been postulated. Here, we test the hypothesis that the putative transporter is the vesicular inhibitory amino acid transporter (VIAAT), a neuronal transmembrane transporter of GABA and glycine. We sequenced the human VIAAT gene and determined that the human and rat proteins share over 98% sequence identity. In vitro expression of VIAAT and immunoblotting of brain and islet lysates revealed two forms of the protein: an ~52-kDa and an ~57-kDa form. By immunoblotting and immunohistochemistry, we detected VIAAT in rat but not human islets. Immunohistochemical staining showed that in rat islets, the distribution of VIAAT expression parallels that of GAD67, with increased expression in the mantle. GABA, too, was found to be present in islet non-β-cells. We conclude that VIAAT is expressed in rat islets and is more abundant in the mantle and that expression in human islets is very low or nil. The rat islet mantle differs from rat and human β-cells in that it contains only GAD67 and relatively increased levels of VIAAT. Cells that express only GAD67 may require higher levels of VIAAT expression.

4.131 Characterization of a new subpopulation of mouse $CD8^+$ $B220^+$ dendritic cells endowed with type 1 interferon production capasity and tolerogenic potential
Martin, P. et al
*Blood, **100(2)**, 383-390 (2002)*

We describe a new $B220^+$ subpopulation of immaturelike dendritic cells ($B220^+$ DCs) with low levels of expression of major histocompatibility complex (MHC) and costimulatory molecules and markedly reduced T-cell stimulatory potential, located in the thymus, bone marrow, spleen, and lymph nodes. $B220^+$ DCs display ultrastructural characteristics resembling those of human plasmacytoid cells and accordingly produce interferon- after virus stimulation. $B220^+$ DCs acquired a strong antigen-presenting cell capacity on incubation with CpG oligodeoxynucleotides, concomitant with a remarkable up-regulation of MHC and costimulatory molecules and the production of interleukin-12 (IL-12) and IL-10. Importantly, our data suggest that nonstimulated $B220^+$ DCs represent a subset of physiological tolerogenic DCs endowed with the capacity to induce a nonanergic state of T-cell unresponsiveness, involving the differentiation of T regulatory cells capable of suppressing antigen-specific T-cell proliferation. In conclusion, our data support the hypothesis that $B220^+$ DCs represent a lymphoid organ subset of immature DCs with a dual role in the immune systemexerting a tolerogenic function in steady state but differentiating on microbial stimulation into potent antigen-presenting cells with type 1 interferon production capacity.

4.132 The volume set point of KCl cotransport in normal and sickle reticulocytes: effects of cell age and sulfhydryl reduction

Joiner, C.H., Rettig, R.K. and Franco, R.S.

Abstracts of papers at the 56th annual meeting of the Society of General Physiologists (2002)

KCl cotransport (KCC) is excessively active in sickle red blood cells (SSRBC) and contributes to pathological dehydration of sickle reticulocytes. To explore the physiological basis for this pathological behavior, methodologies are needed that: (a) reflect KCC activity in the entire reticulocyte population (as opposed to a sub-population selected on the basis of cell density or response to osmotic stimuli) and (b) permit comparison of aged-matched populations of reticulocytes (SS vs. normal, AA). To this end, we have examined the changes in RBC density profile (as a surrogate marker for flux measurements) upon activation of KCC, coupled with flow cytometric detection of reticulocytes to track the density changes of this distinct population. Whole blood (SS or AA) was washed in isotonic HEPES-buffered saline (HBS) and treated with nystatin to adjust hemoglobin concentration (MCHC) to 30 g/dl. After incubation at 37°C under specified conditions and times, cells were washed in isotonic HBS pH 7.4 and subjected to density analysis on stepwise gradients prepared from OptiPrep®. The percentage of RBC at each density level was calculated from cell counts to give the RBC density profile. From the percentage of reticulocytes in each density fraction, the reticulocyte density profile was generated. A density score (DS) was calculated for RBC and reticulocytes as: $DS = \Sigma (N \times P_N)$ where N = gradient fraction number, P_N = percentage of cells in fraction N. There was a linear correlation between DS and MCHC (r = 0.97) when gradients were calibrated using cells treated with nystatin to yield various MCHC. Thus, the DS of a cell population could be used to calculate its MCHC. When KCC was activated by acidification at 37°C to pH 7.0 in HBS, retic density increased rapidly, and then stabilized between 1 and 2 h. Minimal changes occurred in nitrate media. Thus, the rapid increase in MCHC is a manifestation of KCC activity, and the MCHC obtained at 2 h ($MCHC_{final}$) reflects the "volume set point" (VSP) for KCC activity under those conditions. When adjusted to MCHC 30 g/dl, neither SS nor AA reticulocytes changed volume significantly when incubated at pH 7.4. However, when acidified to pH 7.0, both types of RBC became more dense; SS retics had a more rapid change in MCHC than AA retics, and $MCHC_{final}$ was significantly higher in SS than in AA retics (SS, 35.6 ± 1.0 vs. AA 31.2 ± 1.0, $n = 6$, $P < 0.01$ by unpaired t test). Thus, the VSP of SS cells appears to be different from that of AA retics. When the maturity of SS reticulocytes was assessed by fluorescence intensity of reticulum staining by thiazole orange, older SS retics were found to have higher $MCHC_{final}$ than younger cells (35.0 ± 0.2 vs. 34.0 ± 0.4, $n = 6$, $P < 0.04$ by paired t test), suggesting that VSP changes as reticulocytes mature. Finally, treatment of SS cells with the sulfhydryl reducing agent, dithiothreitol, lowered $MCHC_{final}$ (control, 34.4 ± 1.3 vs. treated, 33.4 ± 1.2, $n = 6$, $P < 0.002$, paired t test), suggesting that sulfhydryl oxidation contributes to the abnormally high $MCHC_{final}$ of SS retics. This is plausible in view of the known activation of KCC by sulfhydryl oxidation, and the increased oxidant stress and membrane oxidation seen in SS RBC.

4.133 Marine invertebrate cell lines in the study of coral physiology and pathology

Johnston, C., Larkin, K., Woodley, C. and Morris, P.J.

Marine Biomedicine and Environmental Sciences Annual Research Open House, Medical University of South Carolina. Poster Abstracts (2002)

Coral reefs are among the most productive and diverse ecosystems on earth. The scleractinians, or reef-building corals, like all living organisms, are prone to a variety of different diseases and stressors. Diseases of corals are having significant, negative impacts on the structure and appearance of coral reefs throughout the world. Bacteria, fungi, and cyanobacteria are known to cause diseases in corals, and changing environmental conditions and human impacts are suspected contributors to disease. However, the pathogens responsible for most diseases affecting reef organisms and the underlying mechanisms of pathogenesis are elusive and remain unknown. A coral cell line would be an invaluable tool for the study of coral physiology and disease pathology. Previous attempts at creating coral cell cultures have met with obstacles, and no marine invertebrate cell line exists. In this preliminary study, we assessed the viability of different cell types in culture from a marine invertebrate closely related to scleractinian corals, the sea anemone Aiptasia pallida. Several different cell types were dissociated from the anemone tissue using mechanical and chemical dissociation methods. The cells were separated on an Optiprep density gradient and cultured in Dulbecco's modified Eagle media with heat-inactivated fetal bovine serum, antibiotics, and sterile seawater. Various concentrations of media and heat-inactivated fetal bovine serum were used to optimize primary culture conditions. Primary cultures remained viable up to six days without media change. Future studies will optimize cell viability in media supplemented with trace elements, light, growth factors, and growth substrates.

4.134 Motoneuron death triggered by a specific pathway downstream of Fas: Potentation by ALS-linked SOD1 mutations

Raoul, C. et al

*Neuron, **35**, 1067-1083 (2002)*

Death pathways restricted to specific neuronal classes could potentially allow for precise control of developmental neuronal death and also underlie the selectivity of neuronal loss in neurodegenerative disease. We show that Fas-triggered death of normal embryonic motoneurons requires transcriptional upregulation of neuronal NOS and involves Daxx, ASK1, and p38 together with the classical FADD/caspase-8 cascade. No evidence for involvement of this pathway was found in cells other than motoneurons. Motoneurons from transgenic mice overexpressing ALS-linked SOD1 mutants (G37R, G85R, or G93A) displayed increased susceptibility to activation of this pathway: they were more sensitive to Fas- or NO-triggered cell death but not to trophic deprivation or excitotoxic stimulation. Thus, triggering of a motoneuron-restricted cell death pathway by neighboring cells might contribute to motoneuron loss in ALS.

4.135 Mouse CD11c$^+$ B220$^+$ Gr1$^+$ plasmacytoid dendritic cells develop independently of the T-cell lineage

Ferrero, I., Held, W., Wilson, A., Tacchini-Cottier, F., Radtke, F. and MacDonald, H.R.

Blood, **100**, 2852-2857 (2002)

The developmental origin of dendritic cells (DCs) is controversial. In the mouse CD8α^+ and CD8α^- DC subsets are often considered to be of lymphoid and myeloid origin respectively, although evidence on this point is conflicting. Very recently a novel CD11c$^+$ B220$^+$ DC subset has been identified that appears to be the murine counterpart to interferon alpha (IFNα)-producing human plasmacytoid DCs (PDCs). We show here that CD11c$^+$ B220$^+$ mouse PDCs, like human PDCs, are present in the thymus and express T lineage markers such as CD8α and CD4. However, the intrathymic development of PDCs can be completely dissociated from immature T lineage cells in mixed chimeras established with bone marrow cells from mice deficient for either Notch-1 or T-cell factor 1, two independent mutations that severely block early T-cell development. Our data indicate that thymic PDCs do not arise from a bipotential T/DCprecursor.

4.136 Rehydration of high-density sickle erythrocytes in vitro

Holtzclaw, J.D., Jiang, M., Yasin, Z., Joiner, C.H. and Franco, R.S.

Blood, **100**, 3017-3025 (2002)

Recent studies have identified older low-density sickle red blood cells (SSRBCs) that were resistant to dehydration by valinomycin, a K^+ ionophore. These cells, thought to derive from dense SSRBCs that have rehydrated, may represent a terminal cellular phase. To study rehydration, we subjected dense SSRBCs (ρ >1.107 g/cc) to either oxygenated incubation or rapid oxygenated/deoxygenated (oxy/deoxy) cycling (70 seconds per cycle). Light cells (ρ < 1.087 g/cc) were generated during both oxy incubation (2.9% ± 2.1%; n =42) and oxy/deoxy cycling (5.3%± 2.4%; n = 42). The rehydrated cells were K^+-depleted (K^+ = 20 ± 14 mmol/kg hemoglobin [Hb]) and Na^+ -loaded (Na^+ = 394 ± 106 mmol/kg Hb), and had high levels of external phosphatidylserine. In the presence of external calcium, the generation of rehydrated SSRBCs was inhibited during oxy/deoxy cycling, but the percentage with external phosphatidyl-serine increased. The calcium-mediated inhibition of rehydration was reversed by charybdotoxin, implying that rehydration was delayed in some cells by the Ca^{++} -activated K^+ channel. Pre-incubation of dense SSRBCs with DIDS (4,4'-di-isothiocyanato-2,2'-disulfostilbene)inhibited the generation of light cells during fast oxy/deoxy cycling, but not during oxy incubation. These results suggest that the sickling-induced pathway, previously implicated in SSRBC dehydration, may be involved in the deoxy-dependent component of rehydration for dense, K^+-depleted cells. Light-cell generation was inhibited by 1mM bumetanide during both oxy incubation and oxy/deoxy cycling, providing evidence that a bumetanide-sensitive, deoxy-independent pathway, previously described in circulating light SSRBCs, also contributes to the rehydration of high-density SSRBCs.

4.137 Isolation of purified oocyst walls and sporocysts from *Toxoplasma gondii*

Everson, W.V., Ware, M.W., Dubey, J.P. and Lindquist, H.D.
*J. Eukaryot. Microbiol., **49(4)**, 344-349 (2002)*

Toxoplasma gondii oocysts are environmentally resistant and can infect virtually all warm-blooded hosts, including humans and livestock. Little is known about the biochemical basis for this resistance of oocysts, and mechanism for excystation of *T. gondii* sporozoites. The objective of the present study was to evaluate different methods (mechanical fragmentation, gradients, flow cytometry) to separate and purify *T. gondii* oocyst walls and sporocysts. Oocyst walls were successfully separated and purified using iodixanol gradients. Sporocysts were successfully separated and purified using iodixanol and Percoll gradients. Purification was also achieved by flow cytometry. Flow cytometry with fluorescence-activated cell sorting (FACS) yielded analytical quantities of oocyst walls and intact sporocysts. Flow cytometry with FACS also proved useful for quantitation of purity obtained following iodixanol gradient fractionation. Methods reported in this paper will be useful for analytical purposes, such as proteomic analysis of components unique to this life cycle stage, development of detection methods, or excystation studies.

4.138 Correlation between RBC deformability and CR1 activity

Kamiyama, M., Inada, Y., Berezina, T., Zaets, S., Condon, M., Spolarics, Z., Kim, J., Deitch, E.A. and Machiedo, G.
Proceedings of 25th Annual Conference on Shock, June 2002.

We have previously presented data showing that binding of immune complex (IC) to CR1 on RBC membrane induced a decreased RBC deformability (RBCD). **Objectives:** In this study we investigated correlation between CR1 activity and RBCD by separating younger RBC with higher CR1 activity from older cells with lower CR1 activity. Further, we studied reversibility of RBCD after removal of IC from RBC CR1. **Methods**: RBC were fractionated according to their density using OptiPrep (iodixanol); RBC suspension was layered on the top of discontinuous 4-layered gradient and centrifuged for 30 mm. at 1,000 x g. Four fractions were harvested and EI (elongation index) of each fraction was measured after incubation in the presence and absence of IC (heat-aggregated human IgG) and complement. EI of RBC after removal of IC was also determined. **Results**: CR1 activity and EI at 3 Pa of each fraction are shown in the table:

Fraction	CR1	EI at 3 Pa	
		-IC	+IC
A:1.097 g/ml	++++	0.334±0.008*	0.276±0.016
B:1.099 g/ml	++++	0.289±0.043	0.244±0.042
C:1.101 g/ml	++	0.256±0.029	0.241±0.036
D:1.104 g/ml	-	0.238±0.044	0.230±0.046

*p<005 vs. +IC

After removal of IC, EIs of all fractions were not significantly different from control levels (-IC). Electron microscopy revealed an increase in number of abnormally shaped RBC after incubation with IC and partial recovery of shape after removal of IC from RBC membrane. **Conclusion:** The results confirm that CR1 activity and deformability of RBC are closely related; the higher CR1 activity gives higher basal RBCD but makes the cell more susceptible to damage by IC. Our results also suggest that IC-induced change in RBC deformability is reversible.

4.139 Sperm separation techniques: comparison and evaluation of gradient products

Tucker, K.E. and Jansen, C.A.M.

In: Proceedings 2nd International workshop for Embryologists: Troubleshooting activities in the ART lab. Ed. R. Basuray and D. Mortimer. (2002)

The human ejaculate is comprised of a mixture of seminal plasma, mature and immature spermatozoa, non-reproductive cells, various micro-organisms and non-specific debris. In preparation for intrauterine insemination (IUI) or *in vitro* fertilization (IVF), the motile, and hopefully, the most fertilizable population of sperm must be separated from the surrounding milieu. Many studies have been performed comparing direct semen processing procedures (i.e. simple wash, swim-up, etc.) with gradient separation techniques. Sperm separation using, a polyvinylpyrrolidone (PVP)-coated, silica-based density gradient (Percoll®), has been shown by numerous investigators to be an effective and relatively simple way to produce a viable, highly motile, morphologically normal and fertilizable population of sperm for use in both IUI and IVF (Mohan and Lindsay, 1995).

The demands on sperm separation techniques have increased with our expanding knowledge of sperm physiology and on their contribution to the embryo. Because of this, there has been rising concern over the safety of any sperm separation procedure with respect to not only the viability of the sperm, but to the long-term effects of any resulting pregnancy.

Although extremely effective in sperm separation and apparently safe for clinical use, Percoll (Pharmacia; Sigma Pharmaceutical) has been made unavailable for therapeutic use in human infertility. It has, therefore, become necessary to evaluate and select an alternative product or procedure that will compare favorably in light of all the studies that have advocated the use of Percoll for sperm separation. To address the need for Percoll-substitutes, a new line of density gradient products have been manufactured, specifically, the silane-coated, colloidal silica particle-based density gradients (i.e. PureSperm®, Isolate®, Enhance S+®). This paper will attempt to compare the efficacy of these new products with each other and with what has become the first choice for sperm separation, Percoll.

4.140 Outbreak of cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania, 2000

Ho, A.Y. et al

*Emerging Infectious Diseases, **8(8)**, 783-788 (2002)*

An outbreak of cyclosporiasis occurred in attendees of the wedding reception held in Philadelphia, Pennsylvania, on June 10,2000. In a retrospective cohort study, 54 (68.4%) of the 79 interviewed guests and members of the wedding party met the case definition. The wedding cake, which had a cream filling that included raspberries, was the food item most strongly associated with illness (multivariate relative risk, 5.9; 95% confidence interval, 3.6 to 10.5). Leftover cake was positive for *Cyclospora* DNA by polymerase chain reaction analyses. Sequencing of the amplified fragments confirmed that the organism was *Cyclospora cayetanensis.* The year 2000 was the fifth year since 1995 that outbreaks of cyclosporiasis definitely or probably associated with Guatemalan raspberries have occurred in the spring in North America. Additionally, this is the second documented U.S. outbreak, and the first associated with raspberries, for which *Cyclospora* has been detected in the epidemiologically implicated food item.

4.141 Long-lived immature dendritic cells mediated by TRANCE-RANK interaction

Cremer, I. et al

*Blood, **100(10)**, 3646-3655 (2002)*

Immature dendritic cells (DCs) reside in interstitial tissues (int-DC) or in the epidermis, where they capture antigen and, thereafter, mature and migrate to draining lymph nodes (LNs), where they present processed antigen to T cells. We have identified int-DCs that express both TRANCE (tumor necrosis factor-related activation-induced cytokine) and RANK (receptor activator of NF-κB) and have generated these cells from $CD34^+$ human progenitor cells using macrophage colony-stimulating factor (M-CSF). These $CD34^+$-derived int-DCs, which are related to macrophages, are long-lived, but addition of soluble RANK leads to significant reduction of cell viability and Bcl-2 expression. This suggests that constitutive TRANCE-RANK interaction is responsible for $CD34^+$-derived int-DC longevity. Conversely, $CD1a^+$ DCs express only RANK and are short-lived. However, they can be rescued from cell death either by recombinant soluble TRANCE or by $CD34^+$-derived int-DCs. $CD34^+$-derived int-DCs mature in response to lipopolysaccharide (LPS) plus CD40 ligand (L) and become capable of CCL21/CCL19-mediated chemotaxis and naive T-cell activation. Upon maturation, they lose TRANCE, making them, like $CD1a^+$ DCs, dependent on exogenous TRANCE for survival. These findings provide evidence that TRANCE and RANK play important roles in the homeostasis of DCs.

4.142 ***Toxoplasma gondii*** **induces granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor secretion by human fibroblasts: implications for neutrophil apoptosis**
Channon, J.Y., Miselis, K.A:, Minns, L.A, Dutta, C. and Kasper, L.H.
Infection and Immunity, ***70(11)***, *6048-6057 (2002)*

Human neutrophils are rescued from apoptosis following incubation with once-washed, fibroblast-derived *Toxoplasma gondii* tachyzoites. Both infected and uninfected neutrophils are rescued, implicating a soluble mediator. In this study we investigated the origin and identity of this soluble mediator. Neutrophils were incubated either with purified tachyzoites or with conditioned medium derived from *T. gondii*-infected human fibroblasts. Conditioned medium was found to be a potent stimulus that delayed neutrophil apoptosis up to 72 h, whereas purified and extensively washed tachyzoites had no effect. Delayed apoptosis correlated with up-regulation of the neutrophil antiapoptotic protein, Mcl-1, and the neutrophil interleukin 3 receptor αsubunit (IL-3Rα), suggesting a role for granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF and granulocyte colony-stimulating factor (G-CSF) were measurable in conditioned medium by enzyme-linked immunosorbent assay. Neutralizing antibodies to GM-CSF and G-CSF were additive in abrogating delayed neutrophil apoptosis induced by conditioned medium. Inhibitors of Src family tyrosine kinases, G_i proteins, phosphatidylinositol 3-kinase, $p44^{erk1}$ and $p42^{erk2}$ mitogen-activated protein kinases, and Jak2 kinases partially attenuated the effect of conditioned medium, consistent with a role for G-CSF and/or GM-CSF. Hence, delayed neutrophil apoptosis is mediated by GM-CSF and G-CSF secreted by *T. gondii*-infected human fibroblasts. This enhanced neutrophil survival may contribute to the robust proinflammatory response elicited in the *T. gondii*-infected host.

4.143 **Dynamic perifusion to maintain and assess isolated pancreatic islets**
Sweet, I.R. et al
Diabet. Techn. & Therapeut., ***4(1)***, *67-76 (2002)*

Advances in human islet transplant techniques are hampered by the inability to assess the quality of isolated islets. A flow culture system was developed to perifuse isolated pancreatic islets or cultured beta-cell lines in order to continuously and noninvasively assess cell function and viability with high kinetic resolution. Continuous perifusion of large amounts of islet tissue as isolated from human pancreata enables the use of noninvasive measurement technologies not previously applied to islets. To compare dynamic perifusion of tissue at high density with conventional static cultures, we measured glucose-stimulated insulin secretion and O_2 consumption of large amounts of INS-1 cells ($45\text{-}65 \times 10^6$) to confirm that perifused cells were adequately supplied with oxygen and nutrients and remained functionally responsive. Isolated human and monkey islets that were perifused for 18 h showed robust biphasic insulin secretion in response to a step increase in glucose, demonstrating the ability to maintain islets and the high kinetic resolution of the system. As an example of the system's ability to resolve multiple indicator dilution experiments, the retention of [^{3}H]-glibenclamide was kinetically distinguished from that of an extracellular marker. In summary, the perifusion system is able to maintain healthy cells, assess insulin secretion and metabolite fluxes such as oxygen consumption and lactate production, and characterize the kinetics of the interaction between radiopharmaceuticals and islet cells. The ability to systematically assess the metabolic and functional viability of islets will facilitate the optimization of islet isolation procedures, islet transplantation studies, and islet storage methodologies.

4.144 Isolation of living neurons from human elderly brains using the immunomagnetic sorting DNA-linker system

Konishi, Y., Lindhilm, K., Yang, L-B., Li, R. and Shen, Y.
Am. J. Pathol., ***161(5)***, *1567-1576 (2002)*

Isolation and culture of mature neurons from affected brain regions during diseased states provide a well-suited ***in vitro*** model system to study age-related neurodegeneration under dynamic conditions at cellular levels. We have developed a novel technique to isolate living neurons from rapidly autopsied human elderly brains, and have succeeded in keeping them alive ***in vitro***. Specifically, the parietal cortex blocks were fractionated by density gradients and further enriched for neurons by an immunomagnetic sorting DNA-linker technique. The postmortem interval averaged 2.6 hours. After isolation and purification of neurons using this technology, the cells were maintained ***in vitro*** for 2 weeks. Our evaluation revealed that 80% of the isolated cells were neurons and they exhibited neurotransmitter phenotypes (glutamate and γ-aminobutyric acid) as well as glutamate receptors. Studies on cell viability and calcium influx suggest that these isolated living cortical neurons still retain their typical neuronal functions. Our present study demonstrates that neurons isolated from human elderly brain autopsies can survive ***in vitro*** and maintain their functional properties. Our study has opened an opportunity to apply such neurons to dynamic pharmacological studies of neurological disorders at the single-cell level.

4.145 Expression of programmed death 1 ligands by murine T cells and APC

Yamazaki, T. et al
J. Immunol., ***169***, *5538-5545 (2002)*

Programmed death 1 (PD-1) is a new member of the CD28/CTLA-4 family, which has been implicated in the maintenance of peripheral tolerance. Two ligands for PD-1, namely, B7-H1 (PD-L1) and B7-DC (PD-L2), have recently been identified as new members of the B7 family but their expression at the protein level remains largely unknown. To characterize the expression of B7-H1 and B7-DC, we newly generated an anti-mouse B7-H1 mAb (MIH6) and an anti-mouse B7-DC mAb (TY25). MIH6 and TY25 immunoprecipitated a single molecule of 43 and 42 kDa from the lysate of B7-H1 and B7-DC transfectants, respectively. Flow cytometric analysis revealed that B7-H1 was broadly expressed on the surface of mouse tumor cell lines while the expression of B7-DC was rather restricted. PD-1 was expressed on anti-CD3-stimulated T cells and anti-IgM plus anti-CD40-stimulated B cells at high levels but was undetectable on activated macrophages or DCs. B7-H1 was constitutively expressed on freshly isolated splenic T cells, B cells, macrophages, and dendritic cells (DCs), and up-regulated on T cells by anti-CD3 stimulation on macrophages by LPS, IFN-γ, GM-CSF, or IL-4, and on DCs by IFN-γ, GM-CSF, or IL-4. In contrast, B7-DC expression was only inducible on macrophages and DCs upon stimulation with IFN-γ, GM-CSF, or IL-4. The inducible expression of PD-1 ligands on both T cells and APCs may suggest new paradigms of PD-1-mediated immune regulation.

4.146 Oligodendrocyte progenitor cells in the adult rat CNS express myelin oligodendrocyte glycoprotein (MOG)

Li, G., Crang, A.J., Rundle, J.L. and Blakemore, W.F.
*Brain Pathol., **12**, 463-471 (2002)*

While the effects of high dose X-irradiation on mitotically active progenitor cells and remyelination are well-documented, its effects on myelinating oligodendrocytes are less clear, due in part to divergent views on their mitotic capacity. To examine the effect of X-irradiation on oligodendrocytes, the spinal cord of rats was exposed to 40 Gy of X-irradiation and the number of oligodendrocytes and oligodendrocyte progenitors in the dorsal funiculi at T12 and L1 was determined by in situ hybridization using cRNA-probes for platelet derived growth factor α receptor (PDGFRα) (to identify oligodendrocyte progenitors), exon 3b of proteolipid protein (PLP) (to identify mature oligodendrocytes) and myelin oligodendrocyte glycoprotein (MOG). X-irradiation resulted in no change in the number of PLP positive cells and no loss of myelin internodes, but caused an almost complete loss of PDGFRα-expressing cells, and a reduction in the number of MOG positive cells to a number similar to that found using the PLP exon 3b probe. Importantly, the number of radiation-sensitive MOG-expressing cells was similar to the number of PDGFRα positive cells. To determine if the radiation-sensitive MOG positive cells were the same population as the radiation sensitive PDGFRα-expressing cells, MOG and PDGFRα-expressing cells were isolated from the adult CNS using antibody coated magnetic beads. Twelve to thirteen percent of MOG positive cells were PDGFRα positive and nearly all the PDGFRa isolated cells were MOG and galactocerebroside positive. Double immunofluorescence revealed colocalization of NG2 and MOG on cells in the normal adult rat spinal cord. These results show that in situ in the adult rat spinal cord white matter oligodendrocyte progenitors are MOG positive and indicates that expression of MOG cannot be regarded a marker that only identifies mature myelin-supporting oligodendrocytes in tissue.

4.147 Proteasome-dependent regulation of Syk tyrosine kinase levels in human basophils

Youssef, L.A., Pharm, B., Wilson, B.S. and Oliver, J.M.
*Allergy Clin. Immunol., **110**, 366-373 (2002)*

Background: In human basophils, FcεRI signal initiation, leading to histamine release, relies on activation of Syk protein tyrosine kinase. Basophils from approximately 10% of unselected donors do not degranulate in response to FcεRI cross-linking. Their unresponsiveness has been linked to the absence of Syk protein despite apparently normal levels of Syk mRNA.
Objective: The aim of this study was to explore pathways of Syk protein degradation as a possible posttranslational mechanism for downregulating Syk protein levels in human basophils and other leukocytes.
Methods: Highly purified basophils, lymphocytes, and monocytes were incubated in the presence or absence of a panel of cell-permeable inhibitors of proteolytic degradation pathway(s). Subsequently, the protein level of Syk tyrosine kinase was determined by means of Western blotting. In vitro assays were conducted through use of immunoprecipitated basophil Syk and a rabbit reticulocyte lysate system.
Results: Three inhibitors of proteasome-mediated degradation—PSI, lactacystin, and ALLN—substantially increased Syk levels in releaser basophils and restored Syk expression in nonreleaser basophils. Caspase inhibitors were less effective, and inhibitors of calpain-mediated proteolysis had no effect. Among other leukocytes tested, only naive $CD4^+$ T cells had more Syk after proteasome inhibitor treatment. In vitro ubiquitination assays demonstrated that Syk is readily ubiquitinated in vitro and also that Syk ubiquitination is associated with a substantial decrease in total levels of Syk protein.
Conclusion: These data provide evidence for a ubiquitin/proteasome-dependent mechanism that contributes to Syk regulation in human basophils and might also be relevant to naive T cells. Understanding this regulatory pathway might lead to strategies for suppressing allergic inflammation while preserving essential Syk-mediated functions in other hematopoietic cells.

4.148 Tip-growing cells of the moss *Ceratodon purpureus* are gravitrophic in high-density media
Schwuchow, J.M., Kern, V.D. and Sack, F.D.
*Plant Physiol., **130**, 2095-2100 (2002)*

Gravity sensing in plants and algae is hypothesized to rely upon either the mass of the entire cell or that of sedimenting organelles (statoliths). Protonemata of the moss *Ceratodon purpureus* show upward gravitropism and contain amyloplasts that sediment. If moss sensing were whole-cell based, then media denser than the cell should prevent gravitropism or reverse its direction. Cells that were inverted or reoriented to the horizontal displayed distinct negative gravitropism in solutions of **iodixanol** with densities of 1.052 to 1.320 as well as in bovine serum albumin solutions with densities of 1.037 to 1.184 g cm^{-3}. Studies using tagged molecules of different sizes and calculations of diffusion times suggest that both types of media penetrate through the apical cell wall. Estimates of the density of the apical cell range from 1.004 to 1.085. Because protonemata grow upward when the cells have a density that is lower than the surrounding medium, gravitropic sensing probably utilizes an intracellular mass in moss protonemata. These data provide additional support for the idea that sedimenting amyloplasts function as statoliths in gravitropism.

4.149 Continuous measurement of oxygen consumption by pancreatic islets
Sweet, I.R. et al
*Diabet. Techn. & Therapeut., **4(5)**, 661-672 (2002)*

The rate of oxygen consumption is an important measure of mitochondrial function in all aerobic cells. In pancreatic beta cells, it is linked to the transduction mechanism that mediates glucose-stimulated insulin secretion. However, measurement of oxygen consumption over long periods of time is technically difficult owing to the error resulting from baseline drift and the challenge of measuring small changes in oxygen tension. We have adapted an ultrastable oxygen sensor based on the detection of the decay of the phosphorescent emission from an oxygen-sensitive dye to a previously developed islet flow culture system. The drift of the sensor is approximately 0.3%/24 h, allowing for the continuous measurement of oxygen consumption by 300 islets (or about 6 x 10^5 cells) for hours or days. Rat islets placed in the perifusion chamber for 24 h were well maintained as reflected by membrane integrity, insulin secretion, and oxygen consumption. Both acute changes in oxygen consumption as induced by glucose and chronic changes as induced by sequential pulses of azide were resolved. The features of the flow culture system-aseptic conditions, fine temporal control of the composition of the media, and the collection of outflow fractions for measurement of insulin, and other products-facilitate a systematic approach to assessing metabolic and functional viability in responses to a variety of stimuli. Applications to the measurement of effects of hypoxia on insulin secretion, membrane integrity, and the redox state of cytochromes are demonstrated. The system has particular application to the field of human islet transplantation, where assessment and the study of islet viability have been hampered by a lack of experimental methods.

4.150 Nef protein of human immunodeficiency virus and lipopolysaccharide induce expression of CD14 on human monocytes through differential utilization of interleukin-10
Creery, D. et al
*Clin. Diagnost. Lab. Immunol., **9(6)**, 1212-1221 (2002)*

We investigated the expression of membrane-bound CD14 (mCD14) on monocytes and soluble CD14 (sCD14) released into the culture supernatants of peripheral blood lymphocytes (PBMC) from human immunodeficiency virus (HIV)-infected individuals. Monocytes from HIV-positive individuals exhibited both enhanced mCD14 expression and sCD14 production in the PBMC culture supernatants compared to the levels of mCD14 and sCD14 in HIV-negative individuals. This enhanced mCD14 expression and sCD14 production in HIV-infected individuals may be due to the effects of cytokines, the bacterial product lipopolysaccharide (LPS), and/or the HIV regulatory antigens Tat and Nef. Interleukin-10 (IL-10), an immunoregulatory cytokine, as well as LPS enhanced mCD14 expression and the release of sCD14 in the culture supernatants. HIV-Nef, unlike Tat, enhanced mCD14 expression on monocytes but did not induce the release of sCD14 into the culture supernatants. Studies conducted to investigate the mechanism underlying HIV-Nef-induced mCD14 expression revealed that HIV-Nef upregulated mCD14 expression via a mechanism that does not involve endogenously produced IL-10. In contrast, LPS upregulated the expression of mCD14 and increased the release of sCD14 via a mechanism that involves, at least in part, endogenously produced IL-10. Furthermore, dexamethasone, an anti-inflammatory and immunosuppressive agent, inhibited HIV-Nef-induced CD14 expression in an IL-10-independent manner. In contrast, dexamethasone inhibited IL-10-dependent LPS-induced CD14 expression by interfering with IL-10-induced signals but not by blocking IL-10 production. These results suggest that HIV-Nef and IL-10 constitute biologically important modulators of CD14 expression which may influence immunobiological responses to bacterial infections in HIV disease.

4.151 Insulin treatment of mice recipients preserves β-cell function in porcine islet transplantation
Pakhomov, O. et al
*Cell Transplantation, **11(7)**, 721-728 (2002)*

Encapsulation of islets of Langerhans confers protection against cell-mediated immune destruction and so should allow the transplantation of islets without immunosuppression. Xenotransplantation of encapsulated islets of Langerhans might therefore help overcome problems of human organ donor shortage. Given that islets exposed to sustained hyperglycemia show impaired β-cell function, we set out to determine whether recipient treatment with insulin could improve transplantation success rate. Islets of Langerhans were obtained from Specific Germ-Free (SPF) pig pancreas and cultured overnight. Islets were encapsulated in AN69 fibers and implanted into the peritoneal cavity of diabetic mice. A group of implanted mice was treated with exogenous insulin from day 3 to day 7 after grafting. Islet implantation depressed plasma glucose in all the mice, both insulin treated and untreated. Glycemia slowly increased in the non-insulin-treated mice, whereas the decrease observed in the insulin-treated mice was maintained until day 29 of follow-up. We found significant differences between the two groups ($p < 0.05$ at day 18 and day 20, $p < 0.001$ at day 23 and day 29). No improvement of hyperglycemia was observed in diabetic mice implanted with empty fibers. When islet-containing fibers were removed from the peritoneal cavity of mice 1 month after the graft plasma glucose increased markedly. We demonstrate that treatment of recipients with exogenous insulin in the immediate posttransplantation period has a positive effect on β-cell function in transplanted macroencapsulated porcine islets.

4.152 Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata
Matsumoto, S. et al
Cell Transplantation, ***11(8)***, *769-777 (2002)*

Previous reports indicate that the two-layer method (TLM) of human pancreas preservation is superior to University of Wisconsin solution (UW) when pancreata are preserved for extended periods (i.e., >24 h) prior to islet isolation. In this study, the efficacy of using the TLM for preserving pancreata for short periods (i.e., <13 h) was evaluated using both nonhuman primate and human pancreata preserved with a TLM kit precharged with oxygen. An oxygen precharged TLM (static TLM) was established and compared with the original TLM with continuous oxygen supply. For the static TLM, the perfluorochemical was fully oxygenated and the oxygen supply removed prior to pancreas preservation. In the primate model, pancreata were preserved by the static TLM, the original TLM, and UW for 5 h prior to islet isolation. In the human model, pancreata were preserved with the static TLM or the original TLM or UW for 4–13 h. Both primate and human pancreata were processed by intraductal collagenase injection and digestion followed by continuous density gradient purification to isolate islets. Islets were assessed for islet yield, purity, viability, and in vitro functionality. In the primate model, islet yield, viability, and in vitro functionality were significantly improved by both the static TLM and the original TLM with similar results. Postculture islet yields were 23,877 ± 3619 IE/g in the static TLM, 21,895 ± 3742 IE/g in the original TLM, and 6773 ± 735 IE/g in UW. In the human model, both the static TLM and the original TLM significantly increased islet yield compared with UW with postculture islet yields of 2659 ± 549 IE/g in the static TLM, 2244 ± 557 IE/g in the original TLM, and 1293 ± 451 IE/g in UW. Nonhuman primate and human pancreata stored in the static TLM, immediately upon procurement, yield isolated islets of a substantially higher quantity than when pancreata are stored in UW. Thus, the use of the static TLM should replace the use of UW for storage of pancreata during transport prior to islet isolation.

4.153 The morphology of islets within the porcine donor pancreas determines the isolation result: Successful isolation of pancreatic islets can now be achieved from young market pigs
Krickhahn, M., Bühler, C., Meyer, T., Thiede, A. and Ulrichs, K.
Cell Transplantation, ***11(8)***, *827-838 (2002)*

Clinical islet allotransplantation has become an increasingly efficient “routine” therapy in recent years. Shortage of human donor organs leads to porcine pancreatic islets as a potential source for islet xenotransplantation. Yet it is still very difficult to isolate sufficient numbers of intact porcine islets, particularly from young market pigs. In the following study islets were successfully isolated from retired breeders [4806 ± 720 islet equivalents per gram organ (IEQ/g); $n = 25$; 2–3 years old; RB] and also from young hybrid pigs [2868 ± 260 IEQ/g; $n = 65$; 4–6 months old; HY] using LiberasePI and a modified version of Ricordi’s digestion-filtration technique. As expected, isolations from RB showed significantly better results ($p < 0.002$). A retrospective histological analysis of almost all donor pancreases showed that the majority of organs from RB (80%) contained mainly large islets (diameter >200 μm), in contrast to only 35% of all pancreases from HY. Remarkably, the islet size in situ, regardless whether detected in RB or HY, strongly determined the isolation result. A donor organ with predominantly large islets resulted in significantly higher numbers of IEQs compared with a donor organ with predominantly small islets [$RB^{Large\ Islets}$: 5680 ± 3,318 IEQ/g ($n = 20$); $RB^{Small\ Islets}$: 1353 ± 427 IEQ/g ($n = 5$); $p < 0.02$]. In addition, isolation results were strongly influenced by the quality of the LiberasePI batch, and therefore single batch testing is invariably required. Purification was performed using Ficoll or OptiPrep™ density gradient centrifugation manually or in the COBE cell processor. Although islet purity was highest when OptiPrep™ was used, final islet yields did not differ between the different purification methods. Our study demonstrates that islet size in situ is an extremely critical parameter for highly successful islet isolation; consequently, we are now performing a morphological screening of each donor organ prior to the isolation process. Under these conditions highly successful isolations can reliably be performed even from young market pigs.

4.154 Elevated C-met in thymic dendritic cells of New Zealand black mice
Okada, T. et al
Develop. Immunol., ***9(1)****, 29-34 (2002)*

New Zealand Black (NZB) mice are a well-known animal model of human autoimmune disease. Although the mechanism for development of autoimmunity is unclear, NZB mice are well known for severe thymic microarchitecture abnormalities. It is thought that thymic dendritic cells (DC) may play a role in thymic education and contribute to the autoimmune process. To address this issue and, in particular, that qualitative and/or quantitative differences exist in thymic DC, we took advantage of a novel restriction analysis system that allow definition of differences in the expression of tyrosine kinases using highly enriched populations of thymic DC from NZB compared to BALB/c and C57BL/6 mice. The method chosen, restriction analysis of gene expression, allowed the determination of protein tyrosine kinase transcription profiles. We report herein that NZB mice have a significant upregulation of C-met compared to the control strains. The abnormality of the C-met transcription was confined to thymic DC. We believe that its abnormal expression reflects the resistance of thymic cells to apoptosis, which will ultimately lead to defects and/or abnormal signaling by the interaction of thymic DC and thymocytes. Further studies involving such interactions are under way.

4.155 Comparison of nitric oxide production and motion characteristics after 3-layer Percoll and IxaPrep preparation methods of human sperm
Ding, D-C., Huang, Y-C-, Liu, J-Y and Wu, G-J
Arch. Gynecol. Obstet., ***266****, 210-213 (2002)*

Objektive: To compare two different spermatozoa preparation mediums, the three-layer Percoll (Sigma, St. Louis, MO; salica-based) and the IxaPrep (Medicult, Copenhagen, Denmark; non-salica based, polysucrose medium) method, with respect to recovery of pregressive motile sperm and various sperm motion characteristics. Analysis was determined by computer-aided sperm analysis and nitric oxide (NO) production of the supernatant after centrifugation. *Method:* Thirty-nine semen specimens were obtained from men who presented for semen analysis and each of them was divided into two aliquots for preparation with the two mediums mentioned above. The motile sperm recovery, motility percentage and motion parameters were measured for each semen specimen (n=39) before and after preparation using one of the two above methods. The NO was measured using the chemiluminscence method after centrifugation. *Results:* Recovery rate was higher in the IxaPrep group (Ixaprep: 45.4±28.7% versus Percoll: 32.3±22.7%; $p<0.05$). The other motion characteristics such as average path velocity (VAP) and straight line velocity (VSL) were better than those of fresh semen samples [VAP: 72±17.2 μm/s (Percoll), 62.8±18.2 μm/s (Ixaprep) vs 52.2±9.5 μm/s (fresh); $p<0.05$; VSL: 51.8±13.4 μm/s (Percoll), 44.8±12.9 μm/s (Ixaprep) vs 38.6±7.9 μm/s (fresh); $p<0.05$]. The motility between fresh and post-preparation semen samples had no significant difference. Hyperactivation of the sperm was improved in the IxaPrep group compared with fresh sperm (Percoll: 24.7±16.9% and IxaPrep: 20.5±10.5% versus fresh: 9.2±9.2%; $p<0.05$). NO produced in the IxaPrep method was significantly lower than that in the Percoll method (Ixaprep: 0.24±0.3 μM versus Percoll: 0.54±0.91 μM; $p<0.05$). *Conclusion:* Our data suggests that the IxaPrep method provides a better recovery rate, but that other motion characteristics did not demonstrate any significant difference. The lower level of NO produced in the IxaPrep preparation method may suggest that better sperm quality achieved is due to the decreased NO production.

4.156 Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial
Pecher, G., Häring, A., Kaiser, L. and Thiel, E.
Cancer Immunol. Immunother., ***51****, 669-673 (2002)*

Dendritic cells (DC) derived from peripheral blood monocytes are currently being investigated in clinical trials for their role in stimulating the immune system. We performed a phase I/II clinical trial using human autologous DC transfected with cDNA of the human tumor antigen mucin (MUC1) as a vaccine in 10 patients with advanced breast, pancreatic or papillary cancer. After liposomal transfection, flow cytometry testing showed that 2% to 53% of the DC expressed mucin epitopes. Patients were immunized two or three times with 1 million transfected DC injected subcutaneously (s.c.). A vaccine-specific delayed-type hypersensitivity (DTH) reaction was observed in 3 out of 10 patients. After vaccination, 4 patients showed a 2- to 10-fold increase in the frequency of mucin-specific interferon-gamma (IFN-γ)-secreting $CD8^+$ T cells. We demonstrated the feasibility and safety of a vaccine consisting of autologous gene-transfected DC, and that immunologic responses could be induced even in patients with pretreated and advanced disease.

4.157 Microchimerism and transmission of porcine endogenous retrovirus from a pig cell line or specific pathogen-free pig islets to mouse tissues and human cells during xenografts in nude mice
Clemenceau, B., Jegou, D., Martignat, L, and Saï, P.
*Diabetologia, **45**, 914-923 (2002)*

Aims/hypothesis. Pig islets could transmit porcine endogenous retroviruses (PERV) to diabetic patients. Our previous work showed that pig islets expressed low levels of PERV mRNA and were not likely to transmit PERV to human cells in vitro. The real risk of infection during pig tissue xenografts can only be evaluated by in vivo experiments.
Methods. Nude mice bearing tumours containing human 293 cells were grafted with specific pathogen-free pig islets or PERV-producing pig PK15 cells to determine whether pig cells could transmit PERV to mouse and human cells in vivo. Infection was monitored by PCR, long PCR, RT-PCR and long RT-PCR. As detection of PERV sequences could be due to the presence of residual pig cells, we looked for pig mitochondrial (mt) DNA. Quantitative PCR for PERV and pig mt DNA was done to compare the PERV-to-pig mt (P-to-M) ratio of each sample with the reference ratio for grafted pig cells.
Results. Among 78 mouse tissues from PK15-grafted mice, 54 and 72 were positive for gag and pig mt DNA, respectively. Human tumours developed in these mice were positive for PERV (78%) and pig mt (89%). The P-to-M ratios for mouse tissues and PERV-positive human tumours from PK15-grafted mice were higher than the ratio in PK15 cells. Among 41 tissues from pig islet cell-grafted mice, 7 were positive for PERV (3 lymph nodes, 1 kidney, 2 salivary glands, 1 ovary), and 14 were positive for pig mt DNA. Three of these samples (1 lymph node, 1 kidney and 1 salivary gland) were positive for gag DNA, but negative for pig mt DNA. One human tumour in these mice was positive for PERV DNA. P-to-M reference ratio in grafted islet cells was 0.05+-0.03. The three PERV-positive lymph nodes contained 78 gag/3 mt copies (P-to-M: 26), 101 gag/3 mt copies (P-to-M: 34), and 4 gag/0 mt copies. The two PERV-positive salivary glands contained 14 gag/1 mt copies, and 28 gag/0 mt copies. The ovary and the kidney contained 46 gag/3 mt and 69 gag/0 mt copies, respectively. The PERV-positive human tumour contained 47 gag/3 mt copies.
Conclusions/interpretation. Microchimerism and PERV transmission were frequently observed in both mouse and human tissues during grafting of pig PK15 cells into nude mice bearing human tumours, and sometimes during pig islet xenograft in this model. This strengthens the notion that there is a risk of transmitting PERV during pig islet xenograft.

4.158 Microglia promote glioma migration
Bettinger, I., Thanos, S. and Paulus, W.
*Acta Neuropathol., **103**, 351-355 (2002)*

Diffuse astrocytic gliomas extensively infiltrate brain tissue and contain numerous microglial cells, but it is unknown whether these two characteristic features are pathogenetically related. We therefore studied the effects of murine microglial cells on motility of GL261 mouse glioma cells using Boyden chamber assays. In the presence of microglia, glioma cell migration occurred earlier, and after 48 h it was threefold higher as compared to incubations without microglia. This effect was mediated by substances released from microglia, because similar effects were observed by microglia-conditioned medium, and it was specific to microglia, because oligodendroglia and endothelial cells only weakly stimulated glioma cell migration. Microglia activating substances (GM-CSF, LPS) led to a further increase of motility. These data support the notion that microglia accumulation in diffuse glial tumors does not merely represent a nonspecific reaction to tissue injury, but reflects participation of these cells in supporting and promoting the invasive phenotype of astrocytoma cells.

4.159 The expression of scavenger receptor class B, type I (SR-BI) and caveolin-1 in parenchymal and nonparenchymal liver cells

Malerød, L., Juvet, L.K., Gjøen, T. and Berg, T,
*Cell Tissue Res., **307**, 173-180 (2002)*

The liver is the major site of cholesterol synthesis and metabolism, and the only substantive route for eliminating blood cholesterol. Scavenger receptor class B, type I (SR-BI) has been reported to be responsible for mediating the selective uptake of high-density lipoprotein cholesteryl esters (HDL-CE) in liver parenchymal cells (PC). We analysed the expression of SR-BI in isolated rat liver cells, and found the receptor to be highly expressed in liver PC at both the mRNA and protein levels. We also found SR-BI to be expressed in liver endothelial cells (LEC) and Kupffer cells (KC). SR-BI has not previously been reported to be present in LEC. CD36 mRNA was expressed in all three liver cell types. Since caveolin-1 appears to colocalize with SR-BI and CD36 in caveolae of several cell lines, the distribution and expression of caveolin-1 in the liver cells were investigated. Caveolin-1 was not detected in PC but was found in both LEC and KC. This led to the suggestion that caveolin-1 may be more important in the efflux of cholesterol than in the selective uptake of cholesterol in the liver.

4.160 Feeding NOD mice with pig splenocytes induces transferable mechanisms that modulate cellular and humoral xenogeneic reactions aginst pig spleen or islet cells

You, S., Gouin, E. and Sai, P.
*Clin. Exptl. Immunol., **127**, 412-422 (2002)*

We have reported previously that oral administration of pig cells to NOD mice modified xenogeneic cellular response against pig islet cells (PICs), and hypothesized that it may have induced active suppression. This preliminary report evaluated only the effect of feeding pig cells by 'primary' proliferation, i.e. when splenocytes from fed mice are confronted with pig cells *in vitro*. The present study also considered 'secondary' proliferation and cytokine production after feeding and subsequent *in vivo* graft of pig cells. Additionally, serum IgM and IgG isotypes were quantified by ELISA using pig target cells. Induction of active mechanism by feeding was hypothetical, which led us here to transfer splenocytes from mice fed pig spleen cells (PSC) and evaluate 'primary' (after transfer) and 'secondary' (after transfer and subsequent graft of pig cells) proliferations and cytokine secretions in recipient mice. We also determined whether the effects of feeding pig cells persisted after depression of suppressor mechanisms by cyclophosphamide. Mice fed with PSC displayed increased 'primary' splenocyte proliferation to PSC or PIC ($P < 0.0001$), while 'secondary' responses were decreased ($P < 003$) in those fed PSC and subsequently grafted with PSC. The increased 'primary' and decreased 'secondary' proliferations were reduced ($P < 0.04$) by pretreatment with cyclophosphamide. The IL-l0/ and IL-4/lFNγ ratios produced in response to PSC increased ($P < 004$) in mice fed and grafted with PSC compared to those grafted only with PSC. IgM and IgG levels against pig cells were, respectively, increased ($P < 0.04$) and decreased ($P < 0.04$) in mice fed and grafted with PSC. IgG2a and IgG2b, but not IgG1, levels were lower ($P < 0.01$). These effects of feeding PSC on 'secondary' proliferation, cytokine and antibody productions, were not detected when mice were fed PSC only after graft with PSC. Transfer with splenocytes from mice fed PSC increased 'primary' proliferation of splenocytes from recipient mice in response to PSC ($P < 0.02$) or PIC ($P < 0.05$). After transfer with splenocytes from PSC-fed mice and graft with PSC, 'secondary' proliferation to pig cells were reduced ($P < 0.04$), and the IL-l0/lFNγ ratio produced in response to PSC was increased fourfold. Thus, oral administration of PSC induces active transferable mechanisms, characterized by a biphasic pattern with early increased 'primary' xenogeneic cellular reactions to both PSC and PIC, followed by decreased 'secondary' responsiveness and a concomitant shift of the Thl/Th2 balance towards greater Th2 influence. Decreased responsiveness may be due to active suppression, even though induction of anergy or deletion cannot be excluded.

4.161 High efficiency gene transfer into cultured primary rat and human hepatic stellate cells using baculovirus vectors

Gao, R. et al

*Liver **22**, 15-22 (2002)*

Background/aims: Gene transfer into hepatic stellate cells (HSC) is inefficient when using plasmid-based transfection methods; viral-based systems are therefore being developed. A baculovirus system has recently been shown to be useful for expressing genes in mammalian cells. The aim of this study was to determine if baculovirus vectors can infect and express target genes in rat and human HSC and to assess potential cytotoxic and modulatory effects of infection. *Methods:* A recombinant baculovirus vector (AcCALaZ) carrying the *LacZ* gene was used to infect HSC. β-Ga1actosidase assays and electron microscopy were used to determine efficiency of infection and gene expression. Counting of trypan blue negative cells was used to assess cytotoxic/cytostatic effects of infection. Measurement of protein content of cells and α-smooth muscle actin expression were performed to assess the effects of baculovirus on cell function/phenotype. *Results:* Baculovirus infection of activated HSC was highly efficient (>90%) and provided long-term *LacZ* gene expression (15 days) in the absence of cytotoxic, cytostatic or modulatory effects. Infection of freshly isolated cells was also observed but at lower levels (20%). *Conc/usjons:* Baculovirus vectors can therefore be used to deliver target genes to cultured rat and human HSC with high efficiency and longevity in the absence of detrimental effects on cell function.

4.162 Proteome analysis of rat polymorphonuclear leukocytes: A two-dimensional electrophoresis/ mass spectrometry approach

Piubelli, C., Galvani, M., Hamdan, M., Domenici, E. and Righetti, P.G.

*Electrophoresis, **23(2)**, 298-310 (2002)*

The development of a two-dimensional (2-D) map of rat polymorphonuclear (PMN) leukocytes is here reported for the first time. The map is built up by utilizing a wide immobilized pH gradient (IPG), pH 3–10, in the first dimension and also a narrower IPG pH 4.5–8.5 gradient. In addition, the map is constructed by adopting the most recent protocols in 2-D mapping, which call for reduction and alkylation of the sample prior to the start of any electrophoretic step, including the IPG dimension. Fifty-two major protein spots have been so far identified by utilizing both matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) and electrospray quadrupole (Q)-TOF mass spectrometry. A large number of house-keeping and cytoskeleton proteins were detected, together with proteins which are specific to PMN organelles or related to PMN functions such as phagocytosis and chemotaxis. The results obtained demonstrate the possibility of obtaining a single 2-D gel based proteomic map of PMN with representative proteins from different cellular compartments, also including membrane components, allowing the study of PMN protein expression on a proteome-wide scale. The aim of this project is to build an extensive database of such proteins, to be utilized for future studies where the expression of PMN proteins is used as a disease- or drug treatment marker.

4.163 Cross-presentation of virus-like particles by skin-derived CD8$^-$ dendritic cells: a dispensable role for TAP

Ruedl, C., Storni, T., Lechner, F., Bächl, T. and Bachmann, M.F.

*Eur. J. Immunol., **32(3)**, 818-825 (2002)*

Virus-like particles (VLP) induce efficient CTL responses although they do not carry any genetic information. Here, we analyzed MHC class I associated presentation of VLP-derived CTL-epitopes *in vivo*. After intradermal injection of VLP containing the immunodominant epitope (p33) of lymphocytic choriomeningitis virus (p33-VLP), presentation of peptide p33 in draining lymph nodes was largely restricted to CD8$^-$ skin-derived dendritic cells (DC). Surprisingly, and in contrast to findings with tumor cells, TAP1-deficient DC and macrophages mediated efficient cross-presentation of VLP-derived p33 *in vivo* and *in vitro*. However, the ability of TAP1-deficient DC to cross-present p33-VLP was reduced compared to wild-type DC, indicating that in DC, both TAP-dependent and TAP-independent pathways were operative. In contrast, macrophages cross-presented p33-VLP normally in the absence of TAP. The TAP-dependent pathway of cross-presentation is therefore confined to DC while both macrophages and DC harbor the TAP-independent pathway. In summary, the results show that VLP-derived epitopes are cross-presented by CD8$^-$ DC *in vivo* in a partial TAP-independent fashion and highlight important differences in the processing machinery of DC versus macrophages.

4.164 Effective induction of acquired resistance to *Listeria monocytogenes* by immunizing mice with in vivo-infected dendritic cells

Sashinami, H., Nakane, A., Iwakura, Y. And Sasaki, M.
*Infect.and. Immun.,**71(2)**, 117-125 (2003)*

Splenic dendritic cells (DCs) obtained from mice at 48 h after *Listeria monocytogenes* infection exhibited up-regulation of CD80 and produced higher titers of gamma interferon (IFN-γ) and interleukin-12 (IL-12) than did DCs obtained from uninfected mice. Mice immunized with DCs obtained from mice that had been infected with *L. monocytogenes* 48 h before acquired host resistance to lethal infection with *L. monocytogenes* at 4 and 8 weeks. Immunization with DCs from heat-killed *L. monocytogenes* failed to induce resistance. Acquired antilisterial resistance is specific, since the immunized mice could not be protected from *Salmonella enterica* serovar Typhimurium infection. Infected DCs stimulated proliferation of naive $CD4^+$ and $CD8^+$ cells in vitro, suggesting that in vivo-infected DCs activate $CD8^+$ T cells, which are critical in acquired antilisterial resistance, as well as $CD4^+$ T cells. When wild-type mice were immunized with DCs from IFN-γ-deficient mice, they were protected against a lethal *L. monocytogenes* challenge. In contrast, when mice were immunized with DCs from anti-IL-12 p40 monoclonal antibody-injected mice, they failed to gain acquired antilisterial resistance. These results suggest that DC-derived IL-12, but not IFN-γ, may play a critical role in induction of acquired antilisterial resistance. Our present results suggest that splenic DCs obtained from mice infected with *L. monocytogenes* in vivo may be an effective immunogen with which to induce antigen-specific immunity.

4.165 Acetylcholine promotes the proliferation and collagen gene expression of myofibroblastic hepatic stellate cells

Oben, J.A., Yang, S., Lin, H., Ono, M. and Diehl. A.M.
Biochem. Biophys. Res. Comm., 300, 172-177 (2003)

The mechanisms that initiate and perpetuate the fibrogenic response, during liver injury, are unclear. Animal studies, however, strongly support a role for the autonomic nervous system (ANS) in wound healing. Therefore, the ANS may also mediate the development of cirrhosis. Hepatic stellate cells (HSC), the liver's major matrix-producing cells, are activated by injury to become proliferative, fibrogenic myofibroblasts. HSC respond to sympathetic neurotransmitters by changing phenotype, suggesting that HSC may be the cellular effectors of ANS signals that modulate hepatic fibrogenesis during recovery from liver damage. We show here that the parasympathetic neurotransmitter acetylcholine markedly stimulates the proliferation of myofibroblastic HSC and induces HSC collagen gene expression in these cells. By extending evidence that HSC are direct targets of the ANS, these results support the proposed neuroglial role of HSC in the liver and suggest that interrupting ANS signalling may be useful in constraining the fibrogenic response to liver injury.

4.166 CD4 T cell priming in dendritic cell-deficient mice
Castiglioni, P. et al
Int. Immunol., ***15(1),*** *127-136 (2003)*

Bone marrow (BM) chimeras (BMC) generated from mice carrying a null (–/–) mutation in the *rel*B gene of the NF-κB family represent an ideal model for *in vivo* studies on the role of dendritic cells (DC) in the adaptive immune response. The spleen and lymph nodes (LN) of *rel*B$^{-/-}$ BMC contain a small number of residual DC, mainly CD8α^{+}, that fail to up-regulate MHC class II and co-stimulatory molecules after stimulation *in vitro*. Moreover, residual spleen DC of *rel*B$^{-/-}$ BMC have a 4-fold decrease in the ability to uptake and process soluble model antigen, ovalbumin (OVA), and failed to prime CD4 and CD8 T cells *in vitro* and *in vivo*. In addition, they also failed to present OVA peptide to OT-II transgenic T lymphocytes at a normal 1:10 (stimulator:responder) cell ratio. In spite of these multiple DC defects, *rel*B$^{-/-}$ BMC immunized with plasmid DNA targeted to the spleen as the site of immune induction develop a specific CD4^{+} T cell response comparable to that of *rel*B competent mice. These data demonstrate that CD4^{+} T cells can be primed in the absence of functional DC and suggest that *rel*B may gauge the T cell response *in vivo*.

4.167 Interleukin-6 protects anterior horn neurons from lethal virus-induced injury
Pavelko, K. et al
J. Neurosci., 23(2), 481-492 (2003)

We evaluated the role of interleukin-6 (IL-6) in neuronal injury after CNS infection. IL-6$^{-/-}$ and IL-6$^{+/+}$ mice of resistant major histocompatibility complex (MHC) H-2^{b} haplotype intracerebrally infected with Theiler's virus cleared the infection normally without development of viral persistence, lethal neuronal infection, or late phase demyelination. In contrast, infection of IL-6$^{-/-}$ mice on a susceptible H-2^{q} haplotype resulted in frequent deaths and severe neurologic deficits within 2 weeks of infection as compared with infected IL-6$^{+/+}$ H-2^{q} littermate controls. Morphologic analysis demonstrated dramatic injury to anterior horn neurons of IL-6$^{-/-}$ H-2^{q} mice at 12 d after infection. Infectious viral titers in the CNS (brain and spinal cord combined) were equivalent between IL-6$^{-/-}$ H-2^{q} and IL-6$^{+/+}$ H-2^{q} mice. In contrast, more viral RNA was detected in the spinal cord of IL-6$^{-/-}$ mice compared with IL-6$^{+/+}$ H-2^{q} mice. Virus antigen was localized predominantly to anterior horn cells in infected IL-6$^{-/-}$ H-2^{q} mice. IL-6 deletion did not affect the humoral response directed against virus, nor did it affect the expression of CD4, CD8, MHC class I, or MHC class II in the CNS. Importantly, IL-6 was expressed by astrocytes of infected IL-6$^{+/+}$ mice but not in astrocytes of IL-6$^{-/-}$ mice or uninfected IL-6$^{+/+}$ mice. Furthermore, expression of various chemokines was robust at 12 d after infection in both H-2^{b} and H-2^{q} IL-6$^{-/-}$ mice, indicating that intrinsic CNS inflammatory responses did not depend on the presence of IL-6. Finally, *in vitro* analysis of virus-induced death in neuroblastoma-spinal cord-34 motor neurons and primary anterior horn cell neurons showed that IL-6 exerted a neuroprotective effect. These data support the hypothesis that IL-6 plays a critical role in protecting specific populations of neurons from irreversible injury.

4.168 Adrenaline inhibits lipopolysaccharide-induced macrophage inflammatory protein-1α in human monocytes: the role of β-receptors
Li, C-Y. et al
Anesth. Analg., ***96****, 518-523 (2003)*

Macrophage inflammatory protein-1α (MIP-1α) has an important role in the development of inflammatory responses during infection by regulating leukocyte trafficking and function. Our study was conducted to investigate the effect of adrenaline on lipopolysaccharide (LPS)-induced MIP-1α production by human peripheral blood monocytes and human monocytic THP-1 cells. Monocytes were incubated *in vitro* with LPS for 4 h at 37°C in the presence and absence of adrenaline and/or specific α- and ß-adrenergic receptor antagonists and agonists. The effects of adrenaline on MIP-1α synthesis were studied at the protein level by using enzyme-linked immunosorbent assays and at the messenger RNA level by using reverse transcriptase-polymerase chain reaction. Adrenaline inhibited LPS-induced MIP-1α production in a dose-dependent manner. The suppressive effect could be completely prevented by propranolol, but not by phentolamine. The specific ß-adrenergic agonist isoproterenol produced the same inhibitory effect on LPS-induced MIP-1α production, whereas the α-adrenergic agonist phenylephrine had a minimal effect. In addition, suppression of MIP-1α production was associated with an increase of intracellular cyclic adenosine monophosphate (cAMP) by the cell membrane-permeable cAMP analog dibutyryl-cAMP. Furthermore, we found that adrenaline inhibited LPS-induced MIP-1α messenger RNA expression. These findings suggest that adrenaline can modulate MIP-1α production in inflammatory diseases and sepsis.

4.169 Transcutaneous immunization with cholera toxin B subunit adjuvant suppresses IgE antibody responses via selective induction of Th1 immune responses
Anjuere, F. et al
J. Immunol., ***170****, 1586-1592 (2003)*

Topical application of cholera toxin (CT) onto mouse skin can induce a humoral immune response to CT as well as to coadministered Ags. In this study, we examined the nontoxic cell-binding B subunit of CT (CTB) as a potential adjuvant for cutaneous immune responses when coadministered with the prototype protein Ag, OVA. CTB applied onto skin induced serum Ab responses to itself with magnitudes comparable to those evoked by CT but was poorly efficient at promoting systemic Ab responses to coadministered OVA. However, transcutaneous immunization (TCI) with either CT or CTB and OVA led to vigorous OVA-specific T cell proliferative responses. Furthermore, CTB potentiated Th1-driven responses (IFN-γ production) whereas CT induced both Th1 and Th2 cytokine production. Coadministration of the toxic subunit CTA, together with CTB and OVA Ag, led to enhanced Th1 and Th2 responses. Moreover, whereas TCI with CT enhanced serum IgE responses to coadministered OVA, CTB suppressed these responses. TCI with either CT or CTB led to an increased accumulation of dendritic cells in the exposed epidermis and the underlying dermis. Thus, in contrast to CT, CTB appears to behave very differently when given by the transcutaneous as opposed to a mucosal route and the results suggest that the adjuvanticity of CT on Th1- and Th2-dependent responses induced by TCI involves two distinct moieties, the B and the A subunits, respectively.

4.170 Microtubule-disruption-induced and chemotactic-peptide-induced migration of human neutrophils: implications for differential sets of signaling pathways
Niggli, V.
*J. Cell Sci., **116**, 813-822 (2003)*

Neutrophil granulocytes rely on a functional actin network for directed migration. Microtubule disassembly does not impair receptor-linked chemotaxis, instead it induces development of polarity and chemokinesis in neutrophils concomitant with polarized distribution of α-actinin and F-actin. Cells stimulated with colchicine, which disassembles microtubules, migrate with a speed comparable to cells exposed to chemotactic peptide. We investigated signaling pathways involved in colchicine-induced neutrophil polarization and migration. Colchicine-induced development of polarity was insensitive to treatment with pertussis toxin, in contrast to chemotactic-peptide-induced shape changes, which were completely abolished by this treatment. Thus, colchicine does not appear to act via activating heterotrimeric G_i proteins. Colchicine does also not seem to act via phosphatidylinositol 3-kinase, as it failed to induce phosphorylation of its downstream target Akt and the potent phosphatidylinositol 3-kinase inhibitor wortmannin failed to inhibit colchicine-induced shape changes. By contrast, wortmannin significantly reduced chemotactic-peptide-induced shape changes. However, the Rho-kinase inhibitor Y-27632 (10 μM) inhibited colchicineinduced development of polarity by 95±3% (*n*=5) and chemokinesis by 76±9% (*n*=3), which suggests that the Rho-Rho-kinase pathway has a crucial role in polarity and migration. Indeed, treatment of cells with colchicine induced a significant increase in membrane-bound Rho-kinase II, which is indicative of activation of this protein. This membrane translocation could be prevented by taxol, which stabilizes microtubules. Colchicine also induced a marked increase in myosin light chain phosphorylation, which could be suppressed by Y-27632 and by taxol. In summary, we provide evidence that microtubule disassembly induces in neutrophils a selective activation of Rho-kinase, bypassing activation of heterotrimeric Gi proteins and phosphatidyl-inositol 3-kinase. This process is sufficient for induction of chemokinesis and mediates increased phosphorylation of myosin light chain and accumulation of F-actin and a-actinin in the leading edge.

4.171 Cationic lipid and polymer based gene delivery to human pancreatic cells
Mahato, R.I. et al
*Mol. Therapy, **7**, 89-100 (2003)*

Transplantation of pancreatic islets has great potential for treating Type I diabetes. Ex vivo gene therapy may promote re-vascularization or inhibit apoptosis of the islets and promote graft. In this study, we investigated the feasibility of non-viral gene delivery using Enhanced Green Fluorescent Protein (EGFP) and human Vascular Endothelial Growth Factor ($hVEGF_{165}$) expression plasmids as model reporter and therapeutic genes. LipofectAMINE/pDNA and Superfect/pDNA complexes showed high transfection efficiency in rapidly dividing Jurkat cells, but low transfection in non-dividing human islets. LipofectAMINE/pCAGGS-hVEGF transfected islets showed relatively higher levels of hVEGF than in those transfected with LipofectAMINE/pCMS-EGFP complexes or 5% glucose. To exclude endogenously secreted hVEGF, real time RT-PCR experiment was repeated using pCAGGS vector-specific forward primer and hVEGF gene-specific reverse primer. In this case, both non-transfected islets and the islets transfected with LipofectAMINE/pCMS-EGFP complexes showed negligible amplification of hVEGF. On glucose challenge, insulin release from LipofectAMINE/pCAGGS-hVEGF transfected human islets increased from 10.78±4.56 to 65±5 ng/ml, suggesting little adverse effect on islet β cell response to glucose challenge. The low transfection efficiency is due to the islets being a cluster of approximately 1000 non-dividing cells. *This underscores the importance of experimentation with the actual human islets*

4.172 Granulocyte-macrophage colony stimulating factor is an anti-apoptotic cytokine for thymic dendritic cells and a significant modelator of their accessory function
Vasilijic, S., Cilic, M. and Vucevic, D.
*Immunology letters, **86**, 99-112 (2003)*

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth-promoting factor for myeloid-derived dendritic cells (DC) but not for lymphoid DC. The data about its effect on thymic DC (TDC), which are both of lymphoid and myeloid origin, are very scarce. Using an in vitro model, we demonstrated in this work that GM-CSF significantly increased the survival of rat TDC in culture by inhibiting their apoptosis and the effect correlated with up-regulation of Bcl-2 expression. GM-CSF also stimulated differentiation and maturation of TDC as judged by higher expression of MHC class I and II molecules, CD54, CD80 and CD86. These changes correlated with stronger stimulatory activity of GM-CSF-pulsed TDC in syngeneic thymocyte proliferation assay and MLR. The stimulatory potential of TDC was further increased when thymocytes were cultivated with an anti-αβ TCR (R73) monoclonal antibody (mAb). The influence of unstimulated TDC on proliferation of thymocytes was inhibited by anti-CD86 but not anti-CD80 mAb, whereas in cultures with GM-CSF-treated TDC both mAbs exerted an additive blocking effect. After separation of TDC on $CD11b^+$ and $CD11b^-$ we demonstrated that GM-CSF inhibited apoptosis and potentiated accesory activity of both TDC subsets independently of the myeloid marker expression. Cummulatively, our results suggest that GM-CSF is one of the regulatory cytokine involved in survival, maturation, differentiation and accessory function of TDC.

4.173 IκBα-dependent regulation of low-shear flow-induced NF-κB activity: role of nitric oxide
Mohan, S. et al
*Am. J.Cell Physiol., **284**, C1039-C1047 (2003)*

We have investigated the role of inhibitor κBα (IκBα) in the activation of nuclear factor κB (NF-κB) observed in human aortic endothelial cells (HAEC) undergoing a low shear stress of 2 dynes/cm^2. Low shear for 6 h resulted in a reduction of IκBα levels, an activation of NF-κB, and an increase in κB-dependent vascular cell adhesion molecule 1 (VCAM-1) mRNA expression and endothelial-monocyte adhesion. Overexpression of IκBα in HAEC attenuated all of these shear-induced responses. These results suggest that downregulation of IκBα is the major factor in the low shear-induced activation of NF-κB in HAEC. We then investigated the role of nitric oxide (NO) in the regulation of IκBαNF-κB. Overexpression of endothelial nitric oxide synthase (eNOS) inhibited NF-κB activation in HAEC exposed to 6 h of low shear stress. Addition of the structurally unrelated NO donors *S*-nitrosoglutathione (300 μM) or sodium nitroprusside (1 mM) before low shear stress significantly increased cytoplasmic IB and concomitantly reduced NF-κB binding activity and κB-dependent VCAM-1 promoter activity. Together, these data suggest that NO may play a major role in the regulation of IκBα levels in HAEC and that the application of low shear flow increases NF-κB activity by attenuating NO generation and thus IκBα levels.

4.174 Norepinephrine and neuropeptide Y promote proliferation and collagen gene expression of hepatic myofibroblastic stellate cells
Oben, J.A., Yang, S., Lin, H., Ono, M. and Diehl, A.M.
*Biochem. Biophys. Res. Comm., **302**, 685-690 (2003)*

The mechanisms initiating and perpetuating the fibrogenic response in the injured liver are not well understood. Hepatic stellate cells are activated by liver injury to become proliferative and fibrogenic myofibroblasts. Emerging evidence suggests that the sympathetic nervous system may play a role in the development of cirrhosis. It is not known, however, whether this requires a direct interaction between sympathetic neurotransmitters and stellate cell receptors, or results indirectly, from sympathetic effects on the vasculature. Using cultured hepatic stellate cells, we show that the sympathetic neurotransmitters, norepinephrine and neuropeptide Y, markedly stimulate the proliferation of activated, myofibroblastic, hepatic stellate cells. Norepinephrine, but not neuropeptide Y, also induces collagen gene expression. In conclusion, physiologically relevant concentrations of sympathetic neurotransmitters directly modulate the phenotype of hepatic stellate cells. This suggests that targeted interruption of sympathetic nervous system signaling in hepatic stellate cells may be useful in constraining the fibrogenic response to liver injury.

4.175 A novel I-branching β-1,6-*N*-acetylglucosaminyltransferase involved in human blood group I antigen expression

Inaba, N. et al

*Blood, **101**, 2870-2876 (2003)*

The human blood group i and I antigens are determined by linear and branched poly-*N*-acetyllactosamine structures, respectively. In erythrocytes, the fetal i antigen is converted to the adult I antigen by I-branching β-1,6-*N*-acetylglucosaminyltransferase (IGnT) during development. Dysfunction of the I-branching enzyme may result in the adult i phenotype in erythrocytes. However, the *I* gene responsible for blood group I antigen has not been fully confirmed. We report here a novel human I-branching enzyme, designated *IGnT3*. The genes for *IGnT1* (reported in 1993), *IGnT2* (also presented in this study), and *IGnT3* consist of 3 exons and share the second and third exons. Bone marrow cells preferentially expressed *IGnT3* transcript. During erythroid differentiation using $CD34^+$ cells, *IGnT3* was markedly up-regulated with concomitant decrease in *IGnT1/2*. Moreover, reticulocytes expressed the *IGnT3* transcript, but *IGnT1/2* was below detectable levels. By molecular genetic analyses of an adult i pedigree, individuals with the adult i phenotype were revealed to have heterozygous alleles with mutations in exon 2 (1006G>A; Gly336Arg) and exon 3 (1049G>A; Gly350Glu), respectively, of the *IGnT3* gene. Chinese hamster ovary (CHO) cells transfected with each mutated *IGnT3* cDNA failed to express I antigen. These findings indicate that the expression of the blood group I antigen in erythrocytes is determined by a novel *IGnT3*, not by *IGnT1* or *IGnT2*.

4.176 A chemokine, interferon (IFN)-γ-inducible protein 10 kDa, is stimulated by IFN-τ and recruits immune cells in the ovine endometrium

Hagaoka, K. et al

*Biol. Reprod., **68**, 1413-1421 (2003)*

Proper distribution of immune cells in the uterus is a prerequisite for successful implantation and subsequent placentation, but biochemical signals that govern such events have not been well characterized. In the present study, the cDNA of a chemokine, interferon (IFN)-γ -inducible protein 10 kDa (IP-10), was identified from a cDNA subtraction study between uterine endometrial tissues from Day 17 pregnant and Day 15 cyclic ewes. The effect of IFN-τ on IP-10 expression and the involvement of IP-10 in the recruitment of immune cells were then investigated. Northern blot analysis revealed that large amounts of IP-10 mRNA were present during conceptus attachment to maternal endometrium and early placentation. IP-10 mRNA was localized to monocytes distributed in the subepithelial stroma of pregnant but not cyclic uteri. This finding was supported by the discovery of IP-10 mRNA expression in monocytes but not in lymphocytes, uterine epithelial cells, or stromal cells. Moreover, the expression of IP-10 mRNA by the monocytes was stimulated by IFN-α, IFN-γ, and IFN-τ in a dose-dependent manner, but the expression of IP-10 mRNA by the endometrial explants was most stimulated by IFN-τ. In a chemotaxis assay, migration of peripheral blood mononuclear cells was stimulated by the addition of IFN-τ stimulated-endometrial culture medium, and the effect was significantly reduced by neutralization with an anti-IP-10 antibody. These results suggest that endometrial IP-10 regulated by conceptus IFN-τ regulates recruitment and/or distribution of immune cells seen in the early pregnant uterus.

4.177 PPARγ-dependent anti-flammatory action of rosiglitazone in human monocytes: suppression of TNFα secretion is not mediated by PTEN regulation

Hong, G., Davis, B., Khatoon, N., Baker, S.F. and Brown J.

*Biochem. Biophys. Res. Comm., **303**, 782-787 (2003)*

Thiazolidinediones (TZDs) are insulin-sensitising drugs that are ligands for the nuclear receptor PPARγ. They have been shown to inhibit PMA-stimulated secretion of TNFα from human monocytes, although only at concentrations well in excess of circulating levels observed during TZD therapy, suggesting a mechanism of action independent of PPARγ activation. Here we show that insulin-sensitising concentrations of the TZD rosiglitazone partially inhibit serum- or LPS- (but not PMA-) stimulated TNFα secretion from primary human monocytes, with an IC_{50} of around 50 nM. We also show that the observed effects are independent of PPARγ-mediated regulation of the lipid phosphatase PTEN. Reversed stimulus specificity, IC_{50} in the insulin-sensitising range, and the fact that partial inhibition of TNFα secretion is also observed with a structurally unrelated PPARγ agonist, GW7845, demonstrate a mechanism of action distinct from that observed with higher TZD concentrations. These findings thus represent the first report of a PPARγ-dependent and therapeutically relevant anti-inflammatory action of TZDs in isolated human monocytes.

4.178 Bovine dendritic cells generated from monocytes and bone marrow progenitors regulate immunoglobulin production in peripheral blood B cells

Pinchuk, L.M., Boyd, B.L., Kruger, E.F., Roditi, I. and Furger, A.
*Comp. Immuun. Microbiol. Infect. Dis., **26** 233-249 (2003)*

We examined whether bovine monocyte-derived and bone marrow (BM) dendritic cells (DCs) regulate antibody production in activated peripheral blood B cells. DCs were generated from monocytes and BM progenitors in the presence of bovine recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4). Monocyte-derived DCs promoted B cells activated by the anti-CD3 triggered $CD4^+$ T cells or through immunoglobulin M (IgM) receptor to increase the level of IgG secretion. Furthermore, the addition of DCs triggered B cells activated through IgM receptors to produce IgG2 and IgA, thus inducing an isotype switch. BM-derived DCs increased the production of IgG in B cells activated by the anti-CD3 triggered $CD4^+$ T cells, but unlike monocyte-derived DCs did not have any effect on B cells activated through surface IgM. These data suggest that the regulation of humoral immune responses in cattle depends on the origin of DCs and the mode of B cell activation.

4.179 Monocyte-derived IL12, CD86 (B7-2) and CD40L expression in relapsing and progressive multiple sclerosis

Filion, L.G., Matusevicius, D., Graziani-Bowering, G.M., Kumar, A. and Freedman, M.
*Clin. Immunol., **106** 127-138 (2003)*

Multiple sclerosis has been postulated to be an autoimmune disease in which Th1 immune responses predominate. This response is associated with an increased production of IFNγ and IL12 produced by T cells and by cells of the monocyte (MO) lineage, respectively. An increased expression of costimulatory molecules by T cells and antigen-presenting cells is also observed. We hypothesized that in relapsing–remitting MS (RRMS) (with or without of IFNβ treatment) and in secondary progressive patients (SPMS) IL12 and costimulatory molecules (CD80 [B7-1], CD86 [B7-2], CD28, CD40, CD40L) would be differentially produced or expressed by MO or T cells. We performed cross-sectional and longitudinal flow cytometric studies (at monthly intervals) on peripheral blood mononuclear cells (PBMC) or on MO from SPMS or untreated and IFNβ-treated patients with RRMS. We determined that CD86 and CD40L expression was highest on MO derived from SPMS patients compared to those from RRMS or from healthy controls (HC). In vitro culture of PBMC with recombinant human IL10, a cytokine that may be increased in response to treatment with IFNβ and that down-regulates CD86 expression, reduced the expression of CD86 on MO derived from RRMS patients to a much higher degree compared to cells derived from SPMS or HC. In vitro secreted IL12 levels from freshly isolated MO from SPMS patients were more than 10-fold higher than either the treated or the untreated RRMS or HC. RRMS patients treated with IFNβ demonstrated slightly lower levels of MO IL12 secretion. Our data suggest that a key mechanism in the pathogenesis of MS is the increased expression of CD86 and CD40L and the increased production of IL12 during disease progression. Part of the mechanism of action of IFNβ may be to reduce MO CD86 and CD40L expression and IL12 secretion; failure to do so might signify either a lack of response or a transition to a more progressive phase of illness.

4.180 Monocyte-derived cytokines in multiple sclerosis

Filion, L.G., Graziani-Bowering, G., Matusevicius, D. and Freedman, M.S.
Clin. Exp. Immunol., ***131***, *324-334 (2003)*

MS is an inflammatory, presumably autoimmune, disease mediated by the activation of T cells, B cells and monocytes (MO). Inflammation is thought to occur early during the relapsing-remitting phase of MS (RRMS), whereas in the later phases of MS such as secondary progressive MS (SPMS), inflammation tends to diminish. Our objective was to compare the types and amounts of proinflammatory and regulatory cytokines produced by MO from relapsing-remitting patients with or without treatment with IFN-β ($RRMS^+$ therapy, $RRMS^-$ therapy), respectively, from secondary progressive patients (SPMS) and from healthy controls (HC). MO were isolated by a density-gradient technique and three different techniques (RNase protection assay, ELISA and intracellular cytokine staining) were used to assess cytokine levels. An increase in IL6, IL12 and TNF-α was observed by all three methods for $RRMS^-$ therapy and for SPMS patients compared to HC and $RRMS^+$ therapy patients. We conclude that proinflammatory and regulatory monokines can be derived from MO of MS patients and that these levels are modulated by IFN-β therapy. Although it is believed that inflammation tends to diminish in SPMS patients, our data show that inflammatory cytokines continue to be released at high levels, suggesting that IFN-β or IL10 treatment may be beneficial for this group.

4.181 Improved islet yields from Macaca Nemestrina and marginal human pancreata after two-layer method preservation and endogenous trypsin inhibition

Matsumoto, S., Rigley, T.H., Reems, J.A., Kuroda, Y. and Stevens, R.B.
Am. J. Transplant., ***3***, *53-63 (2003)*

We tested whether two-layer method (TLM) pancreas preservation and trypsin inhibition (Pefabloc) during processing allows longer preservation while retaining or improving viable islet recovery. Non-marginal primate (*Macaca nemestrina*) and marginal human (ischemic or preservation-injured) pancreata were processed with a research-oriented pan technique (Seattle method). Organs were processed upon arrival (± Pefabloc), or after TLM or University of Wisconsin solution (UW) preservation (+ Pefabloc). Islet yield, viability, and function were assessed.
Pefabloc increased *M. nemestrina* islet yields from 9696 ± 1749 IE/g to 15 822 ± 1332 IE/g ($p < 0.01$). Two-layer method preservation (< 6 h) further increased yields, to 23 769 ± 2773 IE/g (vs. + Pefabloc; $p < 0.01$). Similarly, Pefabloc increased marginal human islet yields from 2473 ± 472 IE/g to 4723 ± 1006 IE/g ($p < 0.04$). This increase was maintained after lengthy TLM preservation (> 30 h; 4801 ± 1066 IE/g).
We also tested the applicability of TLM preservation (23.5 ± 3.2 h) to the processing of marginal human pancreata by the Edmonton/Immune Tolerance Network clinical protocol. Islet yield and function approached published results of pancreata processed 4.8 ± 0.8 h after organ recovery ($p = 0.06$).
Pefabloc, and TLM vs. UW preservation, prolonged the tolerable interval between organ recovery and islet isolation. Islet yield, viability, and functionality improved from both marginal and nonmarginal pancreata.

4.182 Liver sinusoidal endothelial cells represent an important blood clearance system in pigs
Nedredal, G.I. et al
*Comp. Hepatol., **2**, 1-14 (2003)*

Background: Numerous studies in rats and a few other mammalian species, including man, have shown that the sinusoidal cells constitute an important part of liver function. In the pig, however, which is frequently used in studies on liver transplantation and liver failure models, our knowledge about the function of hepatic sinusoidal cells is scarce. We have explored the scavenger function of pig liver sinusoidal endothelial cells (LSEC), a cell type that in other mammals performs vital elimination of an array of waste macromolecules from the circulation.
Results: ^{25}I-macromolecules known to be cleared in the rat via the scavenger and mannose receptors were rapidly removed from the pig circulation, 50% of the injected dose being removed within the first 2-5 mm following injection. Fluorescently labeled microbeads (2 μm in diameter) used to probe phagocytosis accumulated in Kupffer cells only, whereas fluorescently labeled soluble macromolecular ligands for the mannose and scavenger receptors were sequestered only by LSEC. Desmin-positive stellate cells accumulated no probes. Isolation of liver cells using collagenase perfusion through the portal vein, followed by various centrifugation protocols to separate the different liver cell populations yielded 280 x 10^7 (range 50-890 x 10^7) sinusoidal cells per liver (weight of liver 237. I g (sd 43.6)). Use of specific anti-Kupffer cell- and anti-desmin antibodies, combined with endocytosis of fluorescently labeled macromolecular soluble ligands indicated that the LSEC fraction contained 62 x $l0^7$ (sd 12 x 10^7) purified LSEC. Cultured LSEC avidly endocytosed ligands for the mannose and scavenger receptors.
Conclusions: We show here for the first time that pig LSEC, similar to what has been found earlier in rat LSEC, represent an effective scavenger system for removal of macromolecular waste products from the circulation.

4.183 Biological sample collection and processing for molecular epidemiological studies
Holland, N.T., Smith, M.T., Eskenazi, B. and Bastaki, M.
*Mutation Res., **543**, 217-234 (2003)*

Molecular epidemiology uses biomarkers and advanced technology to refine the investigation of the relationship between environmental exposures and diseases in humans. It requires careful handling and storage of precious biological samples with the goals of obtaining a large amount of information from limited samples, and minimizing future research costs by use of banked samples. Many factors, such as tissue type, time of collection, containers used, preservatives and other additives, transport means and length of transit time, affect the quality of the samples and the stability of biomarkers and must be considered at the initial collection stage. An efficient study design includes provisions for further processing of the original samples, such as cryopreservation of isolated cells, purification of DNA and RNA, and preparation of specimens for cytogenetic, immunological and biochemical analyses. Given the multiple uses of the samples in molecular epidemiology studies, appropriate informed consent must be obtained from the study subjects prior to sample collection. Use of barcoding and electronic databases allow more efficient management of large sample banks. Development of standard operating procedures and quality control plans is a safeguard of the samples' quality and of the validity of the analyses results. Finally, specific state, federal and international regulations are in place regarding research with human samples, governing areas including custody, safety of handling, and transport of human samples, as well as communication of study results.
Here, we focus on the factors affecting the quality and the potential future use of biological samples and some of the provisions that must be made during collection, processing, and storage of samples, based on our experience in the Superfund Basic Research Program and Children's Environmental Health Center, at the University of California, Berkeley.

4.184 Basal expression of IκBα is controlled by the mammalian transcriptional repressor RBP-J (CBF1) and its activator Notch1
Oakley F. et al
J. Biol. Chem., ***278***, *24359-24370 (2003)*

By using the hepatic stellate cell (HSC) as a paradigm for cells that undergo long term re-programming of NF-κB dependent transcription, we have determined a novel mechanism by which mammalian cells establish their basal NF-κB activity. Elevation of NF-κB activity during HSC activation is accompanied by induction of CBF1 expression and DNA binding activity. We show that the transcriptional repressor CBF1 interacts with a dual NF-κB/ CBF1-binding site (κB2) in the IκBα promoter. Nucleotide substitutions that disrupt CBF1 binding to the κB2 site result in an elevation of IκBα promoter activity and loss of responsiveness of the promoter to a transfected CBF1 reporter vector. Over-expression of CBF1 in COS1 cells was associated with markedly reduced IκBα protein expression and elevated NF-κB DNA binding activity. CBF1-induced repression of IκBα promoter activity was reversed in HSC transfected with the Notch1 intracellular domain (NICD). The ability of NICD to enhance IκBα gene transcription was confirmed in COS1 cells and was found to be dependent on an intact RAM domain of NICD that has been shown previously to help mediate the interaction of NICD with CBF1. One of the mechanisms by which NICD is thought to convert CBF1 into an activator of transcription is via the recruitment of transcriptional co-activators/histone acetylases to gene promoters. Co-transfection of HSC with NICD and p53 caused a diminution of IκBα promoter activity, by contrast overexpression of p300 enhanced IκBα promoter function. Taken together, these data suggest that basal IκBα expression (and as a consequence NF-κB activity) is under the control of the various components of the CBF1/Notch signal transduction pathway.

4.185 Characterization of C5aR expression on murine myeloid and lymphoid cells by the use of a novel monoclonal antibody
Soruri, A., Kim, S., Kiafard, Z. and Zwirner, J.
Immunol. Lett., ***88***, *47-52 (2003)*

The anaphylatoxin C5a is a potent proinflammatory stimulus with immunomodulatory activities. Expression of its receptor C5aR (CD88) has been detected on cells of myeloid origin such as granulocytes and monocytes/ macrophages. However, controversial results exist on the expression of C5aR on T and B lymphocytes as well as on mature dendritic cells (DC). The aim of the present study was to characterize expression of C5aR protein on myeloid and lymphoid cells in the mouse. For this purpose, rat monoclonal antibodies with specificity against the murine C5aR were generated. Using these reagents a distinct amount of C5aR antigen was observed on neutrophils and macrophages. In contrast, C5aR protein was not detectable on resting or stimulated murine T or B lymphocytes. Furthermore, no C5aR protein could be observed on splenic CD11c positive DC which have been classified in the literature as relatively mature. Taken together, our results suggest that in the mouse expression of C5aR protein may be restricted to leukocytes of myeloid origin whereas previous evidence for C5aR expression on lymphoid cells may be reevaluated.

4.186 Neuropeptide Y is neuroproliferative for hippocampal presursor cells
Howell, O.W. et al.
J. Neurochem., ***86***, *646-659 (2003)*

New neurones are produced in the adult hippocampus throughout life and are necessary for certain types of hippocampal learning. Little, however, is known about the control of hippocampal neurogenesis. We used primary hippocampal cultures from early post-natal rats and neuropeptide Y Y1 receptor knockout mice as well as selective neuropeptide Y receptor antagonists and agonists to demonstrate that neuropeptide Y is proliferative for nestin-positive, sphere-forming hippocampal precursor cells and β-tubulin-positive neuroblasts and that the neuroproliferative effect of neuropeptide Y is mediated via its YI receptor. Immunohistochemistry confirmed Y1 receptor staining on both nestin-positive cells and β-tubulin-positive cells in culture and short pulse 5-bromo-2-deoxyuridine studies demonstrated that neuropeptide Y has a proliferative effect on both cell types. These studies suggest that the proliferation of hippocampal neuroblasts and precursor cells is increased by neuropeptide Y and, therefore, that hippocampal learning and memory may be modulated by neuropeptide Y-releasing interneurones.

4.187 Islet transplants and impact on secondary diabetic complications: does C-peptide protect the kidney
Shapiro, A. M. J.
*J. Am. Soc. Nephrol., **14**, 2214-2216 (2003)*

Extract
Hering *et al*. have integrated a number of important steps including: PFC pancreas transportation; careful donor and recipient selection; a new iodixanol non-ficoll based purification gradient; islet culture; thymoglobulin and anti-TNFα (etanercept) induction; less diabetogenic, calcineurin inhibitor-free maintenance immune suppression; and intensive insulin and intravenous heparin in the peri-transplant period. Attention to detail every step of the way led to insulin independence after single donor islet infusions in the first eight recipients, with five of eight maintaining insulin independence in the longer term. With such remarkable progress over the past three years, it is clear that islet transplantation can provide a level of glycemic control that far exceeds intensive insulin or pump therapy. Provided rejection and autoimmune recurrence can be prevented by non-diabetogenic but potent antirejection drugs, glycated HbA1C can remain within the normal range, and emerging outcomes in islet transplantation may soon parallel the results in whole pancreas transplantation, but with less potential risk of pen-transplant morbidity.

4.188 Chemotactically-induced redistribution of CD43 as related to polarity and locomotion of human polymorphonuclear leucocytes
Dehghani Zadeh, A., Seveau, S., Halbwachs-Mecarelli, L. and Keller, H.U.
*Biol. Cell, **95**, 265-273 (2003)*

Leukocyte motility involves pseudopods extension at the leading edge and uropod contraction at the cell rear. Previous studies have shown that the glycoprotein CD43 redistributes to the uropod, when the cells develop polarity and locomotion. The present study addresses the question whether the accumulation of specific membrane molecules, such as CD43 at the contracted uropod precedes or follows development of polarity and locomotion. PMNs were labeled with fluorescent anti-CD43 antibodies and guided to polarize in the direction of a chemoattractant-containing micropipette or, once polarized, they were forced to reverse polarity and movement direction by placing the micropipette behind the uropod. This chemotactically-induced reversal of polarity was used as an efficient tool to analyse the sequence of events. CD43, but not another abundant surface glycoprotein CD45, was concentrated at the uropod. This documents that CD43 redistribution is a selective phenomenon. During reversal of polarity and of locomotion direction, the geometric center of the cell clearly changed direction earlier than the center of anti-CD43 fluorescence intensity. Thus, CD43 redistribution to the new uropod follows rather than precedes reversal of polarity, suggesting that CD43 redistribution is a consequence rather than a prerequisite for polarity and locomotion. PMNs making a U-turn maintained the pre-existing polarity and CD43 remained concentrated at the uropod, even when the front was moving in the opposite direction. Our data show that anterior pseudopod formation, rather than capping of CD43 at the uropod or the position of the uropod determines the direction of locomotion.

4.189 A reliable technique for rodent pancreatic stellate cells isolation
Shek, F.W. et al
*Pancreatology, **3**, 209-269 (2003)*

Introduction: Pancreatic stellate cells (PSC) isolation varies between laboratories. There are increasing pressures to limit animal procedures. Our method which uses unwanted pancreata from other experiments and avoids cannulation (Bachem, 1998), was developed from the Bedayan technique acinar cell isolation and local expertise in HSC isolation.
Methods: Rat pancreata were minced in Hanks Buffer Salt Solution (HBSS) containing calcium and digested in pronase (1mg/ml) and collagenase P (0.5 mg/ml) and shaken 30 minutes at 37^0C. Undigested tissue was filtered using nylon mesh. The filtrate was pelleted and resuspended in OptiPrep density gradient – 12% v/v in HBSS with HBSS buffering layer and centrifuged for 20 minutes at 1,000g at 4^0C. PSC were collected in the OptiPrep/HBSS interface and counted and assessed for viability.
Results: For each preparation, we can reliably obtain between 2-4 million cells with at least 85% viability and 100% purity at passage 1. PSC immunostained positively for desmin (mesenchymal marker) and a-smooth muscle actin (activated stellate cells). This was also confirmed by western blot.
Discussion: Our PSC supplies come from pancreas not required by other researchers. No surgical skills are required. The time-limitations of tissue outgrowth methods are avoided. Local variation of gradient density and centrifugal force may be required to optimize recovery.

4.190 Fas/tumor necrosis factor receptor death signaling is required for axotomy-induced death of motoneurons *in vivo*
Ugolini, G. et al
*J. Neurosci.,**23(24)**, 8526-8531 (2003)*

Activation of the Fas death receptor leads to the death of motoneurons in culture. To investigate the role of Fas in programmed cell death and pathological situations, we used several mutant mice deficient for Fas signaling and made a novel transgenic *FADD-DN* (FAS-associated death domain-dominant-negative) strain. *In vitro*, motoneurons from all of these mice were found to be resistant to Fas activation and to show a delay in trophic deprivation-induced death. During normal development *in vivo*, no changes in motoneuron survival were observed. However, the number of surviving motoneurons was twofold higher in animals deficient for Fas signaling after facial nerve transection in neonatal mice. These results reveal a novel role for Fas as a trigger of axotomy-induced death and suggest that the Fas pathway may be activated in pathological degeneration of motoneurons.

4.191 α-defensins can have anti-HIV activity but are not CD8 cell anti_HIV factors
Mackewicz, C.E. et al
*AIDS, **17**, F23-F32 (2003)*

Background:
CD8 T cells from healthy HIV-infected individuals inhibit HIV replication in infected CD4 T cells by a non-cytotoxic mechanism mediated by a soluble CD8 cell antiviral factor, CAF. Recently, the antimicrobial peptides, α-defensins, were reported to constitute CAF.
Objective:
To examine the antiviral activity of α-defensins and address their potential role in CD8 cell non-cytotoxic antiviral responses.
Design and methods:
A purified mixture of human neutrophil proteins (HNP) 1-3 (α-defensins) was used to examine the effect of α-defensins on HIV virions and on HIV replication in CD4 cells treated prior to or post infection. α-Defensin expression was analyzed at the RNA and protein level in CD8 cells as well as in various other cell types. Antibodies to the defensins were tested for their ability to inhibit CAF activity in CD8 cell culture fluids.
Results:
The α-defensins exhibited anti-HIV activity on at least two levels: directly inactivating virus particles; and affecting the ability of target CD4 cells to replicate the virus. However, while we could demonstrate α-defensins in neutrophils and monocytes, we found no evidence for the production of these peptides by CD8 T cells. No messenger RNA encoding these proteins was detected in purified CD8 T cells, nor did these cells produce intracellular or extracellular α-defensin peptides. Moreover, antibodies specific for human α-defensins 1, 2, and 3 did not block the antiviral activity of CAF-active CD8 cell culture fluids.
Conclusions:
The α-defensins are not produced by CD8 cells but unexpectedly were found to be expressed in monocytes. α-Defensins can have anti-HIV activity but are not CD8 cell antiviral factors.

4.192 Kinetics of increased deformability of deoxygenated sickle cells upon oxygenation
Huang, Z. et al
*Biophys. J., **85**, 2374-2383 (2003)*

We have examined the kinetics of changes in the deformability of deoxygenated sickle red blood cells when they are exposed to oxygen (O_2) or carbon monoxide. A flow-channel laser diffraction technique, similar to ektacytometry, was used to assess sickle cell deformability after mixing deoxygenated cells with buffer that was partially or fully saturated with either O_2 or carbon monoxide. We found that the deformability of deoxygenated sickle cells did not regain its optimal value for several seconds after mixing. Among density-fractionated cells, the deformability of the densest fraction was poor and didn't change as a function of O_2 pressure. The deformability of cells from the light and middle fraction increased when exposed to O_2 but only reached maximum deformability when equilibrated with supraphysiological O_2 concentrations. Cells from the middle and lightest fraction took several seconds to regain maximum deformability. These data imply that persistence of sickle cell hemoglobin polymers during circulation in vivo is likely, due to slow and incomplete polymer melting, contributing to the pathophysiology of sickle

cell disease.

4.193 Prevention of leukocyte migration to inflamed skin with a novel fluorosugar modifier of cutaneous lymphocyte-associated antigen
Dimitroff, C.J., Kupper, T.S. and Sackstein, R.
J. Clin. Invest., ***112(7)***, *1008-1018 (2003)*

E-selectin and P-selectin on dermal postcapillary venules play critical roles in the migration of effector T cells into inflamed skin. P-selectin glycoprotein ligand-1 (PSGL-1) modified by α1,3-fucosyltransferase is the principal selectin ligand on skin-homing T cells and is required for effector T cell entry into inflamed skin. We have previously shown that a fluorinated analog of *N*-acetylglucosamine peracetylated-4-fluorinated-D-glucosamine (4-F-GlcNAc), inhibits selectin ligand expression on human T cell PSGL-1. To analyze 4-F-GlcNAc efficacy in dampening effector T cell migration to inflamed skin, we elicited allergic contact hypersensitivity (CHS) reactions in mice treated with 4-F-GlcNAc. We also investigated 4-F-GlcNAc efficacy on lymphocyte E-selectin ligand expression in LNs draining antigen-sensitized skin and on other immunological processes requisite for CHS responses. Our results showed that 4-F-GlcNAc treatment attenuated lymphocyte E-selectin ligand expression in skin-draining LNs and prevented CHS reactions. Significant reductions in inflammatory lymphocytic infiltrate were observed, while pathways related to antigenic processing and presentation and naive T cell recognition within skin-draining LNs were unaffected. These data indicate that 4-F-GlcNAc prevents CHS by inhibiting selectin ligand activity and the capacity of effector T cells to enter antigen-challenged skin without affecting the afferent phase of CHS.

4.194 Lysophospholipids synergistically promote primitive hematopoitic cell chemotaxis via a mechanism involving Vav1
Whetton, A.D., Lu, Y., Pierce, A., Carney, L. and Spooncer, E.
Blood, ***102***, *2798-2802 (2003)*

Hematopoiesis is sustained by the proliferation and development of an extremely low number of hematopoietic stem cells resident in the bone marrow. These stem cells can migrate from their bone marrow microenvironment and can be found at low levels in the peripheral blood. The factors that regulate egress or ingress of the stem cells from the marrow include cytokines and chemokines. This process of stem cell trafficking is fundamental to both stem cell biology and stem cell transplantation. We show that primitive hematopoietic cells with cobblestone area–forming cell activity express receptors for and display enhanced motility in response to a new class of stem cell agonists, namely lysophospholipids. These agents synergistically promote chemokinestimulated cell chemotaxis, a process that is crucial in stem cell homing. The response to lysophospholipids is mediated by Rac, Rho, and Cdc42 G proteins and the hematopoietic-specific guanyl nucleotide exchange factor Vav 1. Inhibitor studies also show a critical role for phosphatidylinositol 3 kinase (PI3K). Lipid mediators, therefore, regulate the critical process of primitive hematopoietic cell motility via a PI3K- and Vav-dependent mechanism and may govern stem cell movement in vivo. These results are of relevance to understanding stem cell trafficking during bone marrow transplantation.

4.195 An alternative efficient procedure for purification of the obligate intracellular fish bacterial pathogen *Piscirickettsia salmonis*
Henriquez, V., Rojas, M.V., and Marshall, S.H.
Appl, Environ. Microbiol., ***69(10)***, *6268-6271 (2003)*

Piscirickettsia salmonis is an obligate intracellular bacterial pathogen of salmonid fish and the etiological agent of the aggressive disease salmonid rickettsial syndrome. Today, this disease, also known as piscirickettsiosis, is the cause of high mortality in net pen-reared salmonids in southern Chile. Although the bacteria can be grown in tissue culture cells, genetic analysis of the organism has been hindered because of the difficulty in obtaining *P. salmonis* DNA free from contaminating host cell DNA. In this report, we describe a novel procedure to purify in vitro-grown bacteria with iodixanol as the substrate to run differential centrifugation gradients which, combined with DNase I digestion, yield enough pure bacteria to do DNA analysis. The efficiency of the purification procedure relies on two main issues: semiquantitative synchrony of the *P. salmonis*-infected Chinook salmon embryo (CHSE-214) tissue culture cells and low osmolarity of iodixanol to better resolve bacteria from the membranous structures of the host cell. This method resulted in the isolation of intact piscirickettsia organisms and removed salmon and mitochondrial DNA effectively, with only 1.0% contamination with the latter.

4.196 Advances in islet cell biology. From stem cell differentiation to clinical transplantation: conference report

Kandeel, F., Smith, C.V., Tpdorov, I. And Mullen, Y

*Pancreas, **27(3)**, e63-e78 (2003)*

The 3rd Annual Rachmiel Levine Symposium entitled "Advances in Islet Cell Biology-From Stem Cell Differentiation to Clinical Transplantation" was organized by the Department of Diabetes, Endocrinology and Metabolism at the City of Hope National Medical Center, with the support of the Southern California Islet Cell Resources Center, American Diabetes Association-David Shapiro Research Fund, Ross Foundation, the National Center for Research Resources (NCRR), and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health. The symposium was held at the Hilton Anaheim Hotel in Anaheim, CA, in October 2002, and was attended by nearly 400 participants from 23 countries and 30 U.S. states. The symposium consisted of 11 sessions focusing on 3 areas: (1) pancreas and islet cell differentiation and islet generation, (2) β cell biology and insulin synthesis and/or secretion, and (3) pancreatic islet transplantation in patients with type I diabetes. Thirty-nine world experts lectured on the most current information in each field. Fifty-three abstracts were selected for presentation and discussed at the poster session. The first author of each of the top 10 posters received a Young Investigator Travel Award provided by the National Center for Research Resources and the Southern California Islet Cell Resources Center. The symposium also offered special Meet the Professor sessions, which gave the attendees an opportunity to closely interact with the participating speakers of the day.

4.197 T-cell specific immunosuppresion results in more than 53 days survival of porcine islets of Langerhans in the monkey

Rijkelijkhuizen, J.K.R.A. et al

*Transplantation, **76(9)**, 1359-1368 82003)*

Background.
Transplantation of islets of Langerhans can restore insulin production in diabetic patients. Because of the shortage of human donor organs, transplantation of porcine islets may be an alternative solution. The present study was aimed at the characterization of rejection mechanisms of porcine islets transplanted into eight nondiabetic monkeys under the kidney capsule.
Methods.
Cultured adult pig islets were used, which showed no expression of the galactose(α1,3)galactose epitope, major histocompatibility complex class II, or CD45, and no binding of antibodies or complement after exposure to monkey serum. Immunosuppression consisted of cyclophosphamide, cyclosporine A (CsA), and steroids (group 1); or antithymocyte globulin, anti-interleukin-2 receptor antibody, CsA, and steroids (group 2). In three animals of group 2, islets were also transplanted in the portal vein.
Results.
Although all monkeys had preformed anti-pig antibodies, no correlation was found between antibody titers and rejection and no deposition of antibodies or complement was observed in the grafts. Group 1 showed islets up to day 11, followed by T-cell infiltration and rejection at approximately day 14. In group 2, two monkeys showed infiltrates consisting predominantly of T cells starting at approximately day 29, whereas two monkeys showed well-preserved islets without infiltration up to day 53. In the livers of the three monkeys that also received islets intraportally and were resectioned on days 21, 33, and 49, no islets could be detected.
Conclusions.
This study demonstrates that cultured adult pig islets can survive in the monkey for more than 53 days without signs of rejection under standard immunosuppression.

4.198 CD4 expression decrease by antisense oligonucleotides: inhibition of rat $CD4^+$ cell reactivity

Rabanal, N.R. et al

*Oligonucleotides, **13** 217-228 (2003)*

In previous studies, we have demonstrated the inhibition of CD4 expression in rat lymphocytes treated with phorbol myristate acetate (PMA) by antisense oligonucleotides (AS-ODNs) directed against the AUG start region of the *cd*4 gene. The aim of the present study was to inhibit CD4 expression in lymphocytes without promoting CD4 synthesis and to determine the effect of this inhibition on $CD4^+$ T cell function. Four 21-mer ODNs against the rat *cd*4 gene (AS-CD4-1 to AS-CD4-4) were used. Surface CD4 expression was measured by immunofluorescence staining and flow cytometry, and mRNA CD4 expression was measured by RT-PCR. T $CD4^+$ cell function was determined by specific and unspecific proliferative response of rat-primed lymphocytes. After 24 hours of incubation, AS-CD4-2 and AS-CD4-4 reduced lymphocyte surface CD4 expression by 40%. This effect remained for 72 hours and was not observed on other surface molecules, such as CD3, CD5, or CD8. CD4 mRNA expression was reduced up to 40% at 24 hours with AS-CD4-2 and AS-CD4-4. After 48 hours treatment, CD4 mRNA decreased up to 27% and 29% for AS-CD4-2 and AS-CD4-4, respectively. AS-CD4-2 and AS-CD4-4 inhibited T $CD4^+$ cell proliferative response upon antigen-specific and unspecific stimuli. Therefore, AS-ODNs against CD4 molecules inhibited surface and mRNA CD4 expression, under physiologic turnover and, consequently, modulate T $CD4^+$ cell reactivity.

4.199 How to detect *Toxoplasma gondii* oocysts in environmental samples
Dumètre, A. and Dardè, M-L.
*FEMS Microbiol. Rev., **27**, 651-661 (2003)*

Detection of *Toxoplasma gondii* oocysts in environmental samples is a great challenge for researchers as this coccidian parasite can be responsible for severe infections in humans and in animals via ingestion of a single oocyst from contaminated water, soil, fruits or vegetables. Despite field investigations, oocysts have been rarely recovered from the environment due to the lack of sensitive methods. Immunomagnetic separation, fluorescence-activated cell sorting, and polymerase chain reaction have recently shown promising use in detection of protozoa from complex matrices. Such procedures could be applied to *T. gondii* detection, if studies on the antigenic and biochemical composition of the oocyst wall are completed. Using such methods, it will be possible to assess the occurrence, prevalence, viability and virulence of *T. gondii* oocysts in environmental matrices and specify sources of human and animal contamination.

4.200 Clinical islet transplant: current and future directions towards tolerance
Shapiro, A.M.J., Nanij, S. and Lakey, J.R.T.
*Immunological Reviews, **96(2)**, 219-236 (2003)*

The ultimate goal of islet transplantation is to completely correct the diabetic state from an unlimited donor source, without the need for chronic immunosuppressive drug therapy. Although islet transplantation provides an opportunity to develop innovative strategies for tolerance in the clinic, both alloimmune and autoimmune barriers must be controlled, if stable graft function is to be maintained long-term. After islet extraction from the pancreas, the cellular graft may be stored in tissue culture or cryopreserved for banking, providing an opportunity not only to optimally condition the recipient but also to allow *in vitro* immunologic manipulation of the graft before transplantation, unlike solid organ grafts. As such, islets may be considered a 'special case.' Remarkable progress has occurred in the last three years, with dramatic improvements in outcomes after clinical islet transplantation. The introduction of a steroid-free, sirolimus-based, anti-rejection protocol and islets prepared from two (or rarely three) donors led to high rates of insulin independence. The 'Edmonton Protocol' has been successfully replicated by other centers in an international multicenter trial. A number of key refinements in pancreas transportation, processing, purification on non-ficoll-based media, storage of islets in culture for two days and newer immunological conditioning and induction therapies have led to continued advancement through extensive collaboration between key centers. This review outlines the historical development of islet transplantation over the past 30 years, provides an update on current clinical outcomes, and summarizes a series of unique opportunities for development and early testing of tolerance protocols in patients.

4.201 Successful single donor islet transplantation in type 1 diabetes
Hering, B.J. et al

Islet transplantation is emerging as a viable treatment option for selected patients with type 1diabetes. This pilot trial sought to determine whether optimization of pancreas preservation, islet processing, induction and maintenance immunosuppression would facilitate reversal of type 1 diabetes after single-donor islet transplantation.
Pancreases were obtained from stable donors age <50 years and preserved for <8 hrs using the two-layer method. Islets were isolated using the automated method after perfilsion of the pancreas with cold LiberaseTM. Islets were purified on continuous iodixanol gradients, and cultured for 2 days. Islet preparations with viabilities >70%, total tissue volumes <10 cc, negative Gram stain, and endotoxin content <5 FU/kg were considered for transplant. 6,000 to 8,725 cultured islet equivalents/kg prepared from single donors were transplanted intraportally on day 0 following minilaparotomy or percutaneous transhepatic access into 8 C-peptide negative, non-uremic, type 1 diabetic patients with hypoglycemia unawareness. Immunosuppression was initiated 2 days before transplant and included rabbit antithymocyte globulin (through day +2, total dose 6 mg/kg), methylprednisolone (day -2 only), daclizumab, etanercept (through day +10), sirolimus, and reduced-dose tacrolimus (started on day +1). Tacrolimus was gradually replaced with mycophenolate mofetil starting one month posttransplant.
All 8 transplant recipients achieved insulin independence with normal HbA 1 c levels and freedom from hypoglycemia. The time to insulin independence varied from 23 to 122 days. 5 of 8 patients have remained insulin-independent, with follow-up exceeding 1 yr in 2 patients. 3 of 8 patients have resumed exogenous insulin therapy preceded by subtherapeutic sirolimus trough levels (<10 ng/ml) in 3 and subtherapeutic MMF doses (1000 mg/day) in 2 of the 3 patients. Procedural complications, serious infections, or serious, unexpected, and islet- or immunosuppression-related adverse events were not encountered.
The data suggests that a combination of maximized viable islet yield, islet culturing, preemptive induction immunosuppression including agents with anti-inflammatory properties, and nondiabetogenic maintenance immunosuppression can result in successfiil single-donor islet transplantation.

4.202 Iodixanol density gradient preparation in University of Wisconsin solution for porcine islet purification
Van der Burg, M.P.M. and Graham, J.M.
*The Scientific World J., **3**, 1154-1159 (2003)*

Previously published as Graham, J.M. (2002) Purification of Islets of Langerhans from porcine pancreas. TheScientificWorld JOURNAL 2, 1657-1661. ISSN 1537-744X; DOI 10.1100/tsw.2002.847. Generally, prior to the purification of isolated pancreatic islets, the collagenase-digested tissue is incubated in the University of Wisconsin solution (UWS; ~320 mOsm) for osmotic stabilization to preserve or improve the density differences between islets and acinar fragments. The adverse effects arising from the subsequent pelleting and resuspension of the islets in a second, different (often highly hyperosmotic) purification solution are avoided in the protocol described here; preparation of the purification medium is simply achieved by mixing the UWS preincubated islets with a second UWS containing the inert impermeant iodixanol. Flotation of the islets isolated from juvenile porcine pancreases through this mildly hypertonic (~380 mOsm) gradient of iodixanol-UWS achieves a much higher recovery of islets of an improved viability than the customary method using a Ficoll gradient. The method has been extended to human islet purification.

4.203 Improved islet yield and function with ductal injection of university of Wisconsin solution before pancreas preservation
Sawada, T. et al
*Transplantation, **75(12)**, 1965-1969 (2003)*

Background. Ensuring sufficient islet yield from preserved pancreases is a critical step in clinical islet transplantation. The aim of this study was to investigate whether pancreatic ductal injection, performed at procurement, using a small volume of preservation solution before cold storage (ductal preservation method) would improve islet yield and function from rat pancreases preserved for 6 and 24 hr.
Materials and Methods. Islets were isolated from Lewis rats. Pancreases were classified into five groups: fresh (group 1); preserved for 6 hr in University of Wisconsin solution without and with ductal preservation (groups 2 and 3); and preserved for 24 hr in University of Wisconsin solution without and with ductal preservation (groups 4 and 5). We assessed islet yield, function, and viability of pancreatic ductal cells.
Results. Islet yields per pancreas in groups 1 to 5 were 2010±774, 674±450, 1418±528, 527±263, and 1655±618

(islet equivalent) (±SD), respectively. Stimulation indices in groups 1 to 5 were 11.97±3.17, 6.48±4.04, 12.44±5.65, 2.56±2.03, and 5.55±2.71. Functional success rates in groups 1 to 5 were 100%, 0%, 100%, 0%, and 66.7%. Percentages of nonviable pancreatic duct cells in groups 1 to 5 were 3.8±2.7%, 59.7±4.4%, 19.5±7.3%, 64.7±4.5%, and 17.2±2.6%. In all experiments, the differences were significant between the groups without versus the groups with ductal preservation ($P < 0.05$, group 2 vs. group 3 and group 4 vs. group 5). Conclusions. Ductal preservation improved islet yield and function after 6 and 24 hr of preservation. Well-preserved pancreatic ducts maintained good distribution of collagenase solution.
For human islet transplantation to become more widely available and applicable, it will be important to obtain a viable islet mass adequate for diabetes reversal from one donor pancreas. The Edmonton group reported seven successful islet transplants into patients with type 1 diabetes (1). These unprecedented results have since been confirmed in a larger series of patients in Edmonton and, importantly, at other institutions. The islets in these trials were isolated from two or more donors for each recipient. To reverse type 1 diabetes, one donor pancreas is adequate in the setting of vascularized pancreas transplantation (2) but not in the setting of islet transplantation (1). It has been reported, both in a rat and dog model, that islet yield improved from a stored pancreas after intraductal flush of collagenase resuspended in University of Wisconsin (UW) solution at the time of pancreas procurement (3). Pre-cold storage injection of collagenase resuspended in buffer solution into the pancreatic duct of human pancreases from donors less than 30 years old also resulted in increased islet yields (4). We have hypothesized that islet isolation from a preserved pancreas is hampered by the exceptional susceptibility of the pancreatic ductal system to cold ischemic injury and the resulting inability to achieve satisfactory distribution of collagenase by intraductal distention even after short periods of cold storage. We have further hypothesized that improved preservation of the ductal epithelium would produce a better islet yield after pancreas preservation.
To test this hypothesis, we have created a new method in which a small volume of UW preservation solution (without adding collagenase) is injected into the pancreatic duct at the time of procurement (i.e., ductal preservation), thereby preserving the pancreatic duct. We studied the yield and the in vitro and in vivo function of islets isolated from rat pancreases preserved for 6 and 24 hr with standard UW solution immersion and those with ductal preservation combined with pancreas immersion in UW solution. We also assessed the preservation of the pancreatic duct by evaluating the percentage of live cells in it, using trypan blue staining. Ductal preservation improved the yield and function of rat islets after 6 and 24 hr of preservation. Well-preserved pancreatic ducts facilitated good distribution of the collagenase solution.

4.204 Decline in rate of colonization of oligodendrocyte progenitor cell (OPC)-depleted tissue by adult OPCs with age

Chari, D.M., Phil, A., Crang, A.J. and Blakemore, W.F.
J. Neuropathol. Exp. Neurol., ***62(9)***, *908-816 (2003)*

Rates of remyelination decline with age and this has been attributed to slower recruitment of oligodendrocyte progenitor cells (OPCs) into areas of demyelination and slower differentiation of OPCs into remyelinating oligodendrocytes. When considering causes for reduced recruitment rates, intrinsic causes (alterations in biological properties of OPCs) need to be separated from extrinsic causes (age-related differences in the lesion environment). Using 40 Gy of X-irradiation to deplete tissue of its endogenous OPC-population, we examined the effects of age on the rate at which adult rat OPCs colonize OPC-depleted tissue. We found a significant reduction in the rate of colonization between 2 and 10 months of age (0.6 mm/week versus 0.38 mm/week). To determine if this represented an intrinsic property of OPCs or was due to changes in the environment that the cells were recolonizing, OPCs from 10-month-old animals were transplanted into 2-month-old hosts and OPCs from 2-month-old animals were transplanted into 10-month-old hosts. These experiments showed that the transplanted OPCs retained their age-related rate of colonization, indicating that the decline in colonizing rates of OPCs with age reflects an intrinsic property of OPCs. This age-related decline in the ability of OPCs to repopulate OPC-depleted tissue has implications for understanding remyelination failure in multiple sclerosis (MS) and developing therapies for remyelination failure.

4.205 Monocyte chemoattractant protein-1 and CC-chemokine receptor-2 in severe hypercholesterolaemia

Blomquist, H.M. and Olsson, A.G.

*Scand. J. Clin. Lab. Invest., **63**, 513-520 (2003)*

Objectives: To investigate whether plasma concentrations of monocyte chemoattractant protein-1 (MCP-1) and the gene expression of its receptor on the monocyte cell surface CCR-2 were elevated above normal in subjects with asymptomatic, isolated hypercholesterolaemia and if statin treatment could influence this cytokine. *Methods*: The investigation was designed as a cross sectional study followed by a single, blind, treatment study of patients receiving pravastatin 80 mg/day for 8 weeks. The study included 23 patients with severe hypercholesterolaemia (LDL>5.2 mmol/L) and 39 normocholesterolaemic controls. Blood samples were obtained from patients and controls at baseline and from patients at end of the study and analysed for lipoproteins and inflammatory mediators: MCP-1, high-sensitivity C-reactive protein (HS-CRP). Isolated peripheral mononuclear cells were analysed for CCR-2 gene expression. *Results:* Mean plasma LDL-C was significantly higher in patients than in controls. No difference in plasma MCP-1 levels or CCR-2 gene expression was seen between the groups at baseline, nor were there any differences in plasma concentrations of CRP. After treatment with pravastatin, LDL-C decreased by 31%. Treatment did not significantly affect the levels of MCP-1 or CCR-2 gene expression, nor was CRP affected by treatment with pravastatin. *Conclusions:* Our study does not support the view that MCP-1 plasma levels and CCR-2 gene expression in circulating monocytes are directly responsible for the monocyte recruitment into the arterial intima in patients with severe asymptomatic hypercholesterolaemia. In addition, the inflammatory response of a high concentration of LDL-C in isolated asymptomatic hypercholesterolaemia is minute.
Objectives: To investigate whether plasma concentrations of monocyte chemoattractant protein-1 (MCP-1) and the gene expression of its receptor on the monocyte cell surface CCR-2 were elevated above normal in subjects with asymptomatic, isolated hypercholesterolaemia and if statin treatment could influence this cytokine. *Methods*: The investigation was designed as a cross sectional study followed by a single, blind, treatment study of patients receiving pravastatin 80 mg/day for 8 weeks. The study included 23 patients with severe hypercholesterolaemia (LDL>5.2 mmol/L) and 39 normocholesterolaemic controls. Blood samples were obtained from patients and controls at baseline and from patients at end of the study and analysed for lipoproteins and inflammatory mediators: MCP-1, high-sensitivity C-reactive protein (HS-CRP). Isolated peripheral mononuclear cells were analysed for CCR-2 gene expression. *Results:* Mean plasma LDL-C was significantly higher in patients than in controls. No difference in plasma MCP-1 levels or CCR-2 gene expression was seen between the groups at baseline, nor were there any differences in plasma concentrations of CRP. After treatment with pravastatin, LDL-C decreased by 31%. Treatment did not significantly affect the levels of MCP-1 or CCR-2 gene expression, nor was CRP affected by treatment with pravastatin. *Conclusions:* Our study does not support the view that MCP-1 plasma levels and CCR-2 gene expression in circulating monocytes are directly responsible for the monocyte recruitment into the arterial intima in patients with severe asymptomatic hypercholesterolaemia. In addition, the inflammatory response of a high concentration of LDL-C in isolated asymptomatic hypercholesterolaemia is minute.

4.206 Isolation of rat Kupffer cells: a combined methodology for highly purified primary cultures
Valatas, V., Xidakis, C., Roumpaki, H., Kolios, G. And Kouroumalis, E.A.
*Cell Biol Int., **27**, 67-73 (2003)*

We report a four-step procedure that optimizes the methodology for isolation of highly purified rat Kupffer cells (KC). We combined the previously reported techniques of enzymatic tissue treatment, density gradient centrifugation, centrifugal elutriation and selective adherence. ED-2 immunophenotyping and non-specific esterase histochemistry were used for cell identification. This combination resulted in a satisfactorily high yield of 80–100×10^6KCs per liver, over 95% positive for ED-2 and 98% viable cells. Cultures of isolated KCs were functionally intact and exhibited a concentration and time-dependent LPS-induced TNF-α and nitric oxide production.

4.207 Heterogeneity of dendritic cells in the mouse liver: identification and characterization of four distinct populations
Lian, Z-X. et al
*J. Immunol., **170**, 2323-2330 (2003)*

Liver dendritic cells (DC) are believed to play important roles in liver immunity, autoimmunity, and in the regulation of hepatic allograft acceptance. However, limited information is available on the phenotypes and functions of DC in the liver. To address this issue, we isolated DC from murine liver using procedures that do not involve collagenase, and characterized the freshly isolated DC population that had not been subjected to in vitro expansion. Thence, based on the expression of CD4, B220, and CD11b, four subsets or groups of hepatic $NK1.1^-CD11c^+$ DC were identified with the following phenotypes: $B220^+CD4^+$,

$B220^+CD4^-$, $B220^-CD11b^+$, and $B220^-CD11b^-$. Each subset was further characterized both phenotypically and functionally. In addition to unique phenotypic expression, each subset displayed different allostimulation capability in mixed lymphocyte reaction assays. All four groups developed DC morphology following in vitro culture with activation agents and synthesized distinct patterns of cytokines in response to different stimuli. Taken together, our results suggest that groups I and II are IFN-α-producing plasmacytoid DC, group III cells are myeloid-related DC, while group IV is a heterogenous population containing both myeloid- and lymphoid-related DC. Our results demonstrate the highly heterogeneous nature of hepatic DC, which is in agreement with the unique requirements for APC in the complex liver environment.

4.208 Murine thymic plasmacytoid dendritic cells

Okada, T., Lian, Z-X., Naiki, M., Ansari, A.A., Ikehara, S. and Gershwin, M.E.
*Eur. J: Immunol., **33(4),** 1012-1019 (2003)*

We report herein heterogeneous murine thymic cell subsets expressing CD11c and B220 (CD45R). The $CD11c^+B220^+$ subset expresses $Ly6C^{high}$ and MHC class II^{low} in contrast with previously described thymic DC ($CD11c^+B220^-$ cells). Freshly isolated thymic $CD11c^+B220^+$ cells show typical plasmacytoid morphology which differentiates to mature DC, *in vitro* with CpG oligodeoxynucleotides (ODN) 2216; we term this subset thymic plasmacytoid DC (pDC). These thymic pDC are highly sensitive to spontaneous apoptosis *in vitro* and induce low T cell allo-proliferation activity. Thymic pDC express low TLR2, TLR3 and TLR4 mRNA, normally found on human immature DC, and high TLR7 and TLR9 mRNA, normally found on human pDC. Thymic pDC also produce high amounts of IFN-α following culture with CpG ODN 2216 (TLR9 ligands) as compared with the previously defined thymic DC lineage which expresses low TLR9 mRNA and produce high IL-12 (p40) with CpG ODN 2216. These results indicate that thymic pDC are similar to IFN-producing cells as well as human pDC. The TLR and cytokine production profiles are consistent with a nomenclature of pDC. The repertoire of this cell lineage to TLR9 ligands demonstrate that such responses are determined not only by the quantity of expression, but also cell lineage.

4.209 Role of Toll-like receptors in costimulating cytotoxic T cell responses

Schwarz, K., Storni, T., Manolova, V., Didierlaurent, A., Sirard, J-C., Röthlisberger, P. and Bachmann, M.F.
*Eur. J: Immunol., **33(6)**, 1465-1470 (2003)*

Stimulation of Toll-like receptors (TLR) by pathogen-derived compounds leads to activation of APC, facilitating the induction of protective immunity. This phenomenon is the basis of most adjuvant formulations currently in development. Here, we tested the ability of TLR2, 3, 4, 5, 7 and 9 signaling to enhance CTL responses upon vaccination with virus-like particles. Stimulation of TLR2 and 4 failed to increase CTL responses, whereas ligands for TLR3, 5 and 7 exhibited moderate adjuvant function. In contrast, stimulation of TLR9 dramatically increased CTL responses, indicating that ligands for TLR9 are likely to be the most promising candidates for the development of novel adjuvant formulations for stimulating CTL responses.

4.210 Age-related changes in neuronal glucose uptake in response to glutamate and β-amyloid

Patel, J.R. and Brewer, G.J.
*J. Neurosci. Res., **72(4)**, 527-536 (2003)*

Energy supplies that may decline with age are crucial for cells to maintain ionic homeostasis and prevent neuron death. We examined baseline glucose transporter expression and rate of glucose uptake in cultured hippocampal neurons from embryonic, middle-age (12-month-old), and old (24-month-old) rats and exposed the neurons to glutamate, β-amyloid, and mitochondrial inhibitors. Without stress, the rate of glucose uptake was similar in middle-age and old neurons, and the rate of glucose uptake in embryonic neurons was threefold greater than that in middle-age and old neurons. Glucose uptake increased in the presence of mitochondrial inhibitors (FCCP and oligomycin) for embryonic and middle-age neurons. The old neurons failed to increase glucose uptake. In the presence of glutamate, FCCP, and oligomycin, embryonic neurons showed a decrease in glucose uptake and the middle-age and old neurons showed no change in glucose uptake. Middle-age neurons took up significantly more glucose than old neurons when under mitochondrial and glutamate stress. In the presence of β-amyloid, only embryonic neurons increased glucose uptake; middle-age and old neurons did not. Fluorescence imaging of immunoreactive glut3 in response to β-amyloid demonstrated a 16–49% increase in glut3 immunoreactivity at the plasma membrane for the three ages. The results suggest that old neurons were not able to upregulate glucose

uptake to ensure cell survival. Neuron aging does not indicate a defect in normal glut3 function; rather, our results suggest that mechanisms regulating glucose uptake under stress fail to react in time to ensure cell survival.

4.211 Platelets and Granulocytes, in Particular the Neutrophils, Form Important Compartments for Circulating Vascular Endothelial Growth Factor
Kusumanto, Y.H., Dam, W.A., Hospers, G.A.P., Meijer, C. and Mulder, N.H.
*Angiogenesis, **6(4)**, 283-287 (2003)*

The measurement of circulating vascular endothelial growth factor (VEGF) levels as a prognostic factor will gain increasing relevance in the diagnosis and evaluation of treatment in cancer patients. Angiogenesis is an absolute requirement in tumour growth and metastatic disease. In the present study data are presented which indicate that circulating VEGF mainly resides in peripheral blood cells. In 15 healthy volunteers we demonstrated that approximately 34% of the circulating VEGF resides in platelets and approximately 11% in patients with cancer (*n*= 4). An important part namely 58% in healthy volunteers and 69% in patients with cancer of the total circulating VEGF is contained in granulocytes, particular in the neutrophils, as confirmed by fluorescence-activated cell sorting (FACS). Also an increased VEGF level per granulocyte is found in patients with cancer (77 μg VEGF/l) compared with the healthy volunteers (164 μg VEGF/l). In contrast only 2% was present in plasma. The biological significance of platelet- or granulocyte-derived VEGF is not yet known. Liberation of VEGF from these compartments could well be of importance for tumour angiogenesis. Therefore, future studies on the clinical value of circulating VEGF as a prognostic factor in cancer patients should include measurements of VEGF in peripheral blood cells.

4.212 Para/autocrine regulation of estrogen receptors in hippocambal neurons
Prange-Kiel, J., Wehrenberg, U., Jarry, H. and Rune, G.M.
*Hippocampus, **13(2)**, 226-234 (2003)*

Previous studies have shown that estrogens, originating from ovaries, have a wide variety of estrogen receptor (ER)-mediated effects in the hippocampus. In the present study, we have investigated whether estrogens, which are synthesized in the hippocampus, could induce these effects as well. As a parameter, we used ER expression in response to estrogen synthesis, because estrogen receptors are ligandinducible transcription factors. The experiments were carried out with cultures of isolated adult rat hippocampal cells, which contained about 95% neurons and about 5% oligodendrocytes in serum-free and steroidfree medium. Hippocampal neurons express both estrogen receptor isoforms (ER_ and ER_), as shown by reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization. The release of estrogens by hippocampal neurons was quantified by radioimmunoassay (RIA). The ER isoforms (_ and _) were studied by semiquantitative immunocytochemical image analysis. Hippocampal cells precultured for 4 days were found to synthesize 17_-estradiol for the next 8 days. This synthesis was completely inhibited by letrozol, an aromatase inhibitor. Inhibition of estrogen synthesis by letrozol induced a significant decrease in ER_ expression, but an increase in ER_. As a control, supplementation of the medium with 17_-estradiol resulted in a significant increase of ER_ expression, whereas ER_ was downregulated. Our findings provide evidence for a de novo synthesis of estrogens in the hippocampus, differential regulation of estrogen receptor isoforms by estrogen and consequently for a para/autocrine loop of estrogen action in the hippocampus.

4.213 Cord blood from collection to expansion : Feasibility in a regional blood bank
Elias, M., Choudhury, N. and Smit Sibinga, CTh.
*Indian J. Padiatr., **70(4)**, 327-336 (2003)*

This article reviews the various aspect of the experimental phase proceeding the establishment of an umbilical cord blood (UCB) bank within a regular blood bank, a situation totally different from that of de novo establishing a cord blood bank having human and financial resources. An ethically approved two-year study has been conducted to determine the technical feasibility, and the practical problems that might be encountered such as public compliance, the additional workload, introduction of new activities ranging from collection and processing to progenitor expansion, infectious disease testing, development of a quality control system, record keeping and documentation, development of specific procedures and definitions of requirements. The cost benefit aspect, which will ultimately depend on the frequency of units release, was not considered in this study.

4.214 Erythropoietin: a candidate compound for neuroprotection in schizophrenia
Ehrenreich, H. et al
*Mol. Psychiatry., **9**, 42-54 (2004)*

Erythropoietin (EPO) is a candidate compound for neuroprotection in human brain disease capable of combating a spectrum of pathophysiological processes operational during the progression of schizophrenic psychosis. The purpose of the present study was to prepare the ground for its application in a first neuroprotective add-on strategy in schizophrenia, aiming at improvement of cognitive brain function as well as prevention/slowing of degenerative processes. Using rodent studies, primary hippocampal neurons in culture, immunohistochemical analysis of human post-mortem brain tissue and nuclear imaging technology in man, we demonstrate that: (1) peripherally applied recombinant human (rh) EPO penetrates into the brain efficiently both in rat and humans, (2) rhEPO is enriched intracranially in healthy men and more distinctly in schizophrenic patients, (3) EPO receptors are densely expressed in hippocampus and cortex of schizophrenic subjects but distinctly less in controls, (4) rhEPO attenuates the haloperidol-induced neuronal death *in vitro*, and (4) peripherally administered rhEPO enhances cognitive functioning in mice in the context of an aversion task involving cortical and subcortical pathways presumably affected in schizophrenia. These observations, together with the known safety of rhEPO, render it an interesting compound for neuroprotective add-on strategies in schizophrenia and other human diseases characterized by a progressive decline in cognitive performance.

4.215 Differential recruitment of Kv1.4 and Kv4.2 to lipid rafts by PSD-95
Wong, W. and Schlichter, L.C.
*J. Biol. Chem., **279(1)**, 444-452 (2004)*

The activity of voltage-gated potassium (Kv) channels, and consequently their influence on cellular functions, can be substantially altered by phosphorylation. Several protein kinases that modulate Kv channel activity are found in membrane subdomains known as lipid rafts, which are thought to organize signaling complexes in the cell. Thus, we asked whether Kv1.4 and Kv4.2, two channels with critical roles in excitable cells, are found in lipid rafts. Acylation can target proteins to raft regions; however, Kv channels are not acylated, and therefore, a different mechanism must exist to bring them into these membrane subdomains. Because both Kv1.4 and Kv4.2 interact with postsynaptic density protein 95 (PSD-95), which is acylated (specifically, palmitoylated), we examined whether PSD-95 can recruit these channels to lipid rafts. We found that a portion of Kv1.4 and Kv4.2 protein in rat brain membranes is raft-associated. Lipid raft patching and immunostaining confirmed that some Kv4.2 is in Thy-1-containing rafts in rat hippocampal neurons. Using a heterologous expression system, we determined that palmitoylation of PSD-95 was crucial to its localization to lipid rafts. We then assessed the contribution of PSD-95 to the raft association of these channels. Co-expression of PSD-95 increased the amount of Kv1.4, but not Kv4.2, in lipid rafts. Deleting the PSD-95 binding motif of Kv1.4 eliminated this recruitment, as did substituting a palmitoylation-deficient PSD-95 mutant. This work represents the first evidence that PSD-95 binding can recruit Kv channels into lipid rafts, a process that could facilitate interactions with the protein kinases that affect channel activity.

4.216 AMG531 stimulates megakaryopoiesis in vitro by binding to Mpl
Broudy, V.C. and Lin, N.L.
*Cytokine, **25**, 52-60 (2004)*

Thrombopoietin (TPO) plays a pivotal role in megakaryopoiesis. TPO initiates its biological effects by binding to its receptor Mpl. A recombinant protein consisting of a carrier Fc domain linked to multiple Mpl-binding domains was constructed, and is called AMG531. To define the biological activity of AMG531, we examined the ability of AMG531 to support CFU-Meg growth and to promote megakaryocyte maturation in vitro. AMG531 stimulates CFU-Meg growth in a dose-dependent manner, and acts in concert with erythropoietin, stem cell factor, interleukin-3, and interleukin-6 to enhance CFU-Meg growth, similar to parallel experiments with TPO. AMG531-stimulated serum-free liquid cultures support the development of mature polyploid megakaryocytes with a predominant DNA content of 32 N and 64 N, identical to that of parallel TPO-stimulated cultures. Competitive binding experiments show that AMG531 effectively competes with ^{125}I-TPO for binding to BaF3-Mpl cells or normal platelets. Treatment

of BaF3-Mpl cells with AMG531 or with TPO resulted in rapid tyrosine phosphorylation of Mpl, JAK2, and STAT5. These results indicate that AMG531 is a potent stimulant of megakarypoiesis in vitro, and provide support for its further characterization in vivo.

4.217 Systematic screening of potential β-cell imaging agents
Sweet, I.R. et al
*Biochem. Biophys. Res. Comm., **314**, 976-983 (2004)*

The β-cell loss seen in diabetes mellitus could be monitored clinically by positron emission tomography (PET) if imaging agents were sufficiently specific for β-cells to overcome the high ratio of non-β-cell to β-cell tissue in pancreas. In this report, we present a screening assay for identifying β-cell-specific compounds that is based on the relative accumulation and retention by islet, INS-1, and exocrine (PANC-1) cells of candidate molecules. Molecules thought to have a high affinity for β-cells were tested and included glibenclamide, tolbutamide, serotonin, L-DOPA, dopamine, nicotinamide, fluorodeoxyglucose, and fluorodithizone. Glibenclamide and fluorodithizone were the most specific, but the specificity ratios fell well below those needed to attain robust signal to background ratio as a PET imaging agent for quantifying β-cell mass. In vivo tests of the biodistribution of glibenclamide and fluorodithizone in rats indicated that the compounds were not specifically associated with pancreas, bearing out the predictions of the in vitro screen.

4.218 Regulation of ATP/ADP in pancreatic islets
Sweet, I.R. et al
*Diabetes, **53**, 401-409 (2004)*

ATP and ADP levels are critical regulators of glucose-stimulated insulin secretion. In many aerobic cell types, the phosphorylation potential (ATP/ADP/P_i) is controlled by sensing mechanisms inherent in mitochondrial metabolism that feed back and induce compensatory changes in electron transport. To determine whether such regulation may contribute to stimulus-secretion coupling in islet cells, we used a recently developed flow culture system to continuously and noninvasively measure cytochrome c redox state and oxygen consumption as indexes of electron transport in perifused isolated rat islets. Increasing substrate availability by increasing glucose increased cytochrome c reduction and oxygen consumption, whereas increasing metabolic demand with glibenclamide increased oxygen consumption but not cytochrome c reduction. The data were analyzed using a kinetic model of the dual control of electron transport and oxygen consumption by substrate availability and energy demand, and ATP/ADP/P_i was estimated as a function of time. ATP/ADP/P_i increased in response to glucose and decreased in response to glibenclamide, consistent with what is known about the effects of these agents on energy state. Therefore, a simple model representing the hypothesized role of mitochondrial coupling in governing phosphorylation potential correctly predicted the directional changes in ATP/ADP/P_i. Thus, the data support the notion that mitochondrial-coupling mechanisms, by virtue of their role in establishing ATP and ADP levels, may play a role in mediating nutrient-stimulated insulin secretion. Our results also offer a new method for continuous noninvasive measures of islet cell phosphorylation potential, a critical metabolic variable that controls insulin secretion by ATP-sensitive K^+–dependent and –independent mechanisms.

4.219 Interleukin-10 secretion differentiates dendritic cells from human liver and skin
Goddard, S., Youster, J., Morgan, E. and Adams, D.H.
*Am. J. Pathol., **164(2)**, 511-519 (2004)*

Liver dendritic cells (DCs), which may orchestrate the liver's unique immunoregulatory functions, remain poorly characterized. We used a technique of overnight migration from pieces of normal human liver and skin to obtain tissue-derived DCs with minimal culture and no additional cytokine treatment. Liver and skin DCs had a monocyte-like morphology and a partially mature phenotype, expressing myeloid markers, MHCII, and co-stimulatory molecules; but only the skin DCs contained a population of CD1a+ cells. Overnight-migrated liver DCs activated naïve cord blood T cells efficiently. Liver DCs produced interleukin (IL)-10 whereas skin DCs failed to secrete IL-10 even after stimulation and neither skin nor liver-derived DCs secreted significant amounts of IL-12p70. Compared with skin DCs, liver DCs were less effective at stimulating T-cell proliferation and stimulated T cells to produce IL-10 and IL-4 whereas skin DCs were more potent stimulators of interferon-γ and IL-4. Monocyte-derived DCs were down-regulated after culture with liver-conditioned media, suggesting that local microenvironmental factors may be important. Thus we show for the first time clear tissue-specific differences in nonlymphoid DCs. Although

it is not possible to conclude from our data whether liver DCs are more regulatory, or skin DCs more proimmunogenic, the ability of liver DCs to secrete IL-10 may be important for regulating local immune responses within the liver in the face of constant exposure to gut antigens.

4.220 Unraveling the secrets of single donor success in islet transplantation
Shapiro, A.M.J. and Ricordi, C.
*Am. J. Transplant., **4**, 295-298 (2004)*

Islet transplantation is emerging as an attractive alternative to solitary pancreas transplantation for a highly select group of patients with severe, labile forms of Type 1 diabetes that had previously tried and failed on intensive insulin therapy. The year 2000 marked a dramatic shift in clinical success with the introduction of the so-called 'Edmonton Protocol', which built upon many years of intensive research and extensive collaborations between islet groups worldwide (1,2).

4.221 Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody
Hering, B.J. et al
*Am. J. Transplant., **4**, 390-401 (2004)*

We sought to determine whether or not optimizing pancreas preservation, islet processing, and induction immunosuppression would facilitate sustained diabetes reversal after single-donor islet transplants. Islets were isolated from two-layer preserved pancreata, purified, cultured for 2 days; and transplanted into six C-peptide-negative, nonuremic, type 1 diabetic patients with hypoglycemia unawareness. Induction immunosuppression, which began 2 days pretransplant, included the Fc receptor nonbinding humanized anti-CD3 monoclonal antibody hOKT3γ1 (Ala-Ala) and sirolimus. Immunosuppression was maintained with sirolimus and reduced-dose tacrolimus. Of our six recipients, four achieved and maintained insulin independence with normal HbA1c levels and freedom from hypoglycemia; one had partial islet graft function; and one lost islet graft function 2 weeks post-transplant. The four insulin-independent patients showed prolonged $CD4^+$ T-cell lymphocytopenia; inverted CD4:CD8 ratios; and increases in the percentage of $CD4^+CD25^+$ T cells. These cells suppressed the *in-vitro* proliferative response to donor cells and, to a lesser extent, to third-party cells. Severe adverse events were limited to a transient rash in one recipient and to temporary neutropenia in three. Our preliminary results thus suggest that a combination of maximized viable islet yield, pretransplant islet culture, and preemptive immunosuppression can result in successful single-donor islet transplants.

4.222 Transplanting encephalomyocarditis virus-infected porcine islet cells reverses diabetes in recipient mice but also transmits the virus
Brewer, L., LaRue, R., Hering, B., Brown, C. and Njenga, M.K.
*Xenotransplantation, **11**, 160-170 (2004)*

Previous studies demonstrated that porcine encephalomyocarditis virus (EMCV) caused acute and persistent infection in the myocardium, central nervous system, and spleen of non-human primates (cynomolgus macaques); and it productively infected primary human cardiomyocytes, suggesting that the virus may pose a risk in pig-to-human transplantation. Recently, transplantation of myocardial and pancreatic tissues from acutely infected pigs transmitted the virus to recipient mice, resulting in acute fatal EMCV disease. Here, we examined whether porcine islet cells (PICs), which are under clinical trial for treatment of type I diabetes in humans, are susceptible to porcine EMCV, and whether EMCV-infected PICs could function in vivo to reverse diabetes. PICs were infected with EMCV in vitro for 5 h, and resulting insulin production compared with that produced by uninfected PICs. Subsequently, infected PICs were transplanted intra-abdominally or under the kidney capsule of C57BL/6 mice, and both virus transmission and PIC function analyzed. PICs were highly susceptible to porcine EMCV, resulting in a 1500-fold increase in production of infectious virus within 5 h of inoculation and cytolysis that destroyed up to 50% of cells within 96 h. However, as long as they were viable, infected PICs produced insulin at levels comparable with uninfected PICs. Intra-abdominal transplantation of 2000 PICs, infected with one plaque forming unit (pfu) per cell of porcine EMCV, into C57BL/6 mice transmitted the virus resulting in acute fatal EMCV disease characterized by hind limb paresis and paralysis and acute respiratory distress in 40% of recipient mice. More importantly, transplantation of 2500 EMCV-infected PICs under the kidney capsule of diabetic C57BL/6 mice (glucose level $\geq$350 mg/dl) reversed diabetes in 83% of recipient mice

(glucose level ≤170 mg/dl); however these mice succumbed to acute EMCV disease transmitted by the xenograft 5 days after transplantation. EMCV infection does not appear to affect insulin production by PICs, but infected xenografts can transmit the virus to recipient animals, resulting in severe disease.

4.223 Increased CD36 protein as a response to defective insulin signaling in macrophages
Liang, C-P. Et al
J. Clin. Invest., ***113(5)***, *764-773*

Accelerated atherosclerosis is a major cause of morbidity and death in insulin-resistant states such as obesity and the metabolic syndrome, but the underlying mechanisms are poorly understood. We show that macrophages from obese (*ob*/*ob*) mice have increased binding and uptake of oxidized LDL, in part due to a post-transcriptional increase in CD36 protein. Macrophages from *ob*/*ob* mice are also insulin resistant, as shown by reduced expression and signaling of insulin receptors. Three lines of evidence indicate that the increase in CD36 is caused by defective insulin signaling: (a) Treatment of wild-type macrophages with LY294002, an inhibitor of insulin signaling via PI3K, results in an increase in CD36; (b) insulin receptor knockout macrophages show a post-transcriptional increase in CD36 protein; and (c) administration of thiazolidinediones to intact *ob*/*ob* mice and *ob*/*ob*, LDL receptor–deficient mice results in a reversal of macrophage insulin receptor defects and decreases CD36 protein. The last finding contrasts with the increase in CD36 that results from treatment of macrophages with these drugs ex vivo. The results suggest that defective macrophage insulin signaling predisposes to foam cell formation and atherosclerosis in insulin-resistant states and that this is reversed in vivo by treatment with PPAR-γ activators.

4.224 Role of tau phosphorylation by glycogen synthase kinase-3β in the regulation of organelle transport
Tatebayashi, Y., Haque, N., Tung, Y-C., Iqbal, K. and Grundke-Iqbal, I.
J. Cell Sci., ***117***, *1653-1663 (2004)*

Anterograde organelle transport is known to be inhibited by overexpression of the microtubule-associated protein tau in cultured cells. However, the molecular mechanism regulating this function of tau protein has not previously been understood. We found that in PC12 cells treated with NGF or fibroblast growth factor-2, glycogen synthase kinase-3ß and tau were upregulated simultaneously from around day 2 of differentiation, with increasing glycogen synthase kinase-3-mediated tau phosphorylation. This phosphorylation did not alter tau's ability to bind to microtubules but appeared to be required for the maintenance of the anterograde organelle transport in differentiated cells. Lithium, alsterpaullone or valproate, three independent glycogen synthase kinase-3 inhibitors, but not butyrolactone 1, an inhibitor of cyclin-dependent protein kinases, induced mitochondrial clustering in association with tau dephosphorylation. In CHO cells transfected with human tau_{441}, mitochondrial clustering was found in cells in which tau was unphosphorylated. These findings raise the possibility that the phosphorylation of tau by glycogen synthase kinase-3 might be involved in the regulation of organelle transport.

4.225 NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder
Agostini, L. et al
Immunity, ***20***, *319-325 (2004)*

Mutations within the *NALP3/cryopyrin/CIAS1* gene are responsible for three autoinflammatory disorders: Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and CINCA. The NALP3 protein is homologous to NALP1, which is a component of the inflammasome, a molecular platform that activates the proinflammatory caspases-1 and -5. NALP3 (and other members of the NALP family) lacks the C-terminal, CARD-containing sequence of NALP1, and its role in caspase activation is unclear. Here, we report that NALP2 and NALP3 associate with ASC, the CARD-containing protein Cardinal, and caspase-1 (but not caspase-5), thereby forming an inflammasome with high proIL-1β-processing activity. Macrophages from Muckle-Wells patients spontaneously secrete active IL-1β. Increased inflammasome activity is therefore likely to be the molecular basis of the symptoms associated with NALP3-dependent autoinflammatory disorders.

4.226 Octreotide regulates CC but not CXC LPS-induced chemokine secretion in rat Kupffer cells
Valatas, V. et al
Br. J. Pharm., ***141***, *477-487 (2004)*

1 Kupffer cells (KC) and lipopolysaccharide (LPS) interaction is the initial event leading to hepatic

inflammation and fibrosis in many types of liver injury. We studied chemokine secretion by KC activated with LPS and the possible effect of the somatostatin analogue octreotide, in the regulation of this process. **2** KC isolated from Sprague–Dawley rats were cultured in the presence of LPS added alone or with different concentrations of octreotide for 24 and 48 h, and chemokine production was assessed in culture supernatants by ELISA. CC chemokine mRNA expression was assessed by semiquantitative RT–PCR. **3** Vehicle-stimulated KC produced a basal amount of CC and CXC chemokines. LPS-stimulated KC secreted significantly increased amounts of IL-8 (GRO/CINC-1) (P<0.001), MIP-2 (P<0.001), MCP-1 (P<0.001), and RANTES (P<0.01). **4** Octreotide inhibited LPS-induced secretion of the CC chemokines MCP-1 (P<0.05) and RANTES (P<0.05), but not the CXC chemokines IL-8 (GRO/CINC-1) and MIP-2, in a concentration-dependent manner. Downregulation of basal and LPS-induced mRNA expression of the CC chemokines was also observed in the presence of octreotide. **5** Pretreatment with phosphatidylinositol 3 (PI3)-kinase inhibitors reduced chemokine production by LPS-treated KC in both the mRNA and protein level. Furthermore, it prevented the octreotide inhibitory effect on LPS-induced chemokine secretion, indicating a possible involvement of the PI3-kinase pathway. **6** In conclusion, these data demonstrate that chemokine secretion by KC can be differentially regulated by octreotide, and suggest that this somatostatin analogue may have immunoregulatory effects on resident liver macrophages.

4.227 Interleukin-6 deficiency affects bone marrow stromal precursors, resulting in defective hematopoietic support

Del Carmen, M., Bernad, A. And Aracil, M.

*Blood, **103(9)**, 3349-3354 (2004)*

Interleukin-6 (IL-6) is a critical factor in the regulation of stromal function and hematopoiesis. In vivo bromodeoxyuridine incorporation analysis indicates that the percentage of $Lin^{-}Sca\text{-}1^{+}$ hematopoietic progenitors undergoing DNA synthesis is diminished in IL-6-deficient ($IL\text{-}6^{-/-}$) bone marrow (BM) compared with wild-type BM. Reduced proliferation of $IL\text{-}6^{-/-}$ BM progenitors is also observed in $IL\text{-}6^{-/-}$ long-term BM cultures, which show defective hematopoietic support as measured by production of total cells, granulocyte macrophage-colony-forming units (CFU-GMs), and erythroid burst-forming units (BFU-Es). Seeding experiments of wild-type and $IL\text{-}6^{-/-}$ BM cells on irradiated wild-type or IL-6-deficient stroma indicate that the hematopoietic defect can be attributed to the stromal and not to the hematopoietic component. In $IL\text{-}6^{-/-}$ BM, stromal mesenchymal precursors, fibroblast CFUs (CFU-Fs), and stroma-initiating cells (SICs) are reduced to almost 50% of the wild-type BM value. Moreover, $IL\text{-}6^{-/-}$ stromata show increased CD34 and CD49e expression and reduced expression of the membrane antigens vascular cell adhesion molecule-1 (VCAM-1), Sca-1, CD49f, and Thy1. These data strongly suggest that IL-6 is an in vivo growth factor for mesenchymal precursors, which are in part implicated in the reduced longevity of the long-term repopulating stem cell compartment of $IL\text{-}6^{-/-}$ mice. (Blood. 2004;103:3349-3354)

4.228 A syntaxin 1, $G\alpha_o$, and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization

Li, Q. et al

*J. Neurosci., **24(16)**, 4070-4081 (2004)*

Presynaptic $Ca_V2.2$ (N-type) calcium channels are subject to modulation by interaction with syntaxin 1 and by a syntaxin 1-sensitive $G\alpha_O$ G-protein pathway. We used biochemical analysis of neuronal tissue lysates and a new quantitative test of colocalization by intensity correlation analysis at the giant calyx-type presynaptic terminal of the chick ciliary ganglion to explore the association of $Ca_V2.2$ with syntaxin 1 and $G\alpha_O$. $Ca_V2.2$ could be localized by immunocytochemistry (antibody Ab571) in puncta on the release site aspect of the presynaptic terminal and close to synaptic vesicle clouds. Syntaxin 1 coimmunoprecipitated with $Ca_V2.2$ from chick brain and chick ciliary ganglia and was widely distributed on the presynaptic terminal membrane. A fraction of the total syntaxin 1 colocalized with the $Ca_V2.2$ puncta, whereas the bulk colocalized with MUNC18-1. $G\alpha_O$, whether in its trimeric or monomeric state, did not coimmunoprecipitate with $Ca_V2.2$, MUNC18-1, or syntaxin 1. However, the G-protein exhibited a punctate staining on the calyx membrane with an intensity that varied in synchrony with that for both Ca channels and syntaxin 1 but only weakly with MUNC18-1. Thus, syntaxin 1 appears to be a component of two separate complexes at the presynaptic terminal, a minor one at the transmitter release site with $Ca_V2.2$ and $G\alpha_O$, as well as in large clusters remote from the release site with MUNC18-1. These syntaxin 1 protein complexes may play distinct roles in presynaptic biology.

4.229 Postmortem effect of pentobarbital anesthetic on survival of adult coctical neurons in primary culture

Viel, J.J., McManus, D.Q., Brewer, G.J.
*BrainRres.,**1009**, 219-222 (2004)*

We determined whether pentobarbital anesthetic is required to culture postmortem adult rat neurons. Pentobarbital treatment resulted in two-fold increases in neuron survival in culture after 2 and 4 h postmortem compared to non-anesthetic controls, but was not as effective as simple postmortem treatment on ice and therefore not essential.

4.230 Preservation of human pancreatic islet in vivo function after 6-month culture in serum-free media

Rush, B. et al
*Transplantation, **77**, 1147-1154 (2004)*

Background. Culturing human islets in Memphis serum-free media (M-SFM) is associated with excellent postculture recovery, in vitro function, and in vivo survival. The authors investigate the possibility of preserving islet function for extended periods (6 months) in culture and describe the in vitro and in vivo functional outcomes associated with these extended culture times.
Methods. Human islets isolated from three cadaveric donor organs were cultured in M-SFM for 1, 3, or 6 months before transplantation under the kidney capsule of nonobese diabetic (NOD)-severe combined immunodeficiency (SCID) mice. In vitro function was measured by static incubation at the time of transplantation. In vivo function was assessed by measuring human insulin and C-peptide production, and by the ability of 6-month cultured islets to cure streptozotocin-induced diabetes in this mouse model.
Results. Islet recovery ratios after 1 month in culture ranged from 85% to 88% and declined to 28% to 53% after 6 months of culture (P <0.01). Insulin stimulation indices did not differ among the fresh or the 6-month cultured preparations. All preparations cultured for 1 to 3 months functioned in the NOD-SCID mice. After 6 months of culture, two of the three preparations demonstrated in vivo function and were able to cure streptozotocin-induced diabetes.
Conclusions. These data demonstrate that human islets can be cultured in M-SFM for extended periods and still retain in vitro and in vivo function and the ability to cure experimental diabetes. The ability to maintain islets in culture for prolonged periods is an important step toward the development of islet tissue repositories and distribution centers.

4.231 Comparative proteomics of primitive hematopoietic cell populations reveals differences in expression of proteins regulating motility

Evans, C.A. et al
*Blood, **103(10)**, 3751-3759 (2004)*

Lineage-marker depleted (Lin^-) murine bone marrow cells expressing stem cell antigen 1 (Sca-1) were sorted on the basis of stem cell factor receptor (c-kit) expression to obtain $Lin^-Sca^+Kit^+$ or $Lin^-Sca^+Kit^-$ cells. $Lin^-Sca^+Kit^-$ cells have a markedly greater chemotactic response to stromal derived factor-1 (SDF-1). Using a novel fluorescent stain, we show that both populations generate similar levels of a key messenger, phosphatidylinositol 3,4,5 trisphosphate (PIP_3), in response to SDF-1. Differences in motile behavior may therefore lie downstream of phosphatidylinositol 3-kinase (PI3-kinase) activation at the level of cytoskeleton regulation. The 2 cell populations were compared using 2-dimensional difference gel electrophoresis (2D-DIGE), with a maleimide CyDye fluorescent protein labeling technique that has enhanced sensitivity for low abundance samples. Comparative proteomic analysis of Cy3- and Cy5-labeled protein samples allows relative quantification of protein spots present in both cell populations; of these, 73% were common. Key protein differences were adseverin and gelsolin, actin micro-filament splicing proteins, regulated by Rac, downstream of PI3-kinase activation. Adseverin was shown to be acetylated, a novel modification for this protein. Differences in major regulators of cell shape and motility between the 2 populations can explain the differential response to SDF-1.

4.232 Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes

Astley, S.B., Elliott, R.M., Archer, D.B. and Southon, S.
*Br. J. Nutrition, **91**, 63-72 (2004)*

Epidemiological evidence has shown that the habitual consumption of diets high in fruits and vegetables is associated with reduced risk of cancers. The challenge is to identify causal mechanisms of effect. The aim

of the current study was to determine whether an increase in rate of removal of DNA single-strand breaks (SSB) following oxidative challenge could be provoked ex vivo in peripheral blood lymphocytes (PBL). The PBL were isolated from apparently healthy volunteers following dietary intervention with: (1) a mixed carotene capsule; (2) a daily portion of cooked minced carrots; (3) a matched placebo; (4) a portion of mandarin oranges; (5) vitamin C tablets. Single-cell gel electrophoresis was employed to measure baseline levels of SSB and DNA susceptibility to oxidative damage, and to monitor the number of SSB over 4 h, in both unchallenged and H2O2-treated PBL. The enzymatic capacity for repair of different types of DNA oxidative lesions was also measured using two related cell-free assays. There was no evidence that any of the dietary supplementation regimens altered baseline levels of SSB, provided any direct antioxidant protection or altered DNA repair capacity, with two exceptions: the number of SSB following exposure to H2O2 decreased more rapidly in PBL from volunteers given the mixed carotene capsules and repair patch synthesis activity in PBL increased from volunteers given the cooked carrots. These results suggest that carotenoids and carotenoid-rich foods can influence DNA damage:repair by modulation of discrete stages in the DNA repair mechanisms.

4.233 Metabotropic glutamate receptors are expressed in adult human glial progenitor cells
Luyt, K., Varadi, A., Halfpenny, C.A., Scolding, N.J. and Molnar, E.
*Biochem. Biophys. Res. Comm., **319**, 120-129 (2004)*

Glial precursor cells (GPCs) are present in the adult human central nervous system (CNS) and they can be isolated and maintained in culture for in vitro studies. This study analysed expression of mGluR3 and mGluR5 metabotropic glutamate receptor (mGluR) mRNAs in GPCs. A2B5 surface antigen positive GPCs were isolated using immunomagnetic selection from dissociated temporal lobe subcortical white matter cells. The separated GPCs were maintained in cultures and characterised by immunoreactivity for the differentiation markers A2B5 and human platelet-derived growth factor-α receptor (PDGFαR). Reverse transcription followed by multiplex PCR analysis showed that the GPCs expressed both mGluR3 and mGluR5a mRNAs. Double immunostaining for glial progenitor markers and mGluR5 proteins demonstrated that all A2B5 and PDGFαR-positive cells were also positive for mGluR5. The results indicate that GPCs present in the adult human CNS express mGluR3 and mGluR5a. These neurotransmitter receptors may be involved in the proliferation and differentiation of glial cells.

4.234 Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response
Didierlaurent, A. et al
*J. Immunol., **172**, 6922-6930 (2004)*

Activation of dendritic cells (DC) by microbial products via Toll-like receptors (TLR) is instrumental in the induction of immunity. In particular, TLR signaling plays a major role in the instruction of Th1 responses. The development of Th2 responses has been proposed to be independent of the adapter molecule myeloid differentiation factor 88 (MyD88) involved in signal transduction by TLRs. In this study we show that flagellin, the bacterial stimulus for TLR5, drives MyD88-dependent Th2-type immunity in mice. Flagellin promotes the secretion of IL-4 and IL-13 by Ag-specific $CD4^+$ T cells as well as IgG1 responses. The Th2-biased responses are associated with the maturation of DCs, which are shown to express TLR5. Flagellin-mediated DC activation requires MyD88 and induces NF-κB-dependent transcription and the production of low levels of proinflammatory cytokines. In addition, the flagellin-specific response is characterized by the lack of secretion of the Th1-promoting cytokine IL-12 p70. In conclusion, this study suggests that flagellin and, more generally, TLR ligands can control Th2 responses in a MyD88-dependent manner.

4.235 Regulation of tissue inhibitor of metalloproteinase 1 gene transcription by RUNX1 and RUNX2
Bertrand-Philippe, M. Et al
*J. Biol. Chem., **279(23)**, 24530-24539 (2004)*

Tissue inhibitor of metalloproteinase 1 (TIMP1) is a contributory factor to fibrosis of a variety of organs including the liver. UTE-1 is a regulatory DNA motif essential for *TIMP1* promoter activity in a variety of cell types including hepatic stellate cells (HSC), the key profibrogenic cells of the liver. In this study we identify RUNX1 and RUNX2 as UTE-1-binding proteins that are induced at the post-transcriptional level during activation of HSC. RUNX1 is expressed in at least two major isoforms, RUNX1B and RUNX1A. Overexpression of full-length RUNX1B isoform in HSC repressed *TIMP1* promoter activity, whereas the truncated RUNX1A isoform and RUNX2 functioned as stimulators. To gain further understanding of the way in which RUNX1 isoforms differentially regulate TIMP1 transcription, we investigated the

relationship between the UTE-1 site and its adjacent upstream serum-response element (SRE) in the promoter. The UTE-1 and SRE sites cooperate in a synergistic fashion to stimulate transcription of a heterologous minimal active promoter providing that they are in close proximity. The key regulatory sequence within the SRE is an AP-1 site that in HSC directs high level transcription via its interaction with JunD. RUNX1A was shown to interact directly with JunD, and by contrast RUNX1B failed to interact with JunD. Co-expression studies showed that RUNX1B can repress JunD-stimulated *TIMP1* promoter activity. From these observations we propose that JunD and RUNX factors assemble at the adjacent SRE and UTE-1 sites in the *TIMP1* promoter and form functional interactions that stimulate transcription. However, RUNX1B is unable to interact with JunD, and as such its occupancy at the UTE-1 site disrupts the optimal assembly of transcriptional activators required for directing high level *TIMP1* promoter function.

4.236 Secretion of inflammatory mediators by isolated rat Kupffer cells; the effect of octreotide
Valatas, V. et al
*Regulatory Peptides, **120**, 215-225 (2004)*

Aims: We studied the production of inflammatory mediators by rat KC and the possible in vitro effect of the somatostatin analogue octreotide. *Methods*: Primary KC cultures were incubated with LPS added alone or with different concentrations of octreotide. The production of TNFα, IL-6, IL-10, IL-12 and IL-13 was assessed in culture supernatants by ELISA and that of nitric oxide (NO) by a modification of the Griess reaction. *Results*: Isolated KC produced a basal amount of TNFα, IL-6, IL-12, IL-13, and NO but not IL-10. LPS-stimulated KC secreted significantly increased amounts of TNFα (P<0.001), IL-6 (P<0.01), IL-10 (P<0.001), IL-12 (P<0.01), and NO (P<0.001) whereas IL-13 production remained constant. Octreotide reduced IL-12 (P<0.05) and increased IL-13 (P<0.05) production by unstimulated KC. Furthermore, octreotide suppressed TNFα production (P<0.05), without modifying TNFα mRNA expression and decreased iNOS expression and NO (P≈0.05) production by LPS-activated KC. These effects were reversed with Wortmannin pre-treatment suggesting that octreotide may act via interference with phosphatidylinositol 3-kinase pathways. *Conclusions*: These data demonstrate that KC is a source of multiple inflammatory mediators, indicating a critical role in liver inflammatory disorders. Octreotide modulates inflammatory mediator production by isolated KC, suggesting that it might have immunoregulatory and anti-inflammatory effects in liver diseases.

4.237 Establishment of a pure culture of the hitherto uncultured unicellular cyanobacterium *Aphanothece sacrum*, and phylogenetic position of the organism
Fujishiro, H. et al
*Appl, Enviroment. Microbiol., **70(6)**, 3338-3345 (2004)*

Aphanothece sacrum, an edible freshwater unicellular cyanobacterium, was isolated by using novel synthetic media (designated AST and AST-5xNP). The media were designed on the basis of the ratio of inorganic elements contained in *A. sacrum* cells cultured in a natural pond. The isolated strain exhibits unicellular rod-shaped cells ~6 μm in length that are scattered in an exopolysaccharide matrix, a feature similar to that of natural *A. sacrum*. DNA analysis of the isolated strain revealed that it carried two ferredoxin genes whose deduced amino acid sequences were almost identical to previously published sequences of ferredoxins from natural *A. sacrum*. Analysis of the 16S rRNA gene and ferredoxin genes revealed that *A. sacrum* occupies a phylogenetically unique position among the cyanobacteria.

4.238 Apolipoprotein A-I stimulated apolipoprotein E secretion from human macrophages is independent of cholesterol efflux
Kockx, M. et al'
*J. Biol. Chem., **279(25)**, 25966-25977 (2004)*

Apolipoprotein A-I (apoA-I)-mediated cholesterol efflux involves the binding of apoA-I to the plasma membrane via its C terminus and requires cellular ATP-binding cassette transporter (ABCA1) activity. ApoA-I also stimulates secretion of apolipoprotein E (apoE) from macrophage foam cells, although the mechanism of this process is not understood. In this study, we demonstrate that apoA-I stimulates secretion of apoE independently of both ABCA1-mediated cholesterol efflux and of lipid binding by its C terminus. Pulse-chase experiments using ^{35}S-labeled cellular apoE demonstrate that macrophage apoE exists in both relatively mobile (E_m) and stable (E_s) pools, that apoA-I diverts apoE from degradation to secretion, and that only a small proportion of apoA-I-mobilized apoE is derived from the cell surface. The structural requirements for induction of apoE secretion and cholesterol efflux are clearly dissociated, as C-terminal deletions in recombinant apoA-I reduce cholesterol efflux but increase apoE secretion, and deletion of

central helices 5 and 6 decreases apoE secretion without perturbing cholesterol efflux. Moreover, a range of 11- and 22-mer α-helical peptides representing amphipathic α-helical segments of apoA-I stimulate apoE secretion whereas only the C-terminal α-helix (domains 220–241) stimulates cholesterol efflux. Other α-helix-containing apolipoproteins (apoA-II, apoA-IV, apoE2, apoE3, apoE4) also stimulate apoE secretion, implying a positive feedback autocrine loop for apoE secretion, although apoE4 is less effective. Finally, apoA-I stimulates apoE secretion normally from macrophages of two unrelated subjects with genetically confirmed Tangier Disease (mutations C733R and c.5220–5222delTCT; and mutations A1046D and c.4629–4630insA), despite severely inhibited cholesterol efflux. We conclude that apoA-I stimulates secretion of apoE independently of cholesterol efflux, and that this represents a novel, ABCA-1-independent, positive feedback pathway for stimulation of potentially anti-atherogenic apoE secretion by α-helix-containing molecules including apoA-I and apoE.

4.239 Induction of MIP-1α in Kupffer cell by portal venous transfusion
Park, J.K., Cho, K., Johnson, J. and Perez, R.V.
Transplant Immunol., ***13***, *33-38 (2004)*

Introduction: Previous studies have shown that portal venous transfusion (PVT) induces a state of immunosuppression, and Kupffer cells may be involved in the mechanism. *Objective*: This study was aimed to investigate the effect of PVT on Kupffer cell gene expression. *Materials and Methods*: Each BALB/C mouse was subjected to laparotomy and received one of five treatments: PVT, portal venous saline injection (PVS), inferior vena caval transfusion (IVCT), inferior vena caval saline injection (IVCS) or sham operation (S). The blood for PVT and IVCT was sampled from C57BL/6J mice. Kupffer cells were then isolated 1 or 24 h after each of the 5 treatments, for a total of 10 experimental groups (1-h PVT, PVS, IVCT, IVCS and S, and 24-h PVT, PVS, IVCT, IVCS and S) from BALB/C mice. To examine the effect of PVT on Kupffer cell gene expression, RT-PCR differential display was performed. *Results*: Increase in the expression of MIP-1α mRNA post PVT and IVCT was identified by differential display. PVT groups revealed higher levels of serum MIP-1α than any other groups. *Conclusion*: These results suggest that MIP-1α may be involved in a cascade of signaling events associated with the PVT-mediated immunologic modulation in Kupffer cells.

4.240 Estrogen facilitates neurite extension via apolipoprotein E in cultured adult mouse cortical neurons
Nathan, B.P., Barsukova, A.G., Shen, F., McAsey, M. and Struble, R.G.
Endocrinol., ***145(7)***, *3065-3073 (2004)*

Literature review suggests a close relationship between estrogen and apolipoprotein E (ApoE) in the central nervous system. Epidemiology studies show that estrogen replacement therapy (ERT) decreases the morbidity from several chronic neurological diseases. Alleles of ApoE modify the risk for and progression of the same diseases. ApoE levels in the rodent brain vary during the estrous cycle and increase after 17ß-estradiol administration. Both estradiol and ApoE3, the most common isoform of human ApoE, increase the extent of neurite outgrowth in culture. Combined, these observations suggest a common mechanism whereby estrogen may increase ApoE levels to facilitate neurite growth. We tested this hypothesis by characterizing the effects of estradiol and ApoE isoforms on neurite outgrowth in cultured adult mouse cortical neurons. Estradiol increased ApoE levels and neurite outgrowth. ApoE2 increased neurite length more so than ApoE3 in the presence of estradiol. Estradiol had no effect on neurite outgrowth from mice lacking the ApoE gene or when only ApoE4, the isoform of ApoE that is associated with increased risk of neurological disease, was exogenously supplied. Cultures from mice transgenic for human ApoE3 or ApoE4 showed the same isoform-specific effect. Neuronal internalization of recombinant human ApoE3 was greater than ApoE4, and ApoE3 was more effective than ApoE4 in facilitating neuronal uptake of a fatty acid. We conclude that estradiol facilitates neurite growth through an ApoE-dependent mechanism. The effects of ERT on chronic neurological diseases may vary with ApoE genotype. The clinical use of ERT may require ApoE genotyping for optimal efficacy.

4.241 Liver sinusoidal endothelial cells are insufficient to activate T cells
Katz, S.C., Pillarisetty, V.G., Bleier, J.I., Shah, A.B. and DeMatteo, R.P.
J. Immunol., ***173***, *230-235 (2004)*

Liver sinusoidal endothelial cells (LSEC) have been reported to express MHC class II, CD80, CD86, and CD11c and effectively stimulate naive T cells. Because dendritic cells (DC) are known to possess these characteristics, we sought to directly compare the phenotype and function of murine LSEC and DC. Nonparenchymal cells from C57BL/6 mice were obtained by collagenase digestion of the liver followed by

density gradient centrifugation. From the enriched nonparenchymal cell fraction, LSEC ($CD45^-$) were then isolated to 99% purity using immunomagnetic beads. Flow cytometric analysis of LSEC demonstrated high expression of CD31, von Willebrand factor, and FcγRs. However, unlike DC, LSEC had low or absent expression of MHC class II, CD86, and CD11c. LSEC demonstrated a high capacity for Ag uptake in vitro and in vivo. Although acetylated low-density lipoprotein uptake has been purported to be a specific function of LSEC, we found DC captured acetylated low-density lipoprotein to a similar extent in vivo. Consistent with their phenotype, LSEC were poor stimulators of allogeneic T cells. Furthermore, in the absence of exogenous costimulation, LSEC induced negligible proliferation of $CD4^+$ or $CD8^+$ TCR-transgenic T cells. Thus, contrary to previous reports, our data indicate that LSEC alone are insufficient to activate naive T cells.

4.242 Hippocampal synapses depend on hippocampal estrogen synthesis
Kretz, O. et al
J. Neurosci., ***24(26)***, *5913-5921 (2004)*

Estrogens have been described to induce synaptogenesis in principal neurons of the hippocampus and have been shown to be synthesized and released by exactly these neurons. Here, we have focused on the significance of local estrogen synthesis on spine synapse formation and the synthesis of synaptic proteins. To this end, we reduced hippocampal estrogen synthesis *in vitro* with letrozole, a reversible nonsteroidal aromatase inhibitor. In hippocampal slice cultures, letrozole treatment resulted in a dose-dependent decrease of 17β-estradiol as quantified by RIA. This was accompanied by a significant decrease in the density of spine synapses and in the number of presynaptic boutons. Quantitative immunohistochemistry revealed a downregulation of spinophilin, a marker of dendritic spines, and synaptophysin, a protein of presynaptic vesicles, in response to letrozole. Surprisingly, no increase in the density of spines, boutons, and synapses and in spinophilin expression was seen after application of estradiol to the medium of cultures that had not been treated with letrozole. However, synaptophysin expression was upregulated under these conditions. Our results point to an essential role of endogenous hippocampal estrogen synthesis in the maintenance of hippocampal spine synapses.

4.243 Monocyte activation in patients with age-related macular degeneration
Cousins, S.W., Espinosa-Heidelmann, D.G. and Csaky, K.G.
Arch. Ophthalmol., ***122***, *1013-1018 (2004)*

Objective To evaluate the activation state of macrophage function in patients with age-related macular degeneration (AMD) by quantifying the production of the proinflammatory and angiogenic factor tumor necrosis factor α(TNF-α) and by correlating its expression with dry and wet AMD.
Methods Circulating monocytes were obtained from the blood of patients with AMD or age-matched control subjects by gradient centrifugation. The monocytes were then analyzed for either TNF-α release from cultured macrophages in response to retinal pigment epithelium–derived blebs and cytokines or TNF-α messenger RNA content by reverse transcriptase–polymerase chain reaction.
Results In human monocytes obtained from controls and AMD patients, TNF-α was expressed by freshly isolated monocytes and produced by macrophages in culture after stimulation with retinal pigment epithelium–derived blebs. However, wide variability in TNF-α expression was observed among different patients. Patients with monocytes that expressed the greatest amount of TNF-α demonstrated higher prevalence of choroidal neovascularization.
Conclusions Both controls and AMD patients vary in the activation state (defined as TNF-α expression) of circulating monocytes. Partially active monocytes, defined as high TNF-α expression, may be a biomarker to identify patients at risk for formation of choroidal neovascularization.
Clinical Relevance Early diagnostic testing may prove useful to detect those patients who will progress to the more severe complications of the disease.

4.244 Inhibition of experimental asthma by indoleamine 2,3-dioxygenase
Hayashi, T. et al
J. Clin. Invest., ***114(2)***, *270-279 (2004)*

Epidemiological evidence points to the inverse relationship between microbial exposure and the prevalence of allergic asthma and autoimmune diseases in Westernized countries. The molecular basis for this observation has not yet been completely delineated. Here we report that the administration of certain toll-like receptor (TLR) ligands, via the activation of innate immunity, induces high levels of indoleamine 2,3-

dioxygenase (IDO), the rate-limiting enzyme of tryptophan catabolism in various organs. TLR9 ligand–induced pulmonary IDO activity inhibits Th2-driven experimental asthma. IDO activity expressed by resident lung cells rather than by pulmonary DCs suppressed lung inflammation and airway hyperreactivity. Our results provide a mechanistic insight into the various formulations of the hygiene hypothesis and underscore the notion that activation of innate immunity can inhibit adaptive Th cell responses.

4.245 Adenovirus-based vascular endothelial growth factor gene delivery to human pancreatic islets
Cheng, K. et al
*Gene Ther., **11**, 1105-1116 (2004)*

Islet transplantation is limited by islet graft failure due to poor revascularization, host immune rejection and nonspecific inflammatory response. Delivery of human vascular endothelial growth factor (hVEGF) gene to the islets is likely to promote islet revascularization and survival. We used a bicistronic adenoviral vector encoding hVEGF and CpG-free allele of green fluorescent protein (Adv-GFP-hVEGF) and introduced into human pancreatic islets by transfection. We found that transfection efficiency and apoptosis were dependent on the multiplicity of infection (MOI). Compared to Adv-GFP transfected and nontransfected islets, the levels of hVEGF secreted from Adv-GFP-hVEGF transfected islets were higher and exhibit a linear relationship between hVEGF expression and MOI (10–5000). Persistent, but low level expression of hVEGF from nontransfected islets was also observed. This may be due to expression of the endogenous hVEGF gene under hypoxic conditions. The levels of DNA fragmentation determined by ELISA of islet lysates were dependent on the MOI of Adv-GFP-hVEGF. On glucose challenge, insulin release from transfected islets was comparable to nontransfected islets. Immunohistochemical staining for hVEGF was very high in Adv-GFP-hVEGF transfected islets. Weak staining was also observed for hCD31 in both transfected and nontransfected islets. These findings suggest that Adv-GFP-hVEGF is a potential candidate for promoting islet revascularization.

4.246 Potential involvement of gelatinases and their inhibitors in *Mannheimia haemolytica* pneumonia in cattle
Starr, A.E., Dan, T., Minhas, K., Shewen, P.E. and Coomber, B.L.
*Infection and Immunity., **72(8)**, 4393-4400 (2004)*

Mannheimia haemolytica infection of the lower respiratory tract of cattle results in a bronchofibrinous pneumonia characterized by massive cellular influx and lung tissue remodeling and scarring. Since altered levels of gelatinases and their inhibitors have been detected in a variety of inflammatory conditions and are associated with tissue remodeling, we examined the presence of gelatinases in lesional and nonlesional lung tissue obtained from calves experimentally infected with *M. haemolytica*. Lesional tissue had elevated levels of progelatinase A and B and active gelatinase A and B when compared with nonlesional tissue obtained from the same lung lobe. In vitro, *M. haemolytica* products stimulated production of gelatinase B, but not its activation, by bovine monocytes. Alveolar macrophages showed constitutive production of gelatinase B but no change in response to *M. haemolytica* products. Bovine neutrophils exposed to *M. haemolytica* products also released gelatinase B, and there was a significant increase in the activated form of this enzyme. These effects were virtually identical when recombinant *O*-sialoglycoprotease was used to stimulate these cells. *M. haemolytica* products also enhanced the expression by bovine monocytes and alveolar macrophages of the tissue inhibitor of metalloproteinase 1. Our results provide evidence that matrix metalloproteinases are activated in lung lesions from cattle with shipping fever and that *M. haemolytica* virulence products induce production, release, and especially activation of gelatinase B by bovine inflammatory cells in vitro.

4.247 Effect of functionalization of multilayered polyelectrolyte films on motoneuron growth
Vodouche, C. et al
*Biomaterials, **26**, 545-554 (2005)*

We studied in vitro cell–substrate interaction of motoneurons with functionalized polylectrolyte films. Thin polylectrolyte films were built on glass by alternating polycations, poly(ethylene-imine) PEI, poly(L-lysine) PLL, or poly(allylamine hydrochloride) PAH, and polyanions, poly(sodium-4-styrenesulfonate) PSS or poly(L-glutamic acid) (PGA). These architectures were functionalized with Brain Derived Neurotrophic Factor (BDNF) or Semaphorin 3A (Sema3A). We used Optical Waveguide Lightmode Spectroscopy (OWLS) and Atomic Force Microscopy (AFM) to characterize the architectures. The viability of motoneurons was estimated by the acid phosphatase method, and morphometrical measures were performed to analyse the influence of different architectures on cell morphology. Motoneurons

appeared to adhere and spread on all the architectures tested and preferentially on PSS ending films. The viability of motoneurons on polyelectrolyte multilayers was higher compared to polyelectrolyte monolayers. BDNF and Sema3A embedded in the films remained active and thereby create functionalized nanofilms.

4.248 In vitro opioid induced proliferation of peripheral blood immune cells correlates with in vivo cold pressor pain tolerance in humans: a biological marker of pain tolerance
Hutchinson, M.R., La Vincente, S.F. and Somogyi, A.A.
*Pain, **110**, 751-755 (2004)*

There is substantial evidence for bidirectional communication between the immune system and the central nervous system, as the cells and signalling molecules of the immune system influence many central nervous system functions, for instance nociception. Opioids, such as morphine, produce analgesia and numerous other central and peripheral effects including sedation and euphoria, while their effects on the immune system are wide-ranging. There is considerable interindividual variability in basal nociception and response to opioids, however, the physiological and biological mechanisms underlying this are unclear. Therefore, we investigated the relationship between the immune system and basal nociceptive thresholds, using the proliferative response of isolated peripheral blood mononuclear cells and cold pressor pain tolerance. Here we show that the percent increase in proliferation of peripheral immune cells from 13 healthy subjects incubated with morphine ex vivo is highly correlated with the subjects' tolerance to noxious cold stimuli (Pearson r=0.92, P<0.0001). These pilot data provide evidence of a novel objective biological marker of pain tolerance in humans, which also links the immune and opioid systems with basal pain tolerance.

4.249 Experimental infection of horses with culture-derived *Sarcocystis neurona* merozoites as a model for equine protozoal myeloencephalitis
Ellison, S.P., Greiner, E., Brown, K.W. and Kennedy, T.
*Intern. J. Appl. Res. Vet. Med., **2(2)**, 79-89 (2004)*

A study was designed to develop an experimental model to produce Sarcocystis neurona encephalitis in horses. Sarcocystis neurona, isolated from the spinal cord of an ataxic horse, was placed in continuous culture using bovine turbinate cells, and the cultured S. neurona merozoites were used to infect lymphocytes. A horse that was subsequently experimentally infected with 100,000 of the S. neurona-infected lymphocytes developed encephalitis and ataxia. Sarcocystis neurona was isolated from the spinal tissues of the infected horse by in vitro culture. Three horses, each infected with a different number of merozoite-infected lymphocytes, were used to estimate an infective dose of parasites needed to induce clinical signs of equine protozoal myeloencephalitis. This is the first report of histologically confirmed experimental infection of horses with culture-derived merozoites of S. neurona. Both a hematogenous method of distribution in the host and an intracellular location by the parasite in a lymphocyte is sufficient to produce clinical equine protozoal myeloencephalitis.

4.250 Short tandem repeat analysis to monitor chimerism in *Macaca Fasicularis*
Lau, M. et al
*Am. J. Transplant., **4**, 1543-1548 (2004)*

Chimerism assessment following bone marrow transplantation (BMT) in cynomolgus monkeys (cynos) has been hampered by the lack of good engraftment markers. In human BMT, such markers have been provided by short tandem repeat (STR) loci. We tested the idea that techniques effective for detecting human STR could be readily adapted to cynos.
Genomic DNA was extracted from cyno unseparated blood or peripheral cell subsets. With only slight modifications, reagents for detecting human STR alleles were used to amplify and detect cyno STRs and to quantitate allelic mixtures on an automated sequencer.
Of the 15 STR loci tested, only CSF1PO, D18S51, and FGA successfully amplified, with seven, seven and two alleles, respectively. CSF1PO and D18S51 heterozygosity (80% and 55%, respectively) allowed use of these two loci for chimerism quantitation after BMT.
The successful adaptation of human STR reagents to monitor chimerism in transplanted cynos will facilitate the use of this species in preclinical tolerance studies.

4.251 An improved method for isolation of mononuclear cells from peripheral blood
Ahmed, Y., Walton, L.J. and Graham, J.

ICO/FOCIS 2004 abstract no. 1758 (2004)

The ability to isolate mononuclear cells (MNC) from human peripheral blood is important in immunology research and diagnosis. A long-established one step method for MNC isolation (Bøyum, 1968) is in routine use in laboratories worldwide. MNC are sedimented on to a 1.077 g/ml density barrier of sodium diatrizoate and Ficoll from whole blood that has been diluted 1:1 with saline. Good yields of MNC are obtained, however the cells are always contaminated by platelets, which lie on top of the MNC band. Although platelets can be remoced by washing, this may seriously compromise the ultimate use of the MNC, particularly for the culture of monocytes.
In the current study, OptiPrep (a sterile solution of 60% iodixanol) was mixed with EDTA-anticoagulated blood in order to raise the density of the plasma to approx. 1.1 g/ml. The 1.078 g/ml barrier 8produced by diluting OptiPrep with cell culture medium) was layered on top, together with a small volume of culture medium. During the centrifugation at 700g for 30 min at 4^0C, the MNC float through the barrier to form a distinct band at the interface with the culture medium.
May-Grünwald-Giemsa staining of the harvested MNC band shows the presence of few, if any contaminating platelets, which remain predominantly in the plasma layer. The MNC band is also separated from the plasma proteins by the 1.078 g/ml barrier.
Moreover, a comparison between the two isolation methods indicates that the recovery o MNC can be as much as 20% higher using the flotation technique and the 1-2% contamination from erythrocytes which is occasionally observed with some blood samples using the sedimentation format, is not seen with the new flotation technique.
Monocytes from the MNC band, isolated by flotation, have been successfully maintained in culture for 21 days.

4.252 Reversal of diabetes in non-immunosuppressed rhesus macaques by intraportal porcine islet xenografts precedes acute cellular rejection
Kirchhof, N. et al
*Xenotransplantation, **11**, 396-407 (2004)*

Background: The functional response and immunobiology of primarily non-vascularized islet cell xenografts remain poorly defined in non-human primates.
Methods: We transplanted 20 000 adult porcine islet equivalents/kg (purified and cultured for 48-h) intraportally into six streptozotocin-diabetic and two non-diabetic rhesus macaques. Two recipients were killed at various intervals post-transplant for histologic examination of livers bearing xenografts.
Results: Plasma glucose levels in diabetic recipients averaged 94 mg/dl at 12 h, 92 mg/dl at 24 h, 147 mg/dl at 48 h, and 157 mg/dl at 72 h post-transplant. Serum porcine C-peptide was present in eight of eight recipients at 12 h, in five of six at 24 h, in four of four at 48 h, and in one of two at 72 h post-transplant. C3a and SC5b-9 plasma levels increased at 12 h post-transplant and returned to pre-transplant levels by 24 h. IgG, IgM anti-pig and anti-Gal IgG serum antibody levels did not increase post-transplant. Rejection was initiated by IgM and complement deposition on islets. Neutrophils dominated the cellular infiltrate at 12 h; $CD4^+$ and $CD8^+$ T cells were the main infiltrating cells at 24, 48, and 72 h; and macrophages increasingly infiltrated xenografts starting at 24 h post-transplant. Numerous xenoislets were present at all time points; their proportion without intraislet infiltrates decreased from 65% at 24 h to 17% at 72 h post-transplant.
Conclusions: Pig-to-primate intraportal islet xenografts reverse diabetes and the majority of intraportally transplanted xenogeneic islets are not subject to hyperacute rejection. They undergo acute cellular rejection mediated by $CD4^+$- and $CD8^+$ T cells and macrophages.

4.253 Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid sysem and problem drug use
Chiang, K.P., Gerber, A.L., Sipe, J.C. and Cravatt, B.F.
*Human Mol. Gen., **13(18)**, 2113-2119 (2004)*

Fatty acid amide hydrolase (FAAH) inactivates the endogenous cannabinoid (endocannabinoid) anandamide and related lipid transmitters *in vivo*. A single nucleotide polymorphism (SNP) in the human *FAAH* gene (385C to A) has recently been described that, in homozygous form, is over-represented in subjects with problem drug use. This SNP, which converts a conserved proline residue in FAAH to threonine (P129T), suggests a potential role for the FAAH–endocannabinoid system in regulating addictive behavior. Nonetheless, the impact of the 385A mutation on the biochemical and cellular function of FAAH remains unknown. Here, we report that T-lymphocytes isolated from patients homozygous for the P129T-

FAAH variant express less than half of the FAAH protein and activity observed in wild-type (WT) lymphocytes. Transfected COS-7 cells also expressed significantly lower levels of P129T-FAAH compared with WT-FAAH, indicating that the aberrant expression of the former protein is not a cell type-specific phenomenon. A comparison of the transcription/translation efficiencies and cellular stabilities of WT- and P129T-FAAH proteins revealed that the reduced expression of the mutant enzyme is due to a post-translational mechanism that precedes productive folding. These findings indicate that the natural 385A SNP in the human *FAAH* gene produces a mutant enzyme with reduced cellular stability, thus fortifying a potential link between functional abnormalities in the endocannabinoid system and drug abuse and dependence.

4.254 Scavenger properties of cultivated pig liver endothelial cells
Elvevold, K.H., Nedredal, G.I., Revhaug, A. and Smedsrød, B.
*Comp. Mepatol., **3(4)**, 1-11 (2004)*

The liver sinusoidal endothelial cells (LSEC) and Kupffer cells constitute the most powerful scavenger system in the body. Various waste macromolecules, continuously released from tissues in large quantities as a consequence of normal catabolic processes are cleared by the LSEC. In spite of the fact that pig livers are used in a wide range of experimental settings, the scavenger properties of pig LSEC has not been investigated until now. Therefore, we studied the endocytosis and intracellular transport of ligands for the five categories of endocytic receptors in LSEC.
Endocytosis of five ^{125}I-labelled molecules: collagen α-chains, FITC-biotin-hyaluronan, mannan, formaldehyde-treated serum albumin (FSA), and aggregated gamma globulin (AGG) was substantial in cultured LSEC. The endocytosis was mediated via the collagen-, hyaluronan-, mannose-, scavenger-, or IgG Fc-receptors, respectively, as judged by the ability of unlabelled ligands to compete with labelled ligands for uptake. Intracellular transport was studied employing a morphological pulse-chase technique. Ninety minutes following administration of red TRITC-FSA via the jugular vein of pigs to tag LSEC lysosomes, cultures of the cells were established, and pulsed with green FITC-labelled collagen, -mannan, and -FSA. By 10 min, the FITC-ligands was located in small vesicles scattered throughout the cytoplasm, with no co-localization with the red lysosomes. By 2 h, the FITC-ligands co-localized with red lysosomes. When LSEC were pulsed with FITC-AGG and TRITC-FSA together, co-localization of the two ligands was observed following a 10 min chase. By 2 h, only partial co-localization was observed; TRITC-FSA was transported to lysosomes, whereas FITC-AGG only slowly left the endosomes. Enzyme assays showed that LSEC and Kupffer cells contained equal specific activities of hexosaminidase, aryl sulphates, acid phosphatase and acid lipase, whereas the specific activities of α-mannosidase, and glucuronidase were higher in LSEC. All enzymes measured showed considerably higher specific activities in LSEC compared to parenchymal cells.
Pig LSEC express the five following categories of high capacity endocytic receptors: scavenger-, mannose-, hyaluronan-, collagen-, and IgG Fc-receptors. In the liver, soluble ligands for these five receptors are endocytosed exclusively by LSEC. Furthermore, LSEC contains high specific activity of lysosomal enzymes needed for degradation of endocytosed material. Our observations suggest that pig LSEC have the same clearance activity as earlier described in rat LSEC.

4.255 MHC class II expression is differentially regulated in plasmacytoid and conventional dendritic cells
LeibundGut-Landemann, S., Waldburger, J-M., Reis e Sousa, C. Acha-Orbea, H. And Reith, W.
*Nature Immunol., **5(9)**, 899-908 (2004)*

Major histocompatibility complex (MHC) class II–restricted antigen presentation is essential for the function of dendritic cells (DCs). We show here that plasmacytoid DCs (pDCs) differ from all other DC subsets with respect to expression of CIITA, the 'master regulator' of MHC class II genes. The gene encoding CIITA is controlled by three cell type–specific promoters: pI, pIII and pIV. With gene targeting in mice, we demonstrate that pDCs rely strictly on the B cell promoter pIII, whereas macrophages and all other DCs depend on pI. The molecular mechanisms driving MHC class II expression in pDCs are thus akin to those operating in lymphoid rather than myeloid cells.

4.256 The remyelinating potential and *in vitro* differentiation of MOG-expressing oligodendrocyte precursors isolated from the adult rat CNS
Crang, A.J., Gilson, J.M., Li, W.-W. and Blakemore, W.F.
*Eur. J. Neurosci., **20**, 1445-1460 (2004)*

There is a long-standing controversy as to whether oligodendrocytes may be capable of cell division and

thus contribute to remyelination. We recently published evidence that a subpopulation of myelin oligodendrocyte glycoprotein (MOG)-expressing cells in the adult rat spinal cord co-expressed molecules previously considered to be restricted to oligodendrocyte progenitors [G. Li *et al*. (2002) *Brain Pathol*., 12, 463-471]. To further investigate the properties of MOG-expressing cells, anti-MOG-immunosorted cells were grown in culture and transplanted into acute demyelinating lesions. The immunosorting protocol yielded a cell preparation in which over 98% of the viable cells showed anti-MOG- and O1-immunoreactivity; 12-15% of the anti-MOG-immunosorted cells co-expressed platelet-derived growth factor alpha receptor (PDGFRα) or the A2B5-epitope. When cultured in serum-free medium containing EGF and FGF-2, 15-18% of the anti-MOG-immunosorted cells lost anti-MOG- and O1-immunoreactivity and underwent cell division. On removal of these growth factors, cells differentiated into oligodendrocytes, or astrocytes and Schwann cells when the differentiation medium contained BMPs. Transplantation of anti-MOG-immunosorted cells into areas of acute demyelination immediately after isolation resulted in the generation of remyelinating oligodendrocytes and Schwann cells. Our studies indicate that the adult rat CNS contains a significant number of oligodendrocyte precursors that express MOG and galactocerebroside, molecules previously considered restricted to mature oligodendrocytes. This may explain why myelin-bearing oligodendrocytes were considered capable of generating remyelinating cells. Our study also provides evidence that the adult oligodendrocyte progenitor can be considered as a source of the Schwann cells that remyelinate demyelinated CNS axons following concurrent destruction of oligodendrocytes and astrocytes.

4.257 Zinc tolerance, uptake, accumulation and distribution in plants and protoplasts of five European populations of the wertland grass *Glyceria fluitans*
Matthews, D.J., Moran, B.M., McCabe, P.F. and Otte, M.L.
Auatic Botany, ***80***, *39-52 (2004)*

Five populations of *Glyceria fluitans* (L.) R. Br. from metal-contaminated and non-contaminated sites across Europe were investigated for innate zinc tolerance. The plants were grown hydroponically in zinc-amended nutrient solutions. Growth and survival of plants from all five populations occurred at all levels of elevated zinc treatments (2, 300, 600 and 1000 μmol L^{-1} $ZnSO_4 \cdot 7H_2O$). There were only slight differences in growth between the populations from contaminated and non-contaminated sites. Uptake of zinc did differ between populations, but this did not affect tolerance. The findings support the theory that wetland angiosperm species tend to be tolerant to exposure to high levels of metals, regardless of their origin.

4.258 Monocyte adhesion to decidual endothelial cells is increased in pregnancies complicated by type 1 diabetes but not by gestational diabetes
Galettis, A. et al
Diabetes Care, ***27(10)***, *2514-2515 82004)*

Type 1 diabetes complicates ~1 of every 200 pregnancies and gestational diabetes a further 2–3% of pregnancies (1). Systemic atheromatous vascular disease can develop or accelerate during diabetic pregnancy. We and others (2) have observed similar vascular lesions in placental bed vessels (Fig. 1*A*) associated with impaired placental function and fetal growth. Genesis of atheroma involves adherence of peripheral blood monocytes to endothelium (3–5). To determine whether a similar process underlies the placental bed vasculopathy of diabetes, we examined cell adhesion in an in vitro coculture system using decidual endothelial cells from normal pregnancies and monocytes from both normal and diabetic pregnancies. Genesis of atheroma involves adherence of peripheral blood monocytes to endothelium (3–5). To determine whether a similar process underlies the placental bed vasculopathy of diabetes, we examined cell adhesion in an in vitro coculture system using decidual endothelial cells from normal pregnancies and monocytes from both normal and diabetic pregnancies.

4.259 Fibrin stimulates platelets to increase factor VIIIa binding site expression
Phillips, J.E., Lord, S.T. and Gilbert, G.E,
J. Thromb. Haemost., ***2***, *1806-1815 (2004)*

Factor (F)VIII functions as an enzymatic cofactor on the membranes of stimulated platelets. However, thrombin stimulates platelets to express only a small number of binding sites for FVIII. We wished to determine whether molecules that are likely to be present in a developing thrombus stimulate platelets to up-regulate FVIII binding site expression. Flow cytometry was utilized to measure binding of fluorescein-labeled FVIIIa to activated platelets and a FXase assay was utilized to measure platelet-dependent function.

Various agonists as well as normal and mutant fibrinogens and fibrin were evaluated as co-stimuli. Thrombin-stimulated platelets expressed 214 ± 67 binding sites for thrombin-activated FVIII (FVIIIa) and none of the established soluble agonists enhanced binding site exposure. However, the presence of 5 μg mL^{-1} fibrin increased the number of FVIIIa binding sites/platelet three- to eight-fold (1470 ± 130, range 600-1800) with a parallel increase in platelet-based FXase assay. Binding site up-regulation was not stimulated by fibrinogen and was blocked by inhibitors of GPIIbIIIa. Mutant fibrin lacking the γ-chain C-terminal four residues was ineffective while fibrin with altered RGD sequences did stimulate expression of FVIIIa binding sites indicating that co-stimulation is mediated by the fibrin γ-chain termini. Fibrin-enhanced expression of FVIIIa binding sites was not supported by D364H fibrin, which does not aggregate normally, and was blocked by the GPRP peptide, which inhibits fibrin polymerization. Polymerized fibrin can function as a platelet co-stimulus, up-regulating expression of binding sites for FVIIIa.

4.260 Improvement in islet yield from obese donors for human islet transplants

Matsumoto, I. Et al

*Transplantation, **78(6)**, 880-885 (2004)*

Background. The feasibility of human islet transplantations has been firmly established. To increase the number of islet transplants, the suitability of pancreases from organ donors considered inappropriate for pancreas transplantations must be evaluated.
Methods. We isolated islets from 114 human cadaver donor pancreases by the automated Ricordi method, followed by purification using continuous-density gradients. We divided the pancreases into two groups by donor body mass index (BMI)-group 1: n=51, BMI of 30 or more; group 2: n=63, BMI of less than 30. We compared the results of human islet isolation, in vitro potency assays, and a nude mouse bioassay.
Results. In group 1 (vs. group 2), we found a significantly higher mean pancreas weight (109.5+/-30.7 vs. 90.6+/-24.0 g; P=0.0002); higher mean islet equivalents/pancreas, after digestion (442,565+/-238,741 vs. 289,860+/-158,995; P<0.0001) and after purification (319,129+/-164,002 vs. 215,753+/-126,089; P=0.0002); and a higher islet isolation success rate-defined as isolations yielding more than 300,000 islet equivalents/pancreas, with purities of more than 50% (37.3% [19 of 51 pancreases] vs. 15.9% [10 of 63]; P=0.009). Our in vitro potency assays and bioassay uncovered no differences between the two groups. Notably, all except one of the donor BMIs for the successful isolations in group 2 exceeded 26; the mean donor BMI for the successful isolations (27.3+/-3.0, n=10) was significantly higher than for the unsuccessful isolations (24.8+/-3.3, n=53) (P=0.03).
Conclusions. Pancreases from both overweight (BMI >=26 but <30) and obese (BMI >=30) cadaver donors are suitable for islet isolation and transplantations. Their use could increase the size of the islet donor pool.

4.261 In vivo adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin

Anjuere, F. et al

*J. Immunol., **173**, 5103-5111 (2004)*

Although dendritic cells (DCs) regulate immune responses, they exhibit functional heterogeneity depending on their anatomical location. We examined the functional properties of intestinal DCs after oral administration of cholera toxin (CT), the most potent mucosal adjuvant. Two $CD11c^+$ DC subsets were identified both in Peyer's patches and mesenteric lymph nodes (MLN) based on the expression of CD8α ($CD8^+$ and $CD8^-$ DCs, respectively). A third subset of $CD11c^+CD8^{int}$ was found exclusively in MLN. Feeding mice with CT induced a rapid and transient mobilization of a new $CD11c^+CD8^-$ DC subset near the intestinal epithelium. This recruitment was associated with an increased production of the chemokine CCL20 in the small intestine and was followed by a massive accumulation of $CD8^{int}$ DCs in MLN. MLN DCs from CT-treated mice were more potent activators of naive T cells than DCs from control mice and induced a Th2 response. This increase in immunostimulating properties was accounted for by $CD8^{int}$ and $CD8^-$ DCs, whereas $CD8^+$ DCs remained insensitive to CT treatment. Consistently, the $CD8^{int}$ and $CD8^-$ subsets expressed higher levels of costimulatory molecules than $CD8^+$ and corresponding control DCs. Adoptive transfer experiments showed that these two DC subsets, unlike $CD8^+$ DCs, were able to present Ags orally coadministered with CT in an immunostimulating manner. The ability of CT to mobilize immature DCs in the intestinal epithelium and to promote their emigration and differentiation in draining lymph nodes may explain the exceptional adjuvant properties of this toxin on mucosal immune responses.

4.262 **Prevalent human coxsaxkie B-5 virus infects porcine islet cells primarily using the coxsackie-adenovirus receptor**
Myers, S.E. et al
*Xenotransplantation, **11**, 536-546 (2004)*

Background: We have previously demonstrated that transplanting porcine encephalomyocarditis virus (EMCV)-infected porcine islet cells (PICs) results in transmission of the virus to recipient mice, which is manifested by acute fatal infection within 5 to 8 days. Here, we determined PIC susceptibility to a related and highly prevalent human picornavirus, coxsackie B-5 virus (CVB-5).
Methods: PICs were inoculated with CVB-5 in vitro for up to 96 hours and infectivity, level of virus replication, and cellular function determined. Subsequently, monoclonal and polyclonal antibody blocking experiments were used to investigate the receptor CVB-5 uses to enter PICs, and the ability of CVB-5-infected islets to reverse diabetes analyzed in mice.
Results: Adult pig islets inoculated with CVB-5 in vitro showed a typical picornaviral replication cycle with a 2-h lag phase followed by a 4-h exponential phase during which the virus titer increased by 4 logs. However, CVB-5 was less cytolytic to PICs than EMCV, resulting in a persistent productive infection lasting for up to 96 h, with minimal evidence of cell lysis. Double immunostaining confirmed the presence of CVB-5 antigens in insulin-producing islets. Infection of PICs in the presence of antibodies against human coxsackie-adenovirus receptor (CAR) resulted in near complete blockage in production of infectious virus particles whereas blocking with anti-porcine decay-accelerating factor (DAF, also called CD55) or anti-porcine membrane cofactor protein (MCP, also called CD46) only slightly decreased the number of infectious CVB-5 particles produced. Immunofluoresence staining showed CAR and MCP expression on the islet surface, but not DAF. Transplanting CVB-5-infected PICs into diabetic C57BL/6 mice resulted in reversal of diabetes.
Conclusion: Although PICs are susceptible to human CVB-5, the infection does not appear to affect xenograft function in vitro or in vivo in the short term.

4.263 **Comparison of six density gradient media for selection of cryopreserved donor spermatozoa**
Mousset-Simeon, N., Rives, N., Masse, L., Chevallier, F. and Mace, B.
*J. Androl., **25(6)**, 881-884 (2004)*

The aim of our study was to evaluate the efficiency of 4 density gradient media for motile cryopreserved spermatozoa selection to Percoll (Kabi Pharmacia, Uppsala, Sweden) and to Puresperm (J.C.D. International Laboratory, L'Aigle, France). Puresperm was the new medium chosen in our laboratory in 1996 as the substitute for Percoll. The solutions tested were 3 colloidal silane-coated silica particle media (Isolate, SpermGrad-100, Sil-Select Plus) and iodixanol (**Optiprep**). Semen parameters analyzed after selection were concentration, motility, and morphology. Semen parameters after Puresperm gradient had similar values compared to Percoll. **Optiprep** was less efficient with a poor concentration. Isolate had a comparatively better concentration, but the capacity of selection was not satisfactory. SpermGrad-100 and Sil-Select Plus were less effective than Puresperm. In conclusion, Puresperm could be considered a better alternative to Percoll for cryopreserved spermatozoa migration.

4.264 **KCl cotransport mediates abnormal sulfhydryl-dependent volume regulation in sickle reticulocytes**
Joiner, C.H., Rettig, R.K., Jiang, M. And Franco, R.S.
*Blood, **104**, 2954-2960 (2004)*

KCl cotransport (KCC) activation by cell swelling and pH was compared in sickle (SS) and normal (AA) red blood cells (RBCs). KCC fluxes had the same relationship to mean corpuscular hemoglobin concentration (MCHC) in SS and AA RBCs when normalized to the maximal volume-stimulated (VS_{max}) flux (MCHC < 270 g/L [27 g/dL]). Acid-stimulated (pH 6.9) KCC flux in SS RBCs was 60% to 70% of VS_{max} KCC versus 20% in AA RBCs. Density gradients were used to track changes in reticulocyte MCHC during KCC-mediated regulatory volume decrease (RVD). Swelling to MCHC of 260 g/L (26 g/dL) produced Cl-dependent RVD that resulted in higher MCHC in SS than AA reticulocytes. In acid pH, RVD was also greater in SS than AA reticulocytes. Sulfhydryl reduction by dithiothreitol (DTT) lowered VS_{max} KCC flux in AA and SS RBCs by one third but did not alter swelling-induced RVD. DTT lowered acid-activated KCC in SS RBCs by 50% and diminished acid-induced RVD in SS reticulocytes. Thus, swelling activation of KCC is normal in SS RBCs but KCC-mediated RVD produces higher MCHC in SS than AA reticulocytes. Acid activation of KCC is exaggerated in SS RBCs and causes dehydration in SS reticulocytes. KCC response to acid stimulation was mitigated by DTT, suggesting that it arises from sulfhydryl oxidation.

4.265 Factor XIIIA transglutaminase crosslinks AT_1 receptor dimers of monocytes at the onset of atherosclerosis

AbdAlla, S., Lother, H., Langer, A., el Faramaway, Y. and Quitterer, U.
Cell, ***119****, 343-354 (2004)*

Many G protein-coupled receptors form dimers in cells. However, underlying mechanisms are barely understood. We report here that intracellular factor XIIIA transglutaminase crosslinks agonist-induced AT_1 receptor homodimers via glutamine^{315} in the carboxyl-terminal tail of the AT_1 receptor. The crosslinked dimers displayed enhanced signaling and desensitization in vitro and in vivo. Inhibition of angiotensin II release or of factor XIIIA activity prevented formation of crosslinked AT_1 receptor dimers. In agreement with this finding, factor XIIIA-deficient individuals lacked crosslinked AT_1 dimers. Elevated levels of crosslinked AT_1 dimers were present on monocytes of patients with the common atherogenic risk factor hypertension and correlated with an enhanced angiotensin II-dependent monocyte adhesion to endothelial cells. Elevated levels of crosslinked AT_1 receptor dimers on monocytes could sustain the process of atherogenesis, because inhibition of angiotensin II generation or of intracellular factor XIIIA activity suppressed the appearance of crosslinked AT_1 receptors and symptoms of atherosclerosis in ApoE-deficient mice.

4.266 Elastic light scattering from single cells: orientational dynamics in optical trap

Watson, D. et al
Biophys. J, ***87,*** *1298-1306 (2004)*

Light-scattering diagrams (phase functions) from single living cells and beads suspended in an optical trap were recorded with 30-ms time resolution. The intensity of the scattered light was recorded over an angular range of 0.5–179.5° using an optical setup based on an elliptical mirror and rotating aperture. Experiments revealed that light-scattering diagrams from biological cells exhibit significant and complex time dependence. We have attributed this dependence to the cell's orientational dynamics within the trap. We have also used experimentally measured phase function information to calculate the time dependence of the optical radiation pressure force on the trapped particle and show how it changes depending on the orientation of the particle. Relevance of these experiments to potential improvement in the sensitivity of label-free flow cytometry is discussed.

4.267 Type 1 collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma

Armstrong, T. et al
Clin. Can. Res., ***10****, 7427-7437 (2004)*

Purpose: The purpose of this study was to determine the role of functional interactions between pancreatic cancer cells and pancreatic stellate cells (PSCs) in the formation of the desmoplastic reaction (DR) in pancreatic cancer and to characterize the effect of type I collagen (the predominant component of the DR) on pancreatic cancer cell phenotype.
Experimental Design: PSCs and type I collagen were identified in sections of pancreatic cancer using immunohistochemistry, and their anatomic relationship was studied. Interactions among pancreatic cancer cell lines (MIA PaCa-2, Panc-1, and AsPC-1), primary cultures of human PSCs, and type I collagen were investigated in a series of tissue culture models.
Results: In vivo, the DR causes gross distortion of normal pancreas, bringing cancer cells into close contact with numerous PSCs and abundant type I collagen. In tissue culture models of pancreatic cancer, conditioned media from each cell line increased PSC [^{3}H]thymidine incorporation up to 6.3-fold that of controls, and AsPC-1 cells also increased PSC collagen synthesis 1.3-fold. Type I collagen was observed to increase long-term survival of pancreatic cancer cells treated with 5-fluorouracil, by up to 62% in clonogenic assays. This was because type I collagen increased the proliferation of cancer cells ([^{3}H]thymidine incorporation was up to 2.8-fold that of cells cultured on tissue culture plastic) and reduced apoptosis of AsPC-1 cells in response to 5-fluorouracil (by regulating mcl-1).
Conclusions: These experiments elucidate a mechanism by which the DR in pancreatic cancer may form and, via the collagen within it, promote the malignant phenotype of pancreatic cancer cells, suggesting significant detriment to the host.

4.268 Synergy between IL-8 and GM-CSF in reproductive tract epithelial cell secretions promotes enhanced neutrophil chemotaxis

Shen, L. et al

Cellular Immunol., ***230***, *23-32 (2004)*

Neutrophils occur in tissues of the female reproductive tract (FRT) under non-infected conditions. These cells generally enter tissues under the influence of chemoattractants called chemokines. Primary epithelial cells (EC) from FRT were a potent source of chemokines, IL-8 being the chief neutrophil chemoattractant secreted. Blocking with neutralizing anti-IL-8 showed that IL-8 did not account for all of the chemoattraction observed. A mixture of 25 ng/mL rIL-8 and 1 ng/mL rGM-CSF mediated 2.7-fold more chemotaxis than that expected if the two agents were additive. We then found that GM–CSF was produced by EC in amounts that synergised strongly with IL-8 to enhance chemotaxis. Treatment of uterine EC conditioned medium with saturating doses of anti-IL-8 plus anti-GM–CSF antibodies produced an 84% inhibition of chemotaxis, These findings demonstrate that the majority of neutrophil chemoattractant activity produced by FRT EC results from the synergistic effects of IL-8 and GM–CSF.

4.269 **Combined disruption of both the MEK/ERK and the IL-6R/STAT3 pathways is required to induce apoptosis of multiple myeloma cells in the presence of bone marrow stromal cells**
Chatterjee, M. et al
Blood, ***104(12)***, *3712-3721 (2004)*

The interleukin-6 receptor (IL-6R)/signal transducer and activator of transcription 3 (STAT3) pathway contributes to the pathogenesis of multiple myeloma (MM) and protects MM cells from apoptosis. However, MM cells survive the IL-6R blockade if they are cocultured with bone marrow stromal cells (BMSCs), suggesting that the BM microenvironment stimulates IL-6–independent pathways that exert a pro-survival effect. The goal of this study was to investigate the underlying mechanism. Detailed pathway analysis revealed that BMSCs stimulate STAT3 via the IL-6R, and mitogen-activated protein (MAP) kinases via IL-6R–independent mechanisms. Abolition of MEK1,2 activity with PD98059, or ERK1,2 small interfering RNA knockdown, was insufficient to induce apoptosis. However, the combined disruption of the IL-6R/STAT3 and MEK1,2/ERK1,2 pathways led to strong induction of apoptosis even in the presence of BMSCs. This effect was observed with MM cell lines and with primary MM cells, suggesting that the BMSC-induced activation of MEK1,2/ERK1,2 renders MM cells IL-6R/STAT3 independent. Therefore, in the presence of cells from the BM micro-environment, combined targeting of different (and independently activated) pathways is required to efficiently induce apoptosis of MM cells. This might have direct implications for the development of future therapeutic strategies for MM.

4.270 **Isolating pure populations of monocytes from the blood of pregnant women: comparison of flotation in iodixanol with elutriation**
Nutt, J.C., Willis, C.C., Morris, J.M. and Gallery, E.D.M.
J. Immunol. Methods, ***293***, *215-218 (2004)*

Observations that the innate arm of the immune system is upregulated in pregnancy have highlighted the need for methods of isolating pure populations of monocytes for studies into pregnancy and pre-eclampsia without activating them during the isolation process. Density gradient centrifugation using **iodixanol** is a useful method for isolating relatively pure populations of unactivated monocytes from human blood but has not been validated in pregnant subjects. We compared the ability of monocytes isolated from pregnant women by density gradient centrifugation using **iodixanol** (n=6) with monocytes isolated by countercurrent centrifugal elutriation (n=6) in terms of their ability to produce interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) under basal conditions and after stimulation with bacterial lipopolysaccharide (LPS). Under basal conditions, monocytes isolated by density gradient centrifugation produced low amounts of IL-6 and MCP-1. Production of IL-6 and MCP-1 after stimulation of the monocytes with LPS was much greater (p<0.01). There was no statistically significant difference between the two methods in terms of stimulated levels of either cytokine.

4.271 **SIVdrl detection in captive mandrills: are mandrill infected with a third strain of simian immunodeficiency virus?**
Gruters, R.A., Osterhaus, A.D.M.E. and Berhout, B.
Retrovirology, ***1(36)***, *1-5 (2004)*

A pol-fragment of simian immunodeficiency virus (SIV) that is highly related to SIVdrl-pol from drill monkeys (*Mandrillus leucophaeus*) was detected in two mandrills (*Mandrillus sphinx*) from Amsterdam Zoo. These captivity-born mandrills had never been in contact with drill monkeys, and were unlikely to be hybrids. Their mitochondrial haplotype suggested that they descended from founder animals in Cameroon

or northern Gabon, close to the habitat of the drill. SIVdrl has once before been found in a wild-caught mandrill from the same region, indicating that mandrills are naturally infected with a SIVdrl-like virus. This suggests that mandrills are the first primate species to be infected with three strains of SIV: SIVmnd1, SIVmnd2, and SIVdrl.

4.272 Role of myosin VIIa and Rab27a in the motility and localization of RPE melanosomes
Gibbs, D. et al
*J. Cell Sci., **117**, 6473-6483 (2004)*

Myosin VIIa functions in the outer retina, and loss of this function causes human blindness in Usher syndrome type 1B (USH1B). In mice with mutant *Myo7a*, melanosomes in the retinal pigmented epithelium (RPE) are distributed abnormally. In this investigation we detected many proteins in RPE cells that could potentially participate in melanosome transport, but of those tested, only myosin VIIa and Rab27a were found to be required for normal distribution. Two other expressed proteins, melanophilin and myosin Va, both of which are required for normal melanosome distribution in melanocytes, were not required in RPE, despite the association of myosin Va with the RPE melanosome fraction. Both myosin VIIa and myosin Va were immunodetected broadly in sections of the RPE, overlapping with a region of apical filamentous actin. Some 70-80% of the myosin VIIa in RPE cells was detected on melanosome membranes by both subcellular fractionation of RPE cells and quantitative immunoelectron microscopy, consistent with a role for myosin VIIa in melanosome motility. Time-lapse microscopy of melanosomes in primary cultures of mouse RPE cells demonstrated that the melanosomes move in a saltatory manner, interrupting slow movements with short bursts of rapid movement (>1 μm/second). In RPE cells from *Myo7a*-null mice, both the slow and rapid movements still occurred, except that more melanosomes underwent rapid movements, and each movement extended approximately five times longer (and further). Hence, our studies demonstrate the presence of many potential effectors of melanosome motility and localization in the RPE, with a specific requirement for Rab27a and myosin VIIa, which function by transporting and constraining melanosomes within a region of filamentous actin. The presence of two distinct melanosome velocities in both control and *Myo7a*-null RPE cells suggests the involvement of at least two motors other than myosin VIIa in melanosome motility, most probably, a microtubule motor and myosin Va.

4.273 Telomere dynamics in Fancg-deficient mouse and human cells
Franco, S. et al
*Blood, **104**, 3927-3935 (2004)*

A number of DNA repair proteins also play roles in telomere metabolism. To investigate whether the accelerated telomere shortening reported in Fanconi anemia (FA) hematopoietic cells relates to a direct role of the FA pathway in telomere maintenance, we have analyzed telomere dynamics in *Fancg*-deficient mouse and human cells. We show here that both hematopoietic (stem and differentiated bone marrow cells, B and T lymphocytes) and nonhematopoietic (germ cells, mouse embryonic fibroblasts [MEFs]) *Fancg*$^{-/-}$ mouse cells display normal telomere length, normal telomerase activity, and normal chromosome end-capping, even in the presence of extensive clastogen-induced cytogenetic instability (mitomycin C [MMC], gamma-radiation). In addition, telomerase-deficient MEFs with humanlike telomere length and decreased Fancg expression (G5 *Terc*$^{-/-}$*/Fancg* shRNA3 MEFs) display normal telomere maintenance. Finally, early-passage primary fibroblasts from patients with FA of complementation group G as well as primary human cells with reduced FANCG expression (*FANCG* shRNA IMR90 cells) show no signs of telomere dysfunction. Our observations indicate that accelerated telomere shortening in patients with FA is not due to a role of FANCG at telomeres but instead may be secondary to the disease. These findings suggest that telomerase-based therapies could be useful prophylactic agents in FA aplastic anemia by preserving their telomere reserve in the context of the disease.

4.274 Immune function is impaired with a mini nutritional assessment score indicative of malnutrition in nursing home elders with pressure ulcers
Hudgens, J. et al
*J. Parenteral and Enteral Nutrition, **28(6)**, 416-422 (2004)*

Background: Malnutrition is prevalent in elders with pressure ulcers and is associated with increased morbidity and mortality. This study compared nutritional status, assessed by the Mini Nutrition Assessment (MNA), to immune function in nursing home elders with pressure ulcers. *Methods:* Nutritional status was assessed in nursing home residents (>65 years) with a stage II or more severe pressure ulcer. Subjects were

classified as well nourished, at risk of malnutrition, or malnourished according to MNA score. Blood was drawn to assess whole blood mitogen-induced lymphocyte proliferation and neutrophil respiratory burst. Delayed-type hypersensitivity to 3 antigens was measured. MNA status was compared with immune parameters using the Kruskall-Wallis test. *Results:* Of the 24 subjects (23 men, 1 woman) who completed the study protocol, only 4 (17%) were classified as well nourished, whereas 7 (29%) were at risk and 13 (54%) were malnourished according to MNA score. Whole blood lymphocyte proliferation was significantly lower in the malnourished *vs* at risk subjects with both pokeweed (median [25^{th},75^{th} percentile], 0.6 [0.3, 0.9] *vs* 1.8 [1.2, 2.1] disintegrations per minute [dpm]/cell, $p < .05$); and concanavalin A (1.7 [0.9, 2.0] *vs* 2.8 [2.6, 3.9] dpm/cell, $p < .05$) mitogens. Neutrophil respiratory burst normalized to a young control was significantly lower in malnourished subjects *vs* well-nourished subjects (0.8 [0.5, 0.9] *vs* 1.4 [1.0, 1.7], $p < .05$). Total induration to 3 skin-test antigens was 13.4 ± 4.6, 3.5 ± 2.6, and 3.8 ± 1.8 (mean ± SEM) for well-nourished, at risk, and malnourished, respectively ($p = .059$). *Conclusions:* Immune function is impaired with an MNA score indicative of malnutrition in nursing home elders with pressure ulcers.

4.275 Systemic treatment of cerebral cortex lesions in rats with a new secreted phospholipase A2 inhibitor
Cunningham, T.J., Souayah, N., Jameson, B., Mitchell, J. and Yao, L.
*J. Neurotrauma, **21(11)**, 1683-1691 (2004)*

An internal fragment of the human neuroprotective polypeptide DSEP (Diffusible Survival Evasion Peptide) was delivered at 0.4 mg/kg (subcutaneously) 20-30 min after stab wound lesions in the parietal cortex of anesthetized rats. The peptide, CHEASAAQC or CHEC-9, inhibited the inflammatory response to the lesion and the degeneration of neurons adjacent to the wound. Four days after surgery, peptide-treated animals ($n = 6$) had 75% fewer reactive ameboid microglia/brain macrophages in the cortical parenchyma surrounding the lesion compared to vehicle-injected control rats ($n = 6$, $p = 0.004$). The cortical laminae in area 2 adjacent to the lesion were completely obscured in controls because of the increase in inflammatory cells and frank degeneration of neurons, while there was preservation of the neurons and cytoarchitecture after peptide treatment. In parallel experiments, CHEC-9 was found to inhibit the enzymatic activity of secreted phospholipase A2 (sPLA2), including activity present in the serum of peptide-injected rats. Kinetic analysis revealed the peptide increased the average Km for serum by 318% when tested 45 min after treatment (peptide-treated, $n = 6$; control-treated, $n = 6$; $p = 0.0087$), suggesting the principal effect of the peptide was to lower the affinity of serum sPLA2 for substrate. The sPLA2 inhibition by this particular peptide sequence appeared to be highly specific since inversion of a single pair of amino acids eliminated the inhibitory effect. Phorbol-12-myristate-13-acetate stimulated platelet aggregation, a PLA2-regulated activity, was also inhibited by the peptide. The discovery of CHEC-9 makes it possible to study *in vivo* the long appreciated contribution made by PLA2-directed inflammation to both acute and chronic neurodegeneration and may be helpful in designing therapies to limit neuron death in these conditions.

4.276 Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages
Porritt, H.E. et al
*Immunity, **20**, 735-745 (2004)*

The nature of early T lineage progenitors in the thymus or bone marrow remains controversial. Here we assess lineage capacity and proliferative potential among five distinct components of the earliest intrathymic stage (DN1, $CD25^{-}44^{+}$). All of these express one or more hemato-lymphoid lineage markers. All can produce T lineage cells, but only two of them display kinetics of differentiation, proliferative capacity, and other traits consistent with being canonical T progenitors. The latter also appeared limited to producing cells of the T or NK lineages, while B lineage potential derived mainly from the other, less typical T progenitors. In addition to precisely defining canonical early progenitors in the thymus, this work reconciles conflicting results from numerous groups by showing that multiple progenitors with a DN1 phenotype home to the thymus and make T cells, but possess different proliferative potentials and lineage capacities.

4.277 Constitutive expression and alternative splicing of the exons encoding SCRs in *Sp152*, the sea urchin homologue of complement factor B. Implications on the evolution of the Bf/C2 gene family
Terwilliger, D.P., Clow, L.A., Gross, P.S. and Smith, L.C.
*Immunogenetics, **56**, 531-543 (2004)*

The purple sea urchin, *Strongylocentrotus purpuratus*, possesses a non-adaptive immune system including elements homologous to C3 and factor B (Bf) of the vertebrate complement system. SpBf is composed of motifs typical of the Bf/C2 protein family. Expression of *Sp152* (encodes SpBf) was identified in the phagocyte type of coelomocyte in addition to gut, pharynx and esophagus, which may have been due to the presence of these coelomocytes in and on all tissues of the animal. *Sp152* expression in coelomocytes was constitutive and non-inducible based on comparisons between pre- and post-injection with lipopolysaccharide or sterile seawater. The pattern of five short consensus repeats (SCRs) in SpBf has been considered ancestral compared to other deuterostome Bf/C2 proteins that contain either three or four SCRs. Three alternatively spliced messages were identified for *Sp152* and designated *Sp152*Δ*1*, *Sp152*Δ*4*, and *Sp152*Δ*1+*Δ*4*, based on which of the five SCRs were deleted. *Sp152*Δ*4* had an in-frame deletion of SCR4, which would encode a putative SpBf Δ4 protein with four SCRs rather than five. On the other hand, both *Sp152*Δ*1* and *Sp152*Δ*1+*Δ*4* had a frame-shift that introduced a stop codon six amino acids downstream of the splice site for SCR1, and would encode putative proteins composed only of the leader. Comparisons between the full-length SpBf and its several splice variants with other Bf/C2 proteins suggested that the early evolution of this gene family may have involved a combination of gene duplications and deletions of exons encoding SCRs.

4.278 Both soluble and membrane-bound forms of Flt3 ligand enhance tumor immunity following "suicide" gene therapy in a murine colon carcinoma model
Alsheihly, A-R., Zweiri, J., Walmesley, A.J., Watson, A.J.M. and Christmas, S.E.
Cancer Immunol. Immunother, ***53***, *946-954 (2004)*

In prodrug-activated ("suicide") gene therapy, tumor cells are transfected with the gene for an enzyme that converts an inactive prodrug, such as ganciclovir (GCV), to a toxic compound. Transfected cells are killed on administration of GCV, as also are untransfected "bystander" cells. The ability of the dendritic cell stimulatory cytokine Flt3 ligand (Flt3-L) to modulate prodrug-activated gene therapy has been investigated. Transfectants of the murine colon carcinoma MC26 were generated expressing soluble (FLS) and membrane-bound forms of Flt3-L. They were inoculated together with wild-type MC26 cells and cells expressing herpes simplex virus-1 (HSV1) thymidine kinase into BALB/c mice, which were then administered GCV. Expression of Flt3-L or FLS prevented regrowth of tumor in most mice, which was comparable to the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF), while tumors recurred in all mice receiving "suicide" gene therapy alone. Recurring tumor cells were resistant to direct killing by GCV but sensitive to "bystander" killing in vitro. Mice without tumor recurrence were rechallenged with unmodified MC26 cells. Of those mice given transfectants expressing GM-CSF, Flt3-L, or FLS, approximately 50% were immune to rechallenge. These mice also showed cytotoxic and proliferative responses to MC26 cells. These experiments show that both soluble and membrane-bound forms of Flt3-L were able to induce a protective immune response to colon carcinoma cells in a fashion similar to GM-CSF.

4.279 Vascular endothelial growth factor gene delivery for revascularization in transplanted human islets
Narang, A.S., et al
Pharmaceut. Res., ***21(1)***, *15-25 (2004)*

Purpose. Islet transplantation is limited by islet graft failure because of poor revascularization, host immune rejection, and nonspecific inflammatory response. Human vascular endothelial growth factor (hVEGF) gene delivery is likely to promote islet revascularization and survival.
Methods. We evaluated gene expression from a bicistronic plasmid encoding hVEGF and enhanced green fluorescent protein (EGFP) (pCMS-EGFP-hVEGF). Glucose responsiveness of islets was evaluated both *in vitro* and *in vivo*, and revascularization in islet graft was evaluated by immunohistochemistry.
Results. After transfection, hVEGF and EGFP expression levels were comparable with original monocistronic plasmids in Jurkat cells but higher and prolonged hVEGF expression in islets transfected with the bicistronic plasmid was observed, possibly as the result of differences in promoter strength and hypoxia response. The 3:1 w/w complexes showed little toxicity to islets at a dose of 5 μg DNA per 2000 islets. On glucose challenge, insulin release from transfected islets as well as secretion from islets after transplantation under the mouse kidney capsules in response to glucose stimulation, increased with time. Immunohistochemical staining of transplanted islets using mouse anti-human insulin, mouse anti-human von Willebrand factor, and rat anti-mouse CD31 antibodies suggests that islets are functional and there is new blood vessel formation.

Conclusions. These findings suggest that transient hVEGF gene expression by the islets may promote islet revascularization and prolong islet survival after transplantation.

4.280 Sodium channels β1 subunits promote neurite outgrowth in cerebellar granule neurons
Davis, T.H., Chen, C. and Isom, L.L.
J. Biol. Chem., ***279(49)***, *51424-51432 (2004)*

Many immunoglobulin superfamily members are integral in development through regulation of processes such as growth cone guidance, cell migration, and neurite outgrowth. We demonstrate that homophilic interactions between voltage-gated sodium channel β_1 subunits promote neurite extension in cerebellar granule neurons. Neurons isolated from wild-type or β_1(-/-) mice were plated on top of parental, mock-, or β_1-transfected fibroblasts. Wild-type neurons consistently showed increased neurite length when grown on β_1-transfected monolayers, whereas β_1(-/-) neurons showed no increase compared with control conditions. β_1-Mediated neurite extension was mimicked using a soluble β_1 extracellular domain and was blocked by antibodies directed against the β_1 extracellular domain. Immunohistochemical analysis suggests that the β_1 and β_4 subunits, but not β_2 and β_3, are expressed in cerebellar Bergmann glia as well as granule neurons. These results suggest a novel role for β_1 during neuronal development and are the first demonstration of a functional role for sodium channel β subunit-mediated cell adhesive interactions.

4.281 Oleic and docosahexaenoic acid differentially phase separate from lipid raft molecules: a comparative NMR, DSC, AFM, and detergent extraction study
Shaikh, S.R. et al
Biophys. J., ***87***, *1752-1766 (2004)*

We have previously suggested that the ω-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA) may in part function by enhancing membrane lipid phase separation into lipid rafts. Here we further tested for differences in the molecular interactions of an oleic (OA) versus DHA-containing phospholipid with sphingomyelin (SM) and cholesterol (CHOL) utilizing ^{2}H NMR spectroscopy, differential scanning calorimetry, atomic force microscopy, and detergent extractions in model bilayer membranes. ^{2}H NMR and DSC (differential scanning calorimetry) established the phase behavior of the OA-containing 1-[$^2H_{31}$]palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (16:0-18:1PE-d_{31})/SM (1:1) and the DHA-containing 1-[$^2H_{31}$]palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (16:0-22:6PE-d_{31})/SM (1:1) in the absence and presence of equimolar CHOL. CHOL was observed to affect the OA-containing phosphatidylethanolamine (PE) more than the DHA-containing PE, as exemplified by >2x greater increase in order measured for the perdeuterated palmitic chain in 16:0-18:1PE-d_{31}/SM (1:1) compared to 16:0-22:6PE-d_{31}/SM (1:1) bilayers in the liquid crystalline phase. Atomic force microscopy (AFM) experiments showed less lateral phase separation between 16:0-18:1PE-rich and SM/CHOL-rich raft domains in 16:0-18:1PE/SM/CHOL (1:1:1) bilayers than was observed when 16:0-22:6PE replaced 16:0-18:1PE. Differences in the molecular interaction of 16:0-18:1PE and 16:0-22:6PE with SM/CHOL were also found using biochemical detergent extractions. In the presence of equimolar SM/CHOL, 16:0-18:1PE showed decreased solubilization in comparison to 16:0-22:6PE, indicating greater phase separation with the DHA-PE. Detergent experiments were also conducted with cardiomyocytes fed radiolabeled OA or DHA. Although both OA and DHA were found to be largely detergent solubilized, the amount of OA that was found to be associated with raft-rich detergent-resistant membranes exceeded DHA by almost a factor of 2. We conclude that the OA-PE phase separates from rafts far less than DHA-PE, which may have implications for cellular signaling.

4.282 Analysis of transcription factor expression during discrete stages of postnatal thymocyte differentiation
Tabrizifard, S. et al
J. Immunol., ***173***, *1094-1102 (2004)*

Postnatal T lymphocyte differentiation in the thymus is a multistage process involving serial waves of lineage specification, proliferative expansion, and survival/cell death decisions. Although these are believed to originate from signals derived from various thymic stromal cells, the ultimate consequence of these signals is to induce the transcriptional changes that are definitive of each step. To help to characterize

this process, high density microarrays were used to analyze transcription factor gene expression in RNA derived from progenitors at each stage of T lymphopoietic differentiation, and the results were validated by a number of appropriate methods. We find a large number of transcription factors to be expressed in developing T lymphocytes, including many with known roles in the control of differentiation, proliferation, or cell survival/death decisions in other cell types. Some of these are expressed throughout the developmental process, whereas others change substantially at specific developmental transitions. The latter are particularly interesting, because stage-specific changes make it increasingly likely that the corresponding transcription factors may be involved in stage-specific processes. Overall, the data presented here represent a large resource for gene discovery and for confirmation of results obtained through other methods.

4.283 Circulating cytokine profile in anti-neutrophilic cytoplasmatic autoantibody-associated vasculitis: prediction of outcome?
Ohlsson, S., Wieslander, J. and Segelmark, M.
*Mediators of inflammation,**13(4)**, 275-283 (2004)*

AIMS: The anti-neutrophilic cytoplasmatic autoantibody-associated vasculitides (AASV) are diseases of relapsing-remitting inflammation. Here we explore the cytokine profile in different phases of disease, looking for pathogenic clues of possible prognostic value.
Results: Interleukin (IL)-6, IL-8 and IL-10 were significantly elevated in plasma. Patients in the stable phase who subsequently developed adverse events had higher IL-8 values. Patients in the stable phase who relapsed within 3 months had lower IL-10 values and higher IL-6 levels.
Conclusions: Patients with AASV have raised circulating cytokine levels compared with healthy controls, even during remission. Raised IL-8 seems associated with poor prognosis. Lower levels of IL-10 and higher levels of IL-6 herald a greater risk of relapse. Patients with systemic vasculitis in clinical remission have persistent disease activity, kept under control by inhibitory cytokines.

4.284 Inhibition of phenylephrine-induced cardiac hypertrophy by docosahexaenoic acid
Siddiqui, R.A., Shaikh, S.R., Kovacs, R., Stillwell, W. and Zaloga, G.
*J. Cell. Biochem., **92(6)**, 1141-1159 (2004)*

Many of the cardiovascular benefits of fish oil result from the antiarrhythmic actions of the n-3 polyunsaturated lipids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The beneficial effects of DHA/EPA in patients with coronary artery disease and myocardial infarction may also result from modulation of the myocardial hypertrophic response. Hypertrophy was assessed in neonatal cardiomyocytes exposed to phenylephrine (PE) by measuring cell surface area, total protein synthesis (^{14}C leucine incorporation), and the organization of sarcomeric α-actinin and by monitoring expression of atrial natriuretic factor (ANF). We report that PE induced a twofold increase in cell surface area and protein synthesis in cardiomyocytes. The hypertrophied cardiomyocytes also exhibited increased expression of ANF in perinuclear regions and organization of sarcomeric α-actinin into classical z-bands. Treatment of cardiomyocytes with 5 μM DHA effectively prevented PE-induced hypertrophy as shown by inhibition of surface area expansion and protein synthesis, inhibition of ANF expression, and prevention of α-actinin organization into z-bands. DHA treatment prevented PE-induced activation of Ras and Raf-1 kinase. The upstream inhibition of Ras→Raf-1 effectively prevented translocation and nuclear localization of phosphorylated extracellularly regulated kinase 1 and 2 (Erk1/2). These effects consequently led to inhibition of nuclear translocation, and hence, activation of the downstream signaling enzyme p90 ribosomal S6 kinase (p90^{rsk}). These results indicate that PE-induced cardiac hypertrophy can be minimized by DHA. Our results suggest that inhibition of Ras→Raf-1→Erk1/2→p90^{rsk}→hypertrophy is one possible pathway by which DHA can inhibit cardiac hypertrophy. In vivo studies are needed to confirm these in vitro effects of DHA.

4.285 Primary Adipocyte Culture: Adipocyte Purification Methods May Lead to a New Understanding of Adipose Tissue Growth and Development
Fernyhough, M.E., Vierck, J:L., Hausman, G.J., Mir, P.S., Okine, E.K. and Dodson, M.V.
*Cytotechnology, **46(2-3)**, 163-172 (2004)*
In the present manuscript, the methods required to generate purified cultures of mature adipocytes, as well as stromal vascular cells, from the same isolation are detailed. Also, we describe the in vitro conditions for the dedifferentiation of the isolated mature adipocytes. These two types of cells may be used to reevaluate differences between presently available cellular models for lipogenesis/lipolysis and might provide a new

cellular physiological system for studies utilizing the proliferative progeny from mature adipocyte dedifferentiation. Alternative possibilities to the dedifferentiation phenomenon are proposed, as this new area of research is novel.

4.286 The recognition of adsorbed and denatured proteins of different topographics by β_2 integrins and effects on leukocyte adhesion and activation
Brevig, T. et al
*Biomaterials, **26**, 3039-3053 (2005)*

Leukocyte β_2 integrins Mac-1 and p150,95 are promiscuous cell-surface receptors that recognise and mediate cell adhesion to a variety of adsorbed and denatured proteins. We used albumin as a model protein to study whether leukocyte adhesion and activation depended on the nm-scale topography of a protein adlayer. Albumin adsorbed from the native conformation gave rise to different adlayer topographies and different amounts of adsorbed protein on hydrophobic and relatively hydrophilic polystyrene and silanised silicon-wafer surfaces, whereas adsorption of pre-denatured Alb resulted in similar adlayer topographies and similar amounts of adsorbed protein on these surfaces. All three distinct protein-adlayer topographies supported adhesion of in vitro differentiated, macrophage-like U937 and THP-1 cells, but did not support adhesion of their promonocytic precursors. Human monocytes freshly isolated from peripheral blood did not adhere to adsorbed albumin, not even in the presence of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α chemokines. Adhesion of the macrophage-like cells to albumin in any of the three topographies was inhibited by antibodies against β_2 integrins, but not by antibodies against β_1 integrins, and did not induce secretion of the proinflammatory cytokine tumour necrosis factor-α.

4.287 Virus-like particles as carriers for T-cell epitopes: limited inhibition of T-cell priming by carrier-specific antibodies
Ruedl. C. et al
*J. Virol., **79(2)**, 717-724 (2005)*

Virus-like particles (VLPs) are able to induce cytotoxic T-cell responses in the absence of infection or replication. This makes VLPs promising candidates for the development of recombinant vaccines. However, VLPs are also potent inducers of B-cell responses, and it is generally assumed that such VLP-specific antibodies interfere with the induction of protective immune responses, a phenomenon summarized as carrier suppression. In this study, we investigated the impact of preexisting VLP-specific antibodies on the induction of specific cytotoxic T-cell and Th-cell responses in mice. The data show that VLP-specific antibodies did not measurably reduce antigen presentation in vitro or in vivo. Nevertheless, T-cell priming was slightly reduced by antigen-specific antibodies; however, the overall reduction was limited and vaccination with VLPs in the presence of VLP-specific antibodies still resulted in protective T-cell responses. Thus, carrier suppression is unlikely to be a limiting factor for VLP-based T-cell vaccines.

4.288 Brain-derived neurotrophic factor in platelets and airflow limitation in asthma
Lommatzsch, M. et al
*Am. J. Respir. Care Med., **171**, 115-120 (2005)*

Brain-derived neurotrophic factor (BDNF), a key mediator of neuronal plasticity, contributes to airway obstruction and hyperresponsiveness in a model of allergic asthma. BDNF is stored in human platelets and circulates in human plasma, but the significance of BDNF in this compartment is poorly understood. We investigated the relationship between platelet and plasma BDNF levels and pulmonary function in a cohort of 26 adult patients with recently diagnosed allergic asthma. BDNF levels in serum, platelets, and plasma were significantly increased in participants with asthma, as compared with 26 age- and sex-matched control subjects. In steroid-naive patients, but not in patients using inhaled corticosteroids, enhanced platelet BDNF levels correlated with parameters of airway obstruction and airway hyperresponsiveness to histamine. Experiments with activated peripheral blood mononuclear cells revealed that corticosteroids such as fluticasone effectively suppress BDNF secretion. In conclusion, we demonstrate that enhanced platelet BDNF is associated with airflow limitation and airway hyperresponsiveness in asthma. In addition, we provide evidence that corticosteroids suppress BDNF production by activated immune cells.

4.289 IQGAP are differentially expressed and regulated in polarized gastric epithelial cells
Chew, C.S., Okamoto, C.T., Chen, X. and Qin, H.Y.
*Am. J. Physiol., **288**, G376-G387 (2005)*

IQGAPs, GTPase-activating proteins with an IQ motif, are thought to regulate many actin cytoskeleton-based activities through interactions with Cdc42 and Rac. Recently, Cdc42 was implicated in regulation of gastric parietal cell HCl secretion, and IQGAP2 was immunolocalized with Cdc42 to F-actin-rich intracellular canalicular membranes of isolated gastric parietal cells in primary culture. Here we sought to define distribution and localization of IQGAP1 and IQGAP2 in major oxyntic (acid-secreting) gastric mucosal cell types and to determine whether secretory agonists modulate these proteins. Differential staining protocols were used to identify different cell populations (parietal, chief, surface/pit, and mucous neck cells) in semi-intact glands isolated from rabbit gastric mucosae and to characterize these same cells after dispersion and fractionation on isopycnic density gradients with simultaneous staining for F-actin, H^+-K^+-ATPase, and GSII lectin-binding sites. There was a pronounced increase in intracellular F-actin staining in dispersed chief cells, apparently from internalization of F-actin-rich apical membranes that normally abut the gland lumen. Therefore, other membrane-associated proteins might also be redistributed by disruption of cell-cell contacts. Western blot analyses were used to quantitate relative concentrations of IQGAPs in defined mucosal cell fractions, and gastric glands were used for in situ localizations. We detected uniform levels of IQGAP2 expression in oxyntic mucosal cells with predominant targeting to regions of cell-cell contact and nuclei of all cell types. IQGAP2 was not detected in parietal cell intracellular canaliculi. IQGAP1 expression was variable and targeted predominantly to the cortex of chief and mucous neck cells. Parietal cells expressed little or no IQGAP1 vs. other mucosal cell types. Phosphoprotein affinity chromatography, isoelectric focusing, and phosphorylation site analyses indicated that both IQGAP1 and IQGAP2 are phosphoproteins potentially regulated by $[Ca^{2+}]_i$/PKC and cAMP signaling pathways, respectively. Stimulation of glands with carbachol, which elevates $[Ca^{2+}]_i$ and activates PKC, induced apparent translocation of IQGAP1, but not IQGAP2, to apical poles of chief (zymogen) and mucous neck cells. This response was mimicked by PMA but not by ionomycin or by elevation of $[cAMP]_i$ with forskolin. Our observations support a novel, PKC-dependent role for IQGAP1 in regulated exocytosis and suggest that IQGAP2 may play a more general role in regulating cell-cell interactions and possibly migration within the gastric mucosa.

4.290 Microfluid sorting of mammalian cells by optical force switching
Wang, M.M. et al
*Nature Biotech., **23(1)**, 83-87 (2005)*

Microfluidic-based devices have allowed miniaturization and increased parallelism of many common functions in biological assays; however, development of a practical technology for microfluidic-based fluorescence-activated cell sorting has proved challenging. Although a variety of different physical on-chip switch mechanisms have been proposed1, 2, 3, 4, 5, 6, none has satisfied simultaneously the requirements of high throughput, purity, and recovery of live, unstressed mammalian cells. Here we show that optical forces can be used for the rapid (2−4 ms), active control of cell routing on a microfluidic chip. Optical switch controls reduce the complexity of the chip and simplify connectivity. Using all-optical switching, we have implemented a fluorescence-activated microfluidic cell sorter and evaluated its performance on live, stably transfected HeLa cells expressing a fused histone−green fluorescent protein. Recovered populations were verified to be both viable and unstressed by evaluation of the transcriptional expression of two genes, HSPA6 and FOS, known indicators of cellular stress.

4.291 The multifunctional DNA repair/redox enzyme Apel/Ref-1 promotes survival of neurons after oxidative stress
Vasko, M.R., Guo, C. and Kelley, M.R.
*DNA Repair, **4(3)**, 367-379 (2005)*

Although correlative studies demonstrate a reduction in the expression of apurinic/apyrimidinic endonuclease/redox effector factor (Ape1/Ref-1 or Ape1) in neural tissues after neuronal insult, the role of Ape1 in regulating neurotoxicity remains to be elucidated. To address this issue, we examined the effects of reducing Ape1 expression in primary cultures of hippocampal and sensory neurons on several endpoints of neurotoxicity induced by H_2O_2. Ape1 is highly expressed in hippocampal and sensory neurons grown in culture as indicated by immunohistochemistry, immunoblotting and activity. Exposing hippocampal or sensory neuronal cultures to 25 or 50 nM small interfering RNA to Ape1 (Ape1siRNA), respectively, for 48 h, causes a reduction in immunoreactive Ape1 by approximately 65 and 54%, and an equivalent loss in endonuclease activity. The reduced expression of Ape1 is maintained for up to 5 days after the siRNA in the medium is removed, whereas exposing cultures to scrambled sequence siRNA (SCsiRNA) has no effect of Ape1 protein levels. The reduction in Ape1 significantly reduces cell viability in cultures 24 h after a 1-h exposure to 25–300 μM H_2O_2, compared to SCsiRNA treated controls. In cells treated with

SCsiRNA, exposure to 300 μM H_2O_2 reduced cell viability by 40 and 30% in hippocampal and sensory neuronal cultures, respectively, whereas cultures treated with Ape1siRNA lost 93 and 80% of cells after the peroxide. Reduced Ape1 levels also increase caspase-3 activity in the cells, 2–3-fold, 60 min after a 1-h exposure to 100 μM H_2O_2 in the cultures. Exposing neuronal cultures with reduced expression of Ape1 to 65 μM H_2O_2 (hippocampal) or 300 μM H_2O_2 (sensory) for 1 h results in a 3-fold and 1.5-fold increase in the phosphorylation of histone H2A.X compared to cells exposed to SCsiRNA. Overexpressing wild-type Ape1 in hippocampal and sensory cells using adenoviral expression constructs results in significant increase in cell viability after exposure to various concentrations of H_2O_2. The C65A repair competent/redox incompetent Ape1 when expressed in the hippocampal and sensory cells conferred only partial protection on the cells. These data support the notion that both of functions of Ape1, redox and repair are necessary for optimal levels of neuronal cell survival.

4.292 Changes in immune cell distribution and IL-10 production are regulated through endometrial IP-10 expression in the goat uterus

Imakawa, K., Nagaoka, K., Nojima, H., Hara, Y. and Christensen, R.K.
Am. J. Reprod. Immunol., ***53****, 54-64 (2005)*

Problem: Changes in distribution or redistribution of immune cells are required for the establishment and maintenance of pregnancy, but these changes during early pregnancy have been poorly understood in the ruminant ungulates. Expression of a chemokine, interferon-γ (IFN-γ)-inducible protein 10 kDa (IP-10, CXCL10), was identified in the endometrium of pregnant goats. Population and/or distribution of endometrial immune cells and their cytokine productions could be regulated by IP-10 during the period of pregnancy establishment.
Method of study: Using reverse transcriptase-polymerase chain reaction (RT-PCR), expression of IP-10, IFN-γ, tumor necrosis factor-α, interleukin-10 (IL-10), CXCR3 mRNA and leukocyte cell surface markers, CD4, CD8, CD11b and CD45 mRNA during the caprine early pregnancy was investigated. The ability of IP-10 to stimulate peripheral blood mononuclear cells (PBMCs) migration was demonstrated using a chemotaxis assay. Changes in migration of PBMCs' immune cell population and cytokine expressions with IP-10 stimulation were investigated using flow cytometry and RT-PCR respectively.
Results: Levels of IP-10, IL-10, CD4 and CD11b mRNA, and the number of CD4 and CD11b positive cells in pregnant goat endometrium were higher than those of cyclic goat endometrium. Migration of PBMCs was stimulated by recombinant caprine IP-10, and the effect was significantly reduced by neutralization with the use of an anti-IP-10 antibody. In the flow cytometric and RT-PCR analyses, migrated cells stimulated by IP-10 increased the expression of IL-10 and CD11b mRNA. Furthermore, IP-10 could stimulate the expression of IL-10 mRNA from PBMCs.
Conclusion: Endometrial chemokine IP-10 could regulate IL-10 production by resident and possibly migrated cells expressing CD11b, probably natural killer cells, and these changes may result in immune environments of the uterus suitable for conceptus implantation in ruminants.

4.293 Rat embryonic motoneurons in long-term co-culture with Schwann cells – a system to investigate motoneuron diseases on a cellular level in vitro

Haastert, K. et al
J. Neurosci. Methods, ***142(2)****, 275-284 (2005)*

Investigations of motoneuron diseases on a cellular and molecular level require long-term cultivation of primary cells. Here we present a new culture system in which matured motoneurons interact with their physiological partners like interneurons, astroglia and peripheral glia cells. This enables motoneuron-maturation for up to 3 weeks, while motoneurons consistently reached large diameters of their somata of 30–45 μm, occasionally more than 80 μm. Dissociated rat embryonic ventral spinal cord cells were enriched for motoneurons by density gradient centrifugation and seeded on a non-confluent mono-layer of highly enriched neonatal rat Schwann cells. Immunocytochemical visualization of neuron specific βIII-tubulin in all neurons and of motoneuron specific non-phosphorylated neurofilament H/M, respectively, revealed that after 3 days in vitro >70% of all neurons were motoneurons. After 20 days in vitro, a motoneuron fraction of 12% was maintained. Motoneurons were susceptible to transient transfection with green fluorescent protein cDNA when liposomal transfection and an enhancer substance were combined. Synaptic connections enabled formation of spontaneously active neuronal networks which provide a culture model to study glutamate excitotoxicity and calcium deregulation on a molecular level. Both mechanisms are implied in the pathophysiology of amyotrophic lateral sclerosis, a neurodegenerative motoneuron disorder.

4.294 Identification of a novel tumor necrosis factor α-responsive region in the *NCF2* promoter
Gauss, K.A. et al
J. Leukoc. Biol., ***77***, *267-278 (2005)*

The phagocyte reduced nicotinamide adenine dinucleotide phosphate oxidase is a multiprotein enzyme that catalyzes the production of microbicidal oxidants. Although oxidase assembly involves association of several membrane and cytosolic oxidase proteins, one of the cytosolic cofactors, $p67^{phox}$, appears to play a more prominent role in final activation of the enzyme complex. Based on the importance of $p67^{phox}$, we investigated transcriptional regulation of the $p67^{phox}$ gene [neutrophil cytosolic factor 2 (*NCF2*)] and demonstrated previously that activator protein-1 (AP-1) was essential for basal transcriptional activity. As $p67^{phox}$ can be up-regulated by tumor necrosis factor α(TNF-α), which activates AP-1, we hypothesized that TNF-α might regulate *NCF2*transcription via AP-1. In support of this hypothesis, we show here that *NCF2* promoter-reporter constructs are up-regulated by TNF-α but only when AP-1 factors were coexpressed. Consistent with this observation, we also demonstrate that *NCF2* mRNA and $p67^{phox}$ protein are up-regulated by TNF-α in various myeloid cell lines as well as in human monocytes. It was surprising that mutagenesis of the AP-1 site in *NCF2* promoter constructs did not eliminate TNF-α induction, suggesting additional elements were involved in this response and that AP-1 might play a more indirect role. Indeed, we used *NCF2* promoter-deletion constructs to map a novel TNF-α-responsive region (TRR) located between –56 and –16 bp upstream of the translational start site and demonstrated its importance in vivo using transcription factor decoy analysis. Furthermore, DNase footprinting verified specific binding of factor(s) to the TRR with AP-1 binding indirectly to this region. Thus, we have identified a novel *NCF2* promoter/enhancer domain, which is essential for TNF-α-induced up-regulation of $p67^{phox}$.

4.295 Characterization of microglia induced from mouse embryonic stem cells and their migration into brain parenchyma
Tsuchiya, T. et al
J. Neuroimmunol., ***160***, *210-218 (2005)*

We derived microglia from mouse embryonic stem cells (ES cells) at very high density. Using the markers $Mac1^+/CD45^{low}$ and $Mac1^+/CD45^{high}$ to define microglia and macrophages, respectively, we show that $Mac1^+$ cells are induced by GM-CSF stimulation following neuronal differentiation of mouse ES cells using a five-step method. $CD45^{low}$ expression was high and $CD45^{high}$ expression was low on induced cells. We used a density gradient method to obtain a large amount of microglia-like cells, approximately 90% of $Mac1^+$ cells. Microglia-like cells expressed MHC class I, class II, CD40, CD80, CD86, and IFN-γR. The expression level of these molecules on microglia-like cells was barely enhanced by IFN-γ. Intravenously transferred GFP^+ microglia derived from GFP^+ ES cells selectively accumulated in brain but not in peripheral tissues such as spleen and lymph node. GFP^+ cells were detected mainly in corpus callosum and hippocampus but were rarely seen in cerebral cortex, where Iba1, another marker of microglia, is primarily expressed. Furthermore, both GFP^+ and $Iba1^+$ cells exhibited a ramified morphology characteristic of mature microglia. These studies suggest that ES cell-derived microglia-like cells obtained using our protocol are functional and migrate selectively into the brain but not into peripheral tissues after intravenous transplantation.

4.296 Clinical response in Japanese metastatic melanoma patients treated with peptide cocktail-pulsed dendritic cells
Akiyama, Y. et al
J. Transplant. Med., ***3(4)***, *1-10 (2005)*

Background
Metastatic, chemotherapy-resistant melanoma is an intractable cancer with a very poor prognosis. As to immunotherapy targeting metastatic melanoma, $HLA\text{-}A2^+$ patients were mainly enrolled in the study in Western countries. However, $HLA\text{-}A24^+$ melanoma patients-oriented immunotherapy has not been fully investigated. In the present study, we investigated the effect of dendritic cell (DC)-based immunotherapy on metastatic melanoma patients with HLA-A2 or A24 genotype.
Methods
Nine cases of metastatic melanoma were enrolled into a phase I study of monocyte-derived dendritic cell (DC)-based immunotherapy. HLA-genotype analysis revealed 4 cases of HLA-A*0201, 1 of A*0206 and 4 of A*2402. Enriched monocytes were obtained using OptiPrep™ from leukapheresis products, and then incubated with GM-CSF and IL-4 in a closed serum-free system. After pulsing with a cocktail of 5 melanoma-associated synthetic peptides (gp100, tyrosinase, MAGE-2, MAGE-3 and MART-1 or MAGE-

1) restricted to HLA-A2 or A24 and KLH, cells were cryopreserved until used. Finally, thawed DCs were washed and injected subcutaneously (s.c.) into the inguinal region in a dose-escalation manner.
Results
The mean percentage of DCs rated as $lin^{-}HLA\text{-}DR^{+}$ in melanoma patients was 46.4 ± 15.6 %. Most of DCs expressed high level of co-stimulatory molecules and type1 phenotype ($CD11c^{+}HLA\text{-}DR^{+}$), while a moderate number of mature DCs with CD83 and CCR7 positive were contained in DC products. DC injections were well tolerated except for transient liver dysfunction (elevation of transaminases, Grade I-II). All 6 evaluable cases except for early PD showed positive immunological responses to more than 2 melanoma peptides in an ELISPOT assay. Two representative responders demonstrated strong HLA-class I protein expression in the tumor and very high scores of ELISPOT that might correlate to the regression of metastatic tumors. Clinical response through DC injections was as follows : 1CR, 1 PR, 1SD and 6 PD. All 59 DC injections in the phase I study were tolerable in terms of safety, however, the maximal tolerable dose of DCs was not determined.
Conclusions
These results suggested that peptide cocktail-treated DC-based immunotherapy had the potential for utilizing as one of therapeutic tools against metastatic melanoma in Japan.

4.297 Immunomagnetic separation of *Toxoplasma gondii* oocysts using a monoclonal antibody directed against the oocyst wall
Dumetre, A. and Darde, M-L.
*J. Microbiol. Methods, **61**, 209-217 (2005)*

Recent outbreaks of waterborne toxoplasmosis have stimulated the development of sensitive methods to detect *Toxoplasma gondii* oocysts in samples suspected to be contaminated. The immunomagnetic separation (IMS) have been standardised to detect waterborne protozoa, but it did not exist for *Toxoplasma* oocysts. In this study, we describe two monoclonal antibodies (mAbs 3G4 and 4B6) produced against the oocyst wall, and the incorporation of mAb 3G4 in an IMS procedure. We found that an indirect IMS method gave better mean recoveries than a direct one (69.4% and 25.2%, respectively). Dissociation of oocyst_magnetic bead complexes was greatly improved by using a 2% aqueous H_2SO_4 solution instead of a 0.1 N HCl solution (82.8% and 17.4%, respectively). With these parameters, mean recoveries of less than 1000 oocysts ranged from 44.6% to 82.9%, depending on incubating temperature and buffe r. Age of oocysts (1 or 12 months old) does not influence IMS performances. Results of this study indicate that the described IMS is an efficient technique to recover *Toxoplasma* oocysts.

4.298 Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes
Hering, B.J: et al
*JAMA, **293(7)**, 830-835 (2005)*

Context Islet allografts from 2 to 4 donors can reverse type 1 diabetes. However, for islet transplants to become a widespread clinical reality, diabetes reversal must be achieved with a single donor to reduce risks and costs and increase the availability of transplantation.
Objective To assess the safety of a single-donor, marginal-dose islet transplant protocol using potent induction immunotherapy and less diabetogenic maintenance immunosuppression in recipients with type 1 diabetes. A secondary objective was to assess the proportion of islet transplant recipients who achieve insulin independence in the first year after single-donor islet transplantation.
Design, Setting, and Participants Prospective, 1-year follow-up trial conducted July 2001 to August 2003 at a single US center and enrolling 8 women with type 1 diabetes accompanied by recurrent hypoglycemia unawareness or advanced secondary complications.
Interventions Study participants underwent a primary islet allotransplant with 7271 (SD, 1035) islet equivalents/kg prepared from a single cadaver donor pancreas. Induction immunosuppression was with antithymocyte globulin, daclizumab, and etanercept. Maintenance immunosuppression consisted of mycophenolate mofetil, sirolimus, and no or low-dose tacrolimus.
Main Outcome Measures Safety (assessed by monitoring the severity and duration of adverse events) and efficacy (assessed by studying the recipients' insulin requirements, C-peptide levels, oral and intravenous glucose tolerance results, intravenous arginine stimulation responses, glycosylated hemoglobin levels, and hypoglycemic episodes) associated with the study transplant protocol.
Results There were no serious, unexpected, or procedure- or immunosuppression-related adverse events. All 8 recipients achieved insulin independence and freedom from hypoglycemia. Five remained insulin-independent for longer than 1 year. Graft failure in 3 recipients was preceded by subtherapeutic sirolimus exposure in the absence of measurable tacrolimus trough levels.

Conclusions The tested transplant protocol restored insulin independence and protected against hypoglycemia after single-donor, marginal-dose islet transplantation in 8 of 8 recipients. These results may be related to improved islet engraftment secondary to peritransplant administration of antithymocyte globulin and etanercept. These findings may have implications for the ongoing transition of islet transplantation from clinical investigation to routine clinical care.

4.299 Are platelets activated after a rapid, one-step density gradient centrifugation? Evidence from flow cytometric analysis

Bagamery, K., Kvell, K., Barnet, M., Landau, R. and Graham, J.
Clin. Lab. Haem., ***27(1)***, *75-77 (2005)*

This procedure describes the preparation of platelets from whole blood of healthy donors and pregnancy-induced hypertensive (PIH) patients by a rapid, one-step density gradient centrifugation, and the direct immunofluorescence staining of obtained platelets (CD63). Platelets are relatively fragile structures. Consequently, for the investigation of their biochemical properties it is recommended to isolate them by a simple method that does not damage their functional parameters and induce their activation. During platelet activation, several changes occur at the platelet surface. CD63 is the receptor for a lysosomal glycoprotein expressed in activated platelets. Currently, flow cytometry (fluorescence-activated cell sorting) is the most sensitive method to detect increased surface exposure of activation antigens on the platelet surface. The present technical note describes that compared with other whole blood flow cytometric techniques, our one-step density-gradient centrifugation method using OptiPrep(TM) can also prevent artificial, sample manipulation-related platelet activation.

4.300 $CD4^-$ plasmacytoid dendritic cells (pDCs) migrate in lymph nodes by CpG inoculation and represent a potent functional subset of pDCs

Yang, G-X. Et al
J. Immunol., ***174***, *3197-3203 (2005)*

We have recently identified two groups of plasmacytoid dendritic cells (pDCs) isolated from murine liver based on the expression of CD4 and other cell surface markers uniquely expressed by pDCs. Herein, we describe the identification of both $CD4^+$ and $CD4^-$ pDCs that clearly exist in lymph nodes (LNs), spleen, liver, thymus, bone marrow, and lung. Normally, $CD4^+$ pDCs are enriched in LNs. However, after in vivo systemic injection with bacterial CpG, a larger number of $CD4^-$ pDCs are recruited to the LNs and local inoculation by CpG drives $CD4^-$ pDCs migrating into local sentinel LNs, suggesting that $CD4^-$ pDCs are the main subpopulation migrating to the peripheral LNs. Furthermore, although both freshly isolated $CD4^+$ pDCs and $CD4^-$ pDCs appear as an immature plasmacytoid cell and develop into a DC morphology following activation, the two subsets have strikingly different immune features, including differences in the production pattern of cytokines stimulated with CpG and in T cell activation.

4.301 Influence of ESAT-6 secretion system 1 (RD1) of *Mycobacterium tuberculosis* on the interaction between mycobacteria and the host immune system

Majlessi, L. et al
J. Immunol., ***174***, *3570-3579 (2005)*

The chromosomal locus encoding the early secreted antigenic target, 6 kDa (ESAT-6) secretion system 1 of *Mycobacterium tuberculosis*, also referred to as "region of difference 1 (RD1)," is absent from *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). In this study, using low-dose aerosol infection in mice, we demonstrate that BCG complemented with RD1 (BCG::RD1) displays markedly increased virulence which albeit does not attain that of *M. tuberculosis* H37Rv. Nevertheless, phenotypic and functional analyses of immune cells at the site of infection show that the capacity of BCG::RD1 to initiate recruitment/activation of immune cells is comparable to that of fully virulent H37Rv. Indeed, in contrast to the parental BCG, BCG::RD1 mimics H37Rv and induces substantial influx of activated ($CD44^{high}CD45RB^-CD62L^-$) or effector ($CD45RB^-CD27^-$) T cells and of activated $CD11c^+CD11b^{high}$ cells to the lungs of aerosol-infected mice. For the first time, using in vivo analysis of transcriptome of inflammatory cytokines and chemokines of lung interstitial $CD11c^+$ cells, we show that in a low-dose aerosol infection model, BCG::RD1 triggered an activation/inflammation program comparable to that induced by H37Rv while parental BCG, due to its overattenuation, did not initiate the activation program in lung interstitial $CD11c^+$ cells. Thus, products encoded by the ESAT-6 secretion system 1 of *M. tuberculosis* profoundly modify the interaction between mycobacteria and the host innate and adaptive immune system. These modifications can explain the previously described improved protective capacity of

BCG::RD1 vaccine candidate against *M. tuberculosis* challenge.

4.302 A phase I study of α-galactosylceramide (KRN7000) – pulsed dendritic cells in patients with advanced and recurrent non – small cell lung cancer
Ishikawa, A. et al
*Clin. Can. Res., **11**, 1910-1917 (2005)*

Purpose: Human Vα24 natural killer T (NKT) cells bearing an invariant Vα24Jα Q antigen receptor, the counterpart of murine Vα14 NKT cells, are activated by a specific ligand, α-galactosylceramide (αGalCer, KRN7000), in a CD1d-dependent manner. I.v. administration of αGalCer-pulsed dendritic cells (DC) induces significant activation and expansion of Vα14 NKT cells in the lung and resulting potent antitumor activities in mouse tumor metastatic models. We did a phase I dose escalation study with αGalCer-pulsed DCs in lung cancer patients.
Experimental Design: Patients with advanced non–small cell lung cancer or recurrent lung cancer received i.v. injections of αGalCer-pulsed DCs (level 1: $5 \times 10^7/m^2$; level 2: $2.5 \times 10^8/m^2$; and level 3: $1 \times 10^9/m^2$) to test the safety, feasibility, and clinical response. Immunomonitoring was also done in all completed cases.
Results: Eleven patients were enrolled in this study. No severe adverse events were observed during this study in any patient. After the first and second injection of αGalCer-pulsed DCs, dramatic increase in peripheral blood Vα24 NKT cells was observed in one case and significant responses were seen in two cases receiving the level 3 dose. No patient was found to meet the criteria for partial or complete responses, whereas two cases in the level 3 group remained unchanged for more than a year with good quality of life.
Conclusions: In this clinical trial, αGalCer-pulsed DC administration was well tolerated and could be safely done even in patients with advanced disease.

4.303 Nuclear factor-κB1 (p50) limits the inflammatory and fibrogenic responses to chronic injury
Oakley, F. et al
*Am. J. Pathol., **166(3)**, 695-708 (2005)*

In this study we addressed the role of the nuclear factor (NF)-κB1/p50 subunit in chronic injury of the liver by determining the inflammatory and fibrotic responses of ***nfκb1***-null mice in an experimental model that mimics chronic liver disease. Mice received repeated hepatic injuries throughout 12 weeks by intraperitoneal injection of the hepatotoxin carbon tetrachloride. In response ***nfκb1***$^{-/-}$ mice developed more severe neutrophilic inflammation and fibrosis compared to ***nfκb1***$^{+/+}$ mice. This phenotype was associated with elevated hepatic expression of tumor necrosis factor (TNF)-α, which was localized to regions of the liver associated with inflammation and fibrosis. Hepatic stellate cells are important regulators of hepatic inflammatory and fibrogenic events but normally do not express TNF-α. Hepatic stellate cells derived from ***nfκb1***$^{-/-}$ mice expressed TNF-α promoter activity, mRNA, and protein. By contrast the expression of other NF-κB-responsive genes (ICAM1 and interleukin-6) was similar between ***nfκb1***$^{-/-}$ and ***nfκb1***$^{+/+}$ cells. We provide experimental evidence that the inappropriate expression of TNF-α by ***nfκb1***$^{-/-}$ cells is because of lack of a p50-dependent histone deacetylase 1 (HDAC1)-mediated repression of TNF-α gene transcription. Taken together these data indicate that the p50 NF-κB subunit plays a critical protective role in the injured liver by limiting the expression of TNF-α and its recruitment of inflammatory cells.

4.304 Arginine supplementation does not e enhance serum nitric oxide levels in elderly nursing home residents with pressure ulcers
Techmiller, J.K. et al
*Biol. Res. for Nursing, **6(4)**, 289-299 (2005)*

The purpose of this study was to determine whether arginine supplementation enhances in vitro (neutrophil burst and mitogen-induced lymphocyte proliferation) and in vivo (delayed-type hypersensitivity [DTH] and serum nitric oxide) measures of immune function in nursing home elders with pressure ulcers. Twenty-six elders, 65 years of age or older, with one or more pressure ulcers, were randomized to receive 8.5 g of arginine or an isonitrogenous supplement for 4 weeks. Immune function studies and serum arginine, ornithine, citrulline, and nitric oxide were measured at baseline, 4 weeks postsupplementation (Week 4) and after a 6-week washout (Week 10). At Week 4, serum ornithine increased ($p = .01$) *and arginine trended to increase (*$p = .055$), but there was no increase in citrulline or nitric oxide with arginine supplementation. There were no differences in neutrophil burst or DTH responses between groups. Whole blood mitogen–induced proliferation decreased significantly atWeek 10 in the isonitrogenous but not in the arginine-supplemented group. There is mounting concern that arginine supplementation during an inflammatory state could be detrimental due to overwhelming nitric oxide production. A key finding of this

study is that arginine supplementation did not increase serum nitric oxide levels over that observed in elders with pressure ulcers given an isonitrogenous supplement.

4.305 Pancreatic β-cell failure and diabetes in mice with a delition mutation of the endoplastic reticulum molecular chaperone gene $P58^{IPK}$
Ladiges, W. et al
*Diabetes, **54**, 1074-1081 (2005)*

The endoplasmic reticulum (ER) transmits apoptotic signals in the pancreas during ER stress, implicating ER stress–mediated apoptosis in the development of diabetes. $P58^{IPK}$ (*DNAJC3*) is induced during ER stress and functions as a negative feedback component to inhibit eIF-2α signaling and attenuate the later phases of the ER stress response. To gain insight into a more comprehensive role of $P58^{IPK}$ function, we generated deletion mutant mice that showed a gradual onset of glucosuria and hyperglycemia associated with increasing apoptosis of pancreatic islet cells. Lack of $P58^{IPK}$ had no apparent effect on the functional integrity of viable ß-cells. A set of genes associated with apoptosis showed altered expression in pancreatic islets from $P58^{IPK}$-null mice, further substantiating the apoptosis phenotype. The data provide in vivo evidence to support the concept that $P58^{IPK}$ functions as a signal for the downregulation of ER-associated proteins involved in the initial ER stress response, thus preventing excessive cell loss by degradation pathways. Insulin deficiency associated with the absence of $P58^{IPK}$ mimics ß-cell failure associated with type 1 and late-stage type 2 diabetes. $P58^{IPK}$ function and activity may therefore provide a novel area of investigation into ER-mediated mechanistic and therapeutic approaches for diabetes.

4.306 Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus
Howell, O.W. et al
*J. Neurochem., **93**, 560-570 (2005)*

Adult dentate neurogenesis is important for certain types of hippocampal-dependent learning and also appears to be important for the maintenance of normal mood and the behavioural effects of antidepressants. Neuropeptide Y (NPY), a peptide neurotransmitter released by interneurons in the dentate gyrus, has important effects on mood, anxiety-related behaviour and learning and memory. We report that adult NPY receptor knock-out mice have significantly reduced cell proliferation and significantly fewer immature doublecortin-positive neurons in the dentate gyrus. We also show that the neuroproliferative effect of NPY is dentate specific, is Y_1-receptor mediated and involves extracellular signal-regulated kinase (ERK)1/2 activation. NPY did not exhibit any effect on cell survival *in vitro* but constitutive loss of the Y_1 receptor *in vivo* resulted in greater survival of newly generated neurons and an unchanged total number of dentate granule cells. These results show that NPY stimulates neuronal precursor proliferation in the dentate gyrus and suggest that NPY-releasing interneurons may modulate dentate neurogenesis.

4.307 Flow cytometric analysis of CD41-labeled platelets isolated by the rapid, one-step OptiPrep method from human blood
Bagamery, K., Kvell, K., Landau, R. and Graham, J.
*Cytometry Part A, **65A**, 84-87 (2005)*

Although platelet-rich plasma is relatively easy to produce by centrifugation of whole blood, yields of platelets may be variable because many of them are trapped within the erythrocyte layer. Although they can be recovered by washing these cells, it is a general rule that the number of centrifugations should be kept to a minimum to avoid activation of platelets. This work describes the rapid, one-step OptiPrep method for the isolation of highly purified platelets from human blood (buffy coat).
To provide a functionally intact and uncontaminated platelet fraction, a density gradient centrifugation was performed by using a density barrier prepared from OptiPrep. CD41 antibody staining was performed to assess the purity of the obtained platelet population by means of a FACScan flow cytometer. Platelets were identified by a morphologic gate in which events were further studied for CD41 expression. Data were analyzed by CellQuest (Becton Dickinson).
Platelet-specific CD41 antibody staining showed that the purity of the platelet population recovered from this density barrier method was greater than 90%. The platelets showed an excellent morphologic state. The rapid, one-step OptiPrep density gradient centrifugation is a reliable method for obtaining highly purified platelets from human blood that are ready for further pharmacologic investigations.

4.308 Reversal of long-term sepsis-induced immunosuppression by dendritic cells
Benjamin, C.F., Lundy, S.K., Lukacs, N.W., Hogaboam, C.M. and Kunkel, S.L.
*Blood, **105**, 3588-3595 (2005)*

Severe sepsis leads to long-term systemic and local immunosuppression, which is the cause of a number of complications, including pulmonary infection. A therapeutic strategy that reverses this immunosuppression is required, given the ongoing high mortality rate of patients who have survived a severe sepsis. The present study demonstrates that experimental severe sepsis renders the lung susceptible to a normally innocuous *Aspergillus fumigatus* fungus challenge, due to a dominant lung type 2 cytokine profile. Dendritic cells (DCs) obtained from the lungs of mice subjected to cecal ligation and puncture (CLP) model were skewed toward type 2 cytokine profile, which occurred with exaggerated expression of Toll-like receptor 2 (TLR2). The intrapulmonary transfer of bone marrow–derived DCs (BMDCs) in postseptic mice prevented fatal *Aspergillus* infection. This therapy reduced the overall inflammatory response and fungal growth in the lung, and promoted the balance of proinflammatory and suppressive cytokines in the lung. Thus, intrapulmonary DC supplementation appears to restore the pulmonary host response in the postseptic lung in our animal model. These data strongly suggest that lung DCs are profoundly affected as a consequence of the systemic impact of severe sepsis, and the identification of mechanisms that restore their function may serve as a key strategy to reverse sepsis-induced immunosuppression.

4.309 TGF-β signaling regulates CD8+ T cell responses to high- and low-affinity TCR interactions
Mehal, W., Sheikh, S.Z., Gorelik, L. and Flavell, R.A.
*Int. Immunol., **17(5)**, 531-538 (2005)*

Absence of transforming growth factor-ß (TGF-ß) signaling to T cells in mice results in an increase in T cell numbers, an activated CD44 high, CD69–, CD25– T cell phenotype and a T cell-mediated injury to many organs. It is not known if such T cell activation in the absence of TGF-ß signaling is spontaneous or due to aberrant T cell responses to a physiological stimulus. We used adoptive transfer of CD8+ T cells from mice double transgenic for the OT-1 TCR and the TGF-ß1-dominant negative transgene [OT-dominant-negative receptor (DNR)] to investigate the role of TGF-ß in regulating CD8+ T cell activation *in vivo*. The activation and expansion of single-transgenic OT and double-transgenic OT-DNR cells to oral antigens, high-affinity and low-affinity peptides were indistinguishable. Activation with high-affinity peptide and CFA however resulted in greater expansion of OT-DNR cells in comparison to OT cells. Low-affinity peptide and adjuvant did not result in OT cell activation or expansion but results in up-regulation of CD44 on OT-DNR cells. These data show that TGF-ß functions *in vivo* to limit the scale of CD8+ T cell expansion after high-affinity peptide–MHC interactions. TGF-ß also limits T cell activation to the highest affinity peptide–MHC interactions. The increase in T cell number and activation present in TGF-ß-deficient and TGF-ß DNR-expressing mice may be due to the loss of these two phenomena.

4.310 Effect of P-selectin on phosphatidylserine exposure and surface-dependent thrombin generation on monocytes
Del Conde, I. Et al
*Arterioscler. Thromb. Vasc. Biol., **25**, 1065-1070 (2005)*

Objective— Stimulation of monocytes with P-selectin induces the synthesis of an array of mediators of inflammation, as well as the expression of tissue factor (TF), the main initiator of coagulation. Because the membrane-bound reactions of coagulation are profoundly influenced by the presence of phosphatidylserine on the membranes of cells, factors that increase its expression may have an impact on coagulation.
Methods and Results— Using flow cytometry, we studied the effect of P-selectin on phosphatidylserine expression in blood monocytes and in the monocytic cells, THP-1. Soluble P-selectin at biologically relevant concentrations (0.31 to 2.5 μg/mL) induced a time-dependent increase in phosphatidylserine expression, an effect that could be inhibited with an anti–PSGL-1 blocking antibody, and by genistein, a tyrosine kinase inhibitor. Binding of activated platelets to THP-1 cells also resulted in a significant increase in phosphatidylserine expression that was dependent on PSGL-1. Consistent with the role of phosphatidylserine on surface-dependent reactions of coagulation, treatment of monocytic cells with soluble P-selectin led to increased thrombin generation. We excluded P-selectin induced apoptosis of monocyte as a mechanism for the increased phosphatidylserine exposure.
Conclusion— In summary, we show that P-selectin, either soluble or in its membrane-bound form, induces phosphatidylserine exposure in monocytes through a mechanism dependent on PSGL-1. Phosphatidylserine expression on monocytes is an important cofactor in the enzymatic reactions of coagulation. Stimulation of monocytes with P-selectin leads to cell activation and the expression of tissue

factor, the main initiator of coagulation. Here we show that P-selectin induces phosphatidylserine expression on monocytes through a mechanism dependent on PSGL-1 on the monocyte.

4.311 Quantitation and characterization of myosin peptide-specific $CD4^+$ T cells in autoimmune myocarditis

Maier, R. et al

*J. Immunol. Methods, **304**, 117-125 (2005)*

Characterization of autoantigen-specific $CD4^+$ T cells at the single cell level is crucial for understanding the immunopathological mechanisms underlying autoimmune diseases. Cardiac myosin heavy chain (myhca) is the major autoantigen associated with autoimmune myocarditis both in humans and in experimental autoimmune myocarditis (EAM) in mice. In the current study, we evaluated two methods for the enumeration and phenotypic characterization of myhca-specific $CD4^+$ T cells during the course of EAM. Both enzyme-linked immunospot (ELISPOT) and cytokine flow cytometry (CFC) assays were suitable for the detection and characterization of myhca-specific Th cells during acute myocardial inflammation and the late healing phase of the disease. Cytokine production of myhca-specific Th cells was restricted to interferon-γ (IFNγ). Only trace amounts of the Th2 cytokines IL-4 and IL-5 could be detected. Concomitant surface marker analysis in the CFC assay revealed the prototypical effector phenotype of myhca-specific Th1 cells during the acute phase of the disease. Taken together, the combination of both methods appears to be most appropriate for a comprehensive ex vivo single cell analysis of Th cells in heart-specific autoimmune disorders.

4.312 Primary defect in UVB-induced systemic immunomodulation does not relate to immature or functionally impaired APCs in regional lymph nodes

Gorman, S. et al

*J. Immunol., **174**, 6677-6685 (2005)*

UVB irradiation of the shaved dorsal skin of mice can cause both local and systemic suppression of contact hypersensitivity responses; the former demonstrated by administration of the sensitizing Ag/hapten to the irradiated site and the latter by its administration at least 72 h later to distal unirradiated sites. The immunological basis of systemic immunomodulation is not clear. When haptens (trinitrochlorobenzene, FITC) were administered to the shaved ventral skin 4 days after irradiation (8 kJ/m^2) to the shaved dorsum of BALB/c mice, $CD11c^+/FITC^+$ cells in the skin-draining lymph nodes from control and irradiated mice produced on a per cell basis similar levels of IL-12 and PGE_2 were phenotypically mature and efficient at presenting FITC to lymphocytes from FITC-sensitized mice. Ag presentation by FACS-sorted $CD11c^+$ lymph node cells isolated 4 days after UVB irradiation was as efficient as were cells from unirradiated mice at presentation in vitro of an OVA peptide ($OVA_{323–339}$) to $CD4^+$ cells from OVA-TCR-transgenic DO11.10 mice. Further, IFN-γ levels were increased in the cultures containing $CD11c^+$ cells from UVB-irradiated mice, suggesting that inflammation may precede downstream immunosuppression. These results suggest that the primary cause of reduced contact hypersensitivity responses in mice in which UV irradiation and the sensitizing Ag are applied to different sites several days apart must originate from cells other than $CD11c^+$ APCs that directly or by production of soluble mediators (IL-12, PGE_2) affect cellular responses in the nodes of UVB-irradiated mice.

4.313 Identification of a cell population that produces alpha/beta interferon in vitro and in vivo in response to noncytopathic bovine viral diarrhea virus

Brackenbury, L.S. et al

*J.Virol., **79(12)**, 7738-7744 (2005)*

In vitro infection of bovine cells of many origins with the cytopathogenic bovine viral diarrhea virus (cpBVDV) results in the induction of alpha/beta interferon (IFN-α/ß), whereas noncytopathogenic BVDV (ncpBVDV) isolates have been shown not to induce IFN-α/ß in vitro. Similarly, cpBVDV induces IFN-α/ß in the early bovine fetus, but ncpBVDV does not. However, acute infection of naïve cattle with ncpBVDV results in IFN-α/ß production. In this study, we identified and characterized a minor population of cells, present in lymph nodes that produce IFN-α in response to ncpBVDV. These cells expressed the myeloid markers CD14, CD11b, and CD172a but did not express CD4 and CD45RB. We also established that these cells produced IFN-α in the absence of detectable productive infection.

4.314 Islet transplantation: progress and challenge
Gaglia, J.L., Shapiro, A.M.J. and Weir, G.C.
Arch. Med. Res., ***36***, *273-280 (2005)*

For over 30 years, investigators have explored islet transplantation as a logical approach to restoring glucose homeostasis in persons with diabetes. Islet transplantation can currently provide improved glycemic control, relief from recurrent severe hypoglycemia, and potentially insulin independence. In this review, we describe details of the evolution of modern islet transplantation and provide insight into ongoing clinical and basic research efforts to overcome current obstacles for this promising therapy.

4.315 Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells
Magalhaes, A. C. Et al
J. Neurosci., ***25(21)***, *5207-5216 (2005)*

Invasion of the nervous system and neuronal spread of infection are critical, but poorly understood, steps in the pathogenesis of transmissible spongiform encephalopathies or prion diseases. To characterize pathways for the uptake and intraneuronal trafficking of infectious, protease-resistant prion protein (PrP-res), fluorescent-labeled PrP-res was used to infect a neuronally derived murine cell line (SN56) and adult hamster cortical neurons in primary culture. Concurrent with the establishment of persistent scrapie infection, SN56 cells internalized PrP-res aggregates into vesicles positive for markers for late endosomes and/or lysosomes but not synaptic, early endocytic, or raft-derived vesicles. Internalized PrP-res was then transported along neurites to points of contact with other cells. Similar trafficking was observed with dextran, Alzheimer's Aβ1-42 fibrils and noninfectious recombinant PrP fibrils, suggesting that PrP-res is internalized by a relatively nonspecific pinocytosis or transcytosis mechanism. Hamster cortical neurons were also capable of internalizing and disseminating exogenous PrP-res. Similar trafficking of exogenous PrP-res by cortical neurons cultured from the brains of PrP knock-out mice showed that uptake and neuritic transport did not require the presence of endogenous cellular PrP. These experiments visualize and characterize the initial steps associated with prion infection and transport within neuronal cells.

4.316 Increased monocyte transcription of the proteinase 3 gene in small vessel vasculitis
Ohlsson, S. et al
Clin. Exp. Immunol., ***141***, *174-182 (2005)*

Proteinase 3 (PR3) is a pleiotropic and destructive serine protease and it is also a major target for autoantibodies in systemic small vessel vasculitis. We have shown recently that patients in stable remission have increased circulating levels of PR3, independent of autoantibody titre, inflammation, neutrophil degranulation and renal function. Here we explore the possibility of increased PR3 gene transcription. RNA was purified from peripheral blood monocytes from vasculitis patients and controls. Specific mRNA was measured by *Taq*Man real-time polymerase chain reaction (PCR). The monocyte-like cell lines THP-1 and U937 and human peripheral blod monocytes from healthy controls were stimulated with cytokines and lipopolysaccharide (LPS) for different time periods. PR3 protein was measured in plasma with enzyme-linked immunosorbent assay (ELISA). The median result for PR3 mRNA was 9·6 (1·8–680) for 22 patients, compared to 1 (0·1–2·8) for the 15 healthy controls. Elastase expression was also significantly increased, whereas myeloperoxidase and interleukin-8 were not. Stimulation of monocytes with tumour necrosis factor (TNF)-α, interferon (IFN)-γ or LPS did not result in any increase of PR3 or elastase transcription, whereas interleukin (IL)-8 transcription was increased 10-fold. Circulating monocytes from patients with systemic vasculitis display increased PR3 gene transcription compared to healthy controls and patients with sytemic lupus erythematosus (SLE). This may be important for the development of vasculitis. Our results do not favour a role for cytokines, antineutrophil cytoplasmic antibodies (ANCA) or immunosuppressive medication in the upregulation of PR3 transcription in vasculitis.

4.317 Improved success of myoblast transplantation in *mdx* mice by blocking the myostatin signal
Benabdallah, B.F., Bouchentouf, M. and Tremblay, J.P.
Transplantation, ***79(12)***, *1696-1702 (2005)*

Background. Duchenne muscular dystrophy (DMD) is caused by a dystrophin gene mutation. Transplantation of normal myoblasts results in long-term restoration of dystrophin. However, the success of this approach is compromised by the limited time of regeneration following muscle damage. Myostatin is known to be responsible for limiting skeletal muscle regeneration. Our purpose is to verify whether blocking the myostatin signal in mdx host mice or in normal myoblasts transplanted in mdx host mice

would increase the extent of muscle repair and thus allow the formation of more dystrophin-positive fibers.
Methods. Transgenic mdx mice carrying a dominant negative form of myostatin receptor (dnActRIIB) were used to test the fiber resistance to damage and to act as a host for normal myoblast transplantation. Myoblasts obtained from nondystrophic transgenic mice carrying the dominant negative myostatin receptor were also transplanted in nontransgenic mdx mice.
Results. Transgenic mdx mice carrying the dnActRIIB gene have bigger muscles than mdx mice with the normal gene of ActRIIB. Their fiber resistance to exercise-induced damage was also greatly improved. Moreover, the success of normal myoblast transplantation was significantly enhanced in mdx/dnActRIIB mice. Finally, nondystrophic dnActRIIB myoblasts formed more abundant and bigger dystrophin positive fibers when transplanted in mdx mice.
Conclusions. Blocking the myostatin signal in mdx mice allowed the size of muscle fibers to increase, the fiber resistance to damage induced by exercise to increase, and the success of normal myoblast transplantation to improve. The transplantation in mdx mice of dnActRIIB myoblasts formed more abundant and larger dystrophin positive fibers.

4.318 Different platelet activation levels in non-pregnant, normotensive pregnant, pregnancy-induced hypertensive and pre-eclamptic women. A pilot study of flow cytometric analysis
Bagamery, K. and Landau, R.
*Eur. J. Obstet. Gynecol. Reprod. Biol., **121**, 117-123 (2005)*

No abstract available

4.319 Molecular epidemiology biomarkers – sample collection and processing considerations
Holland, N.T., Pfleger, L., Berger, E., Ho, A. and Bastaki, M.
*Tox. Appl. Pharmacol., **206**, 261-268 (2005)*

Biomarker studies require processing and storage of numerous biological samples with the goals of obtaining a large amount of information and minimizing future research costs. An efficient study design includes provisions for processing of the original samples, such as cryopreservation, DNA isolation, and preparation of specimens for exposure assessment. Use of standard, two-dimensional and nanobarcodes and customized electronic databases assure efficient management of large sample collections and tracking results of data analyses. Standard operating procedures and quality control plans help to protect sample quality and to assure validity of the biomarker data. Specific state, federal and international regulations are in place regarding research with human samples, governing areas including custody, safety of handling, and transport of human samples. Appropriate informed consent must be obtained from the study subjects prior to sample collection and confidentiality of results maintained. Finally, examples of three biorepositories of different scale (European Cancer Study, National Cancer Institute and School of Public Health Biorepository, University of California, Berkeley) are used to illustrate challenges faced by investigators and the ways to overcome them. New software and biorepository technologies are being developed by many companies that will help to bring biological banking to a new level required by molecular epidemiology of the 21st century.

4.320 Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells
Basu, S. and Srivastava, P.
*PNAS, **102(14)**, 5120-5125 (2005)*

Capsaicin (CP), the pungent component of chili pepper, acts on sensory neurons to convey the sensation of pain. The CP receptor, vanilloid receptor 1 (VR1), has been shown to be highly expressed by nociceptive neurons in dorsal root and trigeminal ganglia. We demonstrate here that the dendritic cell (DC), a key cell type of the vertebrate immune system, expresses VR1. Engagement of VR1 on immature DCs such as by treatment with CP leads to maturation of DCs as measured by up-regulation of antigen-presenting and costimulatory molecules. This effect is present in DCs of $VR1^{+/+}$ but not $VR1^{-/-}$ mice. In $VR1^{+/+}$ mice, this effect is inhibited by the VR1 antagonist capsazepine. Further, intradermal administration of CP leads to migration of DCs to the draining lymph nodes in $VR1^{+/+}$ but not $VR1^{-/-}$ mice. These data demonstrate a powerful influence of a neuroactive ligand on a central aspect of immune function and a commonality of mechanistic pathways between neural and immune functions.

4.321 Debrin E2 is differentially expressed and phosphorylated in paretal cells in the gastric mucosa
Chew, C.S., Okamoto, C.T., Chen, X. and Thomas, R.
*Am. J. Physiol., **289**, G320-G331 (2005)*

Developmentally regulated brain proteins (drebrins) are highly expressed in brain where they may regulate actin filament formation in dendritic spines. Recently, the drebrin E2 isoform was detected in certain epithelial cell types including the gastric parietal cell. In gastric parietal cells, activation of HCl secretion is correlated with actin filament formation and elongation within intracellular canaliculi, which are the sites of acid secretion. The aim of this study was to define the pattern of drebrin expression in gland units in the intact rabbit oxyntic gastric mucosa and to initiate approaches to define the functions of this protein in parietal cells. Drebrin E2 expression was limited entirely or almost entirely to parietal cells and depended upon the localization of parietal cells along the gland axis. Rabbit drebrin E2 was cloned and found to share 86% identity with human drebrin 1a and to possess a number of cross-species conserved protein-protein interaction and phosphorylation consensus sites. Two-dimensional Western blot and phosphoaffinity column analyses confirmed that drebrin is phosphorylated in parietal cells, and several candidate phosphorylation sites were identified by mass spectrometry. Overexpression of epitope-tagged drebrin E2 led to the formation of microspikes and F-actin-rich ring-like structures in cultured parietal cells and suppressed cAMP-dependent acid secretory responses. In Madin-Darby canine kidney cells, coexpression of epitope-tagged drebrin and the Rho family GTPase Cdc42, which induces filopodial extension, produced an additive increase in the length of microspike projections. Coexpression of dominant negative Cdc42 with drebrin E2 did not prevent drebrin-induced microspike formation. These findings suggest that *1*) drebrin can induce the formation of F-actin-rich membrane projections by Cdc42-dependent and -independent mechanisms; and that *2*) drebrin plays an active role in directing the secretagogue-dependent formation of F-actin-rich filaments on the parietal cell canalicular membrane. Finally, the differential distribution of drebrin in parietal cells along the gland axis suggests that drebrin E2 may be an important marker of parietal cell differentiation and functionality.

4.322 Mitochondrial nitric oxide mediates decreased vulnerability of hippocampal neurons from immature animals to NMDA

Marks, J.D., Boriboun, C. and Wang, J.

*J. Neurosci., **25(28)**, 6561-6575 (2005)*

Mitochondrial membrane potential ($\Delta\Psi_m$)-dependent Ca^{2+} uptake plays a central role in neurodegeneration after NMDA receptor activation. NMDA-induced $\Delta\Psi_m$ dissipation increases during postnatal development, coincident with increasing vulnerability to NMDA. NMDA receptor activation also produces nitric oxide (NO), which can inhibit mitochondrial respiration, dissipating $\Delta\Psi_m$. Because $\Delta\Psi_m$ dissipation reduces mitochondrial Ca^{2+} uptake, we hypothesized that NO mediates the NMDA-induced $\Delta\Psi_m$ dissipation in immature neurons, underlying their decreased vulnerability to excitotoxicity. Using hippocampal neurons cultured from 5- and 19-d-old rats, we measured NMDA-induced changes in $[Ca^{2+}]_{cytosol}$, $\Delta\Psi_m$, NO, and $[Ca^{2+}]_{mito}$. In postnatal day 5 (P5) neurons, NMDA mildly dissipated $\Delta\Psi_m$ in a NO synthase (NOS)-dependent manner and increased NO. The NMDA-induced NO increase was abolished with carbonyl cyanide 4-(trifluoromethoxy)phenyl-hydrazone and regulated by $[Ca^{2+}]_{mito}$. Mitochondrial Ca^{2+} uptake inhibition prevented the NO increase, whereas inhibition of mitochondrial Ca^{2+} extrusion increased it. Consistent with this mitochondrial regulation, NOS and cytochrome oxidase immunoreactivity demonstrated mitochondrial localization of NOS. Furthermore, NOS blockade increased mitochondrial Ca^{2+} uptake during NMDA. Finally, at physiologic O_2 tensions (3% O_2), NMDA had little effect on survival of P5 neurons, but NOS blockade during NMDA markedly worsened survival, demonstrating marked neuroprotection by mitochondrial NO. In P19 neurons, NMDA dissipated $\Delta\Psi_m$ in an NO-insensitive manner. NMDA-induced NO production was not regulated by $\Delta\Psi_m$, and NOS immunoreactivity was cytosolic, without mitochondrial localization. NOS blockade also protected P19 neurons from NMDA. These data demonstrate that mitochondrial NOS mediates much of the decreased vulnerability to NMDA in immature hippocampal neurons and that cytosolic NOS contributes to NMDA toxicity in mature neurons.

4.323 Novel sulfasalazine analogues with enhanced NF-kB inhibitory and apoptosis promoting activity

Habens, F. Et al

*Apoptosis, **10**, 481-491 (2005)*

The NF-kB transcription factor plays a key role in the regulation of apoptosis by modulating expression of a wide range of cell death control molecules. NF-kB also plays an important role in human diseases by promoting inappropriate cell survival. Small molecule inhibitors of NF-kB are therefore likely to provide novel therapeutic opportunities. Sulfasalazine (SFZ) is a synthetic anti-inflammatory comprising an aminosalicylate, 5-amino salicylic acid (5-ASA), linked to an antibiotic, sulfapyridine (SPY). SFZ, but not

5-ASA or SPY, inhibits activation of NF-kB. We synthesised a small number of SFZ analogues and determined their ability to inhibit NF-kB activity and promote apoptosis in chronic lymphocytic leukaemia and hepatic stellate cells, where NF-kB plays an important role in cell survival. Remarkably, 3 of the 6 analogues synthesised were significantly more effective (up to 8-fold) inhibitors of NF-kB dependent transcription and this increased activity was associated with enhanced apoptosis. Therefore, it is possible to readily improve the NF-kB inhibiting activity of SFZ and analogues of SFZ may be attractive therapeutic agents for malignancies and chronic liver disease where NF-kB is thought to play a significant role.

4.324 Blockade of B7-H1 on macrophages suppresses $CD4^+$ T cell proliferation by augmenting IFN-γ-induced nitric oxide production
Yamazaki, T. et al
*J. Immunol., **175**, 1586-1592 (2005)*

PD-1 is an immunoinhibitory receptor that belongs to the CD28/CTLA-4 family. B7-H1 (PD-L1) and B7-DC (PD-L2), which belong to the B7 family, have been identified as ligands for PD-1. Paradoxically, it has been reported that both B7-H1 and B7-DC costimulate or inhibit T cell proliferation and cytokine production. To determine the role of B7-H1 and B7-DC in T cell-APC interactions, we examined the contribution of B7-H1 and B7-DC to $CD4^+$ T cell activation by B cells, dendritic cells, and macrophages using anti-B7-H1, anti-B7-DC, and anti-PD-1 blocking mAbs. Anti-B7-H1 mAb and its Fab markedly inhibited the proliferation of anti-CD3-stimulated naive $CD4^+$ T cells, but enhanced IL-2 and IFN-γ production in the presence of macrophages. The inhibition of T cell proliferation by anti-B7-H1 mAb was abolished by neutralizing anti-IFN-γ mAb. Coculture of $CD4^+$ T cells and macrophages from IFN-γ-deficient or wild-type mice showed that $CD4^+$ T cell-derived IFN-γ was mainly responsible for the inhibition of $CD4^+$ T cell proliferation. Anti-B7-H1 mAb induced IFN-γ-mediated production of NO by macrophages, and inducible NO synthase inhibitors abrogated the inhibition of $CD4^+$ T cell proliferation by anti-B7-H1 mAb. These results indicated that the inhibition of T cell proliferation by anti-B7-H1 mAb was due to enhanced IFN-γ production, which augmented NO production by macrophages, suggesting a critical role for B7-H1 on macrophages in regulating IFN-γ production by naive $CD4^+$ T cells and, hence, NO production by macrophages.

4.325 TLR-4 regulates $CD8^+$ T cell trapping in the liver
John, B. and Crispe, I.N.
*J. Immunol., **175,** 1643-1650 (2005)*

Mammalian TLRs are understood primarily as an activating system for innate and adaptive immunity, but have also been implicated in sensing cellular damage and in promoting intestinal integrity. In this study we show that TLR-4 also controls the in vivo distribution of activated $CD8^+$ T cells. The liver is a site for trapping and apoptosis of activated $CD8^+$ T cells during systemic immune responses, but the reason for this is unknown. In this study we tested the hypothesis that the liver's constant exposure to endotoxin, derived from commensal bacteria in the gut, acts via TLR-4 to promote activated T cell adhesion. In the absence of TLR-4, the liver was compromised in its ability to sequester activated $CD8^+$ T cells, and there was an inverse correlation between the frequency of activated $CD8^+$ T cells trapped in the liver and their frequency in the circulating pool. Thus, in the absence of any inflammation, TLR-4 ligands play a significant role in the ability of the liver to trap activated $CD8^+$ T cells. This provides a new perspective on the regulation of immune responses by TLR-4 under basal conditions.

4.326 Inhibition of inhibitor of κB kinases stimulates hepatic stellate cell apoptosis and accelerated recovery from rat liver fibrosis
Oakley, F. et al
*Gastroenterol., **128**, 108-120 (2005)*

Background & Aims: Resolution of liver fibrosis is associated with clearance of hepatic myofibroblasts by apoptosis; development of strategies that promote this process in a selective way is therefore important. The aim of this study was to determine whether the inhibitor of κB kinase suppresser sulfasalazine stimulates hepatic myofibroblast apoptosis and recovery from fibrosis. *Methods:* Hepatic myofibroblasts were generated by culture activation of rat and human hepatic stellate cells. Fibrosis was established in rat livers by chronic injury with carbon tetrachloride followed by recovery with or without sulfasalazine (150 mg/kg) treatment. *Results:* Treatment of hepatic stellate cells with sulfasalazine (0.5–2.0 mmol/L) induced apoptosis of activated rat and human hepatic stellate cells. A single in vivo administration of sulfasalazine promoted accelerated recovery from fibrosis as assessed by improved fibrosis score, selective clearance of

smooth muscle α-actin-positive myofibroblasts, reduced hepatic procollagen I and tissue inhibitor of metalloproteinase 1 messenger RNA expression, and increased matrix metalloproteinase 2 activity. Mechanistic studies showed that sulfasalazine selectively blocks nuclear factor-κB-dependent gene transcription, inhibits hepatic stellate cell expression of Gadd45β, stimulates phosphorylation of Jun N-terminal kinase 2, and promotes apoptosis by a mechanism that is prevented by the Jun N-terminal kinase inhibitor SP600125. As further evidence for a survival role for the inhibitor of κB kinase/nuclear factor-κB pathway in activated hepatic stellate cells, a highly selective cell-permeable peptide inhibitor of κB kinase activation also stimulated hepatic stellate cell apoptosis via a Jun N-terminal kinase-dependent mechanism. *Conclusions:* Inhibition of the inhibitor of κB kinase/nuclear factor-κB pathway is sufficient to increase the rate at which activated hepatic stellate cells undergo apoptosis both in vitro and in vivo, and drugs that selectively target inhibitor of κB kinase have potential as antifibrotics.

4.327 Dendritic cells acquire tolerogenic properties at the site of sterile granulomatous inflammation
Vasilijic, S. et al
Cell. Immunol., ***233****, 148-157 (2005)*

Subcutaneous implantation of polyvinyl sponges represents a suitable model for studying the mechanisms of acute and chronic inflammation, granulomatous foreign-body reaction, as well as wound healing. Using such a model in rats, we studied the phenotypic and functional characteristics of dendritic cells (DC). DC were purified from the sponge exudate using a combination of separation gradients, adherence to plastics, and immunomagnetic sorting. We have shown that the number of DC progressively increased in the sponges, reaching maximal values at day 10 after implantation, followed by their decrease thereafter. Inflammatory DC expressed MHC class II molecules and myeloid markers CD11b, CD11c, and CD68. A subset of DC expressed CD4, R-MC46, DEC-205, R-MC17, and CCR1. Compared to DC isolated in the early phase of inflammation (day 6 DC), DC in the late stage of inflammation (day 14 DC) had a lower capability to stimulate the proliferation of allogeneic lymphocytes and $CD4^+$ T cells. This finding correlated with the downregulation of CD80, CD86, and CD54 expression and the increased proportion of plasmacytoid MHC class II^+ His 24^+ His 48^+ DC. The suppression of allogeneic lymphocyte proliferation was abrogated by the treatment of DC with lipopolysaccharide. In addition, day 14 DC exerted tolerogenic capability in co-culture with allogenic $CD4^+$ T cells. These results correlated with the increased levels of IL-10 and TGF-β in culture supernatants and the sponge exudate.

4.328 Characteristics of poly-L-ornithine-coated alginate microcapsules
Darrabie, M.D., Kendall, W.F. and Opara, E.C.
Biomaterials, ***26****, 6846-6852 (2005)*

Poly-L-Lysine (PLL) is the most widely used biomaterial for providing perm-selectivity in alginate microcapsules for islet transplantation. We had previously reported that Poly-L-Ornithine (PLO) is less immunogenic than PLL, and in the present study, we have compared the physical characteristics of PLO- and PLL-coated hollow alginate microcapsules. Microspheres made with 1.5% alginate were divided into 2 groups that were first coated with either 0.1% PLO or PLL, followed by a second coating with 0.25% alginate. After liquefaction of the inner alginate core with sodium citrate, the microcapsules were washed with saline and used for experiments. Pore size exclusion studies were performed with FITC-labeled lectins incubated with encapsulated pig islets followed by examination for fluorescence activity. Mechanical strength was assessed by an osmotic pressure test and by 36 h of mechanical agitation of microcapsules with inert soda lime beads. The pore size exclusion limit of microcapsules after 20 min of coating was significantly smaller with PLO. While the mean±SEM diameter of PLL-coated microcapsules increased from 718±17 to 821±17 μm ($p<0.05$) during 14 days incubation at 37 °C, the PLO group did not change in size. Also, PLL group had a higher percentage of broken capsules (52.7±4.9%) compared to 3.1±2.05% for PLO capsules ($p<0.0001, n=6$). We conclude that PLO-coated alginate microcapsules are mechanically stronger and provide better perm-selectivity than PLL-coated microcapsules.

4.329 Microglia Kv1.3 channels contribute to their ability to kill neurons
Fordyce, C.B., Jagasia, R., Zhu, X. and Schlichter, L.C.
J. Neurosci., ***25(31)****, 7139-7149 (2005)*

Many CNS disorders involve an inflammatory response that is orchestrated by cells of the innate immune system: macrophages, neutrophils, and microglia (the endogenous CNS immune cell). Hence, there is considerable interest in anti-inflammatory strategies that target these cells. Microglia express Kv1.3 (KCNA3) channels, which we showed previously are important for their proliferation and the NADPH-

mediated respiratory burst. Here, we demonstrate the potential for targeting Kv1.3 channels to control CNS inflammation. Rat microglia express Kv1.2, Kv1.3, and Kv1.5 transcripts and protein, but only a Kv1.3 current was detected. When microglia were activated with lipopolysaccharide or a phorbol ester, only the Kv1.3 transcript (but not protein) expression changed. Using a Transwell cell-culture system that allows separate drug treatment of microglia or neurons, we found that activated microglia killed postnatal hippocampal neurons through a process that requires Kv1.3 channel activity in microglia but not in neurons. A major neurotoxic molecule in this model was peroxynitrite, which is formed from superoxide and nitric oxide; thus, it is significant that Kv1.3 channel blockers reduced the respiratory burst, but not nitric oxide production, by the activated microglia. In addressing the biochemical pathway affected by Kv1.3 channel activity, we found that Kv1.3 acts via a different cellular mechanism from the broad-spectrum drug minocycline, which is often used in animal models of neuroinflammation. That is, the dose-dependent reduction in neuron killing by minocycline corresponded with a reduction in p38 mitogen-activated protein kinase activation in microglia; however, none of the Kv1.3 blockers affected p38 activation.

4.330 **Agrin promotes synaptic differentiation by counteracting an inhibitory effect of neurotransmitter**
Misgeld, T., Kummer, T.T., Lichtman, J.W. and Sanes, J.R.
*PNAS, **102(31)**, 11088-11093 (2005)*

Synaptic organizing molecules and neurotransmission regulate synapse development. Here, we use the skeletal neuromuscular junction to assess the interdependence of effects evoked by an essential synaptic organizing protein, agrin, and the neuromuscular transmitter, acetylcholine (ACh). Mice lacking agrin fail to maintain neuromuscular junctions, whereas neuromuscular synapses differentiate extensively in the absence of ACh. We now demonstrate that agrin's action *in vivo* depends critically on cholinergic neurotransmission. Using double-mutant mice, we show that synapses do form in the absence of agrin provided that ACh is also absent. We provide evidence that ACh destabilizes nascent postsynaptic sites, and that one major physiological role of agrin is to counteract this "antisynaptogenic" influence. Similar interactions between neurotransmitters and synaptic organizing molecules may operate at synapses in the central nervous system.

4.331 **Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth**
Benson, M.D. et al
*PNAS, **102(30)**, 10694-10699 (2005)*

The inability of CNS axons to regenerate after traumatic spinal cord injury is due, in part, to the inhibitory effects of myelin. The three major previously identified constituents of this activity (Nogo, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein) were isolated based on their potent inhibition of axon outgrowth *in vitro*. All three myelin components transduce their inhibitory signals through the same Nogo receptor/p75 neurotrophin receptor/LINGO-1 (NgR1/p75/LINGO-1) complex. In this study, we considered that molecules known to act as repellants in vertebrate embryonic axonal pathfinding may also inhibit regeneration. In mice, ephrin-B3 functions during development as a midline repellant for axons of the corticospinal tract. We therefore investigated whether this repellant was expressed in the adult spinal cord and retained inhibitory activity. We demonstrate that ephrin-B3 is expressed in postnatal myelinating oligodendrocytes and, by using primary CNS neurons, show that ephrin-B3 accounts for an inhibitory activity equivalent to that of the other three myelin-based inhibitors, acting through p75, combined. Our data describe a known vertebrate axon guidance molecule as a myelin-based inhibitor of neurite outgrowth.

4.332 **Production and characterization of monoclonal antibodies against *Enterocytozoon bieneusi* purified from rhesus macaques**
Zhang, Q. et al
*Infect. Immun., **73(8)**, 5166-5172 (2005)*

Enterocytozoon bieneusi spores derived from rhesus macaque feces were purified by serial salt-Percoll-sucrose-**iodixanol** centrifugation, resulting in two bands with different specific densities of 95.6% and 99.5% purity and with a recovery efficiency of 10.8%. An ultrastructural examination revealed typical *E. bieneusi* spores. Twenty-six stable hybridomas were derived from BALB/c mice immunized with spores and were cloned twice by limiting dilution or growth on semisolid medium. Four monoclonal antibodies (MAbs), reacting exclusively with spores, were further characterized. These MAbs specifically reacted with spores present in stools of humans and macaques, as visualized by immunofluorescence, and with spore walls, as visualized by immunoelectron microscopy. A blocking enzyme-linked immunosorbent

assay and Western blotting revealed that the epitope recognized by 8E2 was different from those recognized by 7G2, 7H2, and 12G8, which identified the same 40-kDa protein. These MAbs will be valuable tools for diagnostics, for epidemiological investigations, for host-pathogen interaction studies, and for comparative genomics and proteomics.

4.333 Production of pro- and anti-fibrotic agents by rat kupffer cells; the effect of octreotide
Xidakis, C. et al
Digest. Dis. Sci., ***50(5)***, *935-941 (2005)*

Kupffer cells may be involved in liver fibrogenesis through production of TGF-1. Their role in fibrinolysis is less clear. Octreotide, a synthetic analogue of somatostatin, is often used in cirrhotic patients. Its effect on Kupffer cells was studied. Isolated rat Kupffer cells were cultured in the presence of lipopolysaccharide and/or octreotide. TGF-1, leptin, collagenase (MMP-1), and urokinase-type plasminogen activator (uPA) were assessed in supernatants by ELISA, and MMP-2 and MMP-9 by zymography. Kupffer cells produced large amounts of MMP-1 and lipopolysaccharide induced a significant ($P < 0.02$) early increase. Octreotide and lipopolysaccharide caused a synergistic effect on MMP-1 secretion. By contrast, MMP-9 production stimulated by lipopolysaccharide was suppressed by octreotide. Kupffer cells produced a basal amount of uPA, significantly increased after lipopolysaccharide or octreotide incubation ($P < 0.001$). Large amounts of TGF-1 were produced in a time-dependent manner by unstimulated Kupffer cells. Lipopolysaccharide and octreotide, alone or in combination, induced a significant inhibition of this production ($P < 0.01$). Kupffer cells did not produce leptin, a recently identified mediator of liver fibrosis, or MMP-2. Kupffer cells may play a significant role in liver fibrinolysis. Octreotide, acting on TGF-1, uPA, and MMP-1 production, may be a useful agent for fibrosis resolution.

4.334 Bone marrow is a major reservoir and site of recruitment for central memory $CD8^+$ T cells
Mazo, I.B: et al
Immunity, ***22***, *259-270 (2005)*

Normal bone marrow (BM) contains T cells whose function and origin are poorly understood. We observed that $CD8^+$ T cells in BM consist chiefly of $CCR7^+$ L-$selectin^+$ central memory cells (T_{CM}s). Adoptively transferred T_{CM}s accumulated more efficiently in the BM than naive and effector T cells. Intravital microscopy (IVM) showed that T_{CM}s roll efficiently in BM microvessels via L-, P-, and E-selectin, whereas firm arrest required the VCAM-1/α4β1 pathway. α4β1 integrin activation did not depend on pertussis toxin (PTX)-sensitive Gαi proteins but was reduced by anti-CXCL12. In contrast, T_{CM} diapedesis did not require CXCL12 but was blocked by PTX. After extravasation, T_{CM}s displayed agile movement within BM cavities, remained viable, and mounted potent antigen-specific recall responses for at least two months. Thus, the BM functions as a major reservoir for T_{CM}s by providing specific recruitment signals that act in sequence to mediate the constitutive recruitment of T_{CM}s from the blood.

4.335 Pancreatic islet transplantation using non-heart-beating donors (NHBDs)
Matsumoto, S. and Tanaka, K.
J. Hepatobiliary Pancreat. Surg., ***12***, *227-230 (2005)*

Recent dramatic improvements in clinical islet cell transplantation demonstrated by the Edmonton group have increased the demand for this treatment, and donor shortage could become a major problem. Utilization of marginal donors could alleviate the donor shortage, and non-heart-beating donors (NHBDs) might be good resources. The University of Pennsylvania group demonstrated that it was possible to isolate islets from NHBDs, and the group actually transplanted islets from NHBDs, for the first time. The patient became insulin-independent; however, there had been no more cases using NHBDs until our group initiated islet transplantations from NHBDs in Japan. In order to utilize NHBDs effectively, we modified the standard islet isolation method. These modifications included minimizing the warm ischemic time, the use of trypsin inhibition during isolation, carrying out density measurement before purification and the use of a less toxic islet purification solution. With these modifications we were able to transplant nine of ten islet preparations from ten NHBDs (90%), into five type-1 diabetic patients. The first transplantation was performed on April 7, 2004 (the first time in Japan), and this patient became insulin-independent after the second islet transplantation (first time in Japan). All patients showed improved glycemic control and reduced insulin requirements, without hypoglycemic events. We also performed living-donor islet transplantation, with our modified islet isolation protocol, on January 19, 2005. The improved islet isolation protocol enabled us to perform effective islet transplantations from NHBDs, and it also enabled us to perform the living-donor islet transplantation.

4.336 Multiple isoforms of the KC1 cotransporter are expressed in sickle and normal erythroid cells
Crable, S.C. et al
*Exp. Hematol., **33**, 624-631 (2005)*

Objective
The KCl cotransporter (KCC) plays an important role in cellular cation and volume regulation and contributes to the process of volume reduction that accompanies reticulocyte maturation. In human red cells containing sickle hemoglobin, KCl cotransporter activity is high compared to normal cells, and contributes to the deleterious dehydration of sickle reticulocytes. To date, genes for four KCC isoforms have been identified. As a step toward determining which isoform(s) is responsible for the Cl-dependent K fluxes in reticulocytes, human erythroid cells were examined for the presence of various KCC isoform transcripts.
Methods
In vitro differentiated erythroid precursors, and reticulocytes isolated from normal individuals and sickle patients, were examined by reverse-transcriptase PCR for the expression of KCC isoforms. Transient transfection experiments were subsequently performed to characterize a novel KCC1 promoter.
Results
Expression of multiple isoforms was detected, with transcripts for KCC1, 3, and 4 detected in all samples of erythroid cells. Two N-terminal splicing variants were detected for both KCC1 and 3. Sickle hemoglobin containing reticulocytes demonstrated KCC isoform expression patterns similar to wild-type cells, except for a consistent difference in the relative abundance of one KCC1 splice variant. This N-terminal variant initiates from a newly described promoter in the KCC1 gene.
Conclusion
Three KCC genes are expressed in human red cells. Splicing variants arising from the KCC1 and 3 genes are also evident. Structure/function studies of mouse KCC1 suggest that these natural variants could profoundly affect overall cotransporter activity in the red cell.

4.337 Decreases in phosphoinositide-3-kinase/Akt and extracellular signal-regulated kinase ½ signaling activate components of spinal motoneuron death
Newbern, J., Taylor, A., Robinson, M., Li, L. and Milligan, C.E.
*J. Neurochem., **94**, 1652-1665 (2005)*

Motoneuron dependence on target-derived trophic factors during development is well established, with loss of trophic support leading to the death of these cells. A complete understanding of the intracellular signal transduction machinery associated with extracellular survival signals requires the examination of individual pathways in various cellular and environmental contexts. In cells deprived of trophic support, and hence compromised for survival, phosphoinositide-3-kinase (PI3K) is decreased when compared with healthy cells supplied with trophic support. Extracellular signal-regulated kinase 1/2 (ERK1/2) signaling is dramatically decreased in deprived cells. We have examined the role of these two pathways to understand how changes in their activity regulate motoneuron survival and death. Pharmacological inhibition of PI3K attenuated motoneuron survival and was important in the regulation of Bcl-2 serine phosphorylation, limited release of cytochrome *c* into the cytoplasm and caspase activation. Bax translocation from cytoplasm to mitochondria was not altered when PI3K was inhibited. High levels of ERK1/2 inhibition robustly attenuated motoneuron survival in cells supplied with trophic support, whereas moderate inhibition of ERK1/2 activation had little effect. ERK1/2 inhibition in these cells decreased Bcl-2 phosphorylation and resulted in release of cytochrome *c* from the mitochondria. Bax translocation and caspase activation were not affected by ERK1/2 inhibition. These data reveal that changes in PI3K and ERK1/2 signaling lead to individual and overlapping effects on the cell-death machinery. Characterizing the role of these pathways is critical for a fundamental understanding of the development and degeneration of specific neuronal populations.

4.338 Long-term preservation of high endocytic activity in primary cultures of pig liver sinusoidal endothelial cells
Elvevold, K., Nedredal, G.I., Revhaug, A., Bertheussen, K. And Smedsrød, B.
*Eur. J. Cell Biol., **84(9)**, 749-764 (2005)*

Together with Kupffer cells, liver sinusoidal endothelial cells (LSECs) constitute the most powerful scavenger system in the body. However, studies on LSEC function are hampered by the fact that the cells lose their scavenger ability and start deteriorating after a few days in culture. The purpose of the present

study was to improve the conditions of cultivation to prolong the survival of pig LSECs in vitro. We used the high capacity receptor-mediated endocytosis of soluble waste molecules as a marker for functionally intact cells in the cultures. Compared with two commercially-, and two other media specifically designed for use with either SECs or hepatocytes from rat, our newly developed serum-free medium, DM 110/SS, devoid of any components of animal origin, was superior in maintaining the endocytic activity. Of six growth factors studied for their effect on endocytosis, basic fibroblast, and recombinant epidermal, but not vascular endothelial growth factor, were found to be most beneficial. After 8 days in DM 110/SS, LSECs maintained endocytosis via the scavenger receptor, mannose receptor, collagen α-chain receptor and the Fc-γ receptor. All endocytosed ligands, except for aggregated IgG were degraded in 8-day-old cultures. Using the new medium, the cells endocytosed ligands for up to 20 days, and survived for at least an additional 10 days, albeit without the high endocytic activity typical of intact LSECs. Importantly, DNA synthesis in prolonged cultures of LSECs was observed only when maintained in DM 110/SS medium. In conclusion, we describe a protocol for the maintenance of LSECs in culture for the longest period yet reported.

4.339 GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyothropic lateral sclerosis

Van Damme, P., Braeken, D., Callewaert, G., Robberecht, W. and Van Den Bosch, L.
J. Neuropathol. Exp. Neurol., ***64(7)***, *605-612 (2005)*

AMPA receptor-mediated excitotoxicity has been implicated in the selective degeneration of motor neurons in amyotrophic lateral sclerosis (ALS). Motor neurons in vitro are particularly vulnerable to excessive AMPA receptor stimulation and one of the factors underlying this selective vulnerability is the presence of a large proportion of Ca2+-permeable (i.e. GluR2-lacking) AMPA receptors. However, the precise role of GluR2-lacking AMPA receptors in motor neuron degeneration remains to be defined. We therefore studied the impact of GluR2 deficiency on motor neuron death in vitro and in vivo. Cultured motor neurons from GluR2-deficient embryos displayed an increased Ca2+ influx through AMPA receptors and an increased vulnerability to AMPA receptor-mediated excitotoxicity. We deleted the GluR2 gene in mutant SOD1G93A mice by crossbreeding them with GluR2 knockout mice. GluR2 deficiency clearly accelerated the motor neuron degeneration and shortened the life span of mutant SOD1G93A mice. These findings indicate that GluR2 plays a pivotal role in the vulnerability of motor neurons in vitro and in vivo, and that therapies that limit Ca2+ entry through AMPA receptors might be beneficial in ALS patients.

4.340 Connective tissue growth factor (CCN2) in rat pancreatic stellate cell function: integrin $\alpha_5\beta_1$ as a novel CCN2 receptor

Gao, R. and Brigstock, D.R.
Gastroenterology, ***129***, *1019-1030 (2005)*

Background & Aims: Pancreatic stellate cells (PSCs) are proposed to play a key role in the development of pancreatic fibrosis. The aim of this study was to evaluate the production by rat activated PSCs of the fibrogenic protein, connective tissue growth factor (CCN2), and to determine the effects of CCN2 on PSC function. *Methods:* CCN2 production was evaluated by immunoprecipitation and promoter activity assays. Expression of integrin $\alpha_5\beta_1$ was examined by immunoprecipitation and Western blot. Binding between CCN2 and integrin $\alpha_5\beta_1$ was determined in cell-free systems. CCN2 was assessed for its stimulation of PSC adhesion, migration, proliferation, DNA synthesis, and collagen I synthesis. *Results:* CCN2 was produced by activated PSCs, and its levels were enhanced by transforming growth factor β1 treatment. CCN2 promoter activity was stimulated by transforming growth factor β1, platelet-derived growth factor, alcohol, or acetaldehyde. CCN2 stimulated integrin $\alpha_5\beta_1$–dependent adhesion, migration, and collagen I synthesis in PSCs. Integrin $\alpha_5\beta_1$ production by PSCs was verified by immunoprecipitation, while direct binding between integrin $\alpha_5\beta_1$ and CCN2 was confirmed in cell-free binding assays. Cell surface heparan sulfate proteoglycans functioned as a partner of integrin $\alpha_5\beta_1$ in regulating adhesion of PSCs to CCN2. PSC proliferation and DNA synthesis were enhanced by CCN2. *Conclusions:* PSCs synthesize CCN2 during activation and after stimulation by profibrogenic molecules. CCN2 regulates PSC function via cell surface integrin $\alpha_5\beta_1$ and heparan sulfate proteoglycan receptors. These data support a role for CCN2 in PSC-mediated fibrogenesis and highlight CCN2 and its receptors as potential novel therapeutic targets.

4.341 Developmental pluripotency of the nuclei of neurons in the cerebral cortex of juvenile mice

Osada, K. et al
J. Neurosci., ***25***, *8368-8374 (2005)*

Nuclei isolated from green fluorescent protein-marked neurons in the cerebral cortex of juvenile mice (14–21 d after birth) were injected into enucleated oocytes that were allowed to develop into blastocysts. Embryonic stem (ES) cell lines were established from the inner cell mass of 76 cloned blastocysts after injecting 2026 neuronal nuclei. Some ES cells were injected individually into enucleated oocytes (nuclear transfer). Other ES cells were transferred into the blastocoeles of tetraploid blastocysts (tetraploid complementation). Two-cell embryos after nuclear transfer were transferred to the oviducts of surrogate mothers. Four (1.5%) of 272 nuclear-transferred two-cell embryos developed to term, and two (0.7%) developed into fertile adults. Nineteen (1.9%) of 992 tetraploid blastocysts receiving ES cells reached term, and 10 (1.0%) developed into adults. These findings demonstrate that some of the nuclei of differentiated neurons in the cerebral cortex of juvenile mice maintain developmental pluripotency.

4.342 Exercise-induced oxidative stress leads hemolysis in sedentary but not trained humans
Sentürk, Ü.K. et al
*J. Appl. Physiol., **99**, 1434-1441 (2005)*

Intravascular hemolysis is one of the most emphasized mechanisms for destruction of erythrocytes during and after physical activity. Exercise-induced oxidative stress has been proposed among the different factors for explaining exercise-induced hemolysis. The validity of oxidative stress following exhaustive cycling exercise on erythrocyte damage was investigated in sedentary and trained subjects before and after antioxidant vitamin treatment (A, C, and E) for 2 mo. Exercise induced a significant increase in thiobarbituric acid-reactive substance and protein carbonyl content levels in sedentary subjects and resulted in an increase of osmotic fragility and decrease in deformability of erythrocytes, accompanied by signs for intravascular hemolysis (increase in plasma hemoglobin concentration and decrease in haptoglobulin levels). Administration of antioxidant vitamins for 2 mo prevented exercise-induced oxidative stress (thiobarbituric acid-reactive substance, protein carbonyl content) and deleterious effects of exhaustive exercise on erythrocytes in sedentary subjects. Trained subjects' erythrocyte responses to exercise were different from those of sedentary subjects before antioxidant vitamin treatment. Osmotic fragility and deformability of erythrocytes, plasma hemoglobin concentration, and haptoglobulin levels were not changed after exercise, although the increased oxidative stress was observed in trained subjects. After antioxidant vitamin treatment, functional and structural parameters of erythrocytes were not altered in the trained group, but exercise-induced oxidative stress was prevented. Increased percentage of young erythrocyte populations was determined in trained subjects by density separation of erythrocytes. These findings suggest that the exercise-induced oxidative stress may contribute to exercise-induced hemolysis in sedentary humans.

4.343 *Helicobacter pylori* outer membrane protein 18 *(Hp1125)* induces dendritic cell maturation and function
Rathinavelu, S., Kao, J.Y., Zavros, Y. and Merchant, J.L.
*Helicobacter, **10(5)**, 424-432 (2005)*

Background. Dendritic cells (DCs) are potent antigen-presenting cells that initiate T-cell responses. A robust adaptive Th1 immune response is crucial to an adaptive (Th2) immune response necessary for vaccine-induced protective immunity against *Helicobacter pylori.* It has been shown that several outer membrane proteins (Omps) induce a robust antibody response. However, it is also known that the antibodies generated are not protective. Moreover there is great variation in the recognition of high molecular weight *H. pylori* proteins by sera from infected patients. In contrast to the high molecular weight proteins, serologic responses to small molecular weight proteins provide assessment of current infection with *H. pylori* and also of its eradication.
Aim. The goal of the study was to analyze the activation of the immune response by a specific low molecular weight Omp that is universally expressed by all *H. pylori* strains. Therefore, we studied interaction of *H. pylori* Omp18 with DCs.
Methods. Activation of murine bone marrow-derived DCs and production of cytokines by Omp18 was assessed by fluorescence-activated cell sorter (FACS) for costimulatory markers and ELISA, respectively. The ability of Omp18 stimulated DCs to induce lymphocyte proliferation was measured in a mixed leukocyte reaction.
Results. Omp18 induced higher expression of the B7 (CD80 and CD86) costimulatory molecule after 18 hours indicating processing and presentation of the antigen on the surface by bone marrow-derived DCs. The maturing DCs also secreted significant levels of IL-12, but was 4-fold less than that stimulated by whole bacteria. Omp18-primed DCs induced proliferation and release of IFNγ by syngeneic splenocytes.
Conclusion. We concluded that Omp18 is capable of activating DCs initiating a Th1 immune response.

4.344 A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function
Okunishi, K. et al
J. Immunol., ***175****, 4745-4753 (2005)*

Hepatocyte growth factor (HGF) plays an important role in many biological events such as angiogenesis, cell proliferation, anti-fibrosis and antiapoptosis. It is well known that HGF promotes tumor progression and suppresses development of fibrosis after tissue injury. In contrast, its role in immune-mediated disorders has not been fully clarified. In the present study, we examined the role of HGF in Ag-specific immune response using in vitro studies and an experimental model of allergic airway inflammation. We first confirmed that dendritic cells (DCs) expressed the receptor for HGF, c-*met*, which was not expressed in T cells. Treatment with HGF both in vitro and in vivo potently suppressed DC functions such as Ag-presenting capacity, thus down-regulating Ag-induced Th1- and Th2-type immune responses. Exogenous administration of the HGF expression plasmid into Ag-primed mice markedly suppressed the development of airway eosinophilia and airway hyperresponsiveness, which was induced by Ag inhalation, with suppression of the Ag-presenting capacity of DCs in the lung. HGF exhibited these immunosuppressive effects without up-regulation of IL-10 or TGF-β. We also found that expression of endogenous HGF in the lung significantly increased following Ag sensitization and inhalation challenges. Finally, neutralization of endogenous HGF in vivo significantly increased airway eosinophilia and airway hyperresponsiveness with up-regulation of the Ag-presenting capacity of DCs in the lung. These results demonstrated a novel, significant, and possibly therapeutic role of HGF as a potent regulator in immune-mediated disorders such as asthma.

4.345 Herpesvirus saimiri-based vector biodistribution using noninvasive optical imaging
Smith, P.G. et al
Gen. Ther., ***12****, 1465-1476 (2005)*

Herpesvirus saimiri (HVS) is capable of infecting a range of human cell types with high efficiency and the viral genome persists as high copy number, circular, nonintegrated episomes which segregate to progeny upon cell division. This allows the HVS-based vector to stably transduce a dividing cell population and provide sustained transgene expression for an extended period of time both *in vitro* and *in vivo*. Here we assess the dissemination of HVS-based vectors *in vivo* following intravenous and intraperitoneal administration. Bioluminescence imaging of an HVS-based vector expressing luciferase demonstrates that the virus can infect and establish a persistent latent infection in a variety of mouse tissues. Moreover, the long-term *in vivo* maintenance of the HVS genome as a nonintegrated circular episome provided sustained expression of luciferase over a 10-week period. A particularly high level of transgene expression in the liver and the ability of HVS to infect and persist in hepatic stellate cells suggest that HVS-based vectors may have potential for the treatment of inherited and acquired liver diseases.

4.346 Superior efficacy of dendritic cell-tumor fusion vaccine compared with tumor lysate-pulsed dendritic cell vaccine in colon cancer
Kao, J.Y., Zhang, M., Chen, C-M. and Chen, J-J.
Immunology Letters, ***101(2)****, 154-159 (2005)*

Dendritic cell (DC)-based tumor vaccine is a promising therapy for malignancies. Recent studies showed greater potency with DC/tumor fusion vaccines against acute myeloid leukemia and melanoma compared with lysate-pulsed DC vaccines. We compared these two vaccine strategies against murine colon cancer and investigated whether DC/tumor fusion cells continue to produce tumor antigens following fusion as a possible explanation for their increased potency. Using a mouse colon cancer model, CT26, we first showed that the DC/CT26 fusion vaccine is more effective in preventing tumor implantation than CT26 lysate-pulsed DC vaccine. Next, CT26 made to constitutively produce bioactive TGF-β, a surrogate of tumor-derived products, was fused to DCs and found to produce bioactive TGF-β 72 h after fusion. Our results suggest the DC/tumor fusion vaccine is more potent against colon cancer than the lysate-pulsed DC vaccine. These fusion cells have the distinct advantage of prolonged interaction with tumor antigens in vivo.

4.347 Activation of bone marrow-resident memory T cells by circulating, antigen-bearing dendritic cells
Cavanagh, L.L. et al
Nature Immunol., ***6(10)****, 1029-1037 (2005)*

Dendritic cells (DCs) carry antigen from peripheral tissues via lymphatics to lymph nodes. We report here that differentiated DCs can also travel from the periphery into the blood. Circulating DCs migrated to the spleen, liver and lung but not lymph nodes. They also homed to the bone marrow, where they were retained better than in most other tissues. Homing of DCs to the bone marrow depended on constitutively expressed vascular cell adhesion molecule 1 and endothelial selectins in bone marrow microvessels. Two-photon intravital microscopy in bone marrow cavities showed that DCs formed stable antigen-dependent contacts with bone marrow–resident central memory T cells. Moreover, using this previously unknown migratory pathway, antigen-pulsed DCs were able to trigger central memory T cell–mediated recall responses in the bone marrow.

4.348 Defects in secretory pathway trafficking during sperm development in *Adam2* knockout mice
Stein, K.K., Go, J.C., Primakoff, P. and Myles, D.G.
Biol. Reprod., ***73***, *1032-1038 (2005)*

Adam2-null and *Adam3*-null male mice exhibit reduced levels of one or more ADAM proteins on mature sperm, in addition to the loss of the genetically targeted protein. ADAM protein loss was believed to occur posttranslationally, although the timing of loss and the mechanism by which the loss occurred were not explored. In this study we have found that in *Adam3*-null mice, fertilin beta (also known as ADAM2) is lost during the formation of testicular sperm. In *Adam2*-null males, most cyritestin (ADAM3) protein is also lost at this stage, but 25% of cyritestin is lost later, during sperm passage through the epididymis. Although normal levels of cyritestin are synthesized and acquire Endoglycosidase H resistance, indicating transit through the Golgi, the protein does not reach the cell surface. We also discovered that the majority of both fertilin beta and cyritestin are found in a Triton X-100 insoluble compartment on testicular sperm, when most of the cyritestin was observed on the cell surface. This insoluble compartment may represent a sorting platform, because in *Adam2*-knockout cells, only a small fraction of the cyritestin becomes Triton X-100 insoluble. Thus, it appears that cyritestin loss in *Adam2*-knockout mice may result, at least in part, from a disruption in protein trafficking.

4.349 Murine plasmacytoid dendritic cells produce IFN-γ upon IL-4 stimulation
Suto, A. et al
J. Immunol., ***175***, *5681-5689 (2005)*

IL-4 plays a key role in inducing IL-4 production in $CD4^+$ T cells, functioning as an important determinant for Th2 cell differentiation. We show here that IL-4 induces IFN-γ production in $B220^+$ plasmacytoid dendritic cells (PDCs). By searching for cell populations that produce IFN-γ upon IL-4 stimulation, we found that PDCs were a major IFN-γ-producing cell upon IL-4 stimulation in wild-type and Rag-$2^{-/-}$ splenocytes. Isolated PDCs, but not $CD11b^+$ DCs or $CD8^+$ DCs, produced IFN-γ upon IL-4 stimulation. In vivo, the depletion of PDCs by anti-Ly6G/C Ab prevented IFN-γ production induced by IL-4 administration. We also found that IL-4 induced IFN-γ production, but not IL-12 or IFN-α production, in PDCs and also strongly enhanced CpG oligodeoxynucleotide-induced IFN-γ production, but not CpG oligodeoxynucleotide-induced IL-12 or IFN-α production. However, IL-4 did not induce IFN-γ production in Stat$6^{-/-}$ PDCs. Moreover, IL-4 induced Stat4 expression in PDCs through a Stat6-dependent mechanism, and only the Stat4-expressing PDCs produced IFN-γ. Furthermore, IL-4 did not induce IFN-γ production in Stat$4^{-/-}$ PDCs. These results indicate that PDCs preferentially produce IFN-γ upon IL-4 stimulation by Stat6- and Stat4-dependent mechanisms.

4.350 A dual role for TGF-β1 in the control and persistence of fungal pneumonia
Shao, X., Rivera, J., Niang, R., Casadevall, A. and Goldman, D.L.
J. Immunol., ***175***, *6757-6763 (2005)*

TGF-β1 (TGF) has been implicated in the pathogenesis of several chronic infections and is thought to promote microbial persistence by interfering with macrophage function. In rats with experimental pulmonary cryptococcosis, increased lung levels of TGF were present at 12 mo of infection. Within the lung, expression of TGF localized to epithelioid cells and foamy macrophages in areas of inflammation. Increased TGF expression was also observed in the lungs of experimentally infected mice and a patient with pulmonary cryptococcosis. TGF reduced Ab and serum-mediated phagocytosis of *Cryptococcus neoformans* by rat alveolar macrophages (AM) and peripheral blood monocytes, and this was associated with decreased chemokine production and oxidative burst. Interestingly, TGF-treated rat AM limited both intracellular and extracellular growth of *C. neoformans.* Control of *C. neoformans* growth by TGF-treated

rat AM was due to increased secretion of lysozyme, a protein with potent antifungal activity. The effects of TGF on the course of infection were dependent on the timing of TGF administration relative to the time of infection. TGF treatment of chronically infected rats resulted in reduced lung fungal burden, while treatment early in the course of infection resulted in increased fungal burden. In summary, our studies suggest a dual role for TGF in persistent fungal pneumonia whereby it contributes to the local control of infection by enhancing macrophage antifungal efficacy through increased lysozyme secretion, while limiting inflammation by inhibiting macrophage/monocyte phagocytosis and reducing associated chemokine production and oxidative burst.

4.351 Kyoto islet isolation method: the optimized one for non-heart-beating donors with highly efficient islet retrieval
Okitsu, T. et al
Transplant. Proc., ***37***, *3391-3392 (2005)*

The availability of pancreata for clinical cadaveric islet transplantation is restricted to non-heart-beating donors (NHBDs) in Japan. This forced us to modify the current standard islet isolation protocol that was made up for brain-dead donors and make it suitable for NHBDs. The Kyoto islet isolation method is the one with induction of several steps based on the ideas both already reported literally and invented originally by ourselves. Using this islet isolation method, we isolated islets from 13 human pancreata of NHBDs and transplanted 11 preparations to six type-1 diabetic patients. The rate to meet release criteria of Edmonton protocol was 84.6%. Establishment of this method allowed us to begin a clinical islet transplantation program in Japan and to continue to perform the preparation of islets from NHBDs with high rate to meet the release criteria of the Edmonton protocol.

4.352 Mullerian inhibiting substance acts as a motor neuron survival factor in vitro
Wang, P-Y. et al
PNAS, ***102(45)***, *16421-16425 (2005)*

The survival of motor neurons is controlled by multiple factors that regulate different aspects of their physiology. The identification of these factors is important because of their relationship to motor neuron disease. We investigate here whether Mullerian Inhibiting Substance (MIS) is a motor neuron survival factor. We find that motor neurons from adult mice synthesize MIS and express its receptors, suggesting that mature motor neurons use MIS in an autocrine fashion or as a way to communicate with each other. MIS was observed to support the survival and differentiation of embryonic motor neurons *in vitro*. During development, male-specific MIS may have a hormone effect because the blood-brain barrier has yet to form, raising the possibility that MIS participates in generating sex-specific differences in motor neurons.

4.353 Nitric oxide scavenging by red blood cells as a function of hematocrit and oxygenation
Azarov, I. et al
J. Biol. Chem., ***280(47)***, *39024-39032 (2005)*

The reaction rate between nitric oxide and intraerythrocytic hemoglobin plays a major role in nitric oxide bioavailability and modulates homeostatic vascular function. It has previously been demonstrated that the encapsulation of hemoglobin in red blood cells restricts its ability to scavenge nitric oxide. This effect has been attributed to either factors intrinsic to the red blood cell such as a physical membrane barrier or factors external to the red blood cell such as the formation of an unstirred layer around the cell. We have performed measurements of the uptake rate of nitric oxide by red blood cells under oxygenated and deoxygenated conditions at different hematocrit percentages. Our studies include stopped-flow measurements where both the unstirred layer and physical barrier potentially participate, as well as competition experiments where the potential contribution of the unstirred layer is limited. We find that deoxygenated erythrocytes scavenge nitric oxide faster than oxygenated cells and that the rate of nitric oxide scavenging for oxygenated red blood cells increases as the hematocrit is raised from 15% to 50%. Our results 1) confirm the critical biological phenomenon that hemoglobin compartmentalization within the erythrocyte reduces reaction rates with nitric oxide, 2) show that extra-erythocytic diffusional barriers mediate most of this effect, and 3) provide novel evidence that an oxygen-dependent intrinsic property of the red blood cell contributes to this barrier activity, albeit to a lesser extent. These observations may have important physiological implications within the microvasculature and for pathophysiological disruption of

nitric oxide homeostasis in diseases.

4.354 Glucose stimulation of cytochrome C reduction and oxygen consumption as assessment of human islet quality
Sweet, I.R. et al
*Transplantation, **80(8)**, 1003-1011 (2005)*

Background. An in vitro method to assess human islets could prevent transplantation of nonviable islets and facilitate the optimization of islet preparation. We hypothesize that glucose-stimulated cytochrome c reduction and oxygen consumption by human islets can be used as predictors of transplant success. Methods. Isolated human islets were obtained from research-grade pancreata. Using a previously developed islet flow culture system, the response of cytochrome c reduction and oxygen consumption to glucose was compared to the ability of islets transplanted into nondiabetic NOD-SCID mice to secrete C-peptide in response to a glucose tolerance test conducted 7 days following transplant (n=10).
Results. In vitro responses by human islets were qualitatively similar to those seen in rat islets: glucose increased both oxygen consumption and cytochrome c reduction. However, the responses were smaller in magnitude and quite variable. Scatter plots of C-peptide and quantiles for ln(C-peptide) indicated that 12 ng/ml could be used as threshold of transplant success with which to evaluate the diagnostic potential of cytochrome c and oxygen consumption. Data was analyzed by generating receiver operating curves and the area under the curve was 0.889 (95% CI: 0.645-1.000) and 0.738 (95% CI: 0.413-1.000) for cytochrome c reuction and oxygen consumption respectively (1 indicates absolute predictive capability and 0.5 indicates no predictive capability).
Conclusions. The detection of glucose-stimulated cytochrome c reduction and oxygen consumption may have utility as criteria for the assessment of human islet quality.

4.355 Targeted expression of human CD1d in transgenic mice reveals independent roles for thymocytes and thymic APCs in positive and negative selection of Vα14i NKT cells
Schäumann, J. et al
*J. Immunol., **175**, 7303-7310 (2005)*

CD1d-dependent invariant Vα14 (Vα14i) NKT cells are innate T lymphocytes expressing a conserved semi-invariant TCR, consisting, in mice, of the invariant Vα14-Jα18 TCR α-chain paired mostly with Vβ 8.2 and Vβ7. The cellular requirements for thymic positive and negative selection of Vα14i NKT cells are only partially understood. Therefore, we generated transgenic mice expressing human CD1d (hCD1d) either on thymocytes, mainly $CD4^+$ $CD8^+$ double positive, or on APCs, the cells implicated in the selection of Vα14i NKT cells. In the absence of the endogenous mouse CD1d (mCD1d), the expression of hCD1d on thymocytes, but not on APCs, was sufficient to select Vα14i NKT cells that proved functional when activated ex vivo with the Ag α-galactosyl ceramide. Vα14i NKT cells selected by hCD1d on thymocytes, however, attained lower numbers than in control mice and expressed essentially Vβ8.2. The low number of $V\beta8.2^+$ Vα14i NKT cells selected by hCD1d on thymocytes was not reversed by the concomitant expression of mCD1d, which, instead, restored the development of $V\beta7^+$ Vα14i NKT cells. $V\beta8.2^+$, but not $V\beta7^+$, NKT cell development was impaired in mice expressing both hCD1d on APCs and mCD1d. Taken together, our data reveal that selective CD1d expression by thymocytes is sufficient for positive selection of functional Vα14i NKT cells and that both thymocytes and APCs may independently mediate negative selection.

4.356 CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival viral infection
Tyner, J.W. et al
*Nature Med., **11(11)**, 1180-1187 (2005)*

Host defense against viruses probably depends on targeted death of infected host cells and then clearance of cellular corpses by macrophages. For this process to be effective, the macrophage must presumably avoid its own virus-induced death. Here we identify one such mechanism. We show that mice lacking the chemokine Ccl5 are immune compromised to the point of delayed viral clearance, excessive airway inflammation and respiratory death after mouse parainfluenza or human influenza virus infection. Virus-inducible levels of Ccl5 are required to prevent apoptosis of virus-infected mouse macrophages *in vivo* and mouse and human macrophages *ex vivo*. The protective effect of Ccl5 requires activation of the Ccr5 chemokine receptor and consequent bilateral activation of $G\alpha_i$-PI3K-AKT and $G\alpha_i$-MEK-ERK signaling pathways. The antiapoptotic action of chemokine signaling may therefore allow scavengers to finally stop

the host cell-to-cell infectious process.

4.357 Nongenotoxic activation of the p53 pathway as a therapeutic strategy for multiple myeloma
Stühmer, T. et al
*Blood, **106(10)**, 3609-3617 (2005)*

Mutation of p53 is a rare event in multiple myeloma, but it is unknown if p53 signaling is functional in myeloma cells, and if targeted nongenotoxic activation of the p53 pathway is sufficient to kill tumor cells. Here, we demonstrate that treatment of primary tumor samples with a small-molecule inhibitor of the p53–murine double minute 2 (MDM2) interaction increases the level of p53 and induces p53 targets and apoptotic cell death. Significantly, given the importance of the bone marrow microenvironment for the support and drug resistance of myeloma cells, tumor cells undergo effective apoptosis also in the presence of stromal cells, which themselves appear to tolerate exposure to nutlin-3. The in vitro toxicity of nutlin-3 was similar to that of the genotoxic drug melphalan. Because nutlin-mediated p53 activation is not dependent on DNA damage, MDM2 antagonists may help to avoid or reduce the severe genotoxic side effects of chemotherapeutic agents currently used to treat multiple myeloma. Therefore, MDM2 antagonists may offer a new treatment option for this disease.

4.358 Elevated plasma arginase levels in hemoglobinopathies
Larkin, S.K., Morris, C.R., Styles, L.A. and kuypers, F.A.
*Blood, **106(11)**, abstract 2346 (2005)*

The decreased bioavailability of arginine (Arg) and the resulting lower nitric oxide (NO) production has been shown to be an important factor in the pathology of sickle cell disease (SCD) and thalassemia. Vascular alterations leading to pulmonary hypertension are important factors of heart failure and death in these hemoglobinopathies. Red blood cell (RBC) hemolysis in these patients will release arginase into the circulation and contribute to the reduction of plasma Arg levels, change the Arg-to-ornithine ratio, and increase other downstream amino acid metabolites. Such compounds, including proline and polyamines, may contribute to vascular and airway remodeling. To study the contribution of arginase released from RBC, we measured the arginase activity and arginase protein concentration in the plasma and RBC lysates of normal controls, SCD patients, and thalassemia patients.

Arginase activity was determined by the conversion of ^{14}C-labeled guanidine-L-arginine to ^{14}C-labeled urea, then to $^{14}CO_2$ by urease and trapped as $Na_2\ ^{14}CO_3$ for scintillation counting. Arginase and hemoglobin concentration were measured by ELISA (BioVendor, Candler, NC and Bethyl Labs Inc., Montgomery, TX respectively). Mean cellular hemoglobin concentration was measured with a Coulter Counter (Beckman Coulter, Fullerton, CA) or Technicon H3 analyzer (Tarrytown, NY). RBC were separated by density gradient centrifugation with **OptiPrep** (Axis Shield, Dundee, UK). Arginase specific activity (SA) was calculated by dividing the activity by the concentration to yield mole of Arg metabolized per g of arginase per hour. We found a higher level of arginase in the plasma and RBC of SCD and thalassemia patients as compared to normal controls. These levels correlated with cell-free hemoglobin content (plasma) or reticulocyte count (RBC). The light density fraction (<1.077 g/ml) of RBC, enriched in reticulocytes, showed higher arginase levels confirming increased arginase levels in young RBC. Interestingly, as the plasma arginase protein and activity increased with cell-free hemoglobin, the SA decreased. Similarly in RBC lysates, the SA decreases with hemoglobin concentration. This suggests that a factor exists within the RBC that negatively modulates arginase activity. Due to the high SA of arginase at low hemoglobin concentrations, low levels of intravascular hemolysis can generate relatively high levels of Arg breakdown. We conclude that low levels of intravascular hemolysis of RBC with increased arginase content in SCD and thalassemia, play a role in the altered Arg metabolism and decreased NO production. While the reason for high levels of arginase in the RBC as well as complete clinical impact of elevated arginase activity in plasma remain to be determined, dysregulated Arg metabolism and a shift toward Arg catabolic products, may be associated with the development of pulmonary hypertension and mortality in SCD and thalassemia.

4.359 Characterization of progesterone receptor isoform expression in fetal membrane
Mills, A., Yonish, B., Feng, L. and Murtha, A.
*Am. J. Obstet. Gynecol., **193(6), Suppl. 1**, abstract 164, (2005)*

Objective
To quantify the levels of expression of progesterone receptor (PR) isoforms A and B in amnion, chorion,

and decidua, and to determine whether expression changes in cell culture.
Study design
After IRB approval, placentas from term gestations delivered by cesarean section without labor were collected. Layers of amnion, chorion, and decidua were separated manually and then digested. Cell layers were further separated using **Opti-prep** (Sigma Aldrich) density gradient. Purity of cell separation was confirmed using immunocytochemistry. RNA was immediately extracted from an aliquot of cells from each cell layer and the remainder was cultured for 48 hours. RNA was then extracted for quantitative RT-PCR using primers specific for PR isoforms A and B, as well as for β-2 microglobulin (β2M), a constituitively expressed gene used to normalize quantification across tissue samples. Standard curves were generated from known concentrations of each transcript. Data were analyzed using t-test and Mann Whitney U.
Results
PR isoforms A and B were identified in the chorion and decidua but were below the lower limits of detection of our assay in the amnion. For both isoforms, decidual expression was higher than expression in the chorion (PR-A/β2M 0.006 vs. 0.001, p = 0.02; PR-B/β2M 0.005 vs. 0.0004, p = 0.02). Ratios of PR-A to PR-B were similar in both decidua and chorion. When fresh cells were compared to cultured cells there was no significant difference in PR expression.
Conclusion
PR isoforms A and B are present in chorion and decidua, with the highest concentrations found in the maternally-derived decidua. PR isoforms appear to be equally expressed in cultured and fresh cells. These findings will aid in our understanding of how progesterone contributes to the pathogenesis of preterm delivery.

4.360 Decrease in Langerhans cells and increase in lymph node dendritic cells following chronic exposure of mice to suberythemal doses of solar simulated radiation
McLoone, P., Woods, G.M. and Norval, M.
Photochem. Photobiol., ***81(5)****, 1168-1173 (2005)*

Exposure of certain strains of mice to ultraviolet radiation (UVR) causes suppression of some innate and adaptive immune responses. One such consequence of acute UVB exposure is a reduction in the number of Langerhans cells (LC) in the epidermis and an increase in dendritic cells (DC) in lymph nodes draining the irradiated skin sites. Exposure to chronic UVB irradiation also has effects on the immune system, but it is unknown what effects are caused by repeated doses of solar simulated radiation (SSR). Consequently, the main aims of the present study were to determine whether repeated exposure to low doses of SSR would lead to similar changes in these cell populations and whether chronic doses of SSR activate a protective photoadaptation mechanism. Groups of C3H/HeN mice were irradiated daily with 3.7 J/cm^2 SSR from Cleo Natural lamps for 2, 10, 20, 30 or 60 days. Further groups of mice received an additional dose of 7.4 J/cm^2 SSR on days 2, 10, 30 or 60 to test for photoadaptation. The numbers of LC in the epidermis and DC in the lymph nodes draining irradiated skin sites were counted 24 h after the final irradiation. With the exception of mice irradiated for only 2 days, LC were significantly reduced throughout the chronic irradiation protocol, and no recovery occurred. DC numbers were significantly increased in the draining lymph nodes of mice irradiated for 20 days and 60 days.

4.361 Blocking intrahepatic deletion of activated CD8$^+$ T cells by an altered peptide ligand
Kuniyasu, Y. et al
Cell. Immunol., ***238****, 31-37 (2005)*

Background
Activated CD8$^+$ T cells are retained by the healthy liver where the majority undergo apoptosis. The intrahepatic apoptosis of activated CD8$^+$ T cells is enhanced by the presence of SIINFEKL peptide. It is of great interest to identify strategies for maintaining intrahepatic T cell number and function in the presence of SIINFEKL peptides.
Aim
Our aim was to test if low affinity peptides can block SIINFEKL peptide induced T cell deletion.
Methods
We used an in vivo model of intrahepatic CD8$^+$ T cell deletion with peptides of different affinities.
Results and discussion
We show that the intrahepatic deletion of CD8$^+$ T cells by SIINFEKL peptide results in loss of in vivo cytotoxic T lymphocyte function. In contrast we show that a low affinity peptide (G4) does not result in intrahepatic deletion of CD8$^+$ T cells. High concentrations G4 peptide can however block intrahepatic

deletion of activated $CD8^+$ T cells, and prevent loss of in vivo cytotoxicity due to SIINFEKL peptide. This is the first demonstration of blocking of SIINFEKL peptide induced $CD8^+$ T cell deletion in the liver, with enhancement of in vivo cytotoxicity.

4.362 Improved Methods for Culturing Rat and Mouse Motor Neurons
Guettier-Sigrist, S et al
*New Methods for Cultering Cells from Nervous Tissues, **1**, 96-108 (2005)*

How synapses are formed and which molecules contribute to this process are essential, but not yet fully understood, aspects of neuronal development. However, identifying the steps of formation of neuromuscular junction would be very important to understand the physiopathology of neuromuscular disorders. Those who want to study this process in vitro need to prepare purified motor neuron suspensions, together with other cells contributing to the formation of neuromuscular junction [1].
Similarly, in order to study neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), it is essential to have these cells at our disposal. With the development of transgenic animal strains displaying such diseases [2], it is now possible to study the behavior of diseased motor neurons in culture and especially follow the biochemical events occurring in these cells [3]. It is also possible to study the effects of putative neurotrophic or neuroprotective drugs [4]. However, it should be underlined that an isolated motor neuron does not have an entire physiological or pathophysiological significance, since, in vivo, it is always connected to muscle fibers. The pertinent unit for studying more precisely motor neuron diseases or neuromuscular disorders is the motor unit, which is defined as the anatomo-functional entity consisting of a motor neuron and the set of muscle fibers innervated by the ramifications of its axon.
We describe here the preparation of the rat and mouse purified motor neuron suspensions.

4.363 Cellular Uptake of Vitamin B_{12} in Patients with Chronic Renal Failure
Obeid, R., Kuhlmann, M., Kirsch, C-M. And Hermann, W.
*Nephron. Clin. Pract., **99**, c42-c48 (2005)*

Background/Aims: Elevated concentration of plasma homocysteine (tHcy) is common in renal patients, however, the reason behind the resistance to vitamin B_{12} and folate therapy are poorly understood. *Methods:* We investigated vitamin B_{12} uptake by mononuclear cells (MC) from predialysis patients (n = 19) as compared to healthy controls (n = 15). Serum levels of tHcy, methylmalonic acid and cystathionine, holotranscobalamin (holoTC), total vitamin B_{12} and folate were also measured. *Results:* The uptake of vitamin B_{12} by MC from renal patients was lower than that by MC from controls (9.3 vs. 12.5 pg/3 × 10^6 cells; p = 0.001). Nonetheless, the receptor-binding capacity was comparable between patients and controls (6.1 vs. 6.5 pg/3 × 10^6 cells; p = 0.627). Average reduction of vitamin B_{12} uptake in patients as compared to the controls was 18.1%. *Conclusions:* Our results show that vitamin B_{12} uptake is impaired in MC from renal patients, with no evidence that the surface receptor is down-regulated. High serum concentrations of holoTC are common in renal patients and might be related to a generalized resistance to this vitamin. Serum concentrations of vitamin B_{12} within the reference range are not likely to ensure vitamin delivery into the cells. Supraphysiological doses of vitamin B_{12} may be necessary to deliver a sufficient amount of the vitamins to the cells via mechanisms largely independent of holoTC receptor.

4.364 Amelioration of rat adjuvant-induced arthritis by Met-RANTES
Shahrara, S., Proudfoot, A.E.I., Woods, J.M., Ruth, J.H., Amin, M.A., Park, C.C., Haas, C.S., Pope, R.M., Haines, G.K., Zha, Y.Y. and Koch, A.E.
*Arthritis & Rheumatism, **52(6)**, 1907-1919 (2005)*

Objective
CC chemokines and their receptors play a fundamental role in trafficking and activation of leukocytes at sites of inflammation, contributing to joint damage in rheumatoid arthritis. Met-RANTES, an amino-terminal–modified methionylated form of RANTES (CCL5), antagonizes the binding of the chemokines RANTES and macrophage inflammatory protein 1α (MIP-1α; CCL3) to their receptors CCR1 and CCR5, respectively. The aim of this study was to investigate whether Met-RANTES could ameliorate adjuvant-induced arthritis (AIA) in the rat.
Methods
Using immunohistochemistry, enzyme-linked immunosorbent assay, real-time reverse transcription–polymerase chain reaction, Western blot analysis, adoptive transfer, and chemotaxis, we defined joint inflammation, bony destruction, neutrophil and macrophage migration, Met-RANTES binding affinity to rat receptors, proinflammatory cytokine and bone marker levels, CCR1 and CCR5 expression and

activation, and macrophage homing into joints with AIA.
Results
Administration of Met-RANTES as a preventative reduced the severity of joint inflammation. Administration of Met-RANTES to ankles with AIA showed decreases in inflammation, radiographic soft tissue swelling, and bone erosion. Met-RANTES significantly reduced the number of neutrophils and macrophages at the peak of arthritis compared with saline-injected controls. Competitive chemotaxis in peripheral blood mononuclear cells demonstrated that Met-RANTES inhibited MIP-1α and MIP-1β at 50% inhibition concentrations of 5 nM and 2 nM, respectively. Furthermore, levels of tumor necrosis factor α, interleukin-1β, macrophage colony-stimulating factor, and RANKL were decreased in joints with AIA in the Met-RANTES group compared with the control group. Interestingly, the expression and activation of CCR1 and CCR5 in the joint were down-regulated in the Met-RANTES group compared with the control group. Functionally, Met-RANTES administration decreased adoptively transferred peritoneal macrophage homing into the joint.
Conclusion
The data suggest that the targeting of Th1-associated chemokine receptors reduce joint inflammation, bone destruction, and cell recruitment into joints with AIA.

4.365 CD46 on glial cells can function as a receptor for viral glycoprotein-mediated cell–cell fusion
Cassiani-Ingoni, R., Greenstone, H.L., Donati, D., Fogdell-Hahn, A., Martinell, E., Refai, D., Martin, R., Berger, E.A. and Jacobson, S.
GLIA, ***52(3)***, *252-258 (2005)*

Membrane cofactor protein (CD46) is a regulator of complement activation that also serves as the entry receptor for human herpes virus 6 (HHV-6) and measles virus (MV) into human cells. While it is clear that oligodendrocytes and astrocytes are cell types commonly infected by these viruses, it is unclear whether oligodendrocytes express CD46, or which are the cellular mechanisms underlying the infection. We show that adult oligodendrocytes, as well as astrocytes and microglial cells, express CD46 on the cellular surface. Moreover, we employed a quantitative fusion assay to demonstrate that HHV-6A infection of T lymphocytes enables cell–cell fusion of these cells to astrocytes or to oligodendroglial cells. This fusion is mediated by the interaction between viral glycoproteins expressed on the membrane of the infected cells and CD46 on the glial targets, and is also observed using cells expressing recombinant MV glycoproteins. These data suggest a mechanism that involves cell–cell fusion by which certain viruses could spread the infection from the periphery to the cells in the nervous system.

4.366 Lineage-negative bone marrow cells travel bidirectionally in the olfactory migratory stream but maintain hematopoietic phenotype
Moore, B.E., Colvin, G.A., Dooner, M.S. and Quesenberry, P.J.
J. Cell. Physiol., ***202(1)***, *147-152 (2005)*

The mammalian olfactory system is a physiologically plastic region of the brain with the potential to support implanted stem cells. We performed direct injection of lineage-negative (lin-neg), green fluorescent protein-positive (GFP+) bone marrow cells into the olfactory bulb to assess cell survival and motility within the central nervous system (CNS). Before direct injection of 100,000 lin-neg cells, some of the C57/Bl mice received 1,000 cGy brain irradiation with the aim of disabling the endogenous reservoir of periventricular neural progenitor cells. Brain harvest took place up to 2 weeks after cell implantation. Brains were evaluated for presence of GFP positivity via fluorescence microscopy. Many GFP+ cells were identified within the turbinate neuroepithelium, olfactory bulb, and frontal lobe. Most of the cells that had traveled from the implantation site adopted an elongated, arborizing morphology consistent with cellular extensions arrayed in the direction of the rostral migratory stream (RMS). No difference was seen in brain-irradiated versus non-irradiated mice. Antibody staining revealed that these cells did not take on a neural, glial, or endothelial phenotype, while largely retaining their hematopoietic lineage as demonstrated by CD45 positivity.

4.367 rAAV-mediated stable expression of heme oxygenase-1 in stellate cells: A new approach to attenuate liver fibrosis in rats
Tsui, T-Y., Lau, C-K., Ma, J., Wu, X., Wang, Y-Q., Farkas, S., Xu, R., Schlitt, H.J. and Fan, S-T.
Hepatology, ***42(2)***, *335-342 (2005)*

Liver fibrosis is the consequence of activation of hepatic stellate cells mediated by persistent or recurrent

liver injury, where oxidative stress or inflammatory response resulting from immune cells and cytokines are involved. Targeting of hepatic stellate cells could be an important strategy for the therapy of liver fibrosis. In this study, we showed a tropism of recombinant adeno-associated virus (rAAV, serotype 2) with high efficiency in transduction of a homeostatic gene, heme oxygenase-1 (HO-1), to activated stellate cells. The binding of rAAVs to stellate cells increased significantly after serum-stimulated activation compared with quiescent status. Portal injection of rAAVs to normal or carbon tetrachloride (CCl4)-induced liver fibrosis showed a distinct distribution of rAAV binding. The majority of injected rAAVs bound to the cells in fibrotic areas that were associated with higher expression levels of fibroblast growth factor receptor-1α at 2 hours after administration. Isolation of different types of cells from CCl4-induced fibrotic livers showed predominant expression of transgene in stellate cells after rAAV/HO-1 administration on day 3 and remained stable for 12 weeks. In addition, HO-1–transduced stellate cells showed reduced transcript levels of type 1 collagen and impaired proliferative ability compared with controls. With this approach, the severity of established micronodular cirrhosis was markedly reduced. In conclusion, these findings suggest a new approach for the treatment of liver fibrosis using adeno-associated virus-mediated gene transfer.

4.368 Expression of a dominant negative form of Daxx *in vivo* rescues motoneurons from Fas (CD95)-induced cell death
Raoul, C., Barthelemy, C., Couzinet, A., Hancock, D., Pettmann, B. and Hueber, A-O.
J. Neurobiol., ***62(2)***, *178-188 (2005)*

Fas-induced death of motoneurons *in vitro* has been shown to involve two signaling cascades that act together to execute the death program: a Fas-Daxx-ASK-1-p38 kinase-nNOS branch, which controls transcriptional and post-translational events, and the second classical Fas-FADD-caspase-8 branch. To analyze the role of Daxx in the developmental motoneuron cell death, we studied Fas-dependent cell death in motoneurons from transgenic mice that overexpress a dominant-negative form of Daxx. Motoneurons purified from these transgenic mice are resistant to Fas-induced death. This protective effect is specific to Fas because ultraviolet irradiation-triggered death is not affected by the transgene. The Daxx and the FADD pathways work in parallel because only Daxx, but not FADD, is involved in the transcriptional control of neuronal nitric oxide synthase and nitric oxide production. Nevertheless, we do not observe involvement of Daxx in developmental motoneuronal cell death, as the pattern of naturally occurring programmed cell death *in vivo* is normal in transgenic mice overexpressing the dominant negative form of Daxx, suggesting that Daxx-independent pathways are used during development.

4.369 CD86 molecule is a specific marker for canine monocyte-derived dendritic cells
Bonnefont-Rebeix, C. et al
Vet. Immunol. Immunopathol., ***109***, *167-176 (2006)*

In this study, canine monocyte-derived dendritic cells (cMo-DC) were produced in presence of canine GM-CSF (cGM-CSF) and canine IL-4 (cIL-4), and they were characterized by their dendritic morphology, MLR functionality and phenotype. We noticed that cMo-DC were labelled with three anti-human CD86 (FUN-1, BU63 and IT2.2 clones), whereas resting and activated lymphocytes or monocytes were not stained. CD86 expression was induced by cIL-4 and was up-regulated during the differentiation of the cMo-DC, with a maximum at day 7. Furthermore, cMo-DC were very potent even in low numbers as stimulator cells in allogeneic MLR, and BU63 mAb was able to completely block the cMo-DC-induced proliferation in MLR. We also observed that cMo-DC highly expressed MHC Class II and CD32, but we failed to determine their maturation state since the lack of commercially available canine markers. Moreover, cMo-DC contained cytoplasmic periodic microstructures, potentially new ultrastructural markers of canine DC recently described. In conclusion, this work demonstrates that the CD86 costimulatory marker is now usable for a better characterization of in vitro canine DC.

4.370 Helminth-primed dendritic cells: alter the host response to enteric bacterial infection
Chen, C-C., Louie, S., McCormick, B.A., Walker, W.A. and Shi, H.N.
J. Immunol., ***176***, *472-483 (2006)*

To examine whether intestinal helminth infection may be a risk factor for enteric bacterial infection, a murine model was established using the intestinal helminth *Heligomosomoides polygyrus* and a murine pathogen C*itrobacter rodentium*, which causes infectious colitis. Using this model we recently have shown that coinfection with the Th2-inducing *H. polygyrus* and *C. rodentium* promotes bacterial-associated disease and colitis. In this study, we expand our previous observations and examine the hypothesis that

dendritic cells (DC) stimulated by helminth infection may play an important role in the regulation of the intestinal immune response to concurrent *C. rodentium* infection as well as in the modulation of the bacterial pathogenesis. We show that *H. polygyrus* infection induces DC activation and IL-10 expression, and that adoptive transfer of parasite-primed DC significantly impairs host protection to *C. rodentium* infection, resulting in an enhanced bacterial infection and in the development of a more severe colonic injury. Furthermore, we demonstrate that adoptive transfer of parasite-primed IL-10-deficient DCs fails to result in the development of a significantly enhanced *C. rodentium*-mediated colitis. Similarly, when the DC IL-10 response was neutralized by anti-IL-10 mAb treatment in mice that received parasite-primed DC, no deleterious effect of the parasite-primed DC on the host intestinal response to *C. rodentium* was detected. Thus, our results provide evidence to indicate that the *H. polygyrus*-dependent modulation of the host response to concurrent *C. rodentium* infection involves IL-10-producing DCs.

4.371 Antitumor efficiacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma

DiJoseph, J.F. et al

Clin. Cancer Res., ***12(1)***, *242-249 (2006)*

Purpose: CMC-544 is a CD22-targeted cytotoxic immunoconjugate, currently being evaluated in B-cell non-Hodgkin's lymphoma (B-NHL) patients. Rituximab is a CD20-targeted antibody commonly used in B-NHL therapy. Here, we describe antitumor efficacy of a combination of CMC-544 and rituximab against B-cell lymphoma (BCL) in preclinical models.
Experimental Design: BCLs were cultured *in vitro* with CMC-544, rituximab, or their combination. BCLs were injected either s.c. or i.v. to establish localized s.c. BCL in nude mice or disseminated BCL in severe combined immunodeficient mice, respectively. I.p. treatment with CMC-544 or rituximab was initiated at various times either alone or in combination and its effect on s.c. BCL growth or survival of mice with disseminated BCL was monitored.
Results: *In vitro* growth-inhibitory activity of CMC-544 combined with rituximab was additive. Rituximab but not CMC-544 exhibited effector functions, such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Rituximab was less effective in inhibiting growth of established BCL xenografts than developing xenografts. In contrast, CMC-544 was equally effective against both developing and established BCL xenografts. Although CMC-544 and rituximab individually caused partial inhibition of the growth of BCL xenografts at suboptimal doses examined, their combination suppressed xenograft growth by >90%. In a disseminated BCL model, 60% of CMC-544-treated mice and 20% of rituximab-treated mice survived for 125 days. In contrast, 90% of mice treated with the combination of CMC-544 and rituximab survived for longer than 125 days.
Conclusion: The demonstration of superior antitumor activity of a combination of CMC-544 and rituximab described here provides the preclinical basis for its clinical evaluation as a treatment option for B-NHL.

4.372 Motor neurone targeting of IGF-1 prevents specific force decline in ageing mouse muscle

Payne, A.M. et al

J. Physiol., ***570(2)***, *283-294 (2006)*

IGF-1 is a potent growth factor for both motor neurones and skeletal muscle. Muscle IGF-1 is known to provide target-derived trophic effects on motor neurones. Therefore, IGF-1 overexpression in muscle is effective in delaying or preventing deleterious effects of ageing in both tissues. Since age-related decline in muscle function stems partly from motor neurone loss, a tetanus toxin fragment-C (TTC) fusion protein was created to target IGF-1 to motor neurones. IGF-1–TTC retains IGF-1 activity as indicated by [^{3}H]thymidine incorporation into L6 myoblasts. Spinal cord motor neurones effectively bound and internalized the IGF-1–TTC *in vitro*. Similarly, IGF-1–TTC injected into skeletal muscles was taken up and retrogradely transported to the spinal cord *in vivo*, a process prevented by denervation of injected muscles. Three monthly IGF-1–TTC injections into muscles of ageing mice did not increase muscle weight or muscle fibre size, but significantly increased single fibre specific force over aged controls injected with saline, IGF-1, or TTC. None of the injections changed muscle fibre type composition, but neuromuscular junction post-terminals were larger and more complex in muscle fibres injected with IGF-1–TTC, compared to the other groups, suggesting preservation of muscle fibre innervation. This work demonstrates that induced overexpression of IGF-1 in spinal cord motor neurones of ageing mice prevents muscle fibre specific force decline, a hallmark of ageing skeletal muscle.

4.373 Differential regulation of neutrophil chemotaxis to IL-8 and fMLP by GM-CSF: lack of direct effect of oestradiol

Shen, L. et al

*Immunology, **117(2)**, 205-212 (2006)*

Neutrophils are a normal constituent of the female reproductive tract and their numbers increase in the late secretory phase of the menstrual cycle prior to menses. Several cytokines are produced in female reproductive tract tissue. In particular granulocyte–macrophage colony-stimulating factor (GM-CSF), a potent activator of neutrophils, is secreted in high concentrations by female reproductive tract epithelia. We previously observed that GM-CSF synergizes strongly with interleukin-8 (IL-8) in enhancing chemotaxis of neutrophils. Thus we investigated whether pretreatment of neutrophils with GM-CSF would prime subsequent chemotaxis to IL-8 in the absence of GM-CSF. Surprisingly, a 3-hr pulse of GM-CSF severely diminished chemotaxis to IL-8, whereas *N*-formyl-methyl-leucyl-phenylalanine (fMLP)-mediated chemotaxis was retained. Conversely, when cells were incubated without GM-CSF they retained IL-8-mediated migration but lost fMLP chemotaxis. These changes in chemotaxis did not correlate with expression of CXCR1, CXCR2 or formyl peptide receptor. However, IL-8-mediated phosphorylation of p44/42 mitogen-activated protein kinase was greatly reduced in neutrophils that no longer migrated to IL-8, and was diminished in cells that no longer migrated to fMLP. Oestradiol, which is reported by some to exert an anti-inflammatory effect on neutrophils, did not change the effects of GM-CSF. These data suggest that neutrophil function may be altered by cytokines such as GM-CSF through modulation of signalling and independently of surface receptor expression.

4.374 Alveolar epithelial cells secrete chemokines in response to IL-1β and lipopolysaccharide but not to ozone

Manzer, R., Wang, J., Nishina, K., McConville, G. and Mason, R.J.

*Am. J. Respir. Cell Mol., **34**, 158-166 (2006)*

Ozone exposure produces acute inflammation and neutrophil influx in the distal lung. Alveolar epithelial cells cover a large surface area, secrete chemokines, and may initiate or modify the inflammatory response. The effect of ozone on chemokine production by these cells has not been defined. Isolated rat type II cells were cultured in different conditions to express the morphologic appearance and biochemical markers for the type I and the type II cell phenotypes. These cells were exposed to ozone at an air/liquid interface. The type I–like cells were more susceptible to injury than the type II cells and showed signs of injury at exposure levels of 100 ppb ozone for 60 min. Both phenotypes showed evidence of lipid peroxidation after ozone exposure as measured by 8-isoprostane production, but neither phenotype secreted increased amounts of MIP-2 (CXCL3), CINC-1 (CXCL1), or MCP-1 (CCL2) in response to ozone. Both cell phenotypes secreted MIP-2 and MCP-1 in response to IL-1β or lipopolysaccharide, but there was no priming or synergy with ozone. It is likely that the inflammatory response to ozone in the alveolar compartment is not due to the direct effect of ozone on epithelial cells.

4.375 Oxygen tension regulates the in vitro maturation of GM-CSF expanded murine bone marrow dendritic cells by modulating class II MHC expression

Goth, S.R., Chu, R.A. and Pessah, I.N.

*J. Immunol. Methods, **308**, 179-191 (2006)*

Conventional culture conditions for GM-CSF expanded murine bone marrow derived dendritic cells (BMDCs) uses ambient (hyperoxic) oxygen pressure (20% v/v, 152 Torr) and medium supplemented with the thiol 2-mercaptoethanol (2-Me). Given the redox activities of O_2 and 2-Me, the effects of 2%, 5%, 10%, and 20% v/v O_2 atmospheres and omitting 2-Me from the medium were tested upon the generation of GM-CSF expanded BMDCs. DC yield, phenotype and function were compared to BMDCs grown using conventional conditions. All cultures yielded DC subsets with $CD11c^+$ MHC II^{NEG}, $CD11c^+$ MHC II^{INT}, $CD11c^+$ MHC II^{HI} expression phenotypes, classed as precursor, immature, and mature DCs (IDC, MDC). Low O_2 tensions generated significantly fewer precursor DCs, and more IDCs and MDCs. Cytometer sorted precursor DCs expressed surface class II MHC after transfer to low, but not high O_2 atmospheres. Expression of myeloid markers was similar between BMDC cultures generated in 5% O_2 or conventional conditions, and MDCs from low O_2 cultures had the morphology typical of mature myeloid DCs. IDCs and MDCs from low O_2 and conventional culture conditions were similarly potent allostimulatory APCs. The O_2 tension (but not 2-Me addition) in vitro significantly influences overall DC subset frequencies and yield, and governs DC maturation by regulating the surface class II MHC expression of GM-CSF expanded BMDC cultures.

4.376 Adjuvant IL-15 does not enhance the efficacy of tumor cell lysate-pulsed dendritic cell vaccines for active immunotherapy of T cell lymphoma
Gatza, E. and Okada, C.Y.
*Cancer Immunol. Immunother., **55**, 420-432 (2006)*

There has been a recent interest in using IL-15 to enhance antitumor activity in several models because of its ability to stimulate $CD8^+$ T cell expansion, inhibit apoptosis and promote memory T cell survival and maintenance. Previously, we reported that C6VL tumor lysate-pulsed dendritic cell vaccines significantly enhanced the survival of tumor-bearing mice by stimulating a potent tumor-specific $CD8^+$ T cell response. In this study, we determined whether IL-15 used as immunologic adjuvant would augment vaccine-primed $CD8^+$ T cell immunity against C6VL and further improve the survival of tumor-bearing mice. We report that IL-15 given after C6VL lysate-pulsed dendritic cell vaccines stimulated local and systemic expansion of NK, NKT and $CD8^+$ $CD44^{hi}$ T cells. IL-15 did not, however, augment innate or cellular responses against the tumor. T cells from mice infused with IL-15 following vaccination did not secrete increased levels of tumor-specific TNF-α or IFN-γ or have enhanced C6VL-specific CTL activity compared to T cells from recipients of the vaccine alone. Lastly, IL-15 did not enhance the survival of tumor-bearing vaccinated mice. Thus, while activated- and memory-phenotype $CD8^+$ T cells were dramatically expanded by IL-15 infusion, vaccine-primed $CD8^+$ T cell specific for C6VL were not significantly expanded. This is the first account of using IL-15 as an adjuvant in a therapeutic model of active immunotherapy where there was not a preexisting pool of tumor-specific $CD8^+$ T cells. Our results contrast the recent studies where IL-15 was successfully used to augment tumor-reactivity of adoptively transferred transgenic $CD8^+$ T cells. This suggests that the adjuvant potential of IL-15 may be greatest in settings where it can augment the number and activity of preexisting tumor-specific $CD8^+$ T cells.

4.377 Migratory monocytes and granulocytes are major lymphatic carriers of *Salmonella* from tissue to draining lymph node
Bonneau, M. et al
*J. Leukoc. Biol., **79**, 268-276 (2006)*

Dendritic cells (DC) are recognized as sentinels, which capture antigens in tissue and migrate to the lymph node, where they initiate immune responses. However, when a vaccine strain of green fluorescent protein-expressing *Salmonella abortusovis* (SAO) was inoculated into sheep oral mucosa, it induced accumulation of myeloid non-DC in the subcapsular sinus and paracortex of the draining lymph node, and SAO was mainly found associated with these cells (granulocytes and macrophages) but rarely with DC. To analyze how bacteria reached lymph nodes, we used cervical pseudo-afferent lymph duct catheterization. We showed that *Salmonella* administered in the oral mucosa were traveling free in lymph or associated with cells, largely with lymph monocytes and granulocytes but less with DC. SAO also induced a strong influx of these phagocytic cells in afferent lymph. Migrating DC presented a semi-mature phenotype, and SAO administration did not alter their expression of major histocompatibility complex type 2 and coactivation molecules. Compared with blood counterparts, lymph monocytes expressed lower levels of CD40, and granulocytes expressed higher levels of CD80. The data suggest that immunity to bacteria may result from the complex interplay between a mixture of phagocytic cell types, which transport antigens and are massively recruited via lymph to decisional lymph nodes.

4.378 Identification and characterization of a novel isoform of the vesicular γ-aminobutyric acid transporter with glucose-regulated expression in rat islets
Suckow, A.T. et al
*J. Mol. Endocrinol., **36**, 187-199 (2006)*

Pancreatic islets are unique outside the nervous system in that they contain high levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), synthesized by the enzyme glutamic acid decarboxylase (GAD). Since the role that GABA plays in the islet and the mechanisms whereby the two major GAD isoforms (GAD65 and GAD67) function as diabetes-associated autoantigens are unknown, continued characterization of the islet GAD–GABA system is important. We previously demonstrated that the GABA and glycine transporter vesicular inhibitory amino acid transporter (VIAAT also known as VGAT) is present in rat islets. Here we identify a novel 52 kDa variant of VIAAT in rat islets: VIAAT-52 (V52). V52 is an amino-terminally truncated form of VIAAT (V57) that likely results from utilization of a downstream start site of translation. V57 and V52 display different patterns of post-translational modification and cellular expression. Our results have indicated that islet content of V52, but not V57, is responsive to

changes in glucose concentration and other extracellular conditions. VIAAT is expressed in the islet α cells, but there have been conflicting findings regarding the presence of VIAAT in the ß cells. Here we have also provided additional evidence for the presence of VIAAT in islet ß cells and show that the ß cell line INS-1 expresses V57. V52 may be better adapted than V57 to the unique rat α cell GAD–GABA system, which lacks GAD65 and in which VIAAT traffics to secretory granules rather than just to synaptic microvesicles.

4.379 Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation
Noguchi, H. et al
*Am. J. Transplant., **6**, 496-504 (2006)*

Islet allotransplantation can achieve insulin independence in patients with type I diabetes. Recent reports show that the two-layer method (TLM), which employs oxygenated perfluorochemical (PFC) and UW solution, is superior to simple cold storage in UW for pancreas preservation in islet transplantation. However, UW solution has several disadvantages, including the inhibition of Liberase activity. In this study, we investigated the features of a new solution, designated M-Kyoto solution. M-Kyoto solution contains trehalose and ulinastatin as distinct components. Trehalose has a cytoprotective effect against stress, and ulinastatin inhibits trypsin. In porcine islet isolation, islet yield was significantly higher in the M-Kyoto/PFC group compared with the UW/PFC group. There was no significant difference in ATP content in the pancreas between the two groups, suggesting that different islet yields are not due to their differences as energy sources. Compared with UW solution, M-Kyoto solution significantly inhibited trypsin activity in the digestion step; moreover, M-Kyoto solution inhibited collagenase digestion less than UW solution. In conclusion, the advantages of M-Kyoto solution are trypsin inhibition and less collagenase inhibition. Based on these data, we now use M-Kyoto solution for clinical islet transplantation from nonheart-beating donor pancreata.

4.380 Variants of the 5'-untranslated region of human *NCF2*: expression and translational efficiency
Gauss, K.A. et al
*Gene, **366**, 169-179 (2006)*

The *NCF2* gene encodes $p67^{phox}$, an essential component of the multi-protein NADPH oxidase enzyme in phagocytic leukocytes, as well as in certain non-phagocytic cells. In humans, the *NCF2* gene is expressed as multiple *NCF2* variants that differ in the 5′-untranslated region (5′-UTR). Previously, we reported the presence of four *NCF2* 5′-UTR mRNA variants (designated as *NCF2* exon 1, intron 1a, intron 1b and intron 1c). As each of the gene variants encodes an identical $p67^{phox}$ protein, the functional significance of these message variants was not apparent. In this study, we investigated the relative expression levels and tissue-specificity of *NCF2* 5′-UTR variant mRNAs and their translation efficiency and stability. *NCF2* 5′-UTR variant transcripts were differentially expressed in various cell lines and human tissues. In vitro translation assays indicated that the *NCF2* 5′-UTR variants also differed in their effects on the translation of a luciferase reporter mRNA and *NCF2* mRNA. Notably, *NCF2* intron 1 5′-UTR variants, which are the predominantly expressed variants found in vivo, strongly inhibited translation when compared to the *NCF2* exon 1 5′-UTR variant. In contrast, RNA decay assays demonstrated that there was no significant difference between stability of *NCF2* intron 1 transcripts and the exon 1 5′-UTR variant in HL-60, MonoMac 6, and U937 cells. Moreover, expression of the variant transcripts remained unchanged after neutrophil phagocytosis, and was similar in normal neutrophils and neutrophils from a patient with X-linked chronic granulomatous disease. These studies suggest that expression of $p67^{phox}$ is regulated through mechanisms that include modulation of transcription and translation.

4.381 Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners
Radaeva, S. et al
*Gastroenterology, **130(2)**, 434-452 (2006)*

Background & Aims: Viral hepatitis infection, which is a major cause of liver fibrosis, is associated with activation of innate immunity. However, the role of innate immunity in liver fibrosis remains obscure. *Methods:* Liver fibrosis was induced either by feeding mice with the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet or by injecting them with carbon tetrachloride. The Toll-like receptor 3 ligand, polyinosinic-polycytidylic acid, was used to activate innate immunity cells and mediators, including natural killer cells and interferon γ. *Results:* In the mouse model of DDC-induced liver fibrosis, natural killer cell activation by polyinosinic-polycytidylic acid induced cell death to activated hepatic stellate cells and attenuated the severity of liver fibrosis. Polyinosinic-polycytidylic acid treatment also ameliorated

liver fibrosis induced by carbon tetrachloride. The observed protective effect of polyinosinic-polycytidylic acid on liver fibrosis was diminished through either depletion of natural killer cells or by disruption of the interferon γ gene. Expression of retinoic acid early inducible 1, the NKG2D ligand, was undetectable on quiescent hepatic stellate cells, whereas high levels were found on activated hepatic stellate cells, which correlated with the resistance and susceptibility of quiescent hepatic stellate cells and activated hepatic stellate cells to natural killer cell lysis, respectively. Moreover, treatment with polyinosinic-polycytidylic acid or interferon γ enhanced the cytotoxicity of natural killer cells against activated hepatic stellate cells and increased the expression of NKG2D and tumor necrosis factor–related apoptosis-inducing ligand on liver natural killer cells. Blocking NKG2D or tumor necrosis factor–related apoptosis-inducing ligand with neutralizing antibodies markedly diminished the cytotoxicity of polyinosinic-polycytidylic acid–activated natural killer cells against activated hepatic stellate cells. *Conclusions:* Our findings suggest that natural killer cells kill activated hepatic stellate cells via retinoic acid early inducible 1/NKG2D-dependent and tumor necrosis factor–related apoptosis-inducing ligand–dependent mechanisms, thereby ameliorating liver fibrosis.

4.382 Somatostatin inhibits dendritic cell responsiveness to *Helicobacter pylori*
Kao, J.Y. et al
*Regulatory Peptides, **134**, 23-29 (2006)*

Somatostatin is a regulatory peptide found in abundance in the stomach. We have previously shown that somatostatin is required for IL-4-mediated resolution of *Helicobacter pylori* gastritis. In the current study, we hypothesize that somatostatin acts directly on antigen-presenting cells in the stomach to lessen the severity of gastritis. To test this hypothesis, we first show that CD11c+ dendritic cells are present in the infected tissue of mice with *H. pylori*-induced gastritis. Pretreatment of bone marrow-derived dendritic cells with somatostatin results in decreased IL-12 production, and lower splenocyte proliferation induced by *H. pylori*-stimulated dendritic cells. Furthermore, octreotide, a somatostatin analogue, is more potent than somatostatin in suppressing IL-12 release by *H. pylori*-stimulated dendritic cells through an NF-kappaB-independent pathway. In addition, IL-4 stimulates somatostatin secretion from dendritic cells. In conclusion, somatostatin inhibits dendritic cell activation by *H. pylori*; a possible mechanism by which IL-4 mediates resolution of gastritis. We suggest that octreotide may be effective in treating immune-mediated diseases of the stomach.

4.383 H2-O expression in primary dendritic cells
Chen, X., Reed-Loisel, L.M., Karlsson, L. and Jensen, P.E.
*J. Immunol., **176**, 3548-3556 (2006)*

H2-O is a nonpolymorphic class II molecule whose biological role remains to be determined. H2-O modulates H2-M function, and it has been generally believed to be expressed only in B lymphocytes and thymic medullary epithelial cells, but not in dendritic cells (DCs). In this study, we report identification of H2-O expression in primary murine DCs. Similar to B cells, H2-O is associated with H2-M in DCs, and its expression is differentially regulated in DC subsets as well as during cell maturation and activation. Primary bone marrow DCs and plasmacytoid DCs in the spleen and lymph nodes express MHC class II and H2-M, but not the inhibitor H2-O. In contrast, myeloid DCs in secondary lymphoid organs express both H2-M and H2-O. In $CD8\alpha\alpha^+$ DCs, the ratio of H2-O to H2-M is higher than in $CD8\alpha\alpha^-$ DCs. In DCs generated from GM-CSF- and IL-4-conditioned bone marrow cultures, H2-O expression is not detected regardless of the maturation status of the cells. Administration of LPS induces in vivo activation of myeloid DCs, and this activation is associated with down-regulation of H2-O expression. Primary splenic DCs from $H2\text{-}O^{-/-}$ and $H2\text{-}O^{+/+}$ mice present exogenous protein Ags to T cell hybridomas similarly well, but $H2\text{-}O^{-/-}$ DCs induce stronger allogeneic CD4 T cell response than the $H2\text{-}O^{+/+}$ DCs in mixed leukocyte reactions. Our results suggest that H2-O has a broader role than previously appreciated in regulating Ag presentation.

4.384 Complete differentiation of CD8$^+$ T cells activated locally within the transplanted liver
Klein, I. and Crispe, I.N.
*J. Exp. Med., **203(2)**, 437-447 (2006)*

The transplanted liver elicits systemic tolerance, and the underlying mechanism may also account for the persistence of liver infections, such as malaria and viral hepatitis. These phenomena have led to the hypothesis that antigen presentation within the liver is abortive, leading to T cell tolerance or apoptosis. Here we test this hypothesis in an optimized orthotopic liver transplantation model. In direct contradiction to this model, the liver itself induces full CD8$^+$ T cell activation and differentiation. The effects of microchimerism were neutralized by bone marrow transplantation in the liver donor, and the lack of liver-derived antigen-presenting cells was documented by eight-color flow cytometry and by sensitive functional assays. We conclude that local antigen presentation cannot explain liver tolerance. On the contrary, the liver may be an excellent priming site for naive CD8$^+$ T cells.

4.385 Effector T cell differentiation and memory T cell maintenance outside secondary lymphoid organs
Obharai, J. et al
*J. Immunol., **176**, 4051-4058 (2006)*

Naive T cell circulation is restricted to secondary lymphoid organs. Effector and memory T cells, in contrast, acquire the ability to migrate to nonlymphoid tissues. In this study we examined whether nonlymphoid tissues contribute to the differentiation of effector T cells to memory cells and the long-term maintenance of memory T cells. We found that CD4, but not CD8, effector T cell differentiation to memory cells is impaired in adoptive hosts that lack secondary lymphoid organs. In contrast, established CD4 and CD8 memory T cells underwent basal homeostatic proliferation in the liver, lungs, and bone marrow, were maintained long-term, and functioned in the absence of secondary lymphoid organs. CD8 memory T cells found in nonlymphoid tissues expressed both central and effector memory phenotypes, whereas CD4 memory T cells displayed predominantly an effector memory phenotype. These findings indicate that secondary lymphoid organs are not necessary for the maintenance and function of memory T cell populations, whereas the optimal differentiation of CD4 effectors to memory T cells is dependent on these organs. The ability of memory T cells to persist and respond to foreign Ag independently of secondary lymphoid tissues supports the existence of nonlymphoid memory T cell pools that provide essential immune surveillance in the periphery.

4.386 Hedgehog signaling maintains resident hepatic progenitors throughout life
Sicklick, J.K. et al
*Am. J. Gastrointest. Liver Physiol., **290**, G859-G870 (2006)*

Hedgehog signaling through its receptor, Patched, activates transcription of genes, including *Patched*, that regulate the fate of various progenitors. Although Hedgehog signaling is required for endodermal commitment and hepatogenesis, the possibility that it regulates liver turnover in adults had not been considered because mature liver epithelial cells lack Hedgehog signaling. Herein, we show that this pathway is essential throughout life for maintaining hepatic progenitors. *Patched*-expressing cells have been identified among endodermally lineage-restricted, murine embryonic stem cells as well as in livers of fetal and adult *Ptc-lacZ* mice. An adult-derived, murine hepatic progenitor cell line expresses *Patched*, and Hedgehog-responsive cells exist in stem cell compartments of fetal and adult human livers. In both species, manipulation of Hedgehog activity influences hepatic progenitor cell survival. Therefore, Hedgehog signaling is conserved in hepatic progenitors from fetal development through adulthood and may be a new therapeutic target in patients with liver damage.

4.387 Effect of donor age on function of isolated human islets
Ihm, S-H. et al
*Diabetes, **55**, 1361-1368 (2006)*

This study intended to evaluate the impact of donor age on the function of isolated islets. Analysis of human islets from cadaveric donors (age 16–70 years) was performed using glucose-stimulated insulin release (GSIR) (n = 93), islet ATP content (n = 27), diabetic nude mouse bioassay (n = 72), and the insulin secretory function after single-donor clinical islet allotransplantation (n = 7). The GSIR index was

significantly higher in younger donors (age ≤40 years) than in older donors and negatively correlated with the donor age (r = –0.535). Islet ATP was higher in younger donors (115.7 ± 17.7 vs. 75.7 ± 6.6 pmol/µg DNA). The diabetes reversal rate of mice with 2,000 IE was significantly higher in younger donors (96 vs. 68%). C-peptide increment to glucose during intravenous glucose tolerance test at days 90–120 after clinical transplantation showed negative correlation with donor age (r = –0.872) and positive correlation with the islet mass (r = 0.832). On the other hand, acute insulin response to arginine only showed correlation with the islet mass and not with donor age. These results show that insulin secretory response to glucose deteriorates with increasing age and that it may be related to changes in ATP generation in ß-cells.

4.388 Pretransplant culture selects for high-quality porcine islets
Rijkelijkhuizen, J.K.R.A., van der Burg, M.P.M., Töns, A., Terpstra, O.T. and Bouwman, E.
*Transplantation, **32(4)**, 403-407 (2006)*

Objectives: The pig is generally considered a suitable alternative donor for clinical islet transplantation. However, adult pig islets are difficult to isolate and culture, often behave variably in in vitro assays, and do not consistently cure diabetic nude mice. In this study, we compared the in vivo function of freshly isolated and cultured adult porcine islets by transplantation in diabetic nude mice.
Methods: Freshly isolated and cultured islets were transplanted in different doses to diabetic nude mice (N = 48).
Results: Average islet yield was 1924 islet-equivalents per gram of pancreas, purity 96%, and the viability that was measured by acridine orange and propidium iodide was greater than 80% in all freshly isolated islet preparations. Grafts of freshly isolated islets failed to reduce hyperglycemia in 17 of 18 recipients. Although after 1 day of culture islet recovery was only 21%, grafts of these islets cured 12 of 17 mice. After 7 to 14 days of culture, the recovery had decreased to 11%; however, these islets reversed hyperglycemia in all mice (13/13) and showed shorter time-to-normoglycemia and more tightly regulated blood glucose.
Conclusions: Although freshly isolated adult porcine islets survive culture and transplantation poorly, islets selected by prolonged culture are of high potential.

4.389 Aberrant T helper cell response in tumor-bearing mice limits the efficacy of dendritic cell vaccine
Kao, J.Y., Zhang, M., Chen, C-M., Pierzchala, A. and Chen, J-J.
*Immunol. Lett., **105**, 16-25 (2006)*

Dendritic cell (DC) vaccine is a promising immunotherapy for malignancies, but its clinical efficacy has been questioned. Here we examined the mechanisms of treatment failure with DC vaccine in a murine colon cancer model. DC vaccination of naive mice prevents tumor implantation, but it is ineffective in tumor-bearing hosts despite the induction of tumor-specific CTL activity. Analyses of tumor-specific T helper cell type 1 (Th1)/T helper cell type 2 (Th2) responses showed that DC vaccine induced a mixed Th1/Th2 response in naive mice. Interestingly, $CD4^+$ T cells from tumor-bearing mice showed a Th1-predominant response before DC vaccination but Th2 after DC vaccination. Furthermore, interleukin-10 production was higher in $CD4^+$ T cells from vaccinated tumor-bearing mice than in $CD4^+$ T cells from unvaccinated tumor-bearing mice. $CD4^+$ T cells from mice treated with lipopolysaccharide (LPS)-matured DC fusion vaccine had lower production of interleukin-10 than $CD4^+$ T cells from mice treated with non-LPS-treated DC vaccine. However, similar to the non-LPS-treated DC vaccine, the LPS-matured DC vaccine failed to suppress tumor growth and induced a Th2 predominant tumor-specific response in tumor-bearing mice. These results suggest that the presence of tumor in the host induces an aberrant $CD4^+$ T cell response to DC vaccine, which may contribute to the failure of the vaccine to eradicate established tumors.

4.390 CCR6-mediated dendritic cell activation of pathogen-specific T cells in Peyer's patches
Salazar-Gonzales, R.M. et al
*Immunity, **24**, 623-632 (2006)*

T cell activation by dendritic cells (DCs) is critical to the initiation of adaptive immune responses and protection against pathogens. Here, we demonstrate that a specialized DC subset in Peyer's patches (PPs) mediates the rapid activation of pathogen specific T cells. This DC subset is characterized by the expression of the chemokine receptor CCR6 and is found only in PPs. $CCR6^+$ DCs were recruited into the dome regions of PPs upon invasion of the follicle associated epithelium (FAE) by an enteric pathogen and were responsible for the rapid local activation of pathogen-specific T cells. CCR6-deficient DCs were unable to respond to bacterial invasion of PPs and failed to initiate T cell activation, resulting in reduced defense against oral infection. Thus, CCR6-dependent regulation of DCs is responsible for localized T cell

dependent defense against entero-invasive pathogens.

4.391 A closed system for the preparation of islets of Langerhans using the COBE2991 cell processor
Lembert, N., Biesemeier, A., Klaffschenkel, R. And Königsrainer, A.
*Cytotherapy,**8, Suppl. 2** (2006)*
Abstracts of the 2nd International Conference "Strategies in Tissue Engineering"
May 31–June 2, 2006 Würzburg, Germany

Introduction: During the isolation of human islets of Langerhans, the digest is repeatedly and directly exposed to the ambient atmosphere. Therefore, the entire cell isolation must be performed in a clean room facility to fulfill the GMP requirements of German authorities. We used the pig model for a modification of the isolation and purification process by performing all islet preparation steps in a closed system consisting of a Ricordi chamber, a cooling device, and the COBE2991 cell processor. This study evaluated whether this entirely closed system to avoid the need of clean room facilities can deliver functional and purified islets. Materials & Methods: Pancreata from 6-month-old market-weight pigs were procured in the local slaughterhouse. After continuous digestion-filtration (Liberase PI, 1.5 mg/g pancreas) the digest was flushed through the cooling device and pumped directly into the COBE2991. Centrifugation (1500 U/min, 1 min), automatic supernatant pump-out, and Ricordi-chamber washing and refilling (HBSS) were repeated five times until a total of 3 l was flushed through the COBE2991, leaving the concentrated digest inside the COBE bag. A discontinuous gradient was applied consisting of a bottom layer (UW/Optiprep, density 1.12), a middle layer (UW/Optiprep, density 1.09), and a top layer (UW solution, density 1.05). Centrifugation (1000 U/min) was stopped after 5 min, and 20-ml fractions were collected. Islet equivalents (IEQ , areal density), insulin (ELISA), amylase activity (kinetic assay), and cell composition (FACS) were determined. Results: The islet yield in six preparations was 116875±17759 IEQ or 1290±118 IEQ/gP (mean9 SEM). Compared with the native organ, the insulin content of the islet fraction increased from 0.5±0.2 to 95±52 mg/mg protein. The insulin content in the islet fraction was 92±32 U. The amylase activity decreased from 2857±1001 to 63±28 U/mg protein. Glucose stimulated insulin output in 4/6 preparations. FACS identified 70% living cells, and 40% living beta cells. The entire preparation was completed within 4 h by two people, which doubled the routine preparation speed. Discussion: The presented technique allows a reproducible and efficient preparation of pure and vital porcine islets in a closed system. The preparation is much faster and less expensive than the traditional islet preparation. This technique may be applicable for human islet preparations without the need of preparations inside clean room facilities.

4.392 Comparison of Methods of Extracting Salmonella enterica Serovar Enteritidis DNA from Environmental Substrates and Quantification of Organisms by Using a General Internal Procedural Control
Klerks, M.M., van Bruggen, A.H.C., Zijlstra, C. And Donnikov, M.
*Appl. Envir. Micorbiol., **72(6)**, 3879-3886 (2006)*

This paper compares five commercially available DNA extraction methods with respect to DNA extraction efficiency of *Salmonella enterica* serovar Enteritidis from soil, manure, and compost and uses an *Escherichia coli* strain harboring a plasmid expressing green fluorescent protein as a general internal procedural control. Inclusion of this general internal procedural control permitted more accurate quantification of extraction and amplification of *S. enterica* serovar Enteritidis in these samples and reduced the possibility of false negatives. With this protocol it was found that the optimal extraction method differed for soil (Mobio soil DNA extraction kit), manure (Bio101 soil DNA extraction kit), and compost (Mobio fecal DNA extraction kit). With each method, as little as 1.2×10^3 to 1.8×10^3 CFU of added serovar Enteritidis per 100 mg of substrate could be detected by direct DNA extraction and subsequent *S. enterica*-specific TaqMan PCR. After bacterial enrichment, as little as 1 CFU/100 mg of original substrate was detected. Finally, the study presents a more accurate molecular analysis for quantification of serovar Enteritidis initially present in soil or manure using DNA extraction and TaqMan PCR.

4.393 Quantitative proteomics reveals posttranslational control as a regulatory factor in primary hematopoietic stem cells
Unwin, R.D. et al
*Blood, **107(12)**, 4687-4694 (2006)*

The proteome is determined by rates of transcription, translation, and protein turnover. Definition of stem cell populations therefore requires a stem cell proteome signature. However, the limit to the number of primary cells available has restricted extensive proteomic analysis. We present a mass spectrometric method using an isobaric covalent modification of peptides for relative quantification (iTRAQ), which was employed to compare the proteomes of approximately 1 million long-term reconstituting hematopoietic stem cells ($Lin^-Sca^+Kit^+$; LSK^+) and non–long-term reconstituting progenitor cells ($Lin^-Sca^+Kit^-$; LSK^-), respectively. Extensive 2-dimensional liquid chromatography (LC) peptide separation prior to mass spectrometry (MS) enabled enhanced proteome coverage with relative quantification of 948 proteins. Of the 145 changes in the proteome, 54% were not seen in the transcriptome. Hypoxia-related changes in proteins controlling metabolism and oxidative protection were observed, indicating that LSK^+ cells are adapted for anaerobic environments. This approach can define proteomic changes in primary samples, thereby characterizing the molecular signature of stem cells and their progeny.

4.394 Helicobacter pylori-secreted factors inhibit dendritic cell IL-12 secretion: a mechanism of ineffective host defense
Kao, J.Y. et al
*Am. J. Physiol Gastrointest. Liver Physiol., **291**, G73-G81 (2006)*

Helicobacter pylori evades host immune defenses and causes chronic gastritis. Immunity against intestinal pathogens is largely mediated by dendritic cells, yet the role of dendritic cells in acute *H. pylori* infection is largely unknown. We observed the recruitment of dendritic cells to the gastric mucosa of *H. pylori*-infected mice. Bone marrow-derived dendritic cells from mice responded to live *H. pylori* by upregulating the expression of proinflammatory cytokine mRNA (i.e., IL-1α, IL-1β, and IL-6). The supernatant from dendritic cells stimulated with *H. pylori* for 18 h contained twofold higher levels of IL-12p70 than IL-10 and induced the proliferation of syngeneic splenocytes and type 1 T helper cell cytokine release (IFN-γ and TNF-α). These responses were significantly lower compared with those induced by *Acinetobacter lwoffi*, another gastritis-causing pathogen more susceptible to host defenses. Analysis of whole *H. pylori* sonicate revealed the presence of a heat-stable factor secreted from *H. pylori* that specifically inhibited IL-12 but not IL-10 release from dendritic cells activated by *A. lwoffi*. Our findings suggest that dendritic cells participate in the host immune response against *H. pylori* and that their suppression by *H. pylori* may explain why infected hosts fail to prevent bacterial colonization.

4.395 Cyclin dependent kinase inhibitors prevent apoptosis of postmitotic mouse motoneurons
Appert-Collin, A. et al
*Life Sciences, **79(5)**, 484-490 (2006)*

Recent evidence suggests that apoptosis in post-mitotic neurons involves an aborted attempt of cells to re-enter the cell cycle which is characterized by increased expression of cyclins, such as cyclin D1, prior to death. However, such cyclins activation prior to apoptotic cell death remains controversial. Many neurological disorders are characterized by neuronal loss, particularly amyotrophic lateral sclerosis (ALS). ALS is a motoneuronal degenerative condition in which motoneuron loss could be due to an inappropriate return of these cells in the cell cycle. In the present study, we observed that deprivation of neurotrophic factor in purified motoneuron cultures induces an apoptotic pathway. After neurotrophic factor withdrawal, DAPI (4,6-diamidin-2-phenylindol dichlorohydrate) staining revealed the presence of nuclear condensation, DNA fragmentation, and perinuclear apoptotic body. Similarly, release of apoptotic microparticles and activation of caspases-3 and -9 were observed within the first hours following neurotrophic factor withdrawal. Next, we tested whether inhibition of cell cycle-related cyclin-dependent kinases (cdks) can prevent motoneuronal cell death. We showed that three cdk inhibitors, olomoucine, roscovitine and flavopiridol, suppress the death of motoneurons. Finally, we observed early increases in cyclin D1 and cyclin E expression after withdrawal of neurotrophic factors. These findings support the hypothesis that after removal of trophic support, post-mitotic neuronal cells die due to an attempt to re-enter the cell cycle in an uncoordinated and inappropriate manner.

4.396 The phyto-chemical (−)-epigallocatechin gallate suppresses gene expression of epidermal growth factor receptor in rat hepatic stellate cells in vitro by reducing the activity of Egr-1
Fu, Y. and Chen, A.
*Biochem. Pharmacol., **72(2)**, 227-238*

Hepatic stellate cells (HSC) are the major effectors in hepatic fibrogenesis. During liver injury, HSC

become activated and proliferative. Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are the potent mitogens for many cell types. We previously demonstrated that (−)-epigallocatechin gallate (EGCG), the major and active component in green tea extracts, inhibited HSC growth, including reducing cell proliferation, and inducing apoptosis. We have reported that EGCG interrupts PDGF signaling by reducing receptor tyrosine phosphorylation and gene expression of PDGF-β receptor. Additional experiments are necessary to elucidate the effect of EGCG on EGF signaling in activated HSC. The aims of this study are to evaluate the effect of EGCG on the expression of EGFR and to elucidate the underlying molecular mechanisms in activated HSC. We hypothesize that EGCG might interrupt EGF signaling by suppressing gene expression of EGF receptor (EGFR) in activated HSC, which, together with the interruption of PDGF signaling, might collectively result in the inhibition of HSC growth. The present report demonstrates that the phyto-chemical dose-dependently suppresses gene expression of EGFR in activated HSC *in vitro*. The Egr-1 binding site located in the *egfr* promoter is found to be cis-activating element in regulating the promoter activity of the gene. EGCG inhibits the *trans*-activation activity of Egr-1 in activated HSC by suppressing gene expression of the transcription factor. The interruption of the ERK signaling pathway by EGCG reduces the *trans*-activation activity of Egr-1 and the promoter activity of EGFR gene in HSC. Taken together, our results demonstrate that EGCG suppresses gene expression of EGFR in rat activated HSC *in vitro* mediated by reducing the *trans*-activation activity of Egr-1.

4.397 A comparison of ovine monocyte-derived macrophage function following infection with *Mycobacterium avium* ssp. *avium* and *Mycobacterium avium* ssp. *paratuberculosis*
Berger, S.T. and Griffin, F.T.
Immunol. Cell Biol., ***84(4)***, *349-356 (2006)*

Mycobacterium avium ssp. paratuberculosis causes Johne's disease in ruminants, whereas the antigenically and genetically similar subspecies Mycobacterium avium ssp. avium is less virulent. In this study, we compared one strain of each subspecies for its ability to survive, induce cytokines, suppress MHC class I and II expression and induce apoptosis or necrosis in ovine monocyte-derived macrophages. Both subspecies survived intracellularly and induced the secretion of IL-10. Low levels of TNF-α were detected after infection with both subspecies at 4 h. IL-12 was not upregulated after infection. Downregulation of MHC class I and II was evident in response to infection with both *M. avium* ssp. *avium* and *M. avium* ssp. *paratuberculosis*. No significant cytotoxicity was detectable in ovine macrophages after the addition of bacteria. *M. avium* ssp. *paratuberculosis* induced slightly more apoptosis than *M. avium* ssp. *avium*. Still the overall rate of apoptosis was very low and both subspecies suppressed LPS-induced macrophage apoptosis.

4.398 Phenotypic and Functional Characterization of Vaginal Dendritic Cells in a Rat Model of Candida albicans Vaginitis
De Bernardis, F. Et al
Infect. Immun., ***74(7)***, *4282-4294 (2006)*

This study analyzes the phenotype of vaginal dendritic cells (VDCs), their antigenic presentation and activation of T-cell cytokine secretion, and their protective role in a rat model of *Candida* vaginitis. Histological observation demonstrated a significant accumulation of $OX62^+$ VDCs in the mucosal epithelium of *Candida albicans*-infected rats at the third round of infection. We identified two subsets of $OX62^+$ VDCs differing in the expression of CD4 molecule in both noninfected and *Candida*-infected rats. The $OX62^+$ $CD4^+$ subset of VDCs displayed a lymphoid cell-like morphology and expressed the T-cell antigen CD5, whereas the $OX62^+$ $CD4^-$ VDC subset exhibited a myeloid morphology and was CD5 negative. *Candida* infection resulted in VDC maturation with enhanced expression of CD80 and CD134L on both $CD4^+$ and $CD4^-$ VDC subsets at 2 and 6 weeks after *Candida* infection. $CD5^-$ $CD4^-$ $CD86^-$ $CD80^-$ $CD134L^+$ VDCs from infected, but not noninfected, rats spontaneously released large amounts of interleukin-12 (IL-12) and tumor necrosis factor alpha, whereas all VDC subsets released comparable levels of IL-10 and IL-2 cytokines. Furthermore, $OX62^+$ VDCs from infected rats primed naïve $CD4^+$ T-cell proliferation and release of cytokines, including gamma interferon, IL-2, IL-6, and IL-10, in response to staphylococcal enterotoxin B stimulation in vitro. Adoptive transfer of highly purified $OX62^+$ VDCs from infected rats induced a significant acceleration of fungal clearance compared with that in rats receiving naive VDCs, suggesting a protective role of VDCs in the anti-*Candida* mucosal immunity. Finally, VDC-mediated protection was associated with their ability to rapidly migrate to the vaginal mucosa and lymph nodes, as assessed by adoptive transfer of $OX62^+$ VDCs labeled with 5 (and 6-)-carboxyfluorescein diacetate succinimidyl ester.

4.399 The antifibrogenic effect of (-)-epigallocatechin gallate results from the induction of de novo synthesis of glutathione in passaged rat hepatic stellate cells
Yumei, F., Zhou, Y., Zheng, S. and Chen, A.
Lab. Invest., ***86***, *697-709 (2006)*

Hepatic stellate cells (HSC) are the major players during hepatic fibrogenesis. Overproduction of extracellular matrix (ECM) is a characteristic of activated HSC. Transforming growth factor-beta (TGF-β) is the most potent fibrogenic cytokine while connective tissue growth factor (CTGF) mediates the production of TGF-β-induced ECM in activated HSC. HSC activation and hepatic fibrogenesis are stimulated by oxidative stress. Glutathione (GSH) is the most important intracellular antioxidant. The aim of this study is to explore the mechanisms of (-)-epigallocatechin-3-gallate (EGCG), the major and most active component in green tea extracts, in the inhibition of ECM gene expression in activated HSC. It is hypothesized that EGCG inhibits ECM gene expression in activated HSC by interrupting TGF-β signaling through attenuating oxidative stress. It is found that EGCG interrupts TGF-β signaling in activated HSC by suppressing gene expression of type I and II TGF-β receptors. EGCG inhibits CTGF gene expression, leading to the reduction in the abundance of ECM, including αI(I) procollagen. Exogenous CTGF dose dependently eliminates the antifibrogenic effect. EGCG attenuates oxidative stress in passaged HSC by scavenging reactive oxygen species and reducing lipid peroxidation. *De novo* synthesis of GSH is a prerequisite for EGCG to interrupt TGF-β signaling and to reduce the abundance of αI(I) procollagen in activated HSC *in vitro*. Taken together, our results demonstrate that the interruption of TGF-β signaling by EGCG results in the suppression of gene expression of CTGF and ECM in activated HSC *in vitro*. In addition, our results, for the first time, demonstrate that the antioxidant property of EGCG derived from *de novo* synthesis of intracellular GSH plays a critical role in its antifibrogenic effect. These results provide novel insights into the mechanisms of EGCG as an antifibrogenic candidate in the prevention and treatment of liver fibrosis.

4.400 Local Intrahepatic CD8+ T Cell Activation by a Non-Self- Antigen Results in Full Functional Differentiation
Wuensch, S.A., Pierce, R.H. and Crispe, I.N.
J. Immunol., ***177***, *1689-1697 (2006)*

The response of T cells to liver Ags sometimes results in immune tolerance. This has been proposed to result from local, intrahepatic priming, while the expression of the same Ag in liver-draining lymph nodes is believed to result in effective immunity. We tested this model, using an exogenous model Ag expressed only in hepatocytes, due to infection with an adeno-associated virus vector. T cell activation was exclusively intrahepatic, yet in contrast to the predictions of the current model, this resulted in clonal expansion, IFN-γ synthesis, and cytotoxic effector function. Local activation of naive $CD8^+$ T cells can therefore cause full $CD8^+$ T cell activation, and hepatocellular presentation cannot be used to explain the failure of CTL effector function against some liver pathogens such as hepatitis C.

4.401 The oncofetal protein glypican-3 is a novel marker of hepatic progenitor/oval cells
Grozdanov, P.N., Yovchev, M.I. and Dabeva, D.
Lab. Invest., ***86***, *1272-1284 (2006)*

Glypican-3 (Gpc3), a cell surface-linked heparan sulfate proteoglycan is highly expressed during embryogenesis and is involved in organogenesis. Its exact biological function remains unknown. We have studied the expression of Gpc3 in fetal and adult liver, in liver injury models of activation of liver progenitor cells: D-galactosamine and 2-acetylaminofluorene (2-AAF) administration followed by partial hepatectomy (PH) (2-AAF/PH); and in the Solt-Farber carcinogenic model: by initiation with a single dose of diethylnitrosamine and promotion with 2-AAF followed by PH treatment. Gpc3 expression was studied using complementary DNA microarrays, reverse transcriptase-polymerase chain reaction, *in situ* hybridization (ISH); ISH combined with immunohistochemistry (IHC) and immunofluorescent microscopy. We found that Gpc3 is highly expressed in fetal hepatoblasts from embryonic days 13 through 16 and its expression gradually decreases towards birth. Dual ISH with Gpc3 and α-fetoprotein (AFP) probes confirmed that only hepatoblasts and no other fetal liver cells express Gpc3. At 3 weeks after birth the expression of Gpc3 mRNA and protein was hardly detected in the liver. Gpc3 expression was highly induced in oval cell of D-gal and 2-AAF/PH treated animals. Dual ISH/IHC with Gpc3 riboprobe and

cytokeratin-19 (CK-19) antibody revealed that Gpc3 is expressed in activated liver progenitor cells. ISH for Gpc3 and AFP performed on serial liver sections also showed coexpression of the two-oncofetal proteins. FACS isolated oval cells with anti-rat Thy1 revealed expression of Gpc3. Gpc3 expression persists in atypical duct-like structures and liver lesions of animals subjected to the Solt-Farber model of initiation and promotion of liver cancer expressing CK-19. In this work we report for the first time that the oncofetal protein Gpc3 is a marker of hepatic progenitor cells and of early liver lesions. Our findings show further that hepatic progenitor/oval cells are the target for malignant transformation in the Solt-Farber model of hepatic carcinogenesis.

4.402 Intramuscular immunization with DNA construct containing Der p 2 and signal peptide sequences primed strong IgE production

Tan, L.K., Huang, C-H., Kuo, I-C., Liew, L.M. and Chua, K.Y.
Vaccine, ***24(29-30)***, *5762-5771 (2006)*

Background
Previous studies demonstrated that allergen gene vaccination induced TH1-skewed responses and inhibited IgE production. This study evaluated and characterized the immune responses induced by three DNA constructs encoding different forms of Der p 2 for safe and efficacious vaccination against mite allergy.
Methods
Mice were immunized intramuscularly with DNA constructs encoding a major mite allergen, Der p 2, without a signal peptide (p2), with a signal peptide (p52), and with a signal peptide plus lysosomal-targeting sequence (p52-LA), respectively, followed by TH2-skewed protein challenge. Antibody and T-cell cytokine responses were assessed by ELISA. Primed dendritic cells (DCs) were adoptively transferred to naïve mice and humoral responses were examined after protein challenge. The circulating Der p 2 protein was detected by sandwich ELISA.
Results
Mice immunized with p52-LA showed strong and clear-cut TH1-type response, as evident by high IFN-γ production and elevated levels of Der p 2-specific IgG2a production whereas construct p2 induced only moderate levels of TH1 response. In contrast, mice immunized with construct p52 showed a mixed TH1/TH2 phenotype and produced substantial circulating Der p 2 protein. Mice adoptively transferred with DCs primed by p52 construct, but not by the p2 or p52-LA constructs, were sensitized to produce high levels of Der p 2-specific IgE.
Conclusions
Immunization with DNA construct encoding a signal peptide could potentially prime TH2-skewed responses and IgE production. The additional inclusion of lysosomal-targeting sequences to such construct could improve the safety and efficacy of DNA vaccination against allergy.

4.403 Effects of cushioned centrifugation on sperm quality in stallion semen stored cooled at 5°C for 24h, and stored cooled for 2h or 24h and then frozen

Sieme, H., Knop, K. And Rath, D.
Animal Reprod. Sci., **94(1-2)**, 99-103 (2006)

Removing seminal plasma from stallion spermatozoa before use in a cooled semen or frozenthawed semen breeding program has been reported to be beneficial. The centrifugation involved is not without detrimental effects on the motility and morphology of spermatozoa, and may lead to loss of spermatozoa. To reduce this, semen can be underlaid with a dense, liquid cushion, on which the spermatozoa float during the centrifugation process (Revell et al., 1997; Ecot et al., 2005). Transportation of stallion spermatozoa for freezing at appropriate facilities would allow stallions to remain at home. Most reports recommend that semen be centrifuged at room temperature to remove seminal plasma, cooled slowly to 5 ◦C, and shipped at 5 ◦C prior to freezing (Crockett et al., 2001; Backman et al., 2004). However, the effects of cushioned centrifugation techniques on stallion spermatozoa after cooled-storage at 5 ◦C for 24 h, and after subsequent cryopreservation are unknown. This study evaluates the effects of high-speed centrifugation (20 min×1000×*g*) with various centrifugation extenders (Eqcellsire®, INRA-82, HBS) with or without a cushion (Cushion-Fluid®, Minit¨ub, Landshut, Germany; Eqcellsire®, IMV, L ´ Aigle, France) in stallions with good and poor semen freezability.

4.404 The Phenotypes of Pluripotent Human Hepatic Progenitors
Schmelzer, E., Wauther, E. and Reid, L.M.
*Stem Cells, **24**, 1852-1858 (2006)*

Human livers contain two pluripotent hepatic progenitors, hepatic stem cells and hepatoblasts, with size, morphology, and gene expression profiles distinct from that of mature hepatocytes. Hepatic stem cells, the precursors to hepatoblasts, persist in stable numbers throughout life, and those isolated from the livers of all age donors from fetal to adult are essentially identical in their gene and protein expression profiles. The gene expression profile of hepatic stem cells throughout life consists of high levels of expression of cytokeratin 19 (CK19), neuronal cell adhesion molecule (NCAM), epithelial cell adhesion molecule (EpCAM), and claudin-3 (CLDN-3); low levels of albumin; and a complete absence of expression of α-fetoprotein (AFP) and adult liver-specific proteins. By contrast, hepatoblasts, the dominant cell population in fetal and neonatal livers, decline in numbers with age and are found as <0.1% of normal adult livers. They express high levels of AFP, elevated levels of albumin, low levels of expression of adult liver-specific proteins, low levels of CK19, and a loss of NCAM and CLDN-3. Mature hepatocytes lack expression altogether of EpCAM, NCAM, AFP, CLDN-3, cytokeratin 19, and have acquired the well-known adult-specific profile that includes expression of high levels of albumin, cytochrome P4503A4, connexins, phosphoenolpyruvate carboxykinase, and transferrin. Thus, hepatic stem cells have a unique stem cell phenotype, whereas hepatoblasts have low levels of expression of both stem cell genes and genes expressed in high levels in mature hepatocytes.

4.405 Cytokine-induced monocyte adhesion to endothelial cells involves platelet-activating factor: Suppression by conjugated linoleic acid
Sneddon, A.A., McLeod, E., Wahle, K.W.J. and Arthur, J.R.
*Biochim. Biophys. Acta, **1761(7)**, 793-801 (2006)*

Monocyte–endothelium interaction is key to many acute and chronic inflammatory diseases. We have investigated the factors regulating monocyte attachment to cytokine-activated human umbilical vein endothelial cells (HUVEC) and the modulatory effect of the polyunsaturated fatty acid (PUFA), conjugated linoleic acid (CLA) in this process. Both TNF-α and IL-1β induced HUVEC platelet-activating factor (PAF) production and PAF was required for subsequent firm THP-1 monocyte adhesion since it was inhibited by both PAF receptor antagonists (BN-52021 or CV-6209) and a PAF synthesis inhibitor (sanguinarine). CLA inhibited the binding of both THP-1 and isolated human peripheral blood monocytes to HUVEC by up to 40% with the CLA t10,c12 isomer suppressing adhesion dose-dependently. Investigation into the mechanism involved demonstrated that with IL-1β, VCAM-1 and ICAM-1 levels and pro-inflammatory cytokine expression were largely unaffected by CLA. Through the use of PAF receptor antagonists and PAF synthesis inhibitors, CLA was shown to inhibit cytokine-induced binding by suppressing PAF production. Direct assay of PAF levels confirmed this result. We conclude that endothelial-generated PAF plays a central role in cytokine-induced monocyte adherence to endothelium and that the anti-inflammatory action of PUFAs such as CLA in suppressing monocyte–endothelial interaction is mediated through attenuation of pro-inflammatory phospholipids such as PAF.

4.406 An α-Glucan of Pseudallescheria boydii Is Involved in Fungal Phagocytosis and Toll-like Receptor Activation
Bittencourt, V.C.B. et al
*J. Biol. Chem., **281(32)**, 22614-22623 (2006)*

The host response to fungi is in part dependent on activation of evolutionarily conserved receptors, including toll-like receptors and phagocytic receptors. However, the molecular nature of fungal ligands responsible for this activation is largely unknown. Herein, we describe the isolation and structural characterization of an α-glucan from *Pseudallescheria boydii* cell wall and evaluate its role in the induction of innate immune response. These analyses indicate that α-glucan of *P. boydii* is a glycogen-like polysaccharide consisting of linear 4-linked α-D-Glc*p* residues substituted at position 6 with α-D-Glc*p* branches. Soluble α-glucan, but not β-glucan, led to a dose-dependent inhibition of conidia phagocytosis. Furthermore, a significant decrease in the phagocytic index occurred when α-glucan from conidial surface was removed by enzymatic treatment with α-amyloglucosidase, thus indicating an essential role of α-glucan in *P. boydii* internalization by macrophages. α-Glucan stimulates the secretion of inflammatory cytokines by macrophages and dendritic cells; again this effect is abolished by treatment with α-amyloglucosidase. Finally, α-glucan induces cytokine secretion by cells of the innate immune system in a mechanism involving toll-like receptor 2, CD14, and MyD88. These results might have relevance in the

context of infections with *P. boydii* and other fungi, and α-glucan could be a target for intervention during fungal infections.

4.407 **The p110δ Isoform of PI3K Differentially Regulates β1 and β2 Integrin-Mediated Monocyte Adhesion and Spreading and Modulates Diapedesis**
Ferreira, A.M., Isaacs, H., Hayflick, J.S., Rogers, K.A. and Sandig, M.
*Microcirculation, **13**, 439-456 (2006)*

Objective: Leukocyte diapedesis is misregulated in inflammatory disease and depends on the binding of monocytic LFA-1 and VLA-4 to endothelial ICAM-1 and VCAM-1, respectively. The authors hypothesized that these different molecular interactions elicit specific signaling cascades within monocytes regulating specific steps in adhesion, motility, and diapedesis.
Methods: The authors employed the PI3K p110δ catalytic subunit specific inhibitor IC87114 (2 μ M) and the broad-spectrum PI3K inhibitory agents LY294002 (50 μ M) and wortmannin (100 nM), to examine the role of PI3Kδ in monocyte diapedesis through endothelial monolayers and its role in monocyte adhesion and spreading upon carpets of ICAM-1 or VCAM-1. They further explored the effects of PI3Kδ inhibition on the activation state of β 1 and β 2 integrins with immunocytochemistry and flow cytometry.
Results: In human peripheral blood monocytes IC87114 was as effective as wortmannin and LY294002 at inhibiting diapedesis, however, in THP-1 cells LY294002 and wortmannin caused a 5-fold reduction in diapedesis, while IC87114 only decreased diapedesis 2-fold. PI3Kδ activity was specifically required for THP-1 cell adhesion and spreading on VCAM-1, but not on ICAM-1 protein substrates. Flow cytometric analysis demonstrated that PI3Kδ inhibition decreased the amount of conformationally active β 1-integrins, while having no effect on the prevalence of conformationally active β 2-integrins expressed on the cell surface. In addition, PI3Kδ inhibition resulted in a 4-fold decrease in the activation state of Rac-1 and Cdc42.
Conclusions: These results demonstrate the specific necessity of PI3Kδ in regulating monocytic integrin activation and the general role of PI3K signaling during diapedesis, implicating PI3K as a target for therapeutic intervention.

4.408 **The efficient isolation of murine splenic dendritic cells and their cytochemical features**
Zarnani, A.H. et al
*Histochem. Cell Biol., **126**, 275-282 (2006)*

Despite their importance in professional antigen presentation and their ubiquitous presence, dendritic cells (DCs) are usually found in such trace amounts in tissues that their isolation with high purity is a difficult task. Because of their scarcity, accurate determination of the purity of isolated dendritic cells is very important. In this study, we purified murine splenic dendritic cells by a three-step enrichment method and evaluated their morphological, cytochemical and functional characteristics. Purity of the isolated cells was determined by established methods such as flow cytometry (FC) and immunocytochemistry (ICC) using anti-CD11c monoclonal antibody. In order to test purified DC functional properties, we used in vivo antigen presentation assay. Our results showed that antigen-pulsed DCs are potent stimulators of antigen-specific lymphocyte proliferation. We studied myeloperoxidase (MPO) and non-specific esterase (NSE) activity in isolated cells to determine the purity of dendritic cells compared to more conventional methods. Our results showed that murine splenic dendritic cells were deficient in both MPO and NSE activity and the percentage of purity obtained by NSE staining on isolated cells was comparable to the results obtained by either FC or ICC. To our knowledge, this is the first report on using NSE activity for determination of the purity of isolated murine splenic dendritic cells. We, therefore, recommend that NSE activity be employed as a simple, inexpensive and yet accurate method for evaluation of the purity of isolated murine splenic dendritic cells.

4.409 **Post-mortem semen cryopreservation and characterization in two different endangered gazelle species (Gazella gazella and Gazella dorcas) and one subspecies (Gazella gazelle acaiae)**
Saragusty, J., Gacitua, H., King, R. and Arav, A.
*Theriogenology, **66(4)**, 775-784 (2006)*

Both *Gazella gazella* and *Gazella dorcas* are endangered species with continually dwindling population size, yet basic knowledge on their spermatozoa is missing. Semen collected post-mortem (PM) from the cauda epididymis of five adult gazelles (three *Gazella gazella gazella*, one *Gazella gazella acaiae* and one *G. dorcas*) was cryopreserved using directional freezing of large volumes (8 mL) with egg-yolk-free extender. Sperm size measurements and SYBR-14/propodium iodide (PI) viability stain validation for use

in gazelles were conducted. Post-thaw characterization included motility, viability, acrosome damage evaluation, computerized motility characterization and morphology and sperm motility index (SMI) was calculated.
Extracted sperm motility was 71.67 ± 11.67% (mean ± S.E.M.). Post-thaw motility ranged between 15% and 63%, viability was 57.49 ± 3.24%, intact acrosome was detected in 63.74 ± 2.6% (median 64.8%, upper/lower quartiles 71.79%, 61.82%), and normal morphology ranged between 41% and 63%. Motility characterization showed two sub-groups—highly active and progressively motile spermatozoa with SMI of 62.75 ± 0.38 and low activity and poorly progressive with SMI of 46.16 ± 1.53. Our results indicate that PM preservation of gazelle spermatozoa with satisfactory post-thaw viability is possible and cryobanking is achievable.

4.410 Arginase 1 Regulation of Nitric Oxide Production Is Key to Survival of Trophic Factor-Deprived Motor Neurons
Estevez, A.G. et al
J. Neurosci., ***26(33)***, *8512-8516 (2006)*

When deprived of trophic factors, the majority of cultured motor neurons undergo nitric oxide-dependent apoptosis. However, for reasons that have remained unclear, 30–50% of the motor neurons survive for several days without trophic factors. Here we hypothesize that the resistance of this motor neuron subpopulation to trophic factor deprivation can be attributed to diminished nitric oxide production resulting from the activity of the arginine-degrading enzyme arginase. When incubated with nor-N^G-hydroxy-nor-L-arginine (NOHA), the normally resistant trophic factor-deprived motor neurons showed a drop in survival rates, whereas trophic factor-treated neurons did not. NOHA-induced motor neuron death was inhibited by blocking nitric oxide synthesis and the scavenging of superoxide and peroxynitrite, suggesting that peroxynitrite mediates NOHA toxicity. When we transfected arginase 1 into motor neurons to see whether it alone could abrogate trophic factor deprivation-induced death, we found that its forced expression did indeed do so. The protection afforded by arginase 1 expression is reversed when cells are incubated with NOHA or with low concentrations of nitric oxide. These results reveal that arginase acts as a central regulator of trophic factor-deprived motor neuron survival by suppressing nitric oxide production and the consequent peroxynitrite toxicity. They also suggest that the resistance of motor neuron subpopulations to trophic factor deprivation may result from increased arginase activity.

4.411 Multiprotein Complexes of the Survival of Motor Neuron Protein SMN with Gemins Traffic to Neuronal Processes and Growth Cones of Motor Neurons
Zhang, H. et al
J. Neurosci., ***26(33)***, *8622-8632 (2006)*

Spinal muscular atrophy (SMA), a progressive neurodegenerative disease affecting motor neurons, is caused by mutations or deletions of the *SMN1* gene encoding the survival of motor neuron (SMN) protein. In immortalized non-neuronal cell lines, SMN has been shown to form a ribonucleoprotein (RNP) complex with Gemin proteins, which is essential for the assembly of small nuclear RNPs (snRNPs). An additional function of SMN in neurons has been hypothesized to facilitate assembly of localized messenger RNP complexes. We have shown that SMN is localized in granules that are actively transported into neuronal processes and growth cones. In cultured motor neurons, SMN granules colocalized with ribonucleoprotein Gemin proteins but not spliceosomal Sm proteins needed for snRNP assembly. Quantitative analysis of endogenous protein colocalization in growth cones after three-dimensional reconstructions revealed a statistically nonrandom association of SMN with Gemin2 (40%) and Gemin3 (48%). SMN and Gemin containing granules distributed to both axons and dendrites of differentiated motor neurons. A direct interaction between SMN and Gemin2 within single granules was indicated by fluorescence resonance energy transfer analysis of fluorescently tagged and overexpressed proteins. High-speed dual-channel imaging of live neurons depicted the rapid and bidirectional transport of the SMN–Gemin complex. The N terminus of SMN was required for the recruitment of Gemin2 into cytoplasmic granules and enhanced Gemin2 stability. These findings provide new insight into the molecular composition of distinct SMN multiprotein complexes in neurons and motivation to investigate deficiencies of localized RNPs in SMA.

4.412 Role of Transcription Factor T-bet Expression by CD4+ Cells in Gastritis Due to Helicobacter pylori in Mice
Eaton, K.A., Benson, L.H., Haeger, J. and Gray, B.M.
Infect. Immun., ***74(8)***, *4673-4684 (2006)*

Gastritis due to *Helicobacter pylori* is induced by a Th1-mediated response that is CD4 cell and gamma interferon (IFN-γ) dependent. T-bet is a transcription factor that directs differentiation of and IFN-γ secretion by $CD4^+$ Th1 T cells. The goal of this study was to use two mouse models to elucidate the role of T-bet in gastritis due to *H. pylori*. C57BL/6J mice, congenic T-bet knockout (KO) mutants, or congenic SCID (severe, combined immunodeficient) mutants were given live *H. pylori* by oral inoculation. SCID mice were given $CD4^+$ splenocytes from C57BL/6J or T-bet KO mice by intraperitoneal injection. Twelve or 24 weeks after bacterial inoculation, C57BL/6J mice developed moderate gastritis but T-bet KO mice and SCID mice did not. In contrast, SCID recipients of either C57BL/6J T cells or T-bet KO T cells developed gastritis 4 or 8 weeks after adoptive transfer. In recipients of C57BL/6J $CD4^+$ cells but not recipients of T-bet KO cells, gastritis was associated with a delayed-type hypersensitivity response to *H. pylori* antigen and elevated gastric and serum IFN-γ, interleukin 6, and tumor necrosis factor alpha. In spite of the absence of IFN-γ expression, indicating failure of Th1 differentiation, $CD4^+$ T cells from T-bet KO mice induce gastritis in *H. pylori*-infected recipient SCID mice. This indicates that Th1-independent mechanisms can cause gastric inflammation and disease due to *H. pylori*.

4.413 Procurement of the Human Pancreas for Pancreatic Islet Transplantation from Marginal Cadaver Donors

Nagata, H. et al

*Transplantation, **82(3)**, 327-331 (2006)*

Background. Recent advances in pancreatic islet transplantation (PIT) have contributed significantly to the treatment of patients with type 1 diabetes. The specific aim of this study was to develop an effective technique for the procurement of pancreas for PIT from nonheart-beating-donor (NHBDs).
Methods. Between January 2004 and August 2004, eight human pancreata were procured and processed for isolation of islets at a cell processing center. After confirmation of brain death status, a double balloon catheter was inserted to prevent warm ischemic damage to the donor pancreas by using an in situ regional organ cooling system that was originally developed for procurement of kidneys. The catheter position of the cooling system was modified specifically for the pancreas and kidney. Furthermore, we worked in cooperation with a kidney procurement team to protect the pancreas during kidney procurement.
Results. Warm ischemic time could be controlled with the modified in situ regional cooling system at 3.0+/-0.8 min (mean+/-SE). The operations for procurement of the kidneys and pancreata lasted 45.6+/-3.6 min and 10.6+/-1.8 min, respectively. Islet yield per isolation was 444,426+/-35,172 IE (islet equivalent). All eight cases met the criteria for PIT based on the Edmonton protocol.
Conclusion. We developed a novel procurement technique in cooperation with our kidney procurement team. This protocol for the procurement of pancreas and kidney from a NHBD enabled us to transplant islets into a type 1 diabetic patient and kidney into a renal failure patient.

4.414 T cell surface redox levels determine T cell reactivity and arthritis susceptibility

Gelderman, K.A., Hultquist, M., Holmberg, J., Olafsson, P. and Holmdahl, R.

*PNAS, **103(34)**, 12831-12836 (2006)*

Rats and mice with a lower capacity to produce reactive oxygen species (ROS) because of allelic polymorphisms in the *Ncf1* gene (which encodes neutrophil cytosolic factor 1) are more susceptible to develop severe arthritis. These data suggest that ROS are involved in regulating the immune response. We now show that the lower capacity to produce ROS is associated with an increased number of reduced thiol groups (–SH) on T cell membrane surfaces. Artificially increasing the number of reduced thiols on T cells from animals with arthritis-protective *Ncf1* alleles by glutathione treatment lowered the threshold for T cell reactivity and enhanced proliferative responses *in vitro* and *in vivo*. Importantly, T cells from immunized congenic rats with an E3-derived Ncf1 allele (DA.$Ncf1^{E3}$ rats) that cannot transfer arthritis to rats with an arthritis-associated Dark Agouti (DA)-derived mutated Ncf1 allele (DA.$Ncf1^{DA}$ rats) became arthritogenic after increasing cell surface thiol levels. This finding was confirmed by the reverse experiment, in which oxidized T cells from DA.$Ncf1^{DA}$ rats induced less severe arthritis compared with controls. Therefore, we conclude that ROS production as controlled by *Ncf1* is important in regulating surface redox levels of T cells and thereby suppresses autoreactivity and arthritis development.

4.415 Human Fallopian Tube Neutrophils – A Distinct Phenotype from Blood Neutrophils

Smith, J.M., Wira, C.R., Fanger, M.W. and Shen, L.

*Am. J. Reprod. Immunol., **56**, 218-229 (2006)*

Problem The role of neutrophils in the human Fallopian tube (FT) is unknown. In order to provide insights into their functions in the FT, we systematically compared neutrophils from normal FT and peripheral blood (PB).
Method of study Flow cytometric analysis of surface receptors, granule proteins, and intracellular cytokines expressed by neutrophils from enzymatically dispersed FT and PB was performed.
Results Fallopian tube neutrophils expressed significantly higher levels of CD64, human class II histocompatibility antigen DR (HLA-DR), γ-interferon, and vascular endothelial growth factor than those from PB. Fewer FT neutrophils expressed IL-8 receptors compared to PB, while more expressed the receptor for the bacterial-derived chemoattractant formyl-Met-Leu-Phe (fMLP). The number of FT neutrophils containing the granule proteins matrix metalloproteinase-9, lactoferrin, and myeloperoxidase was decreased versus PB.
Conclusion Fallopian tube neutrophils exhibit a phenotype distinct from PB neutrophils, suggesting functional activation of innate immune defense in the female reproductive tract as well as a potential role in maintaining normal FT physiology.

4.416 Successful Islet Transplantation from Nonheartbeating Donor Pancreata Using Modified Ricordi Islet Isolation Method
Matsumoto, S. et al
*Transplantation, **82(4)**, 460-465 (2006)*

Background. Current success of islet transplantation has led to donor shortage and the need for marginal donor utilization to alleviate this shortage. The goal of this study was to improve the efficacy of islet transplantation using nonheartbeating donors (NHBDs).
Methods. First, we used porcine pancreata for the implementation of several strategies and applied to human pancreata. These strategies included ductal injection with trypsin inhibitor for protection of pancreatic ducts, ET-Kyoto solution for pancreas preservation, and Iodixanol for islet purification.
Results. These strategies significantly improved both porcine and human islet isolation efficacy. Average 399,469+/-36,411 IE human islets were obtained from NHBDs (n=13). All islet preparations met transplantation criteria and 11 out of 13 cases (85%) were transplanted into six type 1 diabetic patients for the first time in Japan. All islets started to secrete insulin and all patients showed better blood glucose control without hypoglycemic loss of consciousness. The average HbA1c levels of the six recipients significantly improved from 7.5+/-0.4% at transplant to 5.1+/-0.2% currently ($P<0.0003$). The average insulin amounts of the six recipients significantly reduced from 49.2+/-3.3 units at transplant to 11+/-4.4 units ($P<0.0005$) and five out of six patients reduced to less than half dose. The first patient is now insulin free, the first such case in Japan.
Conclusion. This demonstrates that our current protocol makes it feasible to use NHBDs for islet transplant into type 1 diabetic patients efficiently.

4.417 Evidence for epithelial-mesenchymal transitions in adult liver cells
Sicklick, J.K: et al
*AM. J. Physiol. Liver Physiol., **291**, G575-G583 (2006)*

Both myofibroblastic hepatic stellate cells (HSC) and hepatic epithelial progenitors accumulate in damaged livers. In some injured organs, the ability to distinguish between fibroblastic and epithelial cells is sometimes difficult because cells undergo epithelial-mesenchymal transitions (EMT). During EMT, cells coexpress epithelial and mesenchymal cell markers. To determine whether EMT occurs in adult liver cells, we analyzed the expression profile of primary HSC, two HSC lines, and hepatic epithelial progenitors. As expected, all HSC expressed HSC markers. Surprisingly, these markers were also expressed by epithelial progenitors. In addition, one HSC line expressed typical epithelial progenitor mRNAs, and these epithelial markers were inducible in the second HSC line. In normal and damaged livers, small ductular-type cells stained positive for an HSC marker. In conclusion, HSC and hepatic epithelial progenitors both coexpress epithelial and mesenchymal markers, providing evidence that EMT occurs in adult liver cells.

4.418 Evaluation of Islet Transplantation from Non-Heart Beating Donors
Noguchi, H. et al
*Am. J. transplant., **6**, 2476-2482 (2006)*

We evaluated islet transplantation from non-heart beating donors (NHBDs) with our Kyoto Islet Isolation

Method. All patients had positive C-peptide after transplantation. The average HbA_{1C} levels of the five recipients significantly improved from 7.8 ± 0.4% at transplant to 5.2 ± 0.2% currently ($p < 0.01$). Three patients with no or a single autoantibody became insulin independent while the other two patients with double autoantibodies reduced their insulin requirement but did not become insulin independent. C-peptide in patients who became insulin-independent gradually increased after each transplantation whereas C-peptide in patients who did not become insulin-independent from 3 months after the first transplantation to the next transplantation dramatically decreased. The β-score of the three patients who became insulin independent was the best of eight. In conclusion, our method makes it feasible to use NHBDs for islet transplant into type 1 diabetic patients efficiently.

4.419 Immunophenotype and functions of fetal baboon bone-marrow derived dendritic cells
Awasthi, S. and Cropper, J.
Cell. Immunol., ***240(1)***, *31-40 (2006)*

Dendritic cells (DCs) are unique antigen-presenting cells that can take up pathogens, pathogens-derived and stress-antigens and stimulate antigen-specific immune response. Here we investigated the immunobiology of fetal DCs and compared their phenotype and activation status against infectious stimuli with those of young and adult baboons. The DCs were obtained from femoral bone-marrow (BMDCs) of fetus (140 and 175 days of gestation), young (4–5 years old) and mature adult (10–35 years old) baboons. The cells were cultured in the presence of GM-CSF and IL-4. To study phagocytic ability of BMDCs, the cells were harvested on 6th day and incubated with fluorescent-labeled *Escherichia coli* bioparticles. The BMDCs were also treated with *E. coli* O111:B4 lipopolysaccharide (LPS) for 24 h and changes in expression of cell-surface markers and IL-12 were studied using distinct immunoassays. We found that the phenotype and morphology of BMDCs from fetal, young and adult baboons were similar and showed increased expression of HLA-DP, DQ, DR and T cell co-stimulatory molecules upon LPS treatment. However, significant differences were observed in phagocytic activity and IL-12 secretion among BMDCs from these sources. The ability of fetal baboon BMDCs to phagocytose *E. coli* bioparticles was significantly lower and they secreted lower level of LPS-stimulated IL-12 as compared to the BMDCs from adult baboon. These results suggest that compared to adult BMDCs, fetal baboon BMDCs are less efficient in mounting immune response against Gram-negative bacterial stimuli.

4.420 NF-κB/Rel Regulates Inhibitory and Excitatory Neuronal Function and Synaptic Plasticity
O'Mahony, A. et al
Mol. Biol. Cell., ***26(19)***, *7283-7298 (2006)*

Changes in synaptic plasticity required for memory formation are dynamically regulated through opposing excitatory and inhibitory neurotransmissions. To explore the potential contribution of NF-κB/Rel to these processes, we generated transgenic mice conditionally expressing a potent NF-κB/Rel inhibitor termed IκB α superrepressor (IκBα-SR). Using the prion promoter-enhancer, IκBα-SR is robustly expressed in inhibitory GABAergic interneurons and, at lower levels, in excitatory neurons but not in glia. This neuronal pattern of IκBα-SR expression leads to decreased expression of glutamate decarboxylase 65 (GAD65), the enzyme required for synthesis of the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA) in GABAergic interneurons. IκBα-SR expression also results in diminished basal GluR1 levels and impaired synaptic strength (input/output function), both of which are fully restored following activity-based task learning. Consistent with diminished GAD65-derived inhibitory tone and enhanced excitatory firing, IκBα -SR^+ mice exhibit increased late-phase long-term potentiation, hyperactivity, seizures, increased exploratory activity, and enhanced spatial learning and memory. IκBα-SR^+ neurons also express higher levels of the *a*ctivity-*r*egulated, *c*ytoskeleton-associated (Arc) protein, consistent with neuronal hyperexcitability. These findings suggest that NF-κB/Rel transcription factors act as pivotal regulators of activity-dependent inhibitory and excitatory neuronal function regulating synaptic plasticity and memory.

4.421 Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus
Bonasio, R. et al
Nature Immunol., ***7(10)***, *1092-1100 (2006)*

Dendritic cell (DC) presentation of self antigen to thymocytes is essential to the establishment of central tolerance. We show here that circulating DCs were recruited to the thymic medulla through a three-step adhesion cascade involving P-selectin, interactions of the integrin VLA-4 with its ligand VCAM-1, and pertussis toxin–sensitive chemoattractant signaling. Ovalbumin-specific OT-II thymocytes were selectively deleted after intravenous injection of antigen-loaded exogenous DCs. We documented migration of

endogenous DCs to the thymus in parabiotic mice and after painting mouse skin with fluorescein isothiocyanate. Antibody to VLA-4 blocked the accumulation of peripheral tissue–derived DCs in the thymus and also inhibited the deletion of OT-II thymocytes in mice expressing membrane-bound ovalbumin in cardiac myocytes. These findings identify a migratory route by which peripheral DCs may contribute to central tolerance.

4.422 Mutational Analyses of Taiwanese Kindred With X-linked Adrenoleukodystrophy
Chiu, H-C., Liang, J-S., Wang, J-S. and Feng, J-F.
*Pediatric Neurol., **35(4)**, 250-256 (2006)*

X-linked adrenoleukodystrophy is a neurodegenerative disorder with highly variable clinical presentation, including the childhood cerebral form, adult form adrenomyeloneuropathy, and Addison disease. The biochemical hallmark of the disorder is the accumulation of saturated very long chain fatty acids in all tissues and body fluids. This accumulation results from mutations in the ABCD1 gene localized to Xq28. Using polymerase chain reaction and direct sequencing of deoxyribonucleic acid, we identified five novel mutations, including a microdeletion (1624 del ATC), a splicing site mutation (intervening sequence 1 [IVS1] -2a>c), and three missense mutations (1172 T>C, 1520 G>A, and 1754 T>C), from Taiwanese kindred with X-linked adrenoleukodystrophy. A polymorphism involving a single nucleotide deletion in the intervening sequence 5 (IVS5 -6 del c) of the ABCD1 gene, previously misattributed as a mutation in the Chinese population, was also identified. The dinucleotide deletion (1415 del AG) mutation common in Japan and Western countries was not found as frequently in the Chinese and Taiwanese populations. Instead, a higher mutation frequency was observed in exon 6 of the ABCD1 gene among Japanese, Chinese, and Taiwanese kindred with X-linked adrenoleukodystrophy, representing a potential mutational hotspot for future mutational screening among these Asian populations.

4.423 Islet neogenesis associated protein transgenic mice are resistant to hyperglycemia induced by streptozotocin
Taylor-Fishwick, D.A. et al
*J. Endocrinol., **190**, 729-737 (2006)*

Islet neogenesis associated protein (INGAP) is a protein factor that can stimulate new islet mass from adult pancreatic progenitor cells. In models of islet neogenesis, INGAP expression is elevated in pancreatic acinar cells. Using a transgenic model to drive a sustained expression of INGAP in pancreatic acinar cells, we have identified a protection to chemical-induced hyperglycemia. A sustained expression of INGAP during development did not perturb islet development or basal blood glucose homeostasis, although ß-cell mass and pancreatic insulin content were significantly increased in the INGAP transgenic mice. When challenged with a diabetogenic dose of streptozotocin (STZ), mice carrying the INGAP transgene did not become hyperglycemic. In contrast, wild-type mice became and remained hyperglycemic, blood glucose > 550 mg/dl. The serum insulin levels and islet morphology were preserved in the transgenic mice after STZ treatment. These data suggest that the sustained expression of INGAP in the acinar pancreas confers resistance to a diabetogenic insult. The INGAP transgenic mouse provides a new model to uncover factors that are protective to diabetes onset and biomarkers to track ß-cell pathology.

4.424 Characterization of progesterone receptor isoform expression in fetal membranes
Mills, A.A. et al
*Am. J. Obstet. Gynecol., **195**, 998-1003 (2006)*

Objective
To quantify expression of progesterone receptor (PR) messenger RNA (mRNA) isoforms in fetal membranes, and to determine whether these levels change in culture.
Study design
Placentas from women undergoing term cesarean delivery before labor were collected. Layers of amnion, chorion, and decidua were separated manually, enzymatically digested, and separated further with the use of a density gradient. RNA was extracted immediately and after culture for 48 hours, then analyzed by quantitative reverse transcription polymerase chain reaction for PR-A, PR-B, and β-2 microglobulin mRNA expression. Separation of cell types was confirmed by immunohistochemistry.
Results
PR isoform expression was identified in fetal membranes, with levels highest in decidua and below the limits of detection in amnion. The ratio of PR-A/PR-B mRNA was not significantly different between cell layers. PR mRNA isoform levels did not differ significantly in fresh versus cultured cells.

Conclusion
Quantitative reverse transcription polymerase chain reaction was used to quantitate expression of PR mRNA isoforms in cells of fetal membranes and to validate systems for further study of PR with respect to inflammation, infection, and preterm delivery.

4.425 A detailed study of time-dependent changes in human red blood cells: from reticulocyte maturation to erythrocyte senescence
Gifford, S.C., Derganc, J., Shevkoplyas, S.S., Yoshida, T. and Bitensky, M.W.
Br. J. Hematol., ***135***, *395-404 (2006)*

The use of microfabrication technology in the study of biological systems continues to grow rapidly in both prevalence and ascendancy. Customised microdevices that provide superior results than traditional macroscopic methods can be designed in order to investigate specific cell types and cellular processes. This study showed the benefit of this approach in precisely characterising the progressive losses of surface area and haemoglobin (Hb) content by the human red blood cell (RBC), from newborn reticulocyte to senescent erythrocyte. The high-throughput, multiparametric measurements made on individual cells with a specialised microdevice enabled, for the first time, delineation and quantification of the losses that occur during the two stages of the human RBC lifespan. Data acquired on tens of thousands of red cells showed that nearly as much membrane area is lost during the 1–2 d of reticulocyte maturation (*c*. 10–14%) as in the subsequent 4 months of erythrocyte ageing (*c*. 16–17%). The total decrease in Hb over the red cell lifespan is also estimated (*c*. 15%) and a model describing the complete time-course of diminishing mean RBC area and Hb is proposed. The relationship between the losses of Hb and area, and their possible influence on red cell lifespan, are discussed.

4.426 The Src Homology 2 Domain-Containing Leukocyte Protein of 76-kDa Adaptor Links Integrin Ligation with p44/42 MAPK Phosphorylation and Podosome Distribution in Murine Dendritic Cells
Luckashenak, N.A., Ryszkiewizs, R..L., Ramsey, K.D. and Clements, J.L.
J. Immunol., ***177***, *5177-5185 (2006)*

The Src homology 2 domain-containing leukocyte protein of 76 kDa (SLP-76) is an important molecular intermediate in multiple signaling pathways governing immune cell function. In this study, we report that SLP-76 is expressed in $CD11c^{+}B220^{-}$ dendritic cells (DCs) isolated from murine thymus or spleen, and that SLP-76 is rapidly phosphorylated on tyrosine residues upon plating of bone marrow-derived DCs (BMDCs) on integrin agonists. SLP-76 is not required for the in vitro or in vivo generation of DCs, but SLP-76-deficient BMDCs adhere poorly to fibronectin, suggesting impaired integrin function. Consistent with impaired adhesion, cutaneous SLP-76-deficient DCs leave ear tissue at an elevated frequency compared with wild-type DCs. In addition, the pattern and distribution of actin-based podosome formation are visibly altered in BMDCs lacking SLP-76 following integrin engagement. SLP-76-deficient BMDCs manifest multiple signaling defects following integrin ligation, including reduced global tyrosine phosphorylation and markedly impaired phosphorylation of p44/42 MAPK (ERK1/2). These data implicate SLP-76 as an important molecular intermediate in the signaling pathways regulating multiple integrin-dependent DC functions, and add to the growing body of evidence that hemopoietic cells may use unique molecular intermediates and mechanisms for regulating integrin signaling.

4.427 Oestrogen Synthesis in the Hippocampus: Role in Axon Outgrowth
Von Schassen, C. et al
J. Neuroendocrinol., ***18***, *847-856 (2006)*

Ovarian oestrogens have been postulated to be neuroprotective. It has also been shown that considerable amounts of oestrogens are synthesised in hippocampal neurones. In the present study, we focused on a potential role of hippocampus-derived oestradiol compared to gonad-derived oestradiol on axon outgrowth of hippocampal neurones. To address the role of hippocampus-derived oestradiol, we inhibited oestrogen synthesis by treatment of neonatal hippocampal cell cultures with letrozole, a specific aromatase inhibitor. As an alternative, we used siRNA against steroidogenic acute regulatory protein (StAR). Axon outgrowth and GAP-43 expression were significantly down-regulated in response to letrozole and in siRNA-StAR transfected cells. The effects after inhibition of oestrogen synthesis in response to letrozole and in siRNA-StAR transfected cells were reversed by oestrogen supplementation. No difference was found between ovariectomised animals, cycling animals at pro-oestrus and ovariectomised and subsequently oestradiol-treated animals. However, high pharmacological doses of oestradiol promoted axon outgrowth, which was

possible to abolish by the oestrogen receptor antagonist ICI 182,780. Our results show that oestradiol-induced neurite outgrowth is very likely mediated by genomic oestrogen receptors and requires higher doses of oestradiol than physiological serum concentrations derived from the gonads.

4.428 Prion Protein Expression by Mouse Dendritic Cells Is Restricted to the Nonplasmacytoid Subsets and Correlates with the Maturation State
Martinez de Hoya, G., Lopez-Bravo, M., Metharom, P., Ardavin, C. and Aucounterier, P.
*J. Immunol., **177**, 6137-6142 (2006)*

Expression of the physiological cellular prion protein (PrP^C) is remarkably regulated during differentiation and activation of cells of the immune system. Among these, dendritic cells (DCs) display particularly high levels of membrane PrP^C, which increase upon maturation, in parallel with that of molecules involved in Ag presentation to T cells. Freshly isolated mouse Langerhans cells, dermal DCs, and DCs from thymus, spleen, and mesenteric lymph nodes expressed low to intermediate levels of PrP^C. Highest levels of both PrP^C and MHC class II molecules were displayed by lymph node $CD8\alpha^{int}$ DCs, which represent fully mature cells having migrated from peripheral tissues. Maturation induced by overnight culture resulted in increased levels of surface PrP^C, as did in vivo DC activation by bacterial LPS. Studies on Fms-like tyrosine kinase 3 ligand bone marrow-differentiated $B220^-$ DCs confirmed that PrP^C expression followed that of MHC class II and costimulatory molecules, and correlated with IL-12 production in response to TLR-9 engagement by CpG. However, at variance with conventional DCs, $B220^+$ plasmacytoid DCs isolated from the spleen, or in vitro differentiated, did not significantly express PrP^C, both before and after activation by TLR-9 engagement. PrP knockout mice displayed higher numbers of spleen $CD8\alpha^+$ DCs, but no significant differences in their maturation response to stimulation through TLR-4 and TLR-9 were noticed. Results are discussed in relation to the functional relevance of PrP^C expression by DCs in the induction of T cell responses, and to the pathophysiology of prion diseases.

4.429 Lentiviral vector expressing retinoic acid receptor ß2 promotes recovery of function after corticospinal tract injury in the adult rat spinal cord
Yip, P.K. et al
*Hum. Mol. Genet., **15(21)**, 3107-3118 (2006)*

Spinal cord injury often results in permanent and devastating neurological deficits and disability. This is due to the limited regenerative capacity of neurones in the central nervous system (CNS). We recently demonstrated that a transcription factor retinoic acid receptor ß2 (RARß2) promoted axonal regeneration in adult sensory neurones located peripherally. However, it is not known if RARß2 can promote axonal regeneration in cortical neurones of the CNS. Here, we demonstrate that delivery of RARß2 via a lentiviral vector to adult dissociated cortical neurones significantly enhances neurite outgrowth on adult cortical cryosections, which normally provide an unfavourable substrate for growth. We also show that lentiviral-mediated transduction of corticospinal neurones resulted in robust transgene expression in layer V corticospinal neurones and their axonal projections in the corticospinal tract (CST) of the spinal cord. Expression of RARß2 in these neurones enhanced regeneration of the descending CST fibres after injury to these axons in the mid-cervical spinal cord. Furthermore, we observed functional recovery in sensory and locomotor behavioural tests in RARß2-treated animals. These results suggest that a direct and selective delivery of RARß2 to the corticospinal neurones promotes long-distance functional regeneration of axons in the spinal cord and may thus offer new therapeutic gene strategy for the treatment of human spinal cord injuries.

4.430 Preadipocytes Mediate Lipopolysaccharide-Induced Inflammation and Insulin Resistance in Primary Cultures of Newly Differentiated Human Adipocytes
Chung, S. et al
*Endocrinology, **147(11)**, 5340-5351 (2006)*

Recent data suggest that proinflammatory cytokines secreted from adipose tissue contribute to the morbidity associated with obesity. However, characterization of the cell types involved in inflammation and how these cells promote insulin resistance in human adipocytes are unclear. We simulated acute inflammation using the endotoxin lipopolysaccharide (LPS) to define the roles of nonadipocytes in primary cultures of human adipocytes. LPS induction of the mRNA levels of proinflammatory cytokines (*e.g.* IL-6, TNF-α, and IL-1ß) and chemokines (*e.g.* IL-8, monocyte chemoattractant protein-1) occurred primarily in

the nonadipocyte fraction of newly differentiated human adipocytes. Nonadipocytes were characterized as preadipocytes based on their abundant mRNA levels of preadipocyte markers preadipocyte factor-1 and adipocyte enhancer protein-1 and only trace levels of markers for macrophages and myocytes. The essential role of preadipocytes in inflammation was confirmed by modulating the degree of differentiation in the cultures from approximately 0–90%. LPS-induced proinflammatory cytokine/chemokine expression and nuclear factor-κB and MAPK signaling decreased as differentiation increased. LPS-induced cytokine/chemokine expression in preadipocytes was associated with: 1) decreased adipogenic gene expression, 2) decreased ligand-induced activation of a peroxisome proliferator activated receptor (PPAR)-γ reporter construct and increased phosphorylation of PPARγ, and 3) decreased insulin-stimulated glucose uptake. Collectively, these data demonstrate that LPS induces nuclear factor-κB- and MAPK-dependent proinflammatory cytokine/chemokine expression primarily in preadipocytes, which triggers the suppression of PPARγ activity and insulin responsiveness in human adipocytes.

4.431 Naltrexone, an opioid receptor antagonist, attenuates liver fibrosis in bile duct ligated rats
Ebrahimkhani, M.R. et al
*Gut, **55**, 1606-1616 (2006)*

Aim: The aim of this study was to investigate the hypothesis that the opioid system is involved in the development of hepatic fibrosis.
Methods: The effect of naltrexone (an opioid receptor antagonist) on hepatic fibrosis in bile duct ligated (BDL) or sham rats was assessed by histology and hepatic hydroxyproline levels. Liver matrix metalloproteinase 2 (MMP-2) was measured by zymography, and αsmooth muscle actin (α-SMA) and CD45 (leucocyte common antigen) by immunohistochemistry. The redox state of the liver was assessed by hepatic glutathione (GSH)/oxidised glutathione (GSSG) and S-nitrosothiol levels. Subtypes of opioid receptors in cultured hepatic stellate cells (HSCs) were characterised by reverse transcriptase-polymerase chain reaction, and the effects of selective δopioid receptor agonists on cellular proliferation, tissue inhibitor of metalloproteinase 1 (TIMP-1), and procollagen I expression in HSCs determined.
Results: Naltrexone markedly attenuated the development of hepatic fibrosis as well as MMP-2 activity ($p<0.01$), and decreased the number of activated HSCs in BDL rats ($p<0.05$). The development of biliary cirrhosis altered the redox state with a decreased hepatic GSH/GSSG ratio and increased concentrations of hepatic S-nitrosothiols, which were partially or completely normalised by treatment with naltrexone, respectively. Activated rat HSCs exhibited expression of δ1 receptors, with increased procollagen I expression, and increased TIMP-1 expression in response to δ_1 and δ_2 agonists, respectively.
Conclusions: This is the first study to demonstrate that administration of an opioid antagonist prevents the development of hepatic fibrosis in cirrhosis. Opioids can influence liver fibrogenesis directly via the effect on HSCs and regulation of the redox sensitive mechanisms in the liver.

4.432 CLC-3 Channels Modulate Excitatory Synaptic Transmission in Hippocampal Neurons
Wang, X.Q. et al
*Neuron, **52**, 321-333 (2006)*

It is well established that ligand-gated chloride flux across the plasma membrane modulates neuronal excitability. We find that a voltage-dependent Cl^- conductance increases neuronal excitability in immature rodents as well, enhancing the time course of NMDA receptor-mediated miniature excitatory postsynaptic potentials (mEPSPs). This Cl^- conductance is activated by CaMKII, is electrophysiologically identical to the CaMKII-activated CLC-3 conductance in nonneuronal cells, and is absent in *clc-3*$^{-/-}$ mice. Systematically decreasing $[Cl^-]_i$ to mimic postnatal $[Cl^-]_i$ regulation progressively decreases the amplitude and decay time constant of spontaneous mEPSPs. This Cl^--dependent change in synaptic strength is absent in *clc-3*$^{-/-}$ mice. Using surface biotinylation, immunohistochemistry, electron microscopy, and coimmunoprecipitation studies, we find that CLC-3 channels are localized on the plasma membrane, at postsynaptic sites, and in association with NMDA receptors. This is the first demonstration that a voltage-dependent chloride conductance modulates neuronal excitability. By increasing postsynaptic potentials in a Cl^- dependent fashion, CLC-3 channels regulate neuronal excitability postsynaptically in immature neurons.

4.433 A Phase I Study of In vitro Expanded Natural Killer T Cells in Patients with Advanced and Recurrent Non–Small Cell Lung Cancer
Motohashi, S. et al
*Clin. Cancer Res., **12(20)**, 6079-6086 (2006)*

Purpose: Human Vα24 natural killer T (Vα24 NKT) cells bearing an invariant Vα24Jα Q antigen receptor are activated by a glicolipid ligand α-galactosylceramide (αGalCer; KRN7000) in a CD1d-dependent manner. The human Vα24 NKT cells activated with αGalCer and interleukin-2 have been shown to produce large amounts of cytokines, such as IFN-γ, and also exerting a potent killing activity against various tumor cell lines. We did a phase I study with autologous activated Vα24 NKT cell therapy.
Experimental Design: Patients with advanced or recurrent non–small cell lung cancer received i.v. injections of activated Vα24 NKT cells (level 1: 1 x $10^7/m^2$ and level 2: 5 x $10^7/m^2$) to test the safety, feasibility, and clinical response of this therapeutic strategy. Immunomonitoring was also done in all cases.
Results: Six patients were enrolled in this study. No severe adverse events were observed during this study in any patients. After the first and second injection of activated Vα24 NKT cells, an increased number of peripheral blood Vα24 NKT cells was observed in two of three cases receiving a level 2 dose of activated Vα24 NKT cells. The number of IFN-γ-producing cells in peripheral blood mononuclear cells increased after the administration of activated Vα24 NKT cells in all three cases receiving the level 2 dose. No patient was found to meet the criteria for either a partial or a complete response.
Conclusions: The clinical trial with activated Vα24 NKT cell administration was well tolerated and carried out safely with minor adverse events even in patients with advanced diseases.

4.434 Temporospatial coupling of networked synaptic activation of AMPA-type glutamate receptor channels and calcium transients in cultured motoneurons
Jahn, K. et al
Neuroscience, ***142****, 1019-1029 (2006)*

AMPA-type glutamate receptor (GluR) channels provide fast excitatory synaptic transmission in the CNS, but mediate also cytotoxic insults. It could be shown that AMPA-type GluR channel–mediated chronic excitotoxicity leads to an increased intracellular calcium concentration and plays an important role in neurodegenerative diseases like for example amyotrophic lateral sclerosis (ALS). As calcium is an important mediator of various processes in the cell and calcium signals have to be very precise in the temporospatial resolution, excessive intracellular calcium increases can seriously impair cell function. It is still unclear if AMPA-type receptors can directly interact with the intracellular calcium homeostasis or if other mechanisms are involved in this process. The objective of this study was therefore to investigate the calcium homeostasis in rat motoneurons under physiological stimulation of AMPA-type GluR channels using calcium imaging techniques and patch-clamp recordings simultaneously. It was found that spontaneous excitatory postsynaptic currents of cultured motoneurons did not elicit significant intracellular calcium transients. Large intracellular calcium transients occurred only when preceding fast sodium currents were observed. Pharmacological experiments showed that activation of AMPA-type GluR channels during synaptic transmission has a great functional impact on the calcium homeostasis in motoneurons as all kinds of activity was completely blocked by application of the selective kainate- and AMPA-type GluR channel blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Furthermore we suggest from our experiments that calcium transients of several hundred milliseconds' duration result from release of calcium from the endoplasmic reticulum via activation of ryanodine receptors (calcium-induced calcium release, CICR). Our results help to understand the regulatory function of AMPA-type GluR channels in the intracellular calcium homeostasis which is known to be disturbed in neurodegenerative diseases.

4.435 Isolation of an adult blood-derived progenitor cell population capable of differentiation into angiogenic, myocardial and neural lineages
Porat, Y. et al
Br. J. Hematol., ***135****, 703-714 (2006)*

Blood-derived adult stem cells were previously considered impractical for therapeutic use because of their small numbers. This report describes the isolation of a novel human cell population derived from the peripheral blood, termed synergetic cell population (SCP), and defined by the expression of $CD31^{Bright}$, $CD34^{+}$, $CD45^{-/Dim}$ and $CD34^{Bright}$, but not lineage-specific features. The SCP was capable of differentiating into a variety of cell lineages upon exposure to defined culture conditions. The resulting cells exhibited morphological, immunocytochemical and functional characteristics of angiogenic, neural or myocardial lineages. Angiogenic cell precursors (ACPs) expressed CD34, CD133, KDR, Tie-2, CD144, von Willebrand factor, $CD31^{Bright}$, concomitant binding of Ulex-Lectin and uptake of acetylated low density lipoprotein (Ac-LDL), secreted interleukin-8, vascular endothelial growth factor and angiogenin and formed tube-like structures *in vitro*. The majority of $CD31^{Bright}$ ACP cells demonstrated Ac-LDL uptake. Neural cell precursors (NCPs) expressed the neuronal markers Nestin, βIII-Tubulin, and Neu-N,

the glial markers GFAP and O4, and responded to neurotransmitter stimulation. Myocardial cell precursors (MCPs) expressed Desmin, cardiac Troponin and Connexin 43. In conclusion, the simple and rapid method of SCP generation and the resulting considerable quantities of lineage-specific precursor cells makes it a potential source of autologous treatment for a variety of diseases.

4.436 Proinflammatory cytokine secretion from preadipocytes decreases PPARg expression and activity in primary cultures of human adipocytes

Chung, S., LaPoint, K., Kennedy, A., Troy, A. and McIntosh, M.
*FASEB J., **20**, A163 (2006)*

Recent data suggest that proinflammatory cytokines secreted from adipose tissue contribute to the morbidity associated with obesity. However, characterization of the cell types involved in inflammation in adipose tissue is unclear. To delineate the role that non-adipocytes play in inflammation, primary cultures of newly-differentiated human stromal vascular cells containing ~50% adipocytes and ~50% non-adipocytes were stimulated with LPS for 3 h. Subsequently, non-adipocytes were fractionated from adipocytes by centrifugation in 6% **iodixanol** (1.03g/ml) and gene expression in each fraction was quantified by qPCR. LPS induction of proinflammatory cytokine mRNA (IL-6, IL-8, TNFα, IL-1ß, COX-2) occurred primarily in non-adipocytes. Non-adipocytes were characterized as preadipocytes based on high mRNA levels of preadipocyte markers Pref-1 and adipocytes enhancer protein-1*(*AEBP-1*)* and only trace levels of macrophage (CD68 and Mac-1) and myocyte (MyoD) markers. LPS-induced cytokine expression in preadipocytes was associated with suppression of peroxisome proliferator activated receptor (PPAR)γ and adiponectin gene expression in adipocytes. In parallel, LPS treatment increased PPARγ phosphorylation and decreased the activity of a luciferase reporter construct containing a PPARγ response element, indicating that LPS attenuates PPARγ activity. Collectively, these data demonstrate that LPS induces proinflammatory gene expression in preadipocytes, leading to suppression of PPARγ activity in adipocytes, thereby impairing insulin sensitivity.

4.437 Rat liver endothelial cells isolated by anti-CD31 immunomagnetic separation lack fenestrae and sieve plates

DeLeve, L.D., Wang, X., McCuskey, M.K. and McCuskey, R.S.
*Am. J. Physiol. Gastrointest. Liver Physiol., **291**, G1187-G1189 (2006)*

The gold standard for the identification of sinusoidal endothelial cells (SEC) is the presence of fenestrae organized in sieve plates, which is characteristic of SEC in vivo. One of the methods currently in use to isolate SEC is immunomagnetic sorting for CD31. However, there is evidence to suggest that CD31 is not present on the surface of differentiated SEC. The present study used scanning electron microscopy to image rat hepatic endothelial cells isolated by anti-CD31 and immunomagnetic sorting and cells isolated by gradient centrifugation and centrifugal elutriation. Cells isolated by elutriation had well-developed fenestrae and sieve plates, whereas cells isolated by anti-CD31 and immunomagnetic sorting had significantly fewer fenestrae organized in sieve plates. In conclusion, cells isolated by anti-CD31 and immunomagnetic sorting lacked the hallmark features of SEC.

4.438 Distinctive role of donor strain immature dendritic cells in the creation of allograft tolerance

Kim, Y.S. et al
*Int. Immunol., **18(12)**, 1771-1777 (2006)*

Dendritic cells (DCs) are pivotal antigen-presenting cells and serve a unique role in initiating immunity. To test the hypothesis that pre-immunization of recipient with certain DC subsets of donor origin can influence graft outcome, we have studied the effects of immunization with allogeneic $CD4^+CD8^-CD11c^+$ dendritic cell ($CD4^+$DC) and $CD4^-CD8^+CD11c^+$ dendritic cell ($CD8^+$DC) on the allograft response. Although both immature $CD4^+$DC and $CD8^+$DC subsets from DBA/2 were able to prime naive allogeneic C57BL/6 (B6) T cells in mixed lymphocyte reaction (MLR), $CD8^+$DC exerted more vigorous alloimmune responses than $CD4^+$DC did. Also, $CD4^+$DC-driven allogeneic T cell response was attenuated more significantly by anti-CD154 mAb than $CD8^+$DC-driven response. Consistent with the MLR results, combined pre-treatment with $CD4^+$DC, but not $CD8^+$DC, plus anti-CD154 mAb produced donor strain-specific long-term graft survival and induced tolerance while treatment with $CD8^+$DC plus anti-CD154 mAb created minimal prolongation of allograft survival in a pancreas islet transplant model (DBA/2→B6). The beneficial effects exerted by $CD4^+$DC and anti-CD154 mAb pre-treatment were correlated with T_h1 to T_h2 immune deviation and with the amplified donor-specific suppressive capacity by recipient $CD4^+CD25^+$ T cells. These

findings highlight the capacity of $CD4^+$DC to modulate alloimmune responses, and suggest therapeutic approaches for the induction of donor-specific tolerance.

4.439 Contribution of Calcium Influx in Mediating Glucose-Stimulated Oxygen Consumption in Pancreatic Islets

Sweet, I.R. and Gilbert, M.
*Diabetes, **55(12)**, 3509-3519 (2006)*

In brain, muscle, and pancreatic islets, depolarization induces an increase in respiration, which is dependent on calcium influx. The goal of this study was to assess the quantitative significance of this effect in islets relative to glucose-stimulated ATP turnover, to examine the molecular mechanism mediating the changes, and to investigate the functional implications with respect to insulin secretion. Glucose (3–20 mmol/l) increased steady-state levels of cytochrome c reduction (32–66%) in isolated rat islets, reflecting an increased production of NADH, and oxygen consumption rate (OCR) by 0.32 nmol/min/100 islets. Glucose-stimulated OCR was inhibited 30% by inhibitors of calcium influx (diazoxide or nimodipine), whereas a protein synthesis inhibitor (emetine) decreased it by only 24%. None of the inhibitors affected cytochrome c reduction, suggesting that calcium's effect on steady-state OCR is mediated by changes in ATP usage rather than the rate of NADH generation. 3-isobutyl-1-methylxanthine increased insulin secretion but had little effect on OCR, indicating that the processes of movement and exocytosis of secretory granules do not significantly contribute to ATP turnover. At 20 mmol/l glucose, a blocker of sarcoendoplasmic reticulum calcium ATPase (SERCA) had little effect on OCR despite a large increase in cytosolic calcium, further supporting the notion that influx of calcium, not bulk cytosolic calcium, is associated with the increase in ATP turnover. The glucose dose response of calcium influx–dependent OCR showed a remarkable correlation with insulin secretion, suggesting that the process mediating the effect of calcium on ATP turnover has a role in the amplification pathway of insulin secretion.

4.440 Age-related changes in monocyte and platelet cyclooxygenase expression in healthy male humans and rats

Kang, K.B., Van Der Zypp, A., Iannazzo, L. and Majewski, H.
*Translational Res., **148**, 289-294 (2006)*

Cyclooxygenase (COX) catalyses the formation of prostanoids that are crucial in maintaining hemostasis and important in inflammation. Animal studies reveal that COX-1 and COX-2 expression increase in some cell types during aging. This study determined age-related changes in COX expression in platelets and monocytes. Platelets and mononuclear cells were isolated from healthy male human volunteers from 18 to 28 and from 55 to 65 years of age, as well as male rats 8 and 54 weeks old for comparison. Western blot analysis was performed using selective antibodies against COX-1 and COX-2, followed by densitometrical analysis. In humans, an age-related increase in COX-2 expression in mononuclear cells was observed, with a 70% increase in the older age group. In rat studies, a 50% increase of COX-2 protein occurred in mononuclear cells of 54-week-old rats, compared with 8-week-old rats. For COX-1, an age-related increase of 50% occurred in rat platelets, but no difference occurred in the platelets' COX-1 levels between young and elderly human age groups. The increased COX-2 in monocytes of older humans, which is mirrored in rats, may have downstream implications in atherosclerosis and cardiovascular risk as mononuclear prostanoids are implicated in atherosclerotic plaque stability.

4.441 Aminophospholipid Translocase Activity and Phosphatidylserine Externalization in Sickle Red Blood Cell Subpopulations

Arnold, L.E., Palascak, M.B., Ciraolo, P., Joiner, C.H. and Franco, R.S.
*Blood, **108**, Abstract 1241 (2006)*

Red blood cell (RBC) membrane phosphatidylserine (PS) is normally confined to the inner leaflet. In sickle RBC, however, PS externalization has been observed and may contribute to thrombogenesis, endothelial adhesion, and shortened RBC lifespan. Increased calcium leads to PS externalization through inhibition of aminophospholipid translocase (APLT), which normally returns external PS to the inner leaflet, and activation of phospholipid scramblase, which allows nonspecific phospholipid equilibration between the leaflets. Sickle RBC in the light and dense (dehydrated) fractions exhibit increased PS externalization compared to sickle RBC in the normal density fractions. Sickle RBC with modest PS exposure (Type I PS+) occur in both light and dense fractions and, especially in the light fractions, include many reticulocytes. Sickle Cells with high levels of PS exposure (Type II PS+) occur predominantly in the dense fraction. Type II PS+ sickle cells have low levels of HbF, are more adherent than Type I PS+ cells, and

tend to increase in number as sickle cells age in the circulation. Dense sickle cells have decreased APLT activity and this has been considered necessary (but probably not sufficient) for PS externalization. In the current studies we have examined the relationship between PS externalization and translocase activity in sickle cell density fractions. APLT activity and PS externalization were determined flow cytometrically using NBD-PS internalization and annexin V-phycoerythrin binding respectively. Sickle RBC density fractions were isolated using a discontinuous **Optiprep**® gradient, and two fractions were selected for further study, Fx 4 with essentially normal hydration (1.093< g/cc <1.100) and the dehydrated Fx 6 (> 1.120 g/cc). As expected, Fx 6 sickle RBC had a decreased percentage of APLT positive cells (29.9±10.8, N=4) compared to normal RBC (94.5±3.6, N=3) and Fx 4 sickle RBC (92.9±2.3, N=4). Fx 4 contained 5.7±2.8% Type I and 0.3±0.2% Type II PS+ cells. Fx 6 had 9.1±2.5% Type I and 3.2±2.3% Type II PS+ cells. Figure 1 shows the percentage of APLT positive cells in the PS negative (PS-), Type I PS+, and Type II PS+ groups for Fx 4 and Fx 6. Type I PS+ cells in Fx 4 are positive for APLT, whereas those in Fx 6 are mostly negative. In Fx 4, the Type I PS+ group most likely contains many reticulocytes, and in a separate experiment TfR+ reticulocytes had a uniform, slightly elevated APLT activity. The Type II PS+ cells in both Fx 4 and Fx 6 had decreased percentages of APLT positive cells. It thus appears that high levels of PS externalization, regardless of hydration state, are associated with low APLT activity. We therefore conclude:
1.In normally hydrated sickle cells, Type II PS+ cells, but not Type I PS+ cells, are associated with decreased APLT;
2.In dense sickle RBC, both Type I and Type II PS+ cells are associated with decreased APLT. These results further emphasize the pathologic nature of the Type II PS+ sickle cells.

4.442 Advanced methods for Handling and Preparation of Stallion Semen
Loomis, P.R.
*Vet. Clin. Equine, **22**, 663-676 (2006)*

Since the advent of artificial insemination, practitioners and researchers have been concerned with semen handling techniques. Practices that minimize damage and maximize viability, survival, and fertility of spermatozoa are required for assisted reproductive technologies (ARTs). Many procedures for processing semen and ARTs require the separation of spermatozoa from seminal components. Semen is composed of a heterogeneous population of viable and nonviable spermatozoa suspended in secretions from various accessory sex glands. Semen may also contain other cells (eg. Leukocytes, epithelial cells, erythrocytes, immature germ cells) and contaminants (eg. bacteria, viruses, urine). Sperm separation techniques are used by practitioners and researchers for several reasons. One reason is to concentrate spermatozoa and remove seminal plasma before cooling or freezing semen. Another reason is to separate spermatozoa from seminal plasma and to select an enriched population of viable spermatozoa from the ejaculate. The purpose of this article is to outline reasons and procedures for separation and selection of equine spermatozoa.

4.443 Cervical duct cannulation in sheep for collection of afferent lymph dendritic cells from head tissues
Schwartz-Cornil, I., Epardaud, M. and Bonneau, M.
*Nature Protocols, **1(2)**, 874-879 (2006)*

Pseudo-afferent cervical lymph-duct cannulation in a sheep model allows large amounts of lymph cells to be collected under physiological conditions, carrying immune signaling information from the head tissues, including oro-nasal mucosae. Importantly, large quantities of dendritic cells (DCs) of several subtypes are obtained (up to 8 million per overnight collection), as well as many other trafficking leukocytes. The technique includes three steps: removal of all head lymph nodes on one side (2 h), catheterization of cervical lymph ducts after 2 months (2–3 h) and collection/purification of lymph-cell subsets (4 h). The approach is challenging (1 in 3 success rate) but fruitful, and can be used to study DC subsets under immunomodulation, in order to assess lymph-cell subset dynamic changes and antigen transportation from oro-nasal tissues. This protocol is directed to experienced postdoctoral researchers.

4.444 Neuropeptide Urocortin and Its Receptors Are Expressed in Rat Kupffer Cells
Charalampopoulos, I. et al
*Neuroendocrinol., **84**, 49-57 (2006)*

The stress neuropeptides, corticotropin-releasing hormone (CRH) and urocortin (UCN), modulate the inflammatory response via the hypothalamus-pituitary-adrenal axis and locally, in a paracrine manner, act on mast and macrophage cells. Kupffer cells (KCs) are the resident macrophages of the liver. They represent the bulk of tissue macrophages in the body and they are the first to face invading noxious agents

reaching the body via the portal circulation. The aim of the present report was to study the expression of the CRH system in rat KC and test its functionality. Our findings are as follows: (1) In highly purified KCs the transcripts of UCN, of its receptors CRHR1, CRHR2 and that of the pseudoreceptor CRH-binding protein (CRHBP) were present while that of CRH was not detectable. (2) Similarly, immunoreactive UCN, CRHR1, CRHR2 and CRHBP were easily detectable by immunohistochemistry and immunofluorescence in sections of whole rat liver (localized in KC) as well as in purified KC while CRH was again not detectable. (3) Exposure of purified KC to CRH or UCN suppressed lipopolysaccharide-induced tumor necrosis factor alpha production, an effect completely prevented by the CRHR1 and CRHR2 receptor antagonist astressin. Our data demonstrate the presence of UCN and its receptors in rat KC, the absence of CRH, and the functionality of these receptors. We propose that a UCN-based system may affect local inflammatory phenomena in the liver acting in a paracrine manner.

4.445 Lack of UCP2 reduces fas-mediated liver injury in ob/ob mice and reveals importance of cell-specific UCP2 expression

Fülöp, P., Derdak, Z., Sheets, A., Sabo, E., Berthiaume, E.P., Resnick, M.B., Wands, J.R., Pragh, G. and baffy, G.

Hepatology, ***44(3)***, *592-601 (2006)*

Fatty liver is vulnerable to conditions that challenge hepatocellular energy homeostasis. Lipid-laden hepatocytes highly express uncoupling protein-2 (UCP2), a mitochondrial carrier that competes with adenosine triphosphate (ATP) synthesis by mediating proton leak. However, evidence for a link between UCP2 expression and susceptibility of liver to acute injury is lacking. We asked whether absence of UCP2 protects ob/ob mice from Fas-mediated acute liver damage. UCP2-deficient ob/ob mice (ob/ob:ucp2−/−) and UCP2-competent littermates (ob/ob:ucp2+/+) received a single dose of agonistic anti-Fas antibody (Jo2). Low-dose Jo2 (0.15 mg/kg intraperitoneally) caused less serum alanine aminotransferase (ALT) elevation and lower apoptosis rates in ob/ob:ucp2−/− mice. High-dose Jo2 (0.40 mg/kg intraperitoneally) proved uniformly fatal; however, ob/ob:ucp2−/− mice survived longer with less depletion of liver ATP stores, indicating that fatty hepatocytes may benefit from lack of UCP2 during Jo2 challenge. Although UCP2 reportedly controls mitochondrial oxidant production, its absence had no apparent effect on fatty liver tissue malondialdehyde levels augmented by Jo2. This finding prompted us to determine UCP2 expression in Kupffer cells, a major source of intrahepatic oxidative stress. UCP2 expression was found diminished in Kupffer cells of untreated ob/ob:ucp2+/+ mice, conceivably contributing to increased oxidative stress in fatty liver and limiting the impact of UCP2 ablation. In conclusion, whereas UCP2 abundance in fatty hepatocytes exacerbates Fas-mediated injury by compromising ATP stores, downregulation of UCP2 in Kupffer cells may account for persistent oxidative stress in fatty liver. Our data support a cell-specific approach when considering the therapeutic effects of mitochondrial uncoupling in fatty liver disease.

4.446 Proteomic analysis of sperm regions that mediate sperm-egg interactions

Stein, K.K., Go, J.C., Lane, W.S., Primakoff, P. and Myles, D.G.

Proteomics, ***6(12)***, *3533-3543 (2006)*

The sperm interacts with three oocyte-associated structures during fertilization: the cumulus cell layer surrounding the oocyte, the egg extracellular matrix (the zona pellucida), and the oocyte plasma membrane. Each of these interactions is mediated by the sperm head, probably through proteins both on the sperm surface and within the acrosome, a specialized secretory granule. In this study, we have used subcellular fractionation in order to generate a proteome of the sperm head subcellular compartments that interact with oocytes. Of the proteins we identified for which a gene knockout has been tested, a third have been shown to be essential for efficient reproduction *in vivo*. Many of the other presently untested proteins are likely to have a similarly important role. Twenty-five percent of the cell surface fraction proteins are previously uncharacterized. We have shown that at least two of these novel proteins are localized to the sperm head. In summary, we have identified over 100 proteins that are expressed on mature sperm at the site of sperm-oocyte interactions.

4.447 STAT1 inhibits liver fibrosis in mice by inhibiting stellate cell proliferation and stimulating NK cell cytotoxicity

Jeong, W-I., Park, O., Radaeva, S. and Gao, B.

Hepatology, ***44(6)***, *1441-1451 (2006)*

Liver fibrosis, a common scarring response to chronic liver injury, is a precursor to cirrhosis and liver cancer. Here, we identified signal transducer and activator of transcription 1 (STAT1) as an important negative regulator in liver fibrosis. Our findings show that disruption of the STAT1 gene accelerated liver fibrosis and hepatic stellate cell (HSC) proliferation in an *in vivo* model of carbon tetrachloride (CCl_4)-induced liver fibrosis. *In vitro* treatment with IFN-γ inhibited proliferation and activation of wild-type HSCs, but not $STAT1^{-/-}$ HSCs. Moreover, compared to wild-type cells, cellular proliferation stimulated by serum or platelet-derived growth factor (PDGF) was enhanced and accelerated in $STAT1^{-/-}$ HSCs, which was partially mediated via elevated PDGF receptor β expression on such cells. Polyinosinic-polycytidylic acid (poly I:C) or IFN-γ treatment inhibited liver fibrosis in wild-type mice but not in $STAT1^{-/-}$ mice. Induction of NK cell killing of activated HSCs by poly I:C was attenuated in $STAT1^{-/-}$ mice compared to wild-type mice, which was likely due to reduced NKG2D and TRAIL expression on $STAT1^{-/-}$ NK cells. Finally, activation of TGF-β/Smad3 signaling pathway was accelerated, whereas induction of Smad7 was diminished in the liver of $STAT1^{-/-}$ mice after CCl_4 administration compared to wild-type mice. In conclusion, activation of STAT1 attenuates liver fibrosis through inhibition of HSC proliferation, attenuation of TGF-β signaling, and stimulation of NK cell killing of activated HSCs. STAT1 could be a new therapeutic target for treating liver fibrosis.

4.448 Single Cell Analysis on Microfluidic Devices
Culbertson, C.
Methods in Mol. Biol., ***339***, *203-216 (2006)*

There is significant variability among cells of the same type at the single cell level. This variability may be because of external stimuli that vary temporally or spatially among a population of cells. It may also be owing to the nonsynchronized responses of cells to various stimuli. In addition, differences in otherwise similar cells may be generated by genetic mutations acquired by one or more of the cells. Often times multiple biochemical pathways and molecules are involved in such differences. In order to better understand these differences and to detect those rare cells in a large population that may be indicative of early disease states, methods that are capable of rapidly quantifying multiple molecular species in single cells are desired. Microfluidic devices may provide the optimal platform upon which to develop such methods. Microfluidics has the capability of combining the high-throughput manipulation and transport of cells with rapid, high-efficiency separations and high-sensitivity detection. This chapter describes how to fabricate microfluidic devices for the high-throughput manipulation and rapid electrical lysis of single, nonadherent (suspension) cells followed by the injection and separation of the fluorescently labeled cell contents.

4.449 Stem cell continuum: Directed differentiation hotspots
Colvin, G.A. et al
Exp. Hematol., ***35(1)***, *96-107 (2007)*

Objective
The purpose of this study was to evaluate the technique of stem cell-directed differentiation in the context of cell-cycle position. The hypothesis was that stem cells would have different sensitivities to an identical inductive signal through cell-cycle transit and that this would affect the outcome of its progeny.
Materials and Methods
Differentiation of murine marrow $lineage^{negative}rhodamine\text{-}123^{low}\text{-}Hoechst\text{-}33342^{low}$ (LRH) stem cells was determined at different points in cell cycle under stimulation by thrombopoietin, flt3 ligand, and steel factor. LRH stem cells were subcultured in granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and steel factor at different points in cell cycle and differentiation determined 14 days later.
Results
There was a significant, reproducible, and pronounced reversible increase in differentiation to megakaryocytes in early S-phase and to nonproliferative granulocytes in mid S-phase. Megakaryocyte hotspots also were seen on a clonal basis. Elevations of the transcription factor FOG-1 were seen at the hotspot along with increases in Nfe2 and Fli1.
Conclusions
We show that the potential of marrow stem cells to differentiate changes reversibly with cytokine-induced cell-cycle transit, suggesting that stem cell regulation is not based on the classic hierarchical model, but instead on a functional continuum. We propose that there is a tight linkage of commitment to a lineage and a particular phase of cell cycle. Thus, windows of vulnerability for commitment can open and close

depending on the phase of cell cycle. These data indicate that stem cell differentiation occurs on a cell-cycle–related continuum with fluctuating windows of transcriptional opportunity.

4.450 Detection of Mattesia oryzaephili (Neogregarinorida: Lipotrophidae) in grain beetle laboratory colonies with an enzyme-linked immunosorbent assay
Lord, J.C.
J. Invert. Pathol., ***94(1)***, *74-76 (2007)*

An indirect sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of the neogregarine *Mattesia oryzaephili* was developed with monoclonal antibodies. It was used to screen laboratory colonies of *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *C. pusillus*, and *C. turcicus* from the United States, Canada, and Australia. All of the colonies except *C. turcicus* had larvae that tested positive with the percent of positives ranging from 0.2 to 83.9, but only colonies that tested positive had reported population declines. This assay will make possible epizootiological studies to assess the impact of *M. oryzaephili* on pest populations.

4.451 Expression of mRNAs related to connective tissue metabolism in rat hepatic stellate cells and myofibroblasts
Jiroutova, A. et al
Exp. Toxicol. Pathol., ***58(4)***, *263-273 (2007)*

Hepatic stellate cells (HSC) and liver myofibroblasts (MFB) are two cell populations most likely responsible for the synthesis of most connective tissue components in fibrotic liver. They differ in their origin and location, and possibly in patterns of gene expression. Normal and carbon tetrachloride-cirrhotic livers from rats were used to isolate HSC. Liver was perfused with pronase and collagenase solutions, followed by centrifugation of the cell suspension on a density gradient. HSC were quiescent 2 days after plating on plastic but they became activated after another 5 days in culture. When the culture was passaged 5 times, its character changed profoundly as HSC were replaced by MFB. Microarray analysis was used to determine gene expression in quiescent HSC, activated HSC and MFB. The expression of 49 genes coding for connective tissue proteins, proteoglycans, metalloproteinases and their inhibitors, growth factors and cellular markers was determined. The pattern of gene expression changed during HSC activation and there were distinct differences between HSC and MFB. Little difference between normal cells and cells isolated from cirrhotic liver was found.

4.452 Neuroprotection by estradiol: A role of aromatase against spine synapse loss after blockade of GABAA receptors
Zhou, L. et al
Exp. Neurol., ***203(1)***, *72-81 (2007)*

Estrogen has been suggested to be pro-epileptic by reducing GABA synthesis, resulting in increased spine density and a decreased threshold for seizures in the hippocampus, which, once they occur, are characterized by a dramatic spine loss in the affected brain areas. As considerable amounts of estradiol are synthesized in the hippocampus, in this study we focused on aromatase, the rate-limiting enzyme in estrogen synthesis in order to examine the role of locally synthesized estrogens in epilepsy. To this end, we first examined the effects of letrozole, a potent aromatase inhibitor, on GABA metabolism in single interneurons of hippocampal dispersion cultures. Letrozole downregulated estradiol release into the medium, as well as glutamate decarboxylase (GAD) expression and GABA synthesis, and decreased the number of GAD positive cells in the cultures. Next, we counted spine synapses and measured estradiol release of hippocampal slice cultures, in which $GABA_A$ receptors had been blocked by bicuculline, in order to mimic epileptic activity. Treatment of slice cultures with bicuculline resulted in a dramatic decrease in the number of spine synapses and in a significant suppression of estrogen synthesis. The decrease in synapse number in response to bicuculline was restored by combined application of estradiol and bicuculline. Surprisingly, estradiol alone had no effect on either spine synapse number or on GAD expression and GABA synthesis. “Rescue” of synapse number in “epileptic slices” by estradiol and maintenance of GABA metabolism by hippocampus-derived estradiol points to a neuroprotective role of aromatase in epilepsy. Re-filling of estradiol stores after their depletion due to overexcitation may therefore add to therapeutical strategies in epilepsy.

4.453 Cloning and characterization of guinea pig CXCR1
Takahashi, M., Jeevan, A., Sawant, K., McMurray, D.N. and Yoshimuro, T.

Mol. Immunol., ***44(5)***, *878-888 (2007)*

IL-8/CXCL8 plays a critical role in the trafficking and activation of neutrophils via its receptors, CXCR1 and CXCR2, in humans. CXCR1 is highly selective for IL-8, whereas CXCR2 is activated by all CXC chemokines with an ELR motif. In mice and rats, neither IL-8 nor CXCR1 is present, making it difficult to evaluate the in vivo roles of the IL-8/CXCR1 interactions. We previously demonstrated the presence of IL-8 in the guinea pig (gp), suggesting that its specific receptor CXCR1 is also present in this species. Here, we obtained two gp genomic DNA clones, clones 8 and 10, coding for the potential orthologues of CXCR1 and CXCR2, respectively. Transcripts for these genes were expressed in neutrophils, but not in macrophages. Functionally, both gp and human (h) IL-8 induced cell migration and ERK phosphorylation in HEK 293 cells expressing either receptor, whereas hGRO activated only cells expressing the clone 10 protein, confirming that clone 8 indeed coded for gpCXCR1. ^{125}I-labeled hIL-8 bound to gpCXCR1 and addition of unlabeled hIL-8 completely abolished the binding; however, unlabeled gpIL-8 failed to compete against ^{125}I-labeled hIL-8, strongly suggesting that the avidity of hIL-8 to gpCXCR1 is higher than that of gpIL-8. Identification and characterization of CXCR1 in the guinea pig will allow us to use this small animal model to evaluate the role of the IL-8/CXCR1 interactions and to examine the efficacy of CXCR1 antagonists in vivo.

4.454 Ivermectin inhibits AMPA receptor-mediated excitotoxicity in cultured motor neurons and extends the life span of a transgenic mouse model of amyotrophic lateral sclerosis
Andries, M., Van Damme, P., Robberecht, W. and Van Den Bosch, L.
Neurobiol. Disease, ***25(1)***, *8-16 (2007)*

α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated excitotoxicity contributes to the selective motor neuron death in amyotrophic lateral sclerosis (ALS). In this study, we investigated the effect of P2 receptor-influencing substances on kainate-induced motor neuron death in an *in vitro* model for AMPA receptor-mediated excitotoxicity. Complete protection was found after preincubation of the motor neurons with ivermectin or Cibacron Blue 3G-A. Preincubation with both $P2X_4$ modulators did not influence the number or Ca^{2+} permeability of the AMPA receptors and addition during kainate stimulation alone had no effect. Preincubation with a low concentration of ATP, the natural agonist of the $P2X_4$ receptor, also protected the motor neurons against a subsequent excitotoxic stimulation, while high concentrations of ATP were toxic. Moreover, ivermectin increased the toxicity of low ATP concentrations, indicating that ivermectin can potentiate the effect of ATP on its receptor. Ivermectin and ATP also protected against hypoxia/hypoglycemia. To further investigate the relevance of these findings for ALS, we treated SOD1(G93A)-mice, a transgenic animal model for familial ALS, with ivermectin. This resulted in an extension of the life span of these mice with almost 10%. We conclude that ivermectin induces a mechanism in motor neurons, *in vivo* and *in vitro*, that protects against subsequent excitotoxic insults. Our *in vitro* data indicate that this protective mechanism is due to the potentiation by ivermectin of an effect of ATP mediated by the $P2X_4$ receptor.

4.455 Surfactant Protein D Augments Bacterial Association but Attenuates Major Histocompatibility Complex Class II Presentation of Bacterial Antigens
Hansen, S. et al
*Am. J. Respir. Cell Mol. Biol.****36****, 94-102 (2007)*

Surfactant protein D (SP-D) is a secreted pattern recognition molecule associated with lung surfactant and mediates the clearance of pathogens in multiple ways. SP-D is an established part of the innate immune system, but it also modulates the adaptive immune response by interacting with both antigen-presenting cells and T cells. In a previous study, antigen presentation by bone marrow–derived dendritic cells was enhanced by SP-D. As dendritic cell function varies depending on the tissue of origin, we extended these studies to antigen-presenting cells isolated from mouse lung. Flow cytometric studies showed that SP-D binds calcium dependently and specifically to lung CD11c-positive cells. Opsonization of fluorescently labeled *Escherichia coli* by SP-D enhanced uptake by lung dendritic cells. SP-D facilitated the association of *E. coli* and antigen-presenting cells by increasing the frequency of CD11+ cells associated with *E. coli* by up to 10-fold. In contrast to the effect on bone marrow–derived dendritic cells, SP-D decreased the antigen presentation of ovalbumin, expressed in *E. coli*, to ovalbumin-specific major histocompatibility complex class II–specific T-cell hybridomas by 30–50%. The reduction of antigen presentation did not depend on whether the dendritic cells were isolated from the lungs of nonstimulated mice or mice that had been exposed to LPS aerosols. Our results show that SP-D increases the opsonization of pathogens, but decreases the antigen presentation by lung dendritic cells, and thereby, potentially dampens the activation

of T cells and an adaptive immune response against bacterial antigens—during both steady-state conditions and inflammation.

4.456 Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis
Mann, J. et al
*Cell Death and Differentiation, **14**, 275-285 (2007)*

Myofibroblasts are critical cellular elements of wound healing generated at sites of injury by transdifferentiation of resident cells. A paradigm for this process is conversion of hepatic stellate cells (HSC) into hepatic myofibroblasts. Treatment of HSC with DNA methylation inhibitor 5-aza-2'-deoxycytidine (5-azadC) blocked transdifferentiation. 5-azadC also prevented loss of $I^{\kappa}B^{\alpha}$ and $PPAR^{\gamma}$ expression that occurs during transdifferentiation to allow acquisition of proinflammatory and profibrogenic characteristics. ChIP analysis revealed $I^{\kappa}B^{\alpha}$ promoter is associated with transcriptionally repressed chromatin that converts to an active state with 5-azadC treatment. The methyl-CpG-binding protein MeCP2 which promotes repressed chromatin structure is selectively detected in myofibroblasts of diseased liver. siRNA knockdown of MeCP2 elevated $I^{\kappa}B^{\alpha}$ promoter activity, mRNA and protein expression in myofibroblasts. MeCP2 interacts with $I^{\kappa}B^{\alpha}$ promoter via a methyl-CpG-dependent mechanism and recruitment into a CBF1 corepression complex. We conclude that MeCP2 and DNA methylation exert epigenetic control over hepatic wound healing and fibrogenesis.

4.457 Discoidin domain receptor 2 is involved in the activation of bone marrow-derived dendritic cells caused by type I collagen
Lee, J-E. et al
*Biochem. Biophys. Res. Comm., **352(1)**, 244-250 (2007)*

Discoidin domain receptors (DDRs), DDR1 and DDR2, are non-integrin receptor tyrosine kinases for collagen in many cell types. In this study, we investigated the contributions of DDRs to the activation of mouse bone marrow-derived dendritic cells (DCs) by type I collagen (ColI). Our data showed that transcript and protein of DDR2 were expressed constitutively in immature DCs and upregulated in TNF-α-stimulated mature DCs. ColI treatment induced DDR2 phosphorylation and subsequently induced the upregulation of IL-12 production, CD86 expression, and antigen uptake activity by immature DCs. Depletion of DDR2 by specific siRNA attenuated significantly an increase in expression of IL-12 and CD86 in ColI-treated DCs. Additionally, DDR2–ColI interaction upregulated the ability of mature DCs to activate allogeneic T cells. These findings suggest that DDR2 is a critical collagen receptor for DC activation and that DDR2–collagen interaction plays an important role in the functional capacity of DCs regulating immune responses.

4.458 CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ TH-17 cells in relapsing EAE
Bailey, S.I., Schreiner, B., McMahon, E.J. and Miller, S.D.
*Nature Immunol., **8(2)**, 172-180 (2007)*

Peripherally derived $CD11b^{+}$ myeloid dendritic cells (mDCs), plasmacytoid DCs, $CD8\alpha^{+}$ DCs and macrophages accumulate in the central nervous system during relapsing experimental autoimmune encephalomyelitis (EAE). During acute relapsing EAE induced by a proteolipid protein peptide of amino acids 178–191, transgenic T cells (139TCR cells) specific for the relapse epitope consisting of proteolipid protein peptide amino acids 139–151 clustered with mDCs in the central nervous system, were activated and differentiated into T helper cells producing interleukin 17 (T_H-17 cells). CNS mDCs presented endogenously acquired peptide, driving the proliferation of and production of interleukin 17 by naive 139TCR cells *in vitro* and *in vivo*. The mDCs uniquely biased T_H-17 and not T_H1 differentiation, correlating with their enhanced expression of transforming growth factor-β1 and interleukins 6 and 23. Plasmacytoid DCs and $CD8\alpha^{+}$ DCs were superior to macrophages but were much less efficient than mDCs in presenting endogenous peptide to induce T_H-17 cells. Our findings indicate a critical function for CNS mDCs in driving relapses in relapsing EAE.

4.459 Influence of strain and age differences on the yields of porcine islet isolation: extremely high islet yields from SPF CMS miniature pigs
Kim, J.H. et al
*Xenotransplantation, **14**, 60-66 (2007)*

Background: Porcine pancreas is a potential source of material for islet xenotransplantation. However, the difficulty in isolating islets, because of their fragility and the variability of isolation outcome in donor age and breed, represents a major obstacle to porcine islet xenotransplantation. In this study, we compared the islet isolation yield of specific pathogen-free (SPF) Chicago Medical School (CMS) miniature pigs with that of another miniature pig breed and market pigs from a local slaughterhouse.
Methods: Nine adult CMS miniature (ACM) pigs (>12 months), six young CMS miniature (YCM) pigs (6–7 months), four adult Prestige World Genetics (PWG) miniature (APM) pigs (>12 months), and 13 adult market (AM) pigs from a local slaughterhouse were used for islet isolation.
Results: The islet yield per gram of pancreas from ACM pigs (9589 ± 2823 IEQ/g) was significantly higher than that from APM pigs (1752 ± 874 IEQ/g, $P < 0.05$), AM pigs (1931 ± 947 IEQ/g, $P < 0.05$), or YCM pigs (3460 ± 1985 IEQ/g, $P < 0.05$). Isolated islets from ACM pigs were significantly larger than those from AM pigs or YCM pigs. The in vitro and in vivo function of isolated islets showed no difference among experimental groups. The pancreases of ACM pigs contained higher mean islet volume density percentages and larger size of islets than those of AM or APM pigs.
Conclusions: We isolated extremely high yields of well-functioning islets from ACM pigs bred under SPF conditions. SPF CMS miniature pigs should be one of the best porcine islet donors for clinical porcine islet xenotransplantation.

4.460 Control of coronavirus infection through plasmacytoid dendritic-cell–derived type I interferon
Cervantes-Barragan, L. et al
*Blood, **109(3)**, 1131-1137 (2007)*

This study demonstrates a unique and crucial role of plasmacytoid dendritic cells (pDCs) and pDC-derived type I interferons (IFNs) in the pathogenesis of mouse coronavirus infection. pDCs controlled the fast replicating mouse hepatitis virus (MHV) through the immediate production of type I IFNs. Recognition of MHV by pDCs was mediated via TLR7 ensuring a swift IFN-α production following encounter with this cytopathic RNA virus. Furthermore, the particular type I IFN response pattern was not restricted to the murine coronavirus, but was also found in infection with the highly cytopathic human severe acute respiratory syndrome (SARS) coronavirus. Taken together, our results suggest that rapid production of type I IFNs by pDCs is essential for the control of potentially lethal coronavirus infections.

4.461 Selective abrogation of Th1 response by STA-5326, a potent IL-12/IL-23 inhibitor
Wada, Y. et al
*Blood, **109(3)**, 1156-1164 (2007)*

The interleukin-12 (IL-12) cytokine induces the differentiation of naive T cells to the T helper cell type 1 (Th1) phenotype and is integral to the pathogenesis of Th1-mediated immunologic disorders. A more recently discovered IL-12 family member, IL-23, shares the p40 protein subunit with IL-12 and plays a critical role in the generation of effector memory T cells and IL-17–producing T cells. We introduce a novel compound, STA-5326, that down-regulates both IL-12 p35 and IL-12/IL-23 p40 at the transcriptional level, and inhibits the production of both IL-12 and IL-23 cytokines. Oral administration of STA-5326 led to a suppression of the Th1 but not Th2 immune response in mice. In vivo studies using a $CD4^{+}CD45Rb^{high}$ T-cell transfer severe combined immunodeficiency (SCID) mouse inflammatory bowel disease model demonstrated that oral administration of STA-5326 markedly reduced inflammatory histopathologic changes in the colon. A striking decrease in interferon-γ (IFN-γ) production was observed in ex vivo culture of lamina propria cells harvested from animals treated with STA-5326, indicating a down-regulation of the Th1 response by STA-5326. These results suggest that STA-5326 has potential for use in the treatment of Th1-related autoimmune or immunologic disorders. STA-5326 currently is being evaluated in phase 2 clinical trials in patients with Crohn disease and rheumatoid arthritis.

4.462 Astrocyte and Muscle-Derived Secreted Factors Differentially Regulate Motoneuron Survival
Taylor, A.R. et al
*J. Neurosci., **27(3)**, 634-644 (2007)*

During development, motoneurons (MNs) undergo a highly stereotyped, temporally and spatially defined

period of programmed cell death (PCD), the result of which is the loss of 40–50% of the original neuronal population. Those MNs that survive are thought to reflect the successful acquisition of limiting amounts of trophic factors from the target. In contrast, maturation of MNs limits the need for target-derived trophic factors, because axotomy of these neurons in adulthood results in minimal neuronal loss. It is unclear whether MNs lose their need for trophic factors altogether or whether, instead, they come to rely on other cell types for nourishment. Astrocytes are known to supply trophic factors to a variety of neuronal populations and thus may nourish MNs in the absence of target-derived factors. We investigated the survival-promoting activities of muscle- and astrocyte-derived secreted factors and found that astrocyte-conditioned media (ACM) was able to save substantially more motoneurons *in vitro* than muscle-conditioned media (MCM). Our results indicate that both ACM and MCM are significant sources of MN trophic support *in vitro* and *in ovo*, but only ACM can rescue MNs after unilateral limb bud removal. Furthermore, we provide evidence suggesting that MCM facilitates the death of a subpopulation of MNs in a $p75^{NTR}$ - and caspase-dependent manner; however, maturation in ACM results in MN trophic independence and reduced vulnerability to this negative, pro-apoptotic influence from the target.

4.463 Pro-NGF secreted by astrocytes promotes motor neuron cell death
Domeniconi, M., Hempstead, B.L. and Chao, M.V.
Mol. Cell. Neurosci., ***34(2)***, *271-279 (2007)*

It is well established that motor neurons depend for their survival on many trophic factors. In this study, we show that the precursor form of NGF (pro-NGF) can induce the death of motor neurons via engagement of the p75 neurotrophin receptor. The pro-apoptotic activity was dependent upon the presence of sortilin, a p75 co-receptor expressed on motor neurons. One potential source of pro-NGF is reactive astrocytes, which up-regulate the levels of pro-NGF in response to peroxynitrite, an oxidant and producer of free radicals. Indeed, motor neuron viability was sensitive to conditioned media from cultured astrocytes treated with peroxynitrite and this effect could be reversed using a specific antibody against the pro-domain of pro-NGF. These results are consistent with a role for activated astrocytes and pro-NGF in the induction of motor neuron death and suggest a possible therapeutic target for the treatment of motor neuron disease.

4.464 Both Ca2+-dependent and -independent pathways are involved in rat hepatic stellate cell contraction and intrahepatic hyperresponsiveness to methoxamine
Laleman, W. et al
Am. J. Physiol. Gastrointest. Liver Physiol., ***292***, *G556-G564 (2007)*

In chronic liver injury, hepatic stellate cells (HSCs) have been implicated as regulators of sinusoidal vascular tone. We studied the relative role of Ca^{2+}-dependent and Ca^{2+}-independent contraction pathways in rat HSCs and correlated these findings to in situ perfused cirrhotic rat livers. Contraction of primary rat HSCs was studied by a stress-relaxed collagen lattice model. Dose-response curves to the Ca^{2+} ionophore A-23187 and to the calmodulin/myosin light chain kinase inhibitor W-7 served to study Ca^{2+}-dependent pathways. Y-27632, staurosporin, and calyculin (inhibitors of Rho kinase, protein kinase C, and myosin light chain phosphatase, respectively) were used to investigate Ca^{2+}-independent pathways. The actomyosin interaction, the common end target, was inhibited by 2,3-butanedione monoxime. Additionally, the effects of W-7, Y-27632, and staurosporin on intrahepatic vascular resistance were evaluated by in situ perfusion of normal and thioacetamide-treated cirrhotic rat livers stimulated with methoxamine (n = 25 each). In vitro, HSC contraction was shown to be actomyosin based with a regulating role for both Ca^{2+}-dependent and -independent pathways. Although the former seem important, an important auxiliary role for the latter was illustrated through their involvement in the phenomenon of "Ca^{2+} sensitization." In vivo, preincubation of cirrhotic livers with Y-27632 (10^{-4} M) and staurosporin (25 nM), more than with W-7 (10^{-4} M), significantly reduced the hyperresponsiveness to methoxamine (10^{-4} M) by –66.8 ± 1.3%, –52.4 ± 2.7%, and –28.7 ± 2.8%, respectively, whereas in normal livers this was significantly less: –43.1 ± 4.2%, –40.2 ± 4.2%, and –3.8 ± 6.3%, respectively. Taken together, these results suggest that HSC contraction is based on both Ca^{2+}-dependent and -independent pathways, which were shown to be upregulated in the perfused cirrhotic liver, with a predominance of Ca^{2+}-independent pathways.

4.465 Immunological characterization of a bacterial protein isolated from salmonid fish naturally infected with *Piscirickettsia salmonis*
Marshall, S.H. et al
Vaccine, ***25***, *2095-2102 (2007)*

The Salmon Rickettsia syndrome (SRS) remains a major infectious disease in the Chilean aquaculture. A limited number of *Piscirickettsia salmonis* proteins have been characterized so far for their use as potential candidates for vaccines studies. In this study, we identified and expressed a highly immunogenic protein of *P. salmonis* extracted by selective hydrophobicity from crude-cell macerates of naturally infected salmonid fish. One and two-D PAGE gels followed by Western blot analysis with a battery of polyclonal anti-*P. salmonis* antibodies have allowed the isolation of the target protein. Basic local alignment search (BLAST) done after partial sequencing of the pure protein identified it as a member of the heat-shock protein (HSP) family of prokaryotes. The protein, named ChaPs, was cloned as a single open reading frame encoding 545 amino acid residues with a predicted molecular mass of 57.3 kDa. The amplicon representing the entire novel gene was expressed *in vitro* in different heterologous systems: the PurePro *Caulobacter crescentus* expression system from where most of the characterization was attained, and also in the *Escherichia coli* BL-21 CodonPlus model for commercially potential purposes. The immunologic potential of ChaPs was determined with serum from naturally infected fish.

4.466 DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27
Sigmundsdottir, H. et al
Nature Immunol., ***8(3)***, *285-293 (2007)*

During adaptive immune responses, dendritic cells activate T cells and endow them with specific homing properties. Mechanisms that 'imprint' specific tropisms, however, are not well defined. We show here that 1,25(OH)2D3, the active form of vitamin D3, signaled T cells to express CC chemokine receptor 10, which enabled them to migrate to the skin-specific chemokine CCL27 secreted by keratinocytes of the epidermis. In contrast, 1,25(OH)2D3 suppressed the gut-homing receptors $\alpha 4\beta 7$ and CCR9. Vitamin D3, the inactive prohormone naturally generated in the skin by exposure to the sun, was processed by dendritic cells and T cells to the active metabolite, providing a mechanism for the local regulation of T cell 'epidermotropism'. Our findings support a model in which dendritic cells process and 'interpret' locally produced metabolites to 'program' T cell homing and microenvironmental positioning.

4.467 Gene Gun-delivered *p*GM-CSF Adjuvant Induces Enhanced Emigration of two Dendritic Cell Subsets from the Skin
Matthews, K et al
Scand. J. Immunol., ***65***, *231-239 (2007)*

Two subsets of sheep afferent lymph dendritic cells (DC) are defined by the differential expression of CD172a and CD45RA. The majority (~70%) of $CD172a^+$ subset is CD45RA/$CD11c^+$/$CD207^+$/$TLR4^+$. The CD172a− DC are $CD45RA^+$/CD207− and express low levels of CD11c and CD86. Real-time RT-PCR showed that $CD172^+$ DC produce IL-1β and IL-10 and high levels of IL-18 but almost no IL-12*p40*; CD172a− DC express IL-12*p40* but no IL-10 and low levels of IL-1β and IL-18. Gene gun-delivered granulocyte-macrophage colony-stimulating factor (*p*GM-CSF) caused an early rise in the output of $CD172a^+$ DC, changes to DC phenotype and significant increases in the levels of expression cytokine transcripts. However, *p*GM-CSF did not affect any qualitative changes to cytokine expression, $CD172a^+$ DC remained IL-10^+/IL-12*p40*− and the CD172− DC remained IL-10−/IL-12$p40^+$.

4.468 Comparative immunobiology of thymic DC mRNA in autoimmune-prone mice
Okada, T. et al
J. Autoimmunity, ***28***, *41-45 (2007)*

New Zealand Black (NZB) mice have multiple defects in both innate and acquired immunity. A fundamental defect, described more than 25years ago, is premature thymic involution. Subsequent studies have disclosed multiple defects in thymic epithelial cells, and it has been proposed that thymic dendritic cells (DCs) play an important role not only in thymic involution but also in the appearance of immunopathology. However, the number of available thymic DCs makes this population extremely difficult to study. We have taken advantage of our ability to isolate pure populations of thymic DCs and have examined several key mRNA levels of enzymes involved in signal transduction. Our data on NZB mice was compared to that of NZB x NZW F1 (B/WF1), BXSB-Yaa, MRL/lpr, NOD and control mice. Importantly, we demonstrate herein that a common feature in autoimmune-prone mice is an increase of thymic DC c-met mRNA. Indeed, the increase in c-met mRNA levels appeared specific to the thymus and was not noted in the spleen. Additionally, we demonstrate that E-cadherin, a downstream molecule of c-met, is also reduced. Finally, we note that the levels of HGF mRNA are normal in the autoimmune strains

examined herein, confirming that the abnormality of c-met mRNA is not due to primary defects in thymic stromal cells. We submit that these results highlight the possibility of a selective defect in thymic DCs which will be a pivotal step in loss of tolerance, and suggest that future studies focus on adoptive cell transfer involving this population.

4.469 Evaluation of a cushioned method for centrifugation and processing for freezing boar semen
Matas, C., Decuadro, G., Martinez-Miro, S. and Gadea, J.
*Theriogenology, **67**, 1087-1091 (2007)*

The purpose of this investigation was to evaluate the use of an iodixanol cushion during centrifugation on sperm recovery and yield after centrifugation (sperm recovery, sperm motility, viability, membrane lipid disorder, acrosome reaction and ROS generation); and to investigate how this procedure affects sperm function after freezing–thawing (sperm motility, membrane lipid disorder, acrosomal status and homologous in vitro penetration test). The sperm-rich fractions from fertile boars were centrifuged under two centrifugation régimes: 800×*g* for 10min (standard method) and 1000×*g* for 20min with an iodixanol (60% w/v) cushion at the bottom of the centrifuge tubes (Cushion method). The highest recovery was achieved using the cushion method (sperm loss for cushion method was 0.50%±0.18 *versus* 2.97%±0.43 for standard method, $P<0.01$) and sperm quality was not significantly affected by the centrifugation régime. The motion parameters (% progressive motility, % motility, VCL, VSL, VAP, ALH, BCF, $P<0.05$) of frozen–thawed samples showed higher values using the standard method. However, a higher number of viable spermatozoa with lower lipid disorders were found in spermatozoa processed with the cushion method. The in vitro penetration assay showed that the individual boar influenced the parameters studied but there were no differences between the two centrifugation régimes used. Our results support the hypothesis that the proportion of sperm loss in frozen–thawed semen was significantly influenced by the centrifugation régime. Therefore, the iodixanol cushion method is a suitable tool for cryopreservation of boar semen in order to reduce sperm loss without affecting sperm quality.

4.470 Nitroflurbiprofen, a Nitric Oxide-Releasing Cyclooxygenase Inhibitor, Improves Cirrhotic Portal Hypertension in Rats
Laleman, W. et al
*Gastroenterol., **132(2)**, 709-719 (2007)*

Background & Aims: We studied whether administration of nitroflurbiprofen (HCT-1026), a cyclooxygenase inhibitor with nitric oxide (NO)-donating properties, modulates the increased intrahepatic vascular tone in portal hypertensive cirrhotic rats. *Methods:* In vivo hemodynamic measurements (n = 8/condition) and evaluation of the increased intrahepatic resistance by in situ perfusion (n = 5/condition) were performed in rats with thioacetamide-induced cirrhosis that received either nitroflurbiprofen (45 mg/kg), flurbiprofen (30 mg/kg, equimolar concentration to nitroflurbiprofen), or vehicle by intraperitoneal injection 24 hours and 1 hour prior to the measurements. Additionally, we evaluated the effect of acute administration of both drugs (250 μmol/L) on the intrahepatic vascular tone in the in situ perfused cirrhotic rat liver (endothelial dysfunction and hyperresponsiveness to methoxamine) and on hepatic stellate cell contraction in vitro. Typical systemic adverse effects of nonsteroidal anti-inflammatory drugs, such as gastrointestinal ulceration, renal insufficiency, and hepatotoxicity, were actively explored. *Results:* In vivo, nitroflurbiprofen and flurbiprofen equally decreased portal pressure (8 ± 0.8 and 8.4 ± 0.1 mm Hg, respectively, vs 11.8 ± 0.6 mm Hg) and reduced the total intrahepatic vascular resistance. Systemic hypotension was not aggravated in the different treatment groups ($P = .291$). In the perfused cirrhotic liver, both drugs improved endothelial dysfunction and hyperresponsiveness. This was associated with a decreased hepatic thromboxane A_2-production and an increased intrahepatic nitrate/nitrite level. In vitro, nitroflurbiprofen, more than flurbiprofen, decreased hepatic stellate cells contraction. Flurbiprofen-treated rats showed severe gastrointestinal ulcerations (bleeding in 3/8 rats) and nefrotoxicity, which was not observed in nitroflurbiprofen-treated cirrhotic rats. *Conclusions:* Treatment with nitroflurbiprofen, an NO-releasing cyclooxygenase inhibitor, improves portal hypertension without major adverse effects in thioacetamide-induced cirrhotic rats by attenuating intrahepatic vascular resistance, endothelial dysfunction, and hepatic hyperreactivity to vasoconstrictors.

4.471 Progesterone protects fetal chorion and maternal decidua cells from calcium-induced death
Murtha, A.P., Feng, L., Yonish, B., Leppert, P.C. and Schomberg, D.W.
*Am. J. Obstet. Gynecol., **196(3)**, 257.e1-257.e5*

Objective

The purpose of this study was to determine whether progesterone exerts a protective effect in chorion and decidua cells when exposed to calcimycin.
Study design
Fetal membrane samples were collected from term elective repeat cesarean deliveries and chorion and decidua cells that are separated and cultured. Cells were pretreated with progesterone and exposed to calcimycin. Cell viability was determined, and percent cell viability was calculated.
Results
Exposure to calcimycin resulted in a reduction of cell viability in both chorion and decidua cells in a dose-dependent fashion. In chorion and decidua cells, progesterone pretreatment followed by calcimycin increased cell viability compared with calcimycin treatment alone (chorion, 67%, vs controls, 24%; $P < .001$; decidua, 58%, vs controls, 35%; $P < .001$). The progesterone receptor antagonist, RTI 6413-49a, blocked the protective effect of progesterone in both chorion and decidua cells.
Conclusion
These preliminary results suggest that progesterone may provide a protective effect in fetal membrane cells and that this effect may be mediated through the progesterone receptor.

4.472 Innate Immune Response to Adenoviral Vectors Is Mediated by both Toll-Like Receptor-Dependent and -Independent Pathways
Zhu, J., Huang, X. and Yang, Y.
J. Virol., ***81(7)***, *3170-3180 (2007)*

Recombinant adenoviral vectors have been widely used for gene therapy applications and as vaccine vehicles for treating infectious diseases such as human immunodeficiency virus disease. The innate immune response to adenoviruses represents the most significant hurdle in clinical application of adenoviral vectors for gene therapy, but it is an attractive feature for vaccine development. How adenovirus activates innate immunity remains largely unknown. Here we showed that adenovirus elicited innate immune response through the induction of high levels of type I interferons (IFNs) by both plasmacytoid dendritic cells (pDCs) and non-pDCs such as conventional DCs and macrophages. The innate immune recognition of adenovirus by pDCs was mediated by Toll-like receptor 9 (TLR9) and was dependent on MyD88, whereas that by non-pDCs was TLR independent through cytosolic sensing of adenoviral DNA. Furthermore, type I IFNs were pivotal in innate and adaptive immune responses to adenovirus in vivo, and type I IFN blockade diminished immune responses, resulting in more stable transgene expression and reduction of inflammation. These findings indicate that adenovirus activates innate immunity by its DNA through TLR-dependent and -independent pathways in a cell type-specific fashion, and they highlight a critical role for type I IFNs in innate and adaptive immune responses to adenoviral vectors. Our results that suggest strategies to interfere with type I IFN pathway may improve the outcome of adenovirus-mediated gene therapy, whereas approaches to activate the type I IFN pathway may enhance vaccine potency.

4.473 The Use of Iodixanol for the Purification of Rat Pancreatic Islets
Delle, H., Saito, M.H., Yoshimoto, P.M. and Noronha, I.L.
Transplant. Proceed., ***39(2)***, *467-469 (2007)*

Transplantation of pancreatic islets is a promising therapeutic treatment for type 1 diabetes mellitus. For clinical and experimental transplantation, a large number of pure pancreatic islets are required for transplantation. Thus, the improvement of islet isolation and purification techniques are crucial. In this context, iodixanol-based solution, successfully used for the purification of porcine islets, seems to be a possible alternative to Ficoll for purification of islets. The aim of this study was to test the efficacy of iodixanol compared with Ficoll density gradients for the purification of rat pancreatic islets. Twelve Wistar rats were used for isolation and purification of pancreatic islets. Pancreata were digested with Liberase R1 and islets purified by two gradients: Ficoll or iodixanol gradient. The number and the purity of the pancreatic islets were assessed. To analyze the response of isolated pancreatic islet to glucose challenge, in vitro experiments were performed by measuring the insulin concentration in the Supernatant. The results demonstrated that the iodixanol gradient provided a higher purity of pancreatic islets compared to the Ficoll gradient. In addition, the rat islet yield by iodixanol gradient was significantly higher compared to a Ficoll gradient (751 ± 16 versus 464 ± 19 pancreatic islets, respectively; $P < .001$). The viability of pancreatic islets isolated by an iodixanol gradient was confirmed by high glucose challenge, with more than twofold higher increase in insulin secretion. The present study demonstrated that iodixanol density gradient overcomes Ficoll density gradient, providing a greater number of pure and functional rat pancreatic islets.

4.474 Establishment and characterization of porcine Sertoli cell line for the study of xenotransplantation
Lee, H-M. et al
*Xenotransplantation, **14**, 112-118 (2007)*

Background: An understanding of the main mechanism that determines the ability of immune privilege related to Sertoli cells (SC) will provide clues for promoting a local tolerogenic environment. In this report, we established neonatal porcine SC line and evaluated their characteristics.
Methods: SC line was established following the transfection of primary SC (NPSC) from the testis of neonatal pig with plasmid pRNS-1 carrying genes for neomycin resistance and the SV40 large T antigen. Immunohistochemistry and RT-PCR were performed to evaluate the character of immortalized SC lines.
Results: Our immortalized SC line (iPS) proliferated stably and had a phenotype similar to NPSC, as indicated by the immunoexpression of follicle stimulating hormone receptor (FSHR), and mRNA expression of androgen receptor (AR), and Wilms' tumor antigen (WT1). Interestingly, NPSC and iPS expressed mRNA of complement regulatory proteins (CRP) such as membrane cofactor protein (CD46), decay accelerating factor (DAF or CD55), and protectin (CD59), but CD59 mRNA expression was negligible in iPS.
Conclusion: These results suggest that iPS, immortalized by the introduction of SV40 T, retain their original characteristics, except for the relatively low expression of CD59, and that they may be useful for future in vitro and in vivo studies of immune privilege mechanisms related to SC.

4.475 BMP6 is axonally transported by motoneurons and supports their survival in vitro
Wang, P-Y., Koishi, K. and McLennan, I.S.
*Mol. Cell. Neurosci., **34(4)**, 653-661 (2007)*

The regulation of motoneuron survival is only partially elucidated. We have sought new survival factors for motoneuron by analyzing which receptors they produce. We report here that the type II bone morphogenetic receptor (BMPRII) mRNA is one of the most abundant receptor mRNAs in laser microdissected motoneurons. Motoneurons were intensely stained by an anti-BMPRII antibody, indicating the presence of BMPRII protein. One of its ligands (BMP6) supported the survival of motoneurons in vitro. BMP6 was produced by myotubes and mature Schwann cells and was retrogradely transported in mature motor axons. BMP6 thus joins a list of known Schwann-cell-derived regulators of motoneurons, which includes GDNF, CNTF, LIF and TGF-β2. The control of the production of these factors by Schwann cells and the direction of their movement in motor axons is diverse. This suggests that the multiplicity of motoneuron factors is because cells use different factors to regulate different aspects of motoneuron function.

4.476 Costimulatory ligand CD70 is delivered to the immunological synapse by shared intracellular trafficking with MHC class II molecules
Keller, A.M. et al
*PNAS, **104(14)**, 5989-5994 (2007)*

TNF family member CD70 is the ligand of CD27, a costimulatory receptor that shapes effector and memory T cell pools. Tight control of CD70 expression is required to prevent lethal immunodeficiency. By selective transcription, CD70 is largely confined to activated lymphocytes and dendritic cells (DC). We show here that, in addition, specific intracellular routing controls its plasma membrane deposition. In professional antigen-presenting cells, such as DC, CD70 is sorted to late endocytic vesicles, defined as MHC class II compartments (MIIC). In cells lacking the machinery for antigen presentation by MHC class II, CD70 travels by default to the plasma membrane. Introduction of class II transactivator sufficed to reroute CD70 to MIIC. Vesicular trafficking of CD70 and MHC class II is coordinately regulated by the microtubule-associated dynein motor complex. We show that when maturing DC make contact with T cells in a cognate fashion, newly synthesized CD70 is specifically delivered via MIIC to the immunological synapse. Therefore, we propose that routing of CD70 to MIIC serves to coordinate delivery of the T cell costimulatory signal in time and space with antigen recognition.

4.477 IS-741 Attenuates Local Migration of Monocytes and Subsequent Pancreatic Fibrosis in Experimental Chronic Pancreatitis Induced by Dibutyltin Dichloride in Rats
Kaku, T. et al
*Pancreas, **34(3)**, 299-309 (2007)*

Objectives: Chronic pancreatitis consists of excessive leukocyte infiltration and fibrosis. IS-741 has been

reported to be an antiinflammatory drug through an inhibitory action on cell adhesion. In this study, we investigated whether IS-741 could inhibit the progression of pancreatic fibrosis through monocyte infiltration. Moreover, we investigated the effect of IS-741 on rat pancreatic stellate cells (PSCs).
Methods: Chronic pancreatitis was induced by dibutyltin dichloride in rats. From days 7 to 28 after dibutyltin dichloride application, IS-741 or distilled water was administered. At days 14 and 28, histological [hematoxylin-eosin stain and immunostain for ED1 and [alpha] smooth muscle actin ([alpha]-SMA)] and biochemical evaluations (intrapancreatic amylase, protein, cytokines, chemokines, and [alpha]-SMA) were performed. In vitro, rat PSCs were incubated with cytokine, chemokine, and growth factor simultaneously with IS-741, and their proliferation and activation were examined.
Results: Histologically, IS-741 inhibited pancreatic fibrosis and decreased the number of ED1- and [alpha]-SMA-positive cells. The intrapancreatic expression of cytokines, chemokine, and [alpha]-SMA were also decreased. In vitro, IS-741 has no direct effect on the proliferation, [alpha]-SMA expression, and collagen synthesis of PSCs.
Conclusions: These results suggest that IS-741 suppressed macrophage infiltration and subsequent pancreatic fibrosis and that the infiltration of monocytes into pancreas is essential for pancreatic fibrosis.

4.478 Gamma-Glutamyltransferase Activity and Total Antioxidant Status in Serum and Platelets of Patients with Community-acquired Pneumonia
Laskaj, R., Slavica, D., Cepelak, I. and Kuzman, I.
Arc. Med. Res., ***38(4)***, *424-431 (2007)*

Background
We undertook this study to analyze serum and platelet γ-glutamyltransferase (GGT) activity and total antioxidant status (TAS) concentration during the course of pneumonia and to compare them between patients with normal platelet count and those who developed reactive thrombocytosis.
Methods
Platelet count, GGT activity and TAS concentration in serum (S) and platelet (Plt) isolates were measured in 60 patients with community-acquired pneumonia (CAP) on admission and at discharge.
Results
At the end of treatment, platelet count increased significantly from the value recorded on admission. By the end of treatment, 42% of patients developed reactive thrombocytosis. Serum and platelet GGT activity was higher, whereas (S)TAS was significantly lower in CAP patients than in control subjects. On admission, (Plt)TAS was significantly higher in CAP patients as compared with control subjects; at discharge, (Plt)TAS was lower in comparison with either patient admission and control subjects. GGT activity and TAS concentration in serum and platelet isolate on admission did not differ significantly between patients with and without thrombocytosis. At discharge, (S)GGT activity showed no significant changes, whereas (Plt)GGT decreased significantly in patients with thrombocytosis as compared with those without thrombocytosis. In patients with thrombocytosis, (S)TAS concentration showed no significant difference, whereas (Plt)TAS concentration measured at discharge was significantly lower in patients with thrombocytosis as compared to those with normal platelet count.
Conclusions
The pattern of changes in (Plt)GGT catalytic activity and TAS concentration might be indicative of a certain role of thrombocytosis during treatment in patients with CAP. Further investigations are necessary to clarify these changes.

4.479 Tertiary Lymphoid Tissues Generate Effector and Memory T Cells That Lead to Allograft Rejection
Nasr, I.W. et al
Am. J. Transplant., ***7***, *1071-1079 (2007)*

Tertiary lymphoid tissues are lymph node-like cell aggregates that arise at sites of chronic inflammation. They have been observed in transplanted organs undergoing chronic rejection, but it is not known whether they contribute to the rejection process by supporting local activation of naïve lymphocytes. To answer this question, we established a murine transplantation model in which the donor skin contains tertiary lymphoid tissues due to transgenic expression of lymphotoxin-α(*RIP-LT*α), whereas the recipient lacks all secondary lymphoid organs and does not mount primary alloimmune responses. We demonstrate in this model that *RIP-LT*α allografts that harbor tertiary lymphoid tissues are rejected, while wild-type allografts that lack tertiary lymphoid tissues are accepted. Wild-type allografts transplanted at the same time as *RIP-LT*α skin or 60 days later were also rejected, suggesting that tertiary lymphoid tissues, similar to secondary lymphoid organs, generate both effector and memory immune responses. Consistent with this observation, naive T cells transferred to *RIP-LT*α skin allograft but not syngeneic graft recipients proliferated and

differentiated into effector and memory T cells. These findings provide direct evidence that tertiary lymphoid structures perpetuate the rejection process by supporting naïve T-cell activation.

4.480 Cutting Edge: Conventional Dendritic Cells Are the Critical APC Required for the Induction of Experimental Cerebral Malaria
DeWalick, S. et al
*J. Immunol., **178**, 6033-6037 (2007)*

Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection, causing significant morbidity and mortality among young children and nonimmune adults in the developing world. Although previous work on experimental CM has identified T cells as key mediators of pathology, the APCs and subsets therein required to initiate immunopathology remain unknown. In this study, we show that conventional dendritic cells but not plasmacytoid dendritic cells are required for the induction of malaria parasite-specific $CD4^+$ T cell responses and subsequent experimental CM. These data have important implications for the development of malaria vaccines and the therapeutic management of CM.

4.481 Regulation by Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase Substrate-1 of α-Galactosylceramide-Induced Antimetastatic Activity and Th1 and Th2 Responses of NKT Cells
Okajo, J. et al
*J. Immunol., **178**, 6164-6172 (2007)*

Interaction of α-galactosylceramide (α-GalCer) presented by CD1d on dendritic cells (DCs) with the invariant TCR of NKT cells activates NKT cells. We have now investigated the role of Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1), a transmembrane protein abundantly expressed on DCs, in regulation of NKT cells with the use of mice that express a mutant form of SHPS-1. The suppression by α-GalCer of experimental lung metastasis was markedly attenuated in SHPS-1 mutant mice compared with that apparent in wild-type (WT) mice. The antimetastatic effect induced by adoptive transfer of α-GalCer-pulsed DCs from SHPS-1 mutant mice was also reduced compared with that apparent with WT DCs. Both the production of IFN-γ and IL-4 as well as cell proliferation in response to α-GalCer in vitro were greatly attenuated in splenocytes or hepatic mononuclear cells from SHPS-1 mutant mice compared with the responses of WT cells. Moreover, $CD4^+$ mononuclear cells incubated with α-GalCer and $CD11c^+$ DCs from SHPS-1 mutant mice produced markedly smaller amounts of IFN-γ and IL-4 than did those incubated with α-GalCer and $CD11c^+$ DCs from WT mice. SHPS-1 on DCs thus appears to be essential for α-GalCer-induced antimetastatic activity and Th1 and Th2 responses of NKT cells. Moreover, our recent findings suggest that SHPS-1 on DCs is also essential for the priming of $CD4^+$ T cells by DCs.

4.482 Inaugural Article: Toxicity from different SOD1 mutants dysregulates the complement system and the neuronal regenerative response in ALS motor neurons
Lobsiger, C.S., Boilee, S. and Cleveland, D.W.
*PNAS, **104(18)**, 7319-7326 (2007)*

Global, age-dependent changes in gene expression from rodent models of inherited ALS caused by dominant mutations in superoxide-dismutase 1 (SOD1) were identified by using gene arrays and RNAs isolated from purified embryonic and adult motor neurons. Comparison of embryonic motor neurons expressing a dismutase active ALS-linked mutant SOD1 with those expressing comparable levels of wild-type SOD1 revealed the absence of mutant-induced mRNA changes. An age-dependent mRNA change that developed presymptomatically in adult motor neurons collected by laser microdissection from mice expressing dismutase active ALS-linked mutants was dysregulation of the D/L-serine biosynthetic pathway, previously linked to both excitotoxic and neurotrophic effects. An unexpected dysregulation common to motor neurons expressing either dismutase active or inactive mutants was induction of neuronally derived components of the classic complement system and the regenerative/injury response. Alteration of these mutant SOD1-induced pathways identified a set of targets for therapies for inherited ALS.

4.483 A Host Lipase Detoxifies Bacterial Lipopolysaccharides in the Liver and Spleen
Shao, B. et al
*J. Biol. Chem., **282(18)**, 13726-13735 (2007)*

Much of the inflammatory response of the body to bloodborne Gram-negative bacteria occurs in the liver and spleen, the major organs that remove these bacteria and their lipopolysaccharide (LPS, endotoxin) from

the bloodstream. We show here that LPS undergoes deacylation in the liver and spleen by acyloxyacyl hydrolase (AOAH), an endogenous lipase that selectively removes the secondary fatty acyl chains that are required for LPS recognition by its mammalian signaling receptor, MD-2-TLR4. We further show that Kupffer cells produce AOAH and are required for hepatic LPS deacylation *in vivo*. AOAH-deficient mice did not deacylate LPS and, whereas their inflammatory responses to low doses of LPS were similar to those of wild type mice for ~3 days after LPS challenge, they subsequently developed pronounced hepatosplenomegaly. Providing recombinant AOAH restored LPS deacylating ability to *Aoah*$^{-/-}$ mice and prevented LPS-induced hepatomegaly. AOAH-mediated deacylation is a previously unappreciated mechanism that prevents prolonged inflammatory reactions to Gram-negative bacteria and LPS in the liver and spleen.

4.484 Modulation of virulence factors in Francisella tularensis determines human macrophage responses
Carlson Jr., P.E., Carroll, J.A., O'Dee, D.M. and Nau, G.J.
*Microbial Pathogenesis, **42(5-6)**, 204-214 (2007)*

Francisella tularensis, the causative agent of tularemia and Category A biodefense agent, is known to replicate within host macrophages, though the pathogenesis of this organism is incompletely understood. We have isolated a variant of *F. tularensis* live vaccine strain (LVS) based on colony morphology and its effect on macrophages. Human monocyte-derived macrophages produced more tumor necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, and IL-12 p40 following exposure to the variant, designated the activating variant (ACV). The immunoreactivity of the lipopolysaccharide (LPS) from both LVS and ACV was comparable to the previously described blue variant and was distinct from the gray variant of LVS. We found, however, the soluble protein fractions of LVS and ACV differed. Further investigation using two-dimensional gel electrophoresis demonstrated higher levels of several proteins in the parental LVS isolate. The differentially expressed proteins featured several associated with virulence in *F. tularensis* and other pathogens, including intracellular growth locus C (IglC), a σ^{54}-modulation protein family member (YhbH), and aconitase. ACV reverted to the LVS phenotype, indicated by low cytokine induction and high IglC expression, after growth in a chemically defined medium. These data provide evidence that the levels of virulence factors in *F. tularensis* are modulated based on culture conditions and that this modulation impacts host responses. This work provides a basis for investigation of *Francisella* virulence factor regulation and the identification of additional factors, co-regulated with IglC, that affect macrophage responses.

4.485 Suppression of reactive oxygen species production enhances neuronal survival in vitro and in vivo in the anoxia-tolerant turtle Trachemys scripta
Milton, S.L., Nayak, G., Kesaraju, S., Kara, L. and Prentice, H.M.
*J. Neurochem., **101**, 993-1001 (2007)*
Hypoxia-ischemia with reperfusion is known to cause reactive oxygen species-related damage in mammalian systems, yet, the anoxia tolerant freshwater turtle is able to survive repeated bouts of anoxia/reoxygenation without apparent damage. Although the physiology of anoxia tolerance has been much studied, the adaptations that permit survival of reoxygenation stress have been largely ignored. In this study, we examine ROS production in the turtle striatum and in primary neuronal cultures, and examine the effects of adenosine (AD) on cell survival and ROS. Hydroxyl radical formation was measured by the conversion of salicylate to 2,3-dihydroxybenzoic acid (2,3-DHBA) using microdialysis; reoxygenation after 1 or 4 h anoxia did not result in increased ROS production compared with basal normoxic levels, nor did H_2O_2 increase after anoxia/reoxygenation in neuronally enriched cell cultures. Blockade of AD receptors increased both ROS production and cell death *in vitro*, while AD agonists decreased cell death and ROS. As turtle neurons proved surprisingly susceptible to externally imposed ROS stress (H_2O_2), we propose that the suppression of ROS formation, coupled to high antioxidant levels, is necessary for reoxygenation survival. As an evolutionarily selected adaptation, the ability to suppress ROS formation could prove an interesting path to investigate new therapeutic targets in mammals.

4.486 Bacterially Derived 400 nm Particles for Encapsulation and Cancer Cell Targeting of Chemotherapeutics
MacDiamid, J.A. et al
*Cancer Cell, **11(5)**, 431-445 (2007)*

Systemic administration of chemotherapeutic agents results in indiscriminate drug distribution and severe toxicity. Here we report a technology potentially overcoming these shortcomings through encapsulation and cancer cell-specific targeting of chemotherapeutics in bacterially derived 400 nm minicells. We

discovered that minicells can be packaged with therapeutically significant concentrations of chemotherapeutics of differing charge, hydrophobicity, and solubility. Targeting of minicells via bispecific antibodies to receptors on cancer cell membranes results in endocytosis, intracellular degradation, and drug release. This affects highly significant tumor growth inhibition and regression in mouse xenografts and case studies of lymphoma in dogs despite administration of minute amounts of drug and antibody; a factor critical for limiting systemic toxicity that should allow the use of complex regimens of combination chemotherapy.

4.487 Cell cooperation in coelomocyte cytotoxic activity of Paracentrotus lividus coelomocytes
Arizza, V., Giaramita, F.T., Parinello, D., Cammarata, M. and Parinello, N.
*Comp. Biochem. Physiol. Part A, **147(2)**, 389-394 (2007)*

The coelomic fluid from the sea urchin *Paracentrotus lividus* contains several coelomocyte types including amoebocytes and uncoloured spherulocytes involved in immune defences. In the present paper, we show a Ca^{2+}-dependent cytotoxic activity for the unfractionated coelomocytes assayed *in vitro*, with rabbit erythrocytes and the K562 tumour cell line. In a plaque-forming assay, whole coelomocyte preparations as well as density gradient separated coelomocyte populations revealed that cell populations enriched in uncoloured spherulocytes, exerted high cytotoxic activity by releasing lysins in the presence of amoebocytes. This cooperative effect could be dependent on soluble factors released by amoebocytes. With regard to this, we show that an enhanced cytotoxic activity was found by adding the supernatant from sonicated amoebocytes or hemocyte culture medium into spherulocyte preparations.

4.488 Differentiated Human Alveolar Epithelial Cells and Reversibility of their Phenotype In Vitro
Wang, J. et al
*Am. J. Respir. Cell Mol. Biol., **36**, 661-668 (2007)*

Cultures of differentiating fetal human type II cells have been available for many years. However, studies with differentiated adult human type II cells are limited. We used a published method for type II cell isolation and developed primary culture systems for maintenance of differentiated adult human alveolar epithelial cells for *in vitro* studies. Human type II cells cultured on Matrigel (basolateral access) or a mixture of Matrigel and rat tail collagen (apical access) in the presence of keratinocyte growth factor, isobutylmethylxanthine, 8-bromo-cyclicAMP, and dexamethasone (KIAD) expressed the differentiated type II cell phenotype as measured by the expression of surfactant protein (SP)-A, SP-B, SP-C, and fatty acid synthase and their morphologic appearance. These cells contain lamellar inclusion bodies and have apical microvilli. In both systems the cells appear well differentiated. In the apical access system, type II cell differentiation markers initially decreased and then recovered over 6 d in culture. Lipid synthesis was also increased by the addition of KIAD. In contrast, type II cells cultured on rat tail collagen (or tissue culture plastic) slowly lose their lamellar inclusions and expression of the surfactant proteins and increase the expression of type I cell markers. The expression of the phenotypes is regulated by the culture conditions and is, in part, reversible *in vitro*.

4.489 Role of mitochondria in kainate-induced fast Ca^{2+} transients in cultured spinal motor neurons
Grosskreutz, J. et al
*Cell Calcium, **42**, 59-69 (2007)*

Motor neuron death in amyotrophic lateral sclerosis (ALS) has been linked to selective vulnerability towards AMPA receptor-mediated excitotoxicity. We investigated intracellular mechanisms leading to impairment of motor neuron Ca^{2+} homeostasis with near physiological AMPA receptor activation. Using fast solution exchange on patch-clamped cultured neurons, kainate (KA) was applied for 2 s. This induced a transient increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) for seconds. Inhibition of the mitochondrial uniporter by RU-360 abolished the decay of the Ca^{2+} transient and caused immediate $[Ca^{2+}]_c$ overload. Repetitive short KA stimulation caused a slowing of the decay of the Ca^{2+} transient and a gradual increase in peak and baseline $[Ca^{2+}]_c$ in motor neurons, but not in other neurons, indicating saturation of the mitochondrial buffer. Furthermore, mitochondrial density was lower in motor neurons and, in a network of neurons with physiological synaptic AMPA receptor input, RU-360 acutely induced an increase in Ca^{2+} transients. We conclude that motor neurons have an insufficient mitochondrial capacity to buffer large Ca^{2+} elevations which is partly due to a reduced mitochondrial density per volume compared to non-motor neurons. This may exert deleterious effects in motor neuron disease where mitochondrial function is thought to be compromised.

4.490 Effector and regulatory T-cell function is differentially regulated by RelB within antigen-presenting cells during GVHD
MacDonald, K.P.A. et al
*Blood, **109**, 5049-5057 (2007)*

Antigen-presenting cells (APCs) are critical for the initiation of graft-versus-host disease (GVHD), although the responsible APC subset and molecular mechanisms remain unclear. Because dendritic cells (DCs) are the most potent APCs and the NF-kB/Rel family member RelB is associated with DC maturation and potent APC function, we examined their role in GVHD. Within 4 hours of total body irradiation, RelB nuclear translocation was increased and restricted to $CD11c^{hi}$ DCs within the host APC compartment. Furthermore, the transient depletion of $CD11c^{hi}$ donor DCs that reconstitute in the second week after transplantation resulted in a transient decrease in GVHD severity. By using $RelB^{-/-}$ bone marrow chimeras as transplant recipients or $RelB^{-/-}$ donor bone marrow, we demonstrate that the induction and maintenance of GVHD is critically dependent on this transcription factor within both host and donor APCs. Critically, RelB within APCs was required for the expansion of donor helper T cell type 1 (Th1) effectors and subsequent alloreactivity, but not the peripheral expansion or function of donor $FoxP3^{+}$ regulatory T cells. These data suggest that the targeted inhibition of nuclear RelB translocation within APCs represents an attractive therapeutic strategy to dissociate effector and regulatory T-cell function in settings of Th1-mediated tissue injury.

4.491 Osteopontin prevents monocyte recirculation and apoptosis
Burdo, T.H., Wood, M.R. and Fox, H.S.
*J. Leukoc. Biol., **81**, 1504-1511 (2007)*

Cells of the monocyte/macrophage lineage have been shown to be the principal targets for productive HIV-1 replication within the CNS. In addition, HIV-1-associated dementia (HAD) has been shown to correlate with macrophage abundance in the brain. Although increased entry of monocytes into the brain is thought to initiate this process, mechanisms that prevent macrophage egress from the brain and means that prevent macrophage death may also contribute to cell accumulation. We hypothesized that osteopontin (OPN) was involved in the accumulation of macrophages in the brain in neuroAIDS. Using in vitro model systems, we have demonstrated the role of OPN in two distinct aspects of macrophage accumulation: prevention from recirculation and protection from apoptosis. In these unique mechanisms, OPN would aid in macrophage survival and accumulation in the brain, the pathological substrate of HAD.

4.492 Trophic factors counteract elevated FGF-2-induced inhibition of adult neurogenesis
Chen, H., Tung, Y-C., Li, B., Iqbal, K. and Grundke-Iqbal, I.
*Neurobioliology of Aging, **28**, 1148-1162 (2007)*

The dentate gyrus of adult mammalian brain contains neural progenitor cells with self-renewal and multi-lineage potential. The lineage and maturation of the neural progenitors are determined by the composition and levels of the trophic factors in their microenvironment. In Alzheimer disease (AD) brain, especially the hippocampus, the level of basic fibroblast growth factor (FGF-2) is markedly elevated. Here we show that elevated FGF-2 enhances the division and nestin levels of cultured adult rat hippocampal progenitors but impairs neuronal lineage determination and maturation of these cells in culture. The trophic factors ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), and insulin-like growth factors-1 and -2 (IGF-1, IGF-2) as well as an Alzheimer peptidergic drug, Cerebrolysin® (CL), in which we found these neurotrophic activities, counteract the effect of FGF-2 in inducing neuronal lineage (early neurogenesis). Whereas CNTF is the most active of the neurotrophic factors studied in promoting neurogenesis, CL, probably because of a combined effect of these factors, induces similar changes but without inhibiting cell proliferation. These findings suggest that CNTF, GDNF, IGF-1, and IGF-2 are promising therapeutic targets for AD and other diseases in which neurogenesis is probably inhibited.

4.493 Identification of an ADAM2-ADAM3 Complex on the Surface of Mouse Testicular Germ Cells and Cauda Epididymal Sperm
Nishimura, H., Myles, D.G. and Primakoff, P.
*J. Biol. Chem., **282(24)**, 17900-17907 (2007)*

Male mice lacking ADAM2 (fertilin β) or ADAM3 (cyritestin) are infertile; cauda epididymal sperm (mature sperm) from these mutant mice cannot bind to the egg zona pellucida. ADAM3 is barely present in *Adam2*-null sperm, despite normal levels of this protein in *Adam2*-null testicular germ cells (TGCs; sperm

precursor cells). Here, we have explored the molecular basis for the loss of ADAM3 in *Adam2*-null TGCs to clarify the biosynthetic and functional linkage of ADAM2 and ADAM3. A small portion of total ADAM3 was found present on the surface of wild-type and *Adam2*$^{-/-}$ TGCs at similar levels. In the *Adam2*-null TGCs, however, surface-localized ADAM3 exhibited an increased amount of an endoglycosidase H-resistant form that may be related to instability of ADAM3. Moreover, we found a complex between ADAM2 and ADAM3 on the surface of TGCs and sperm. The intracellular chaperone calnexin was a component of the testicular ADAM2-ADAM3 complex. Our findings suggest that the association with ADAM2 is a key element for stability of ADAM3 in epididymal sperm. The presence of the ADAM2-ADAM3 complex in sperm also suggests a potential role of ADAM2 with ADAM3 in sperm binding to the egg zona pellucida.

4.494 Binding of Pleomorphic Adenoma Gene-like 2 to the Tumor Necrosis Factor (TNF)-α-responsive Region of the NCF2 Promoter Regulates p67phox Expression and NADPH Oxidase Activity
Ammons, M.C., Siemsen, D.W., Nelson-Overton, L.K., Quinn, M.T. and Gauss, K.A.
J. Biol. Chem., ***282(24)***, *17941-17952 (2007)*

NCF2, the gene encoding the NADPH oxidase cytosolic component p67^{phox}, is up-regulated by TNF-α, and we recently mapped a region in the *NCF2* promoter that was required for this TNF-α-dependent response. Because this TNF-α-responsive region (TRR) lacked recognizable transcription factor binding elements, we performed studies to identify factors involved in regulating *NCF2* via the TRR. Using the TRR sequence as bait in a yeast one-hybrid screen, we identified the zinc finger transcription factor Pleomorphic Adenoma Gene-Like 2 (PLAGL2) as a candidate regulator of *NCF2* expression. PLAGL2-specific antibodies were generated that detected the native and SUMO1-modified forms of endogenous PLAGL2. EMSA and DNA-binding protein affinity purification analyses demonstrated specific binding of *in vitro*-translated as well as endogenously expressed PLAGL2 to the TRR, and chromatin immunoprecipitation assays demonstrated enhanced binding of endogenous PLAGL2 to the TRR *in vivo* with TNF-α treatment. Knockdown of PLAGL2 protein inhibited up-regulation of *NCF2* transcript, p67^{phox} protein expression, and subsequent superoxide production in response to TNF-α. Furthermore, relative levels of native and SUMO1-modified endogenous PLAGL2 protein were modulated in a time-dependant manner in response to TNF-α treatment. These data clearly identify PLAGL2 as a novel regulator of *NCF2* gene expression as well as NADPH oxidase activity and contribute to a greater understanding of the transcriptional regulation of *NCF2*.

4.495 Inhibition of human neutrophil degranulation by transforming growth factor-β1
Shen, L. et al
Clin. Exp. Immunol., ***149***, *155-161 (2007)*

Neutrophils enter tissues including the uterus and are found in the endometrium in increased numbers prior to menses. In this environment, they are exposed to transforming growth factor (TGF)-β1 produced by endometrial stromal and epithelial cells. We observed that incubation of neutrophils *in vitro* with TGF-β1 at 1 pg/ml significantly reduced their secretion of lactoferrin in response to lipopolysaccharide (LPS). This effect was achieved with as little as 15 min of pretreatment with TGF-β1. Inhibition of lactoferrin release by TGF-β1 was observed irrespective of whether neutrophils were stimulated by ligands for Toll-like receptor (TLR)-2, TLR-4 or FPR, the G protein-coupled receptor for formylated peptides. Inhibition by TGF-β1 was negated by SB-431542, a small molecule inhibitor that specifically blocks the kinase activity of the type I TGF-β receptor (ALK5) In contrast to lactoferrin release, another important neutrophil function, interleukin (IL)-8 driven chemotaxis, was not affected by TGF-β1 at 1 pg/ml or 100 pg/ml. We conclude that in tissues of the female reproductive tract, TGF-β1 inhibition of neutrophil degranulation may prevent these cells from initiating an inflammatory response or releasing degradative enzymes that could potentially damage the oocyte or fetus.

4.496 Isolation and culture of adult neurons and neurospheres
Brewer, G,J. and Torricelli, J.R.
Nature Protocols, ***2(6)***, *1490-1498 (2007)*

Here we present a protocol for extraction and culture of neurons from adult rat or mouse CNS. The method proscribes an optimized protease digestion of slices, control of osmolarity and pH outside the incubator

with Hibernate and density gradient separation of neurons from debris. This protocol produces yields of millions of cortical, hippocampal neurons or neurosphere progenitors from each brain. The entire process of neuron isolation and culture takes less than 4 h. With suitable growth factors, adult neuron regeneration of axons and dendrites in culture proceeds over 1–3 weeks to allow controlled studies in pharmacology, electrophysiology, development, regeneration and neurotoxicology. Adult neurospheres can be collected in 1 week as a source of neuroprogenitors ethically preferred over embryonic or fetal sources. This protocol emphasizes two differences between neuron differentiation and neurosphere proliferation: adhesion dependence and the differentiating power of retinyl acetate.

4.497 **In vitro methods to prepare astrocyte and motoneuron cultures for the investigation of potential in vivo interactions**
Taylor, A.R., Robinson, M.B. and Milligan, C.E.
*Nature protocols, **2(6)**, 1499-1507 (2007)*

This protocol details methods to isolate and purify astrocytes and motoneurons (MNs) from the chick lumbar spinal cord. In addition, an approach to study the influences of astrocyte secreted factors on MNs is provided. Astrocytes are isolated between embryonic days 10 and 12 (E10–12), propagated in serum (2–3 h) and differentiated in chemically defined medium (3–4 h). When prepared according to this protocol, astrocyte cultures are more than 98% pure when assessed using the astrocyte-specific markers glial fibrillary acidic protein (GFAP) and S100β. MNs are isolated between E5.5 and 6.0 (3–4 h) using a procedure that takes selective advantage of the large size of these cells. These cultures can be maintained using individual trophic factors, target-derived factors or astrocyte-derived factors, the preparation of which is also described (5–6 h). All or part of these techniques can be used to investigate a variety of processes that occur during nervous system development and disease or after injury.

4.498 **Tocotrienols Induce Apoptosis and Autophagy in Rat Pancreatic Stellate Cells Through the Mitochondrial Death Pathway**
Rickmann, M., Vaquero, E.C., Ramon, J., Malagelada, J.R. and Molero, X.
*Gastroenterology, **132**, 2518-2532 (2007)*

Background & Aims: Selective removal of activated pancreatic stellate cells (PSCs) through induction of their own programmed death is a goal of therapeutic interest in patients with chronic pancreatitis. Here, we investigated the effects of tocotrienols on PSC death outcomes. Methods: Activated and quiescent PSCs and acinar cells from rat pancreas were treated with vitamin E derivatives α-tocopherol; individual α-, β-, γ-, and δ-tocotrienols; and a tocotrienol rich fraction (TRF) from palm oil. Results: TRF, but not α-tocopherol, reduced viability of activated PSC by setting up a full death program, independent of cell cycle regulation. Activated PSCs died both through apoptosis, as indicated by increased DNA fragmentation and caspase activation, and through autophagy, as denoted by the formation of autophagic vacuoles and LC3-II accumulation. In contrast to α-tocopherol, TRF caused an intense and sustained mitochondrial membrane depolarization and extensive cytochrome c release. Caspase inhibition with zVAD-fmk suppressed TRF-induced apoptosis but enhanced autophagy. However, mitochondrial permeability transition pore blockade with cyclosporin A completely abolished the deadly effects of TRF. β-, γ-, and δ-tocotrienol, but not α-tocotrienol nor α-tocopherol, reproduced TRF actions on activated PSCs. TRF death induction was restricted to activated PSCs because it did not cause apoptosis either in quiescent PSCs or in acinar cells. <u>*Conclusions:*</u> Tocotrienols selectively trigger activated pancreatic stellate cell death by targeting the mitochondrial permeability transition pore. Our findings unveil a novel potential for tocotrienols to ameliorate the fibrogenesis associated with chronic pancreatitis.

4.499 **A Key Role for Itk in Both IFN-γ and IL-4 Production by NKT Cells**
Byron, B., Au-Yeung, and Fowell, D.J.
*J. Immunol., **179**, 111-119 (2007)*

NKT cells rapidly secrete cytokines upon TCR stimulation and thus may modulate the acquired immune response. Recent studies suggest that signaling for development and effector function in NKT cells may differ from conventional T cells. The tyrosine kinase Itk is activated downstream of the TCR, and its absence in $CD4^+$ T cells results in impaired Th2, but not Th1 responses. In this study, we investigated NKT cell function in the absence of Itk as impaired type 2 responses in vivo could be manifest through IL-4 defects in a number of cell types. We show that Itk-deficient NKT cells up-regulate IL-4 mRNA in the thymus and express constitutive IL-4 and IFN-γ transcripts in peripheral organs. Thus, Itk is not required for the developmental activation of cytokine loci in NKT cells. Nevertheless, Itk-deficient NKT cells are

severely impaired in IL-4 protein production. Strikingly, unlike conventional $CD4^+$ T cells, Itk-deficient NKT cells also have profound defects in IFN-γ production. Furthermore, both IL-4 and IFN-γ production were markedly impaired following in vivo challenge with α-galactosyl ceramide. Function can be restored in Itk-deficient NKT cells by provision of calcium signals using ionomycin. These results suggest that NKT cells are highly dependent on Itk for IL-4- and IFN-γ-mediated effector function. Thus, the pattern of cytokine genes that are affected by Itk deficiency appears to be cell lineage-specific, likely reflecting differences in activation threshold between immune effectors. The severe defect in NKT cell function may underlie a number of the Th1 and Th2 immune defects in Itk-deficient mice.

4.500 Early Intrahepatic Accumulation of CD8+ T Cells Provides a Source of Effectors for Nonhepatic Immune Responses
Poløakos, N.K. et al
*J. Immunol., **179**, 201-210 (2007)*

Interactions between the liver and $CD8^+$ T cells can lead to tolerance, due in part to $CD8^+$ T cell death. To test whether this was the case in an extrahepatic infection, we investigated the fate and effector capacity of intrahepatic $CD8^+$ T cells during lung-restricted influenza infection in mice. Virus-specific T cells accumulated in livers without detectable intrahepatic presentation of viral Ags, and this accumulation was not restricted to the contraction phase, but was apparent as early as day 5. Intrahepatic influenza-specific cells were functionally similar to those recovered from the bronchioalveolar lavage, based on ex vivo cytokine production and specific target lysis. Both adoptive transfer of liver lymphocytes and orthotopic liver transplant of organs containing accumulated effector T cells revealed that activated CD8s from the liver were viable, expanded during reinfection, and generated a memory population that trafficked to lymphoid organs. Thus, intrahepatic $CD8^+$ T cells re-enter circulation and generate functional memory, indicating that the liver does not uniformly incapacitate activated $CD8^+$ T cells. Instead, it constitutes a substantial reservoir of usable Ag-specific effector $CD8^+$ T cells involved in both acute and recall immune responses.

4.501 Resistance to Experimental Autoimmune Encephalomyelitis and Impaired T Cell Priming by Dendritic Cells in Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase Substrate-1 Mutant Mice
Tomizawa, T. et al
*J. Immunol., **179**, 869-877 (2007)*

Src homology 2 domain-containing protein tyrosine phosphatase (SHP) substrate-1 (SHPS-1) is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 through its cytoplasmic region and is expressed on the surface of $CD11c^+$ dendritic cells (DCs) and macrophages. In this study, we show that mice that express a mutant form of SHPS-1 lacking most of the cytoplasmic region are resistant to experimental autoimmune encephalomyelitis (EAE) in response to immunization with a peptide derived from myelin oligodendrocyte glycoprotein (MOG (35–55)). The MOG (35–55)-induced proliferation of, and production of IFN-γ, IL-2, and IL-17, by T cells from immunized SHPS-1 mutant mice were reduced compared with those apparent for wild-type cells. The abilities of splenic DCs from mutant mice to stimulate an allogenic MLR and to prime Ag-specific T cells were reduced. Both IL-12-stimulated and TLR-dependent cytokine production by DCs of mutant mice were also impaired. Finally, SHPS-1 mutant mice were resistant to induction of EAE by adoptive transfer of MOG (35–55)-specific T cells. These results show that SHPS-1 on DCs is essential for priming of naive T cells and the development of EAE. SHPS-1 is thus a potential therapeutic target in inflammatory disorders of the CNS and other autoimmune diseases.

4.502 Expression of Tyk2 in dendritic cells is required for IL-12, IL-23, and IFN-γ production and the induction of Th1 cell differentiation
Tokumasa, N. et al
*Blood, **110(2)**, 553-560 (2007)*

It is well documented that dendritic cells (DCs), representative antigen-presenting cells, are important sources of Th1-promoting cytokines and are actively involved in the regulation of T-helper–cell differentiation. However, the intracellular event that regulates this process is still largely unknown. In this study, we examined the role of Tyk2, a JAK kinase that is involved in the signaling pathway under IL-12 and IL-23, in DC functions. While the differentiation and maturation of DCs was normal in Tyk2-deficient ($Tyk2^{-/-}$) mice, IL-12–induced Stat4 phosphorylation was diminished in $Tyk2^{-/-}$ DCs. IL-12–induced IFN-

γ production was also significantly diminished in Tyk2$^{-/-}$ DCs to levels similar to those in Stat4$^{-/-}$ DCs. Interestingly, Tyk2$^{-/-}$ DCs were defective in IL-12 and IL-23 production upon stimulation with CpG ODN. Furthermore, Tyk2$^{-/-}$ DCs were impaired in their ability to induce Th1-cell differentiation but not Th2-cell differentiation. Taken together, these results indicate that the expression of Tyk2 in DCs is crucial for the production of Th1-promoting cytokines such as IL-12 and IFN-γ from DCs and thereby for the induction of antigen-specific Th1-cell differentiation.

4.503 Characterization of Heme as Activator of Toll-like Receptor 4
Figueiredo, R.T. et al
J. Biol. Chem., ***282(28)***, *20221-20229 (2007)*

Heme is an ancient and ubiquitous molecule present in organisms of all kingdoms, composed of an atom of iron linked to four ligand groups of porphyrin. A high amount of free heme, a potential amplifier of the inflammatory response, is a characteristic feature of diseases with increased hemolysis or extensive cell damage. Here we demonstrate that heme, but not its analogs/precursors, induced tumor necrosis factor-α (TNF-α) secretion by macrophages dependently on MyD88, TLR4, and CD14. The activation of TLR4 by heme is exquisitely strict, requiring its coordinated iron and the vinyl groups of the porphyrin ring. Signaling of heme through TLR4 depended on an interaction distinct from the one established between TLR4 and lipopolysaccharide (LPS) since anti-TLR4/MD2 antibody or a lipid A antagonist inhibited LPS-induced TNF-α secretion but not heme activity. Conversely, protoporphyrin IX antagonized heme without affecting LPS-induced activation. Moreover, heme induced TNF-α and keratinocyte chemokine but was ineffective to induce interleukin-6, interleukin-12, and interferon-inducible protein-10 secretion or co-stimulatory molecule expression. These findings support the concept that the broad ligand specificity of TLR4 and the different activation profiles might in part reside in its ability to recognize different ligands in different binding sites. Finally, heme induced oxidative burst, neutrophil recruitment, and heme oxygenase-1 expression independently of TLR4. Thus, our results presented here reveal a previous unrecognized role of heme as an extracellular signaling molecule that affects the innate immune response through a receptor-mediated mechanism.

4.504 c-Jun N-terminal kinase signaling regulates events associated with both health and degeneration in motoneurons
Newbern, J., Taylor, A., Robinson, M., Livel, M.O. and Milligan, C.E.
Neuroscience, ***147(3)***, *680-692 (2007)*

The c-Jun N-terminal kinases (JNKs) are activated by various stimuli and are critical for neuronal development as well as for death following a stressful stimulus. Here, we have evaluated JNK activity in both healthy and dying motoneurons from developing chick embryos and found no apparent difference in overall JNK activity between the conditions, suggesting that this pathway maybe critical in both circumstances. Pharmacological inhibition of JNK in healthy motoneurons supplied with trophic support resulted in decreased mitochondrial membrane potential, neurite outgrowth, and phosphorylation of microtubule-associated protein 1B. On the other hand, in motoneurons deprived of trophic support, inhibition of JNK attenuated caspase activation, and nuclear condensation. We also examined the role of JNK's downstream substrate c-Jun in mediating these events. While c-Jun expression and phosphorylation were greater in cells supplied with trophic support as compared with those deprived, inhibition of c-Jun had no effect on nuclear condensation in dying cells or neurite outgrowth in healthy cells, suggesting that JNK's role in these events is independent of c-Jun. Together, our data underscore the dualistic nature of JNK signaling that is critical for both survival and degenerative changes in motoneurons.

4.505 Effects of Menstrual Cycle Status and Gender on Human Neutrophil Phenotype
Smith, J.M., Shen, Z., Wira, C.R., Fanger, M.W. and Shen, L.
Am. J. Reprod. Immunol., ***58***, *111-119 (2007)*

The effects of gender and fluctuating ovarian hormones on neutrophil phenotype have yet to be characterized.
Method of study
Neutrophils from females at days 7, 14, 21, and 28 of the menstrual cycle were analyzed by flow cytometry for surface receptor, granule protein, and intracellular cytokine expression. Comparisons were made to neutrophils from males isolated at 7-day intervals during 1 month.
Results

Decreased MMP-9 and TNF-α expression by neutrophils from females was observed during the periovulatory period. Comparing the genders, cells from females during the periovulatory period expressed less CD11b and CD18 than those from males. CXCR1 surface levels were higher on neutrophils from female donors.
Conclusions
Neutrophil phenotype varies minimally during the menstrual cycle and between the genders. Our data provide support for a potential anti-inflammatory effect of ovarian hormones on neutrophils.

4.506 Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response in vitro
Vucevic, D. et al
*Int. Immunopharmacol., **7**, 1211-1220 (2007)*

Royal jelly (RJ), especially its protein components, has been shown to possess immunomodulatory activity. However, almost nothing is known about the influence of RJ fatty acids on the immune system. In this work we studied the effect of 10-hydroxy-2-decanoic acid (10-HDA) and 3,10-dihydroxy-decanoic acid (3,10-DDA), isolated from RJ, on the immune response using a model of rat dendritic cell (DC)–T-cell cocultures. Both fatty acids, at higher concentrations, inhibited the proliferation of allogeneic T cells. The effect of 10-HDA was stronger and was followed by a decrease in interleukin-2 (IL-2) production and down-regulation of IL-2 receptor expression. Spleen DC, cultivated with 10 μg/ml of fatty acids down-regulated the expression of CD86 and the production of IL-12, but up-regulated the production of IL-10. In contrast, DC, pretreated with 100 μg/ml of 3,10-DDA, up-regulated the expression of CD86 and augmented the proliferation of allogeneic T cells. The highest dose (200 μg/ml) of both fatty acids which was non-apoptotic for both T cells and DC, down-regulated the expression of MHC class II and CD86, decreased the production of IL-12 and made these DC less allostimulatory. The immunosuppressive activity of 3,10-DDA was also confirmed in vivo, using a model of Keyhole lymphet hemocyanine immunization of rats. In conclusion, our results showed the immunomodulatory activity of RJ fatty acids and suggest that DC are a significant target of their action.

4.507 Evaluation of nonleukoreduced red blood cell transfusion units collected at delivery from the placenta
Widing, L., Bechensteen, A.G., Mirlashari, M.R., Vetlesen, A. and Kjeldsen-Kragh, J.
*Transfusion, **47**, 1481-1487 (2007)*

BACKGROUND: The objective of this study was to evaluate the suitability of cord blood (CB) as a source of red blood cells (RBCs) for autologous transfusion.
STUDY DESIGN AND METHODS: CB was collected in 150-mL storage containers with citrate phosphate dextrose (CPD) as anticoagulant and stored in either saline, adenine, glucose, and mannitol (SAG-M; n = 18) or phosphate, adenine, glucose, guanosine, saline, and mannitol (PAGGS-M; n = 18) for 35 days at 4°C. Hematologic status and hemolysis were studied. The lipopolysaccharide (LPS)-induced production of tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 from CB monocytes was analyzed after incubation with addition of weekly sampled supernatants from the CB RBC units. Five additional units (PAGGS-M) were leukoreduced and thereafter analyzed as indicated above.
RESULTS: Hemolysis increased significantly over time, in SAG-M more than in PAGGS-M. During storage in both media, the number of white blood cells (WBCs) decreased, and the LPS-induced production of TNF-α and TGF-β1 decreased and increased, respectively. There were no significant changes in the LPS-induced production of TNF-α and TGF-β1 in the leukoreduced CB RBC units.
CONCLUSION: Hemolysis in CB RBC units increased significantly over time, and PAGGS-M appears to be superior to SAG-M as a preservation solution for CB RBC. The changes in LPS-induced TNF-α and TGF-β1 production over time were probably caused by substances released from apoptotic and/or necrotic WBCs. Further studies are needed to identify both which substances are responsible for the changes in LPS-induced cytokine release and the clinical significance hereof.

4.508 RT-PCR and immunocytochemistry studies support the presence of somatostatin, cortistatin and somatostatin receptor subtypes in rat Kupffer cells
Xidakis, C. et al
*RegulatoryPeptides, **143(1-3)**, 76-82 (2007)*

The present study investigated the presence of somatostatin receptor subtypes (ssts) and the endogenous peptides somatostatin and cortistatin in rat Kupffer cells, since modulation of these cells by somatostatin may be important for the beneficial effect of somatostatin analogues in a selected group of hepatocellular

carcinoma patients. Kupffer cells were isolated from rat liver in agreement with national and EU guidelines. RT-PCR was employed to assess the expression of somatostatin, cortistatin and ssts in Kupffer cells. Western blot analysis and immunocytochemistry were employed to assess the expression and the localization of the receptors, respectively. Quiescent Kupffer cells were found to express sst_{1-4} mRNA, while immunocytochemical studies supported the presence of only the sst_3 and sst_4 receptors, which were found to be internalized. However, sst_1 and sst_{2A} receptors were detected by western blotting. RT-PCR and RIA measurements support the presence of both somatostatin and cortistatin. Stimulation of the cells with LPS activated the expression of the sst_2, sst_3 and sst_4 receptors. The present data provide evidence to support the presence of ssts and the endogenous neuropeptides somatostatin and CST in rat Kupffer cells. Both peptides may act in an autocrine manner to regulate sst receptor distribution. Studies are in progress in order to further characterize the role of ssts in Kupffer cells and in hepatic therapeutics.

4.509 Human hepatic stem cells from fetal and postnatal donors
Schmelzer, E. et al
J. Exp. Med., ***204(8)***, *1973-1987 (2007)*

Human hepatic stem cells (hHpSCs), which are pluripotent precursors of hepatoblasts and thence of hepatocytic and biliary epithelia, are located in ductal plates in fetal livers and in Canals of Hering in adult livers. They can be isolated by immunoselection for epithelial cell adhesion molecule-positive (EpCAM+) cells, and they constitute ~0.5-2.5% of liver parenchyma of all donor ages. The self-renewal capacity of hHpSCs is indicated by phenotypic stability after expansion for >150 population doublings in a serum-free, defined medium and with a doubling time of ~36 h. Survival and proliferation of hHpSCs require paracrine signaling by hepatic stellate cells and/or angioblasts that coisolate with them. The hHpSCs are ~9 μm in diameter, express cytokeratins 8, 18, and 19, CD133/1, telomerase, CD44H, claudin 3, and albumin (weakly). They are negative for α-fetoprotein (AFP), intercellular adhesion molecule (ICAM) 1, and for markers of adult liver cells (cytochrome P450s), hemopoietic cells (CD45), and mesenchymal cells (vascular endothelial growth factor receptor and desmin). If transferred to STO feeders, hHpSCs give rise to hepatoblasts, which are recognizable by cordlike colony morphology and up-regulation of AFP, P4503A7, and ICAM1. Transplantation of freshly isolated EpCAM+ cells or of hHpSCs expanded in culture into NOD/SCID mice results in mature liver tissue expressing human-specific proteins. The hHpSCs are candidates for liver cell therapies.

4.510 Cytoskeletal Rearrangements via Vasodilator-Stimulated Phosphoprotein (VASP) Phosphorylation and Redistribution in Human Retinal Endothelial Cells (HREC), Endothelial Precursor Cells(EPC) and Platelets: Implications for Retinal Vessel Repair
Calzi, S.L. et al
Invest. Ophthalmol. Vis. Sci., ***48***, *E-abstract 4096 (2007)*

Purpose: The actin motor protein VASP powers cell migration, vasodilatation, and inhibition of platelet aggregation- essential processes for maintaining retinal vascular health. VASP has three phosphorylation sites (Ser-157, Ser-239, and Thr-278) that are regulated by cGMP and cAMP protein kinases. Therefore, we examined the effect of the vasoactive angiogenic agents CO, NO and stromal derived factor-1 (SDF-1) on cell migration, VASP phosphorylation, and VASP localization.
Methods:CD34+ EPCs were isolated from human peripheral blood using magnetic microbeads and platelets using **Opti-Prep**™. HREC were prepared as previously described (Grant MB 1991). After 1, 5 and 15 min exposure to the NO donor DETA/NO, the CO donor $Ru(II)Cl_2(CO)_3$ dimer or SDF-1, VASP phosphorylation in platelets and endothelial cells was evaluated by Western analysis. FACS analysis was used to evaluate EPCs. Phosphospecific anti-pSer-157 antibody or anti-pSer-239 antibody was used for analysis. Immunohistochemistry was performed to evaluate VASP redistribution in HREC and EPCs using anti-VASP antibody following exposure to CO, NO or SDF-1. Migration to CO, NO and SDF-1 was performed using the modified Boyden chamber assay in HREC and EPC.
Results: Upon CO stimulation in human platelets, VASP was phosphorylated on Ser-157, but not Ser-239, while NO exposure resulted in phosphorylation mainly of Ser-239. In endothelial cells, VASP is phosphorylated on Ser-239 in response to both CO and NO exposure. In EPC and HREC, VASP was redistributed to filopodia after incubation with either CO, NO or SDF-1. At the concentrations tested, all three agents induced EPC and endothelial cell migration in a dose-dependent manner.
Conclusion: In the retinal vasculature, NO, CO and SDF-1 may compensate for one another or work together to promote cytoskeletal changes through site-specific phosphorylation of VASP. Vasoactive agents may promote retinal vascular repair and improved tissue perfusion by increasing EPC and endothelial cell migration and by preventing platelet aggregation.

4.511 Guinea Pig Neutrophils Infected with Mycobacterium tuberculosis Produce Cytokines Which Activate Alveolar Macrophages in Noncontact Cultures
Sawant, K.T. and McMurray, D.N.
Infect. Immun., ***75(4)***, *1870-1877 (2007)*

The early influx of neutrophils to the site of infection may be an important step in host resistance against *Mycobacterium tuberculosis*. In this study, we investigated the effect of *M. tuberculosis* infection on the ability of guinea pig neutrophils to produce interleukin-8 (IL-8; CXCL8) and tumor necrosis factor alpha (TNF-α) and to activate alveolar macrophages. Neutrophils and alveolar macrophages were isolated from naïve guinea pigs, cultured together or alone, and infected with virulent *M. tuberculosis* for 3, 12, and 24 h. IL-8 protein production in cocultures, as measured by using an enzyme-linked immunosorbent assay, was found to be additive at 24 h and significantly greater in *M. tuberculosis*-infected cocultures than in uninfected cocultures and in cultures of the infected neutrophils or macrophages alone. The IL-8 mRNA levels, determined by real-time reverse transcription-PCR, were elevated at 24 h in infected cocultures and infected cells cultured alone. In order to elucidate the contributions of neutrophils and their soluble mediators to the activation of alveolar macrophages, neutrophils and alveolar macrophages were cultured in a contact-independent manner by using a Transwell insert system. Neutrophils were infected with virulent *M. tuberculosis* in the upper wells, and alveolar macrophages were cultured in the lower wells. The release of hydrogen peroxide from alveolar macrophages exposed to soluble products from infected neutrophils was significantly increased compared to that from unexposed alveolar macrophages. Significant up-regulation of IL-1ß and TNF-α mRNA levels in alveolar macrophages was observed at 24 and 30 h, respectively, compared to those in cells not exposed to soluble neutrophil products. Treatment with anti-guinea pig TNF-α polyclonal antibody completely abolished the response of alveolar macrophages to neutrophil products. This finding suggests that TNF-α produced by infected neutrophils may be involved in the activation of alveolar macrophages and hence may contribute to the containment of *M. tuberculosis* infection during the early period of infection.

4.512 The Blood Contains Multiple Distinct Progenitor Populations with Clonogenic B and T Lineage Potential
Umland, O., Mwangi, W.N., Anderson, B.M., Walker, J.C. and Petrie, H.T.
J. Immunol., ***178***, *4147-4152 (2007)*

The thymus is seeded by bone marrow-derived progenitors that circulate in the blood. Multiple cell types can be found in the thymus early after i.v. administration or in steady state, but most fail to satisfy the known characteristics of true T progenitors. Cells that do conform to classical definitions retain multilineage potential, but surprisingly, cannot make B cells. Because acquisition of the T lineage fate among noncommitted progenitors is a lengthy process, the absence of B cell potential in early thymocytes suggests that B and T lineages diverge prethymically. To test this suggestion, we screened numerous presumptive progenitor populations for T cell growth and differentiation potential, as well as for clonogenic T or B cell development. We find that blood and marrow each contain multiple distinct subsets that display growth and differentiation potential consistent with being canonical T progenitors. Assessment of clonogenic potential further shows that although all blood and marrow populations have high T cell cloning potential, no T/non-B cells are apparent. These data suggest that either true thymic reconstitution potential derives from a small T/non-B cell subset of one of these populations, or that most of the cells defined as canonical progenitors within the thymus do not, in fact, reside in the mainstream of T progenitor differentiation.

4.513 Effect of fetuin, a TGFβ antagonist and pentoxifylline, a cytokine antagonist on hepatic stellate cell function and fibrotic parameters in fibrosis
Verma-Gandhu, M., Peterson, M.R. and Peterson, T.C.
Eur. J. Pharmacol., ***572***, *220-227 (2007)*

We have previously shown that monocyte conditioned medium (MCM) from patients with liver fibrosis stimulated proliferation of hepatic stellate cells (HSCs), the major cell involved in hepatic fibrosis. To investigate the potential role of fetuin and pentoxifylline in fibrosis we used MCM samples obtained from patients with biopsy proven hepatic fibrosis related to Hepatitis C (HCV). Our results indicate that the MCM obtained from patients with HCV-related liver fibrosis significantly stimulated collagen synthesis in HSCs as assessed by tritiated proline incorporation into a collagenase sensitive trichloroacetic acid (TCA)

precipitate. Collagen synthesis was also stimulated in HSCs using transforming growth factor beta (TGFβ) and this effect was neutralized using TGFβ antibody. Incubation of HSCs with fetuin (but not TGFβ antibody) significantly inhibited collagen synthesis in HSCs that were stimulated by HCV MCM samples. Patient MCM samples would also stimulate proliferation of HSCs as assessed by tritiated thymidine uptake but this effect was not attenuated by fetuin. Likewise the significant stimulatory effect of platelet derived growth factor (PDGF) on HSC proliferation and collagen synthesis was not inhibited by fetuin but could be significantly reduced by 70% and 40% respectively, when treated with pentoxifylline. We also investigated the ability of samples obtained from patients with hepatic fibrosis to inhibit HSC apoptosis, as determined by okadaic acid-induced 4-hydroxynonenal immunocytochemistry in HSCs. We have previously reported that okadaic acid induces apoptosis in HSCs as assessed by Hoescht and TUNEL. Okadaic acid treatment produced a positive 4-hydroxynonenal (4-HNE) immunoreactivity in HSCs and treatment with HCV patient MCM or TGFβ decreased the 4-HNE positive immunoreactivity in HSCs treated with okadaic acid. Our results suggest that fetuin may be beneficial in hepatic fibrosis and suggest that combination of fetuin and pentoxifylline may target the two key events in hepatic fibrosis by modifying the effects of TGFβ and PDGF, the two major growth factors in fibrosis.

4.514 Pancreatic Islet Cell Transplantation: Update and New Developments
Onaca, N. et al
Nutr. Clin. Pract., ***22***, *485-493 (2007)*

Pancreatic islet cell transplantation is a treatment alternative for patients with type 1 diabetes who experience hypoglycemic unawareness despite maximal care. The good results obtained by the group from Edmonton and other centers, with 80% insulin independence at 1 year posttransplant, are not sustainable over time, with 5-year insulin independence achieved in only 10% of patients. However, persistent graft function, even without insulin independence, results in improved glucose control and avoidance of hypoglycemic events. Changes in organ preservation, islet processing technique, and immunosuppression regimens can result in improvement of results in the future. Islet autotransplantation is an option for patients who undergo total pancreatectomy for chronic pancreatitis with debilitating pain, in which reinfusion of the islets from the resected pancreas can result in avoidance of postsurgical diabetes or enhanced glucose control.

4.515 Plasmodium falciparum glycosylphosphatidylinositol induces limited apoptosis in liver and spleen mouse tissue
Wichmann, D. et al
Apoptosis, ***12***, *1037-1041 (2007)*

Plasmodium falciparum malaria affects about 500 million people worldwide and is responsible for approximately 2.5 million deaths per year. Glycosylphosphatidylinositol (GPI) is the major anchor for membrane-associated proteins of *P. falciparum* and GPI plays a major role as a toxin in the pathology of malaria. Therefore, we tested the hypothesis that GPI, like LPS, induces apoptosis *in vitro* and in vital organs of mice. Our data does not provide evidence for direct cardiomyocyte apoptosis induced by GPI *in vitro*. However, *in vivo* injection of GPI induced limited apoptosis in mouse liver and spleen tissue. Apoptosis may be due to a direct GPI apoptotic effect or to an indirect effect via the induction of TNFα and nitric oxide production.

4.516 Retinoic acid signaling sensitizes hepatic stellate cells to NK cell killing via upregulation of NK cell activating ligand RAE1
Radaeva, S. et al
Am. J. Physiol. Gastrointest. Liver Physiol., ***293***, *G809-G816 (2007)*

Hepatic stellate cells (HSCs) store 75% of the body's supply of vitamin A (retinol) and play a key role in liver fibrogenesis. During liver injury, HSCs become activated and susceptible to natural killer (NK) cell killing due to increased expression of the NK cell activating ligand retinoic acid early inducible gene 1 (RAE-1). To study the mechanism by which RAE-1 is upregulated in HSCs during activation, an in vitro model of cultured mouse HSCs was employed. RAE-1 was detected at low levels in quiescent HSCs but upregulated in 4- and 7-day cultured HSCs (early activated HSCs), whereas 21-day cultured HSCs (fully activated HSCs) lost RAE-1 expression. High levels of RAE-1 in 4- and 7-day cultured HSCs correlated with their susceptibility to NK cell killing, which was diminished by treatment with RAE-1 neutralizing antibody. Furthermore, retinoic acid (RA) and retinal dehydrogenase (Raldh) levels were upregulated in early activated HSCs compared with quiescent or fully activated HSCs. Blocking RA synthesis by the

Raldh inhibitor or blocking RA signaling by the retinoic acid receptor antagonist abolished upregulation of RAE-1 whereas treatment with RA induced RAE-1 expression in HSCs. In conclusion, during activation, HSCs lose retinol, which is either secreted out or oxidized into RA; the latter stimulates RAE-1 expression and sensitizes early activated HSCs to NK cell killing. In contrast, fully activated HSCs become resistant to NK cell killing because of lack of RAE1 expression, leading to chronic liver fibrosis and disease.

4.517 Methods in Cell Separations
Dainiak, M.B., Kumar, A., Galaev, I.Y. and Matthiasson, B.
Adv. Biochem. Engin/Biotechnol., Springer Berlin/Heidelberg (2007)

Research in the field of cell biology and biomedicine relies on technologies that fractionate cell populations and isolate rare cell types to high purity. A brief overview of methods and commercially available products currently used in cell separations is presented. Cell fractionation by size and density and highly selective affinity-based technologies such as affinity chromatography, fluorescence-activated cell sorting (FACS) and magnetic cell sorting are discussed in terms of throughput, yield, and purity.

4.518 The Long Form of Fas Apoptotic Inhibitory Molecule Is Expressed Specifically in Neurons and Protects Them against Death Receptor-Triggered Apoptosis
Segura, M.F. et al
*J. Neurosci., **27(42)**, 11228-11241 (2007)*

Death receptors (DRs) and their ligands are expressed in developing nervous system. However, neurons are generally resistant to death induction through DRs and rather their activation promotes neuronal outgrowth and branching. These results suppose the existence of DRs antagonists expressed in the nervous system. Fas apoptosis inhibitory molecule ($FAIM_S$) was first identified as a Fas antagonist in B-cells. Soon after, a longer alternative spliced isoform with unknown function was identified and named $FAIM_L$. $FAIM_S$ is widely expressed, including the nervous system, and we have shown previously that it promotes neuronal differentiation but it is not an anti-apoptotic molecule in this system. Here, we demonstrate that $FAIM_L$ is expressed specifically in neurons, and its expression is regulated during the development. Expression could be induced by NGF through the extracellular regulated kinase pathway in PC12 (pheochromocytoma cell line) cells. Contrary to $FAIM_S$, $FAIM_L$ does not increase the neurite outgrowth induced by neurotrophins and does not interfere with nuclear factor κB pathway activation as $FAIM_S$ does. Cells overexpressing $FAIM_L$ are resistant to apoptotic cell death induced by DRs such as Fas or tumor necrosis factor R1. Reduction of endogenous expression by small interfering RNA shows that endogenous $FAIM_L$ protects primary neurons from DR-induced cell death. The detailed analysis of this antagonism shows that $FAIM_L$ can bind to Fas receptor and prevent the activation of the initiator caspase-8 induced by Fas. In conclusion, our results indicate that $FAIM_L$ could be responsible for maintaining initiator caspases inactive after receptor engagement protecting neurons from the cytotoxic action of death ligands.

4.519 The Role of Dendritic Cells in the Development of Acute Dextran Sulfate Sodium Colitis
Berndt, B.E., Zhang, M., Chen, G-H., Huffnagle, G.B. and Kao, J.Y.
*J. Immunol., **179**, 6255-6262 (2007)*

Dendritic cells (DCs) are essential mediators of the host immune response to surrounding microbes. In this study, we investigate the role of DCs in the pathogenesis of a widely used colitis model, dextran sulfate sodium-induced colitis. The effect of dextran sulfate sodium on the production of proinflammatory cytokines and chemokines by bone marrow-derived DCs (BM-DCs) was analyzed. BM-DCs were adoptively transferred into C57BL/6 mice or DCs were ablated using transgenic CD11c-DTR/GFP mice before treatment with 5% dextran sulfate sodium in drinking water. We found that dextran sulfate sodium induced production of proinflammatory cytokines (IL-12 and TNF-α) and chemokines (KC, MIP-1α, MIP-2, and MCP-1) by DCs. Adoptive transfer of BM-DCs exacerbated dextran sulfate sodium colitis while ablation of DCs attenuated the colitis. We conclude that DCs are critical in the development of acute dextran sulfate sodium colitis and may serve a key role in immune balance of the gut mucosa.

4.520 Estradiol Attenuates Lipopolysaccharide-Induced CXC Chemokine Ligand 8 Production by Human Peripheral Blood Monocytes
Pioli, P.A. et al
*J. Immunol., **179**, 6284-6290 (2007)*

Regulation of the inflammatory response is imperative to the maintenance of immune homeostasis.

Activated monocytes elaborate a broad variety of proinflammatory cytokines that mediate inflammation, including CXCL8. Release of this chemokine attracts neutrophils to sites of bacterial invasion and inflammation; however, high levels of CXCL8 may result in excessive neutrophil infiltration and subsequent tissue damage. In this study, we demonstrate that 17β-estradiol (E2) attenuates LPS-induced expression of CXCL8 in human peripheral blood monocytes. Treatment of monocytes with estradiol before administration of LPS reduces CXCL8 message and protein production through an estrogen receptor-dependent mechanism, and luciferase reporter assays demonstrate that this inhibition is mediated transcriptionally. Importantly, the ability of estradiol-pretreated LPS-activated monocytes to mobilize neutrophils is impaired. These results implicate a role for estradiol in the modulation of the immune response, and may lead to an enhanced understanding of gender-based differences in inflammatory control mechanisms.

4.521 Thymosin β4 Upregulates the Expression of Hepatocyte Growth Factor and Downregulates the Expression of PDGF-β Receptor in Human Hepatic Stellate Cells
Barnaeva, E., Nadezhda, A., Hannappell, E., Sjogren, M.H: and Rojkind, M.
*Ann. N.Y. Acad. Sci., **1112**, 154-160 (2007)*

Hepatic stellate cells (HSCs) are the main producers of type I collagen in the liver, and therefore are responsible, in part, for the fibrous scar observed in cirrhotic livers. Although there is no approved treatment for this deadly disease, drugs inducing HSC apoptosis in animals (gliotoxin) and hepatocyte regeneration in man (hepatocyte growth factor [HGF]), have been used successfully in ameliorating liver fibrosis. In this communication we investigated whether thymosin β_4 (Tβ_4), an actin-sequestering peptide that prevents scarring of the heart after a myocardial infarction and that prevents kidney fibrosis in animals, has the potential to be used to treat liver fibrosis. To this end we studied whether the administration of Tβ_4 to HSCs could alter the expression of genes encoding for extracellular matrix components, as well as those required for differentiation of HSCs. Our preliminary findings show that Tβ_4 had no effect on the expression of α2 (I) collagen, tissue inhibitor of metalloproteinases-1, and matrix metalloproteinase-2 mRNAs. However, it upregulated the expression of HGF and downregulated the expression of platelet-derived growth factor-β receptor mRNAs in these cells. Overall, these findings suggest that Tβ_4 has antifibrogenic potential.

4.522 Conventional dendritic cells regulate the outcome of colonic inflammation independently of T cells
Abe, K. et al
*PNAS, **104(43)**, 17022-17027 (2007)*

We explored the physiological role of conventional dendritic cells (cDCs) in acute colitis induced by a single cycle of dextran sodium sulfate administration. Depending on their mode of activation and independently of T cells, cDCs can enhance or attenuate the severity of dextran sodium sulfate-induced colitis. The latter beneficial effect was achieved, in part, by IFN-1 induced by Toll-like receptor 9-activated cDCs. IFN-1 inhibits colonic inflammation by regulating neutrophil and monocyte trafficking to the inflamed colon and restraining the inflammatory products of tissue macrophages. These data highlight a novel role of cDCs in the regulation of other innate immune cells and position them as major players in acute colonic inflammation.

4.523 A closed system for islet isolation and purification using the COBE2991 cell processor may reduce the need of clean room facilities
Klaffschenkel, R.A. et al
*Cell Transplant., **16(6)**, 587-594 (2007)*

During the isolation of human islets of Langerhans the digest has repeated direct contact with the ambient atmosphere. In order to fulfill GMP requirements in clinical applications, the entire cell preparation must be performed in clean room facilities. We hypothesized that the use of a closed system, which avoids the direct exposure of tissue to the atmosphere, would significantly ease the preparation procedure. To avoid the direct atmosphere exposure we tested a modification of the isolation and purification process by performing all islet preparation steps in a closed system. In this study we compared the isolation outcome of the traditional open preparation technique with the new closed system. Pancreata from 6-month-old hybrid pigs were procured in the local slaughterhouse. After digestion/filtration the digest was cooled, collected, and concentrated in centrifugation containers and purified thereafter in the COBE2991 by top

loading (control). In the control group 502±253 IEQ per gram pancreas were purified. The total preparation time amounted to 12 h. In the closed system the digest were cooled and directly pumped into the COBE2991 for centrifugation followed by supernatant expelling. Bag filling, centrifugation and expelling were repeated several times. Islets in pellet form were than purified by adding a gradient (bottom loading). Using this closed system 1098±489 IEQ per gram pancreas were purified with a total cell viability of 67±10% and a β-cell viability of 41±13%. The total preparation time reduced to 6 h. After 24 h of cell culture the viability of β-cells was still 56±10% and was only reduced after the addition of proapoptotic IL-1 and TNF-α to 40±4%, indicating that freshly isolated islets are not apoptotic. In conclusion, the closed system preparation is much faster, more effective, and less expensive than the traditional islet preparation. The closed system may be applicable for human islets preparations to restrict the need of clean room facilities for islets preparations to a minimum and may open the way for islet preparations without clean room demand.

4.524 Interleukin-22 but Not Interleukin-17 Provides Protection to Hepatocytes during Acute Liver Inflammation
Zenewicz, L.A. et al
Immunity, ***27(4)***, *647-659 (2007)*

The cytokine interleukin-22 (IL-22) is primarily expressed by T helper 17 (Th17) $CD4^+$ T cells and is highly upregulated during chronic inflammatory diseases. IL-22 receptor expression is absent on immune cells, but is instead restricted to the tissues, providing signaling directionality from the immune system to the tissues. However, the role of IL-22 in inflammatory responses has been confounded by data suggesting both pro- and anti-inflammatory functions. Herein, we provide evidence that during inflammation, IL-22 played a protective role in preventing tissue injury. Hepatocytes from mice deficient in IL-22 were highly sensitive to the detrimental immune response associated with hepatitis. Additionally, IL-22-expressing Th17 cells provided protection during hepatitis in IL-22-deficient mice. On the other hand, interleukin-17 (IL-17), which is coexpressed with IL-22 and can induce similar cellular responses, had no observable role in liver inflammation. Our data suggest that IL-22 serves as a protective molecule to counteract the destructive nature of the immune response to limit tissue damage.

4.525 Generation of islets from stem cells
Soria, B., Hmadcha, A., Bedoya, F.J. and Tejedo, J.R.
Tissue Eengineering, 3rd Ed., 605-618 (2007)

No abstract available

4.526 Directional Freezing of Equine Semen in Large Volumes
Saragusty, J., Gacitua, H., Petit, M.T. and Arav, A.
Reprod. Dom. Anim., ***42***, *610-615 (2007)*

Despite its potential impact on the horse industry, sperm cryopreservation is not an established technology throughout the industry, for a number of reasons that include a reduction in pregnancy rate and increased cost per pregnancy. We have evaluated a novel directional freezing technique, based on a multi-thermal gradient (MTG), by comparing it with the conventional, controlled-rate cryopreservation method (CRCM). Ninety-seven ejaculates with ≥50% motility, collected from 31 stallions were each divided into two parts and subsequently frozen by either MTG or CRCM. Frozen samples were then stored in liquid nitrogen until thawing. The two treatments were evaluated by three methods: progressive linear motility (PLM), viability stain and hypoosmotic swelling (HOS) test. High correlation was found between the three evaluation methods for all post-thaw samples. Eighty-eight per cent of the ejaculates frozen by MTG had post-thaw PLM ≥35%, whereas only 59% of the ejaculates frozen by CRCM had such motility. Post-thaw evaluations of samples frozen by MTG and CRCM were: PLM – 50.2 ± 1.5% and 37.4 ± 1.5%, respectively; viability – 53.6 ± 1.5% and 39.5 ± 1.4%, respectively; membrane integrity, as evaluated by HOS – 36.2 ± 1.3% and 26.5 ± 1.1%, respectively. The differences according to all the evaluation methods were highly significant ($p < 0.001$), and the results indicate that freezing stallion semen by MTG is superior to CRCM.

4.527 Confocal light absorption and scattering spectroscopic microscopy monitors organelles in live cells with no exogenous labels
Itzkan, I. et al
PNAS, ***104(44)***, *17255-17260 (2007)*

This article reports the development of an optical imaging technique, confocal light absorption and scattering spectroscopic (CLASS) microscopy, capable of noninvasively determining the dimensions and other physical properties of single subcellular organelles. CLASS microscopy combines the principles of light-scattering spectroscopy (LSS) with confocal microscopy. LSS is an optical technique that relates the spectroscopic properties of light elastically scattered by small particles to their size, refractive index, and shape. The multispectral nature of LSS enables it to measure internal cell structures much smaller than the diffraction limit without damaging the cell or requiring exogenous markers, which could affect cell function. Scanning the confocal volume across the sample creates an image. CLASS microscopy approaches the accuracy of electron microscopy but is nondestructive and does not require the contrast agents common to optical microscopy. It provides unique capabilities to study functions of viable cells, which are beyond the capabilities of other techniques.

4.528 Neurotrophins Redirect p75^{NTR} from a Clathrin-Independent to a Clathrin-Dependent Endocytic Pathway Coupled to Axonal Transport
Deinhardt, K., Reversi, A., Berninghausen, O., Hopkins, C.R. and Schiavo, G.
Traffic, ***8***, *1736-1749 (2007)*

The p75 neurotrophin receptor (p75^{NTR}) plays multiple roles in neuronal physiology through interactions with many ligands and coreceptors. However, its intracellular neuronal trafficking prior to and after neurotrophin activation is still poorly characterized. We have previously shown that in response to nerve growth factor (NGF), p75^{NTR} is retrogradely transported along the axons of motor neurons (MNs) in carriers shared with NGF, brain-derived neurotrophic factor and the tyrosine kinase receptor TrkB. Here, we report that NGF does not enhance the internalization or degradation of p75^{NTR}, which undergoes a rapid dynamin-dependent and clathrin-independent recycling process in MNs. Instead, incubation of cells with NGF leads to the redirection of a pool of plasma membrane p75^{NTR} into clathrin-coated pits. The subsequent internalization of p75^{NTR} via clathrin-mediated endocytosis, as well as the activity of Rab5, are essential for the sorting of the p75^{NTR}-containing endosomes to the axonal retrograde transport pathway and for the delivery of p75^{NTR} to the soma. Our findings suggest that the spatial regulation of p75^{NTR} signalling is controlled by these ligand-driven routes of endocytosis.

4.529 Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid ß-oxidation: functional role of peroxisome proliferator–activated receptor α in human peripheral blood mononuclear cells
Bouwens, M., Afman, L.A. and Müller, M.
Am. J. Clin. Nutr., ***86***, *1515-1523 (2007)*

Background: Peripheral blood mononuclear cells (PBMCs) are the only readily available cells in healthy humans. Various studies showed disease-characteristic gene expression patterns in PBMCs. However, little is known of nutritional effects on PBMC gene expression patterns. Fatty acids are nutrients that regulate gene expression by activating the nuclear receptor peroxisome proliferator–activated receptor α (PPARα). PBMCs express PPARα, making these cells interesting to study FA-dependent gene expression.
Objective: The aim of this study was to elucidate whether PBMC gene expression profiles also reflect nutrition-related metabolic changes. Furthermore, we focused on the specific role of PPARα in regulation of PBMC gene expression during fasting, when plasma free fatty acids are elevated.
Design: Four healthy male volunteers fasted for 48 h. PBMC RNA was hybridized on Affymetrix whole genome microarrays. To elucidate the role of PPARα, PBMCs of 9 blood donors were incubated with the specific PPARα ligand Wy14643.
Results: After 24 and 48 h of fasting, 1200 and 1386 genes were changed >1.4-fold, respectively. Many of those genes were involved in fatty acid ß-oxidation and are known PPARα target genes. Incubation of PBMCs with Wy14643 resulted in up-regulation of genes that were also up-regulated during fasting.
Conclusions: We conclude that PBMC gene expression profiles reflect nutrition-related metabolic changes such as fasting and that part of the fasting-induced changes are likely regulated by PPARα.

4.530 Endothelial protein C receptor is overexpressed in rheumatoid arthritic (RA) synovium and mediates the anti-inflammatory effects of activated protein C in RA monocytes
Xue, M., March, L., Sambrook, P.N., Fukudome, K. and Jackson, C.J.
Ann. Rheum. Dis., ***66***, *1574-1580 (2007)*

Objectives: (1) To investigate whether inflammatory synovial tissues from patients with rheumatoid

arthritis (RA) express endothelial protein C receptor (EPCR) and (2) to determine the major cell type(s) that EPCR is associated with and whether EPCR functions to mediate the effects of activated protein C (APC) on these cells.
Methods: EPCR, CD68 and PC/APC in synovial tissues were detected by immunostaining and in situ PCR. Monocytes were isolated from peripheral blood of patients with RA and treated with APC, lipopolysaccharide (LPS), and/or EPCR blocking antibody RCR252. Cells and supernatants were collected for RT-PCR, western blotting, enzyme-linked immuosorbent assay and chemotaxis assay.
Results: EPCR was expressed by both OA and RA synovial tissues but was markedly increased in RA synovium. EPCR was colocalised with PC/APC mostly on CD68 positive cells in synovium. In RA monocytes, APC upregulated EPCR expression and reduced monocyte chemoattractant protein-1-induced chemotaxis of monocytes by approximately 50%. APC also completely suppressed LPS-stimulated NF-κB activation and attenuated TNF-α protein by more than 40% in RA monocytes. The inhibitory effects of APC were reversed by RCR252, indicating that EPCR is required.
Conclusions: Our results demonstrate for the first time that EPCR is expressed by synovial tissues, particularly in RA, where it co-localises with PC/APC on monocytes/macrophages. In addition, APC inhibits the migration and activation of RA monocytes via EPCR. These inhibitory effects on RA monocytes suggest that PC pathway may have a beneficial therapeutic effect in RA.

4.531 Rat coronaviruses infect rat alveolar type I epithelial cells and induce expression of CXC chemokines
Miura, T.A., Wang, J., Holmes, K.V. and Mason, R.J.
*Virology, **369(2)**, 288-298 (2007)*

We analyzed the ability of two rat coronavirus (RCoV) strains, sialodacryoadenitis virus (SDAV) and Parker's RCoV (RCoV-P), to infect rat alveolar type I cells and induce chemokine expression. Primary rat alveolar type II cells were transdifferentiated into the type I cell phenotype. Type I cells were productively infected with SDAV and RCoV-P, and both live virus and UV-inactivated virus induced mRNA and protein expression of three CXC chemokines: CINC-2, CINC-3, and LIX, which are neutrophil chemoattractants. Dual immunolabeling of type I cells for viral antigen and CXC chemokines showed that chemokines were expressed primarily by uninfected cells. Virus-induced chemokine expression was reduced by the IL-1 receptor antagonist, suggesting that IL-1 produced by infected cells induces uninfected cells to express chemokines. Primary cultures of alveolar epithelial cells are an important model for the early events in viral infection that lead to pulmonary inflammation.

4.532 The use of test gradient s to determine the bottom layer density for subsequent continuous isopycnic purification of human islets on a COBE 2991 cell processor
Yonekawa, Y. et al
*Xenotransplantation, **14(5)**, poster PJ1007, 449-549 (2007)*

Objectives: The standard human islet purification method utilizes fixed densities of 1.075 to 1.095 g/cm$_3$ for continuous density gradients and 1.095 g/cm$_3$ for bottom layers. We hypothesized that acinar tissue densities vary among donor organs and that test gradients (TGs) measuring acinar tissue density improve our understanding of the dynamics of tissue density during pancreas processing and thereby separation of islet from acinar tissue during subsequent continuous density gradient separation.
Materials and Methods: We determined the acinar tissue density of tissue digests prepared from pancreata from 50 deceased donors and 4 donors who underwent total pancreatectomy and islet autotransplantation. 5-ml iodixanol gradients with densities of 1.085, 1.090, 1.095, 1.100, 1.105, and 1.110 g/cm$_3$ were transferred to six 15-ml conical tubes. Each gradient was overlaid with 1 ml of cap solution (1.035). Pancreatic digest (200 ll), obtained at the start of phase 2, was top-loaded onto each of the 6 gradients. These TGs were centrifuged at 430xg for 3 min at 8 _C. The density chosen as the density of the bottom layer for the subsequent COBE run was 0.005 g/cm$_3$ higher than the highest density that still allowed sedimentation of digest to the bottom of the tube.
Results: The mean ± SD density selected as the bottom layer for autoislet purification was 1.106 ± 0.005 (range: 1.100-1.110). This density was significantly higher ($p<0.001$) than the density selected for deceased donor islet purification, which was 1.091 ± 0.005 (range: 1.070-1.100). For autoislet purification, fractions excluding the COBE bag and denser than 1.095g/cm$_3$ contained 27.0 ± 23.8% of the total islet volume and only 1.7 ± 2.3 ml (range: 0.5-6.0) tissue volume.
Conclusions: TGs appear useful for facilitating the separation of acinar tissue with densities >1.095 g/cm$_3$, as found typically in preparations intended for islet autotransplantation. Using TGs, we were able to return to the pancreatectomized patient a large proportion of autoislets that would have otherwise been lost

in the COBE bag. Interventions that maintain the high density of acinar tissue throughout pancreas preservation and processing are likely to improve density gradient purification of deceased donor islets.

4.533 Excitotoxicity mediated by non-NMDA receptors causes distal axonopathy in long-term cultured spinal motor neurons
King, A.E. et al
Eur. J. Neurosci., ***26***, *2151-2159 (2007)*

Excitotoxicity has been implicated as a potential cause of neuronal degeneration in amyotrophic lateral sclerosis (ALS). It has not been clear how excitotoxic injury leads to the hallmark pathological changes of ALS, such as the abnormal accumulation of filamentous proteins in axons. We have investigated the effects of overactivation of excitatory receptors in rodent neurons maintained in long-term culture. Excitotoxicity, mediated principally via non-N-methyl-D-aspartate (NMDA) receptors, caused axonal swelling and accumulation of cytoskeletal proteins in the distal segments of the axons of cultured spinal, but not cortical, neurons. Axonopathy only occurred in spinal neurons maintained for 3 weeks in vitro, indicating that susceptibility to axonal pathology may be related to relative maturity of the neuron. Excitotoxic axonopathy was associated with the aberrant colocalization of phosphorylated and dephosphorylated neurofilament proteins, indicating that disruption to the regulation of phosphorylation of neurofilaments may lead to their abnormal accumulation. These data provide a strong link between excitotoxicity and the selective pattern of axonopathy of lower motor neurons that underlies neuronal dysfunction in ALS.

4.534 Transition from enhanced T cell infiltration to inflammation in the myelin-degenerative central nervous system
Grundtner, R. et al
Neurobiology of Disease, ***28(3)***, *261-275 82007)*

Myelin degeneration in the central nervous system (CNS) is often associated with elevated numbers of T cells in brain and spinal cord (SC). In some degenerative diseases, this T cell immigration has no clinical relevance, in others, it may precede severe inflammation and tissue damage. We studied T cells in the myelin-degenerative SC of transgenic (tg) Lewis rats overexpressing the proteolipid protein (PLP). These lymphocytes are T_H1/T_C1 cells and represent different T cell clones unique to individual animals. The SC-infiltrating $CD8^+$ T cell pool is more restricted than its $CD4^+$ counterpart, possibly due to constrictions in the peripheral $CD8^+$ T cell repertoire. Some SC-infiltrating T cells are highly motile and cover large distances within their target tissue, others are tethered to MHC class II^+ microglia cells. The activation of the tethered cells may trigger the formation of inflammatory foci and could pave the way for inflammation in degenerative CNS disease.

4.535 Fibrillar beta-amyloid (Aβ) (1–42) elevates extracellular Aβ in cultured hippocampal neurons of adult rats
Maja, S., Rastegar, K., Zarifkar, A. and Takhshid, M.A.
Brain Res., ***1185***, *321-327 (2007)*

Alzheimer's disease (AD) is a chronic disorder with progressive neurodegeneration associated with aging and is characterized by fibrillar beta-amyloid (Aβ) deposits in the brain. Although the increased production of Aβ seems to play a noticeable role in AD pathogenesis and its progression, all the mechanisms which are involved in this extracellular Aβ elevation are not known completely. In the present study, we used adult hippocampal neuronal culture as an in vitro model which is favorable for adult neurodegenerative diseases' studies. We introduced a toxic concentration for fibrillar Aβ1–42 in adult neurons which was much lower from the toxic concentration in embryonic neurons. To determine the effect of fibrillar Aβ1–42 which is the most toxic part of amyloid plaques, on extracellular Aβ1–40, as the main part of βAPP proteolysis products, we treated the neurons with fibrillar Aβ1–42 at nontoxic concentrations of 2×10^{-6}, 2×10^{-5} and 2×10^{-4} μM and measured extracellular Aβ1–40. Our findings show that even very low levels of fibrillar Aβ1–42 can contribute to subsequent extracellular Aβ elevation in a dose dependent manner. These results suggest that even low levels of fibrillar Aβ may have deleterious actions if it remains in extracellular space for a period of time.

4.536 Defective T Helper Response of Hepatocyte-Stimulated CD4 T Cells Impairs Antiviral CD8 Response and Viral Clearance
Wiegard, C. et al

*Gasteroenterology, **133**, 2010-2018 (2007)*

<u>*Background & Aims:*</u> In hepatitis, hepatocytes gain the ability to express major histocompatibility complex (MHC) class II molecules and to present antigen to CD4 T cells. Here, we investigated whether MHC class II-expressing hepatocytes influence in vitro the differentiation of CD4 T cells and in vivo the T-cell response to and control of viral infection. <u>*Methods:*</u> Class II transactivator-transgenic hepatocytes that constitutively express MHC class II molecules were used to stimulate CD4 T cells in vitro, and the effector response type of the stimulated CD4 T cells was determined. The in vivo relevance of the obtained findings was confirmed by infecting nontransgenic or class II transactivator-transgenic mice with lymphocytic choriomeningitis virus. <u>*Results:*</u> MHC II-expressing hepatocytes induced T helper cell (Th) 2 differentiation of uncommitted CD4 T cells and abrogated the ability of previously differentiated Th1 to secrete interferon-γ, even in the presence of proinflammatory microbial signals. The suppression of Th1 responses by hepatocytes was associated with poor expression levels of Th1-promoting Delta-like Notch ligands. In vivo, MHC II expression by hepatocytes impaired the interferon-γ production by lymphocytic choriomeningitis virus-specific CD4 and CD8 T cells and prolonged viral persistence. <u>*Conclusions:*</u> By instructing infiltrating CD4 T cells to differentiate into a less inflammatory phenotype, MHC II-expressing hepatocytes seem to impair antiviral CD8 T-cell responses and viral clearance. Thus, hepatocytes may contribute to the chronicity of hepatitis virus infection.

4.537 Expression of transforming growth factor-β by human islets: Impact on inslets viability and function
Sabek, O.M. et al
*Cell Transplant., **16**, 775-785 (2007)*

Transforming growth factor-β1 (TGF-β1) is a pleotrophic cytokine that promotes angiogenesis and extracellular matrix protein synthesis in addition to its immunosuppressive effects. The purpose of this study is to identify optimal conditions for in vivo expression of TGF-β1 by human islets to exploit the possible beneficial effects and minimize undesirable side effects. We transduced human islets with adenoviral vectors encoding the active form of Ad-TGF-β1 or Ad-LacZ to test the effects of TGF-β1 gene expression on islet in vivo function following their transplantation into a NOD-SCID mouse model. Islets were transduced with multiplitic y of infection (MOI) of 20, 10, 5 and 2.5 per islet cell. At a MOI ranging from 2.5 to 20, expression of TGF-β1 in islet supernatant persisted for 1-2 months and ranged from 153 ± 5 to 2574 ± 1299 pg/ml, respectively. Transduction with the lowest MOI (2.5) did not comprise the in vivo production of human C-peptide. We conclude that TGF-β1 expression in transplanted islets does not compromise viability and that adenoviral transduction with the TGF-β1 gene has a dose-dependent effect, with larger MOIs being used successfully to evaluate the nonimmune effects of gene transduction.

4.538 Kupffer cell heterogeneity: functional properties of bone marrow–derived and sessile hepatic macrophages
Klein, I. et al
*Blood, **110**, 4077-4085 (2007)*

Kupffer cells form a large intravascular macrophage bed in the liver sinusoids. The differentiation history and diversity of Kupffer cells is disputed; some studies argue that they are derived from blood monocytes, whereas others support a local origin from intrahepatic precursor cells. In the present study, we used both flow cytometry and immunohistochemistry to distinguish 2 subsets of Kupffer cells that were revealed in the context both of bone marrow transplantation and of orthotopic liver transplantation. One subset was radiosensitive and rapidly replaced from hematogenous precursors, whereas the other was relatively radioresistant and long-lived. Both were phagocytic but only the former population was recruited into inflammatory foci in response to $CD8^+$ T-cell activation. We propose the name "sessile" for the radioresistant Kupffer cells that do not participate in immunoinflammatory reactions. However, we found no evidence that these sessile Kupffer cells arise from immature intrahepatic precursors. Our conclusions resolve a long-standing controversy and explain how different experimental approaches may reveal one or both of these subsets.

4.539 Surface Modification of RGD-Liposomes for Selective Drug Delivery to Monocytes/Neutrophils in Brain
Qin, J. et al
*Chem. Pharm. Bul., **55(8)**, 1192-1197 (2007)*

In the present study, RGD peptide was coupled with ferulic acid (FA) liposomes for binding to monocytes

and neutrophils in peripheral blood for brain targeting in response to leukocyte recruitment. Cholesterol (Ch) was esterified with succinic anhydride to introduce a carboxylic end group (Ch-COOH). Soybean phosphatidylcholine, cholesterol and Ch-COOH were in a molar ratio of 1 : 0.23 : 0.05. FA was loaded into liposomes with 80.2±5.2% entrapment efficiency (EE) using a calcium acetate gradient method since it was difficult to load FA by other methods. RGD peptide was a novel compound coupled with Ch-COOH *via* carbodiimide and *N*-hydroxysulfosuccinimide. The results of the *in vitro* flow cytometric study showed that RGD conjugation liposomes (RGD-liposomes) could bind to monocytes/neutrophils efficiently. The rats were subjected to intrastriatal microinjections of 100 μl of human recombinant IL-1β to produce brain inflammation and subsequently sacrificed after 15, 30, 60 and 120 min of administration of three formulations (FA solution, FA liposome, RGD-coated FA liposome). The body distribution results showed that RGD-liposomes could be directed to the target site, *i.e.* the brain, by cell selectivity in case of an inflammatory response. For RGD coated liposomes, the concentration of FA in brain was 6-fold higher than that of FA solution and 3-fold higher than that of uncoated liposomes. MTT assay and flow cytometry were used in the pharmacodynamic studies where it was found that FA liposomes exhibited greater antioxidant activity to FA solution on U937 cell.

4.540 Body Distributioin of RGD-mediated Liposome in Brain-targeting Drug Delivery
Qin, J. et al
Yakugaku Zasshi, ***127(9)***, *1497-1501 (2007)*

RGD conjugation liposomes (RGD-liposomes) were evaluated for brain-targeting drug delivery. The flow cytometric *in vitro* study demonstrated that RGD-liposomes could bind to monocytes and neutrophils effectively. Ferulic acid (4-hydroxy-3-methoxycinnamic, FA) was loaded into liposomes. Rats were subjected to intrastriatal microinjections of 100 units of human recombinant IL-1β to produce brain inflammation and caudal vein injection of three formulations (FA solution, FA liposome and RGD-coated FA liposome). Animals were sacrificed 15, 30, 60 and 120 min after administration to study the body distribution of the FA in the three formulations. HPLC was used to determine the concentration of FA *in vivo* with salicylic acid as internal standard. The results of body distribution indicated that RGD-coated liposomes could be mediated into the brain with a 6-fold FA concentration compared to FA solution and 3-fold in comparison to uncoated liposome. Brain targeted delivery was achieved and a reduction in dosage might be allowed.

4.541 Coronavirus Non-Structural Protein 1 Is a Major Pathogenicity Factor: Implications for the Rational Design of Coronavirus Vaccines
Züst, R. et al
PloS Pathogens, ***3(8)***, *1062-1072 (2007)*

Attenuated viral vaccines can be generated by targeting essential pathogenicity factors. We report here the rational design of an attenuated recombinant coronavirus vaccine based on a deletion in the coding sequence of the non-structural protein 1 (nsp1). In cell culture, nsp1 of mouse hepatitis virus (MHV), like its SARS-coronavirus homolog, strongly reduced cellular gene expression. The effect of nsp1 on MHV replication in vitro and in vivo was analyzed using a recombinant MHV encoding a deletion in the nsp1-coding sequence. The recombinant MHV nsp1 mutant grew normally in tissue culture, but was severely attenuated in vivo. Replication and spread of the nsp1 mutant virus was restored almost to wild-type levels in type I interferon (IFN) receptor-deficient mice, indicating that nsp1 interferes efficiently with the type I IFN system. Importantly, replication of nsp1 mutant virus in professional antigen-presenting cells such as conventional dendritic cells and macrophages, and induction of type I IFN in plasmacytoid dendritic cells, was not impaired. Furthermore, even low doses of nsp1 mutant MHV elicited potent cytotoxic T cell responses and protected mice against homologous and heterologous virus challenge. Taken together, the presented attenuation strategy provides a paradigm for the development of highly efficient coronavirus vaccines.

4.542 Novel hepatic progenitor cell surface markers in the adult rat liver
Yovchev, M.I., Groszdanov, P.N., Hoseph, B., Gupta, S.and Daabeva, M.D.
Hepatology, ***45(1)***, *139-149 (2007)*

Hepatic progenitor/oval cells appear in injured livers when hepatocyte proliferation is impaired. These cells can differentiate into hepatocytes and cholangiocytes and could be useful for cell and gene therapy applications. In this work, we studied progenitor/oval cell surface markers in the liver of rats subjected to 2-acetylaminofluorene treatment followed by partial hepatectomy (2-AAF/PH) by using rat genome 230

2.0 Array chips and subsequent RT-PCR, immunofluorescent (IF), immunohistochemical (IHC) and *in situ* hybridization (ISH) analyses. We also studied expression of the identified novel cell surface markers in fetal rat liver progenitor cells and FAO-1 hepatoma cells. Novel cell surface markers in adult progenitor cells included tight junction proteins, integrins, cadherins, cell adhesion molecules, receptors, membrane channels and other transmembrane proteins. From the panel of 21 cell surface markers, 9 were overexpressed in fetal progenitor cells, 6 in FAO-1 cells and 6 are unique for the adult progenitors (CD133, claudin-7, cadherin 22, mucin-1, ros-1, Gabrp). The specificity of progenitor/oval cell surface markers was confirmed by ISH and double IF analyses. Moreover, study of progenitor cells purified with Ep-CAM antibodies from D-galactosamine injured rat liver, a noncarcinogenic model of progenitor cell activation, verified that progenitor cells expressed these markers. *Conclusion*: We identified novel cell surface markers specific for hepatic progenitor/oval cells, which offers powerful tool for their identification, isolation and studies of their physiology and pathophysiology. Our studies also reveal the mesenchymal/epithelial phenotype of these cells and the existence of species diversity in the hepatic progenitor cell identity.

4.543 Immune role of hepatic TLR-4 revealed by orthotopic mouse liver transplantation
John, B., Klein, I. and Crispe, I.N.
Hepatology, ***45(1)***, *178-186 (2007)*

Activated CD8+ T cells migrate to the liver at the end of an immune response and go through apoptosis there, but this mechanism is impaired in mice lacking Toll-like receptor-4. This allowed us to test the importance of liver trapping in an ongoing immune response. In the absence of Toll-like receptor-4, reduced liver accumulation was associated with an increase in the circulating CD8+ T cell pool, more long-lived memory T cells and increased CD8+ T cell memory responses. Using experimental orthotopic liver transplantation, we showed that the effect of Toll-like receptor-4 on the formation of the CD8+ T cell memory resides in the liver. Conclusion: These studies reveal a new function for the liver, which is to regulate the magnitude of T cell memory responses through a Toll-like receptor-4–dependent mechanism.

4.544 Murine liver plasmacytoid dendritic cells become potent immunostimulatory cells after Flt-3 ligand expansion
Kingham, T.P., Chaudhry, U.I., Plitas, G., Katz, S.C., Raab, J. and DeMatteo, R.P.
Hepatology, ***45(2)***, *445-454 (2007)*

The liver has unique immunological properties. Although dendritic cells (DCs) are central mediators of immune regulation, little is known about liver DCs. Plasmacytoid DCs (pDCs) are a recently identified subtype of murine liver DC. We sought to define the function of freshly isolated murine liver pDCs. We found that normal liver pDCs were weak in stimulating T cells, yet they possessed a proinflammatory cytokine profile with high tumor necrosis factor-α and low IL-10 secretion. To facilitate the investigation of murine liver pDCs, we expanded them in vivo with fms-like tyrosine kinase 3 ligand (Flt3L). After Toll-like receptor-9 ligation, expanded liver pDCs secreted high levels of IFN-α and were able to stimulate NK cells, NKT cells, and antigen-specific CD8+ T cells in vitro. In addition, Flt3L expansion alone generated pDCs capable of activating antigen-specific CD8+ T cells in vivo. Conclusion: Unstimulated liver pDCs exist in a latent state with the potential to become potent activators of the innate and adaptive immune systems through their interactions with other immune effectors. Our findings have implications for understanding the role of the liver in tolerance and immunity.

4.545 Transdifferentiation of hepatocyte-like cells from the human hepatoma HepaRG cell line through bipotent progenitor
Cerec, V., glaise, D., Garnier, D., Morosan, S., Turlin, B., Drenou, B., Gripon, P., Kremsdorf, D., Guguen-Guillouzo, C. and Corlu, A.
Hepatology, ***45(4)***, *957-967 (2007)*

Hepatic tumors, exhibiting mature hepatocytes and undifferentiated cells merging with cholangiocyte and hepatocyte phenotypes, are frequently described. The mechanisms by which they occur remain unclear. We report differentiation and transdifferentiation behaviors of human HepaRG cells isolated from a differentiated tumor developed consecutively to chronic HCV infection. We demonstrate that, in vitro, proliferating HepaRG cells differentiate toward hepatocyte-like and biliary-like cells at confluence. If hepatocyte-like cells are selectively isolated and cultured at high cell density, they proliferate and preserve their differentiation status. However, when plated at low density, they transdifferentiate into hepatocytic and biliary lineages through a bipotent progenitor. In accordance, transplantation of either undifferentiated

or differentiated HepaRG cells in uPA/SCID mouse damaged liver gives rise mainly to functional human hepatocytes infiltrating mouse parenchyma. Analysis of the differentiation/transdifferentiation process reveals that: (1) the reversible differentiation fate of HepaRG cells is related to the absence of p21CIP1 and p53 accumulation in differentiated cells; (2) HepaRG bipotent progenitors express the main markers of in vivo hepatic progenitors, and that cell differentiation process is linked to loss of their expression; (3) early and transient changes of β-catenin localization and HNF3β expression are correlated to Notch3 upregulation during hepatobiliary commitment of HepaRG cells. Conclusion: Our results demonstrate the great plasticity of transformed hepatic progenitor cells and suggest that the transdifferentiation process could supply the pool of hepatic progenitor cells. Moreover, they highlight possible mechanisms by which transdifferentiation and proliferation of unipotent hepatocytes might cooperate in the development of mixed and differentiated tumors.

4.546 Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase

Trebicka, J., Hennenberg, M., Laleman, W., Shelest, W., Biecker, E., Schepke, M., Nevens, F., Sauerbruch, T. and Heller, J.

Hepatology, ***46(1)***, *242-253 (2007)*

In cirrhosis, increased RhoA/Rho-kinase signaling and decreased nitric oxide (NO) availability contribute to increased intrahepatic resistance and portal hypertension. Hepatic stellate cells (HSCs) regulate intrahepatic resistance. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) inhibit synthesis of isoprenoids, which are necessary for membrane translocation and activation of small GTPases like RhoA and Ras. Activated RhoA leads to Rho-kinase activation and NO synthase inhibition. We therefore investigated the effects of atorvastatin in cirrhotic rats and isolated HSCs. Rats with secondary biliary cirrhosis (bile duct ligation, BDL) were treated with atorvastatin (15 mg/kg per day for 7 days) or remained untreated. Hemodynamic parameters were determined in vivo (colored microspheres). Intrahepatic resistance was investigated in in situ perfused livers. Expression and phosphorylation of proteins were analyzed by RT-PCR and immunoblots. Three-dimensional stress-relaxed collagen lattice contractions of HSCs were performed after incubation with atorvastatin. Atorvastatin reduced portal pressure without affecting mean arterial pressure in vivo. This was associated with a reduction in intrahepatic resistance and reduced responsiveness of in situ–perfused cirrhotic livers to methoxamine. Furthermore, atorvastatin reduced the contraction of activated HSCs in a 3-dimensional stress-relaxed collagen lattice. In cirrhotic livers, atorvastatin significantly decreased Rho-kinase activity (moesin phosphorylation) without affecting expression of RhoA, Rho-kinase and Ras. In activated HSCs, atorvastatin inhibited the membrane association of RhoA and Ras. Furthermore, in BDL rats, atorvastatin significantly increased hepatic endothelial nitric oxide synthase (eNOS) mRNA and protein levels, phospho-eNOS, nitrite/nitrate, and the activity of the NO effector protein kinase G (PKG). Conclusion: In cirrhotic rats, atorvastatin inhibits hepatic RhoA/Rho-kinase signaling and activates the NO/PKG-pathway. This lowers intrahepatic resistance, resulting in decreased portal pressure. Statins might represent a therapeutic option for portal hypertension in cirrhosis.

4.547 Vaccination with plasmacytoid dendritic cells induces protection against infection with *Leishmania major* in mice

Remer, K., Apetrei, C., Schwarz, T., Linden, C. and Moll, H.

Eur. J. Immunol., ***37(9)***, *2463-2473 (2007)*

DC-based vaccination against *Leishmania major* induces a parasite-specific Th1 response and long-lasting protective immunity in susceptible mice. Since distinct DC subsets have been proposed to direct the predominant development of either Th1 or Th2 cells, we analyzed the capability of plasmacytoid DC (pDC) to induce protection and elicit a Th1 response against *L. major*. Pulsing with *L. major* lysate induced the activation and maturation of semi-mature murine pDC that had been isolated from the spleen, as indicated by up-regulation of the co-stimulatory molecules CD86 and CD80, but did not enhance the level of IFN-α secretion by pDC. Vaccination of susceptible mice with *L. major* lysate-pulsed pDC induced highly effective T cell-mediated immunity against subsequent infection with *L. major* parasites. Surprisingly, the protection was not accompanied by a polarized Th1 cytokine profile. Co-activation of pDC with CpG-containing oligodeoxynucleotides, which has been shown to be critical for activating the protective potential of myeloid DC, was not required for the protective effect of *L. major* antigen-pulsed pDC. These findings demonstrate that antigen-loaded pDC are able to induce T cell-mediated protection against a parasite disease and that experimental leishmaniasis is a suitable model to elucidate the mechanisms underlying DC-based vaccination against infections.

4.548 Simultaneous age-related depolarization of mitochondrial membrane potential and increased mitochondrial reactive oxygen species production correlate with age-related glutamate excitotoxicity in rat hippocampal neurons
Parihar, M.S. and Brewer, G.J.
J. Neurosci. Res., ***85(5)***, *1018-1032 (2007)*

Mitochondria are implicated in glutamate excitotoxicity by causing bioenergetic collapse, loss of Ca2+ homeostasis, and generation of reactive oxygen species (ROS), all of which become increasingly important clinically with age. Little is known about how aging affects the relative importance of mitochondrial membrane potential (ΔΨm) and ROS production. To determine aging affects on ΔΨm and ROS production in individual somal and axonal/dendritic mitochondria, we compared ROS production while simultaneously monitoring ΔΨm before and after glutamate treatment of live neurons from embryonic (day 18), middle-aged (9–12 months), and old (24 months) rats. At rest, old neuronal mitochondria 1) showed a higher rate of ROS production that was particularly strong in axonal/dendritic mitochondria relative to that in middle-age neurons, 2) were more depolarized in comparison with neurons of other ages, and 3) showed no differences in ROS or ΔΨm as a function of distance from the nucleus. All ΔΨm grouped into three classes of high (less than –120 mV), medium (–85 to –120 mV), and low (greater than –85 mV) polarization that shifted toward the lower classes with age at rest. Glutamate exposure dramatically depolarized the ΔΨm in parallel with greatly increased ROS production, with a surprising absence of an effect of age or distance from the nucleus on these mitochondrial parameters. These data suggest that old neurons are more susceptible to glutamate excitotoxicity because of an insidious depolarization of ΔΨm and rate of ROS generation at rest that lead to catastrophic failure of phosphorylative and reductive energy supplies under stress.

4.549 Differential regulation of matrix metalloproteinase 2 and matrix metalloproteinase 9 by activated protein C: Relevance to inflammation in rheumatoid arthritis
Xue, M., March, L., Sambrook, P.N. and Jackson, C.J.
Arthritis & Rheumatism, ***56(9)***, *2864-2874 (2007)*

Objective
To investigate the in vitro effect of activated protein C (APC), a natural anticoagulant and novel antiinflammatory agent, on the regulation of the gelatinases matrix metalloproteinase 2 (MMP-2) and MMP-9.
Methods
Synovial fibroblasts and peripheral blood monocytes isolated from patients with rheumatoid arthritis (RA) or osteoarthritis (OA) and Mono Mac6 cells were used in this study. After treatment, cells and culture supernatants were collected for zymography, enzyme-linked immunosorbent assay, reverse transcription–polymerase chain reaction, and Western blot analysis.
Results
Fibroblasts and monocytes from RA patients produced substantially more MMP-9 than did those from OA patients; however, there was no difference in MMP-2 production. The addition of recombinant APC markedly reduced MMP-9 at the gene and protein levels. In contrast, APC up-regulated and activated MMP-2. Using a blocking antibody to the endothelial protein C receptor (EPCR), we showed that the inhibition of MMP-9 by APC was EPCR-dependent. Furthermore, APC directly suppressed the production of tumor necrosis factor (TNF) and the activation of NF-κB and MAP kinase p38, and inhibitors of NF-κB or p38 reduced the production of MMP-9, suggesting that APC inhibits MMP-9 by blocking TNF, NF-κB, and p38. Thus, APC acts on MMP-9 by binding to EPCRs on the cell surface and, subsequently, inhibiting the intracellular activation of the proinflammatory signaling molecules NF-κB and p38.
Conclusion
APC appears to be the first physiologic agent to inhibit the production of proinflammatory MMP-9, yet increase antiinflammatory MMP-2 activity. Our results provide the initial evidence that APC may be beneficial in the prevention of inflammation and joint destruction in RA.

4.550 Platelet imidazoline receptors as state marker of depressive symptomatology
Piletz, J., Baker, R. and Halaris, A.
J. Psychiatric Res., ***42***, *41-49 (2008)*

Objective
Previous studies have shown that imidazoline receptors (IR-1) are increased in platelets and frontal cortex

of depressed patients, and this up-regulation is normalized (down-regulated) after antidepressant drug treatments. It has been hypothesized that IR-1 up-regulation during the depressive episode may be a state marker for depressive symptomatology. The goal of the present study was to address the state versus trait question.
Method
Twelve healthy subjects (six males and six females) met stringent inclusion and exclusion criteria for physical and mental health. They received desipramine for 6 weeks in order to simulate the length of time and dosing used previously to obtain an IR-1 down-regulation and a therapeutic response in depressed patients. Outcome and safety measures included clinical, psychological, and cardiovascular assessments obtained throughout the study. Plasma concentrations of desipramine were measured throughout the 6 weeks of treatment and again after 2 weeks following tapered discontinuation of desipramine. Platelet receptors were assessed by Western blotting and radioligand binding assays.
Results
Healthy subjects taking desipramine experienced mild dysphoric effects but there were no adverse events. The binding of 8 nM p-[^{125}I]clonidine to IR-1 and α_2-adrenoceptors in healthy subjects did not change during desipramine treatment. The immunodensity of the 33 kDa band associated with IR-1 gradually increased to a maximum, by week-6, of 26% higher than baseline ($p < 0.01$ compared to baseline). Two weeks after desipramine discontinuation, there was a decline in α_2-adrenoceptor binding and 33 kDa band's immunodensity ($p = 0.04$).
Conclusions
The findings support the hypothesis that platelet IR-1 binding sites are a marker of mood state rather than of antidepressant-induced pharmacological regulation. By comparison, platelet α_2-adrenoceptors appear to be regulated by desipramine as a pharmacological effect independent of mood state.

4.551 Abrogation of the Antifibrotic Effects of Natural Killer Cells/Interferon-γ Contributes to Alcohol Acceleration of Liver Fibrosis
Jeong, W-L., Park, O. and Gao, B.
*Gastroenterology, **134**, 248-258 (2008)*

Background & Aims: Chronic alcohol drinking accelerates liver fibrosis in patients with viral hepatitis that cannot be fully explained by ethanol-enhanced liver damage. Here, we identified a novel mechanism by which alcohol accelerates liver fibrosis: inhibition of the antifibrotic effects of natural killer (NK) cells and interferon-γ (IFN-γ). Methods: Alcohol administration was achieved by feeding mice with a liquid diet containing 5% ethanol for 8 weeks. Liver fibrosis was induced by administration of carbon tetrachloride (CCl_4) for 2 weeks. Hepatic stellate cells (HSCs) were also isolated and cultured for in vitro studies. Results: CCl_4 treatment induced greater fibrosis and less apoptosis of HSCs in ethanol-fed mice compared with pair-fed mice. Polyinosinic-polycytidylic acid (Poly I:C) or IFN-γ treatment inhibited liver fibrosis in pair-fed but not in ethanol-fed mice. Poly I:C activation of NK cell cytotoxicity against HSCs was attenuated in ethanol-fed mice compared with pair-fed mice, which was due to reduced natural killer group 2 member D (NKG2D), tumor necrosis factor-related apoptosis-inducing ligand, and IFN-γ expression on NK cells from ethanol-fed mice. In vitro, HSCs from ethanol-fed mice were resistant to IFN-γ–induced cell cycle arrest and apoptosis compared with pair-fed mice. Such resistance was due to diminished IFN-γ activation of signal transducer and activator of transcription 1 (STAT1) in HSCs from ethanol-fed mice caused by the induction of suppressors of cytokine signaling proteins and the production of oxidative stress. Finally, HSCs from ethanol-fed mice were resistant to NK cell killing, which can be reversed by transforming growth factor-β1 (TGF-β1) neutralizing antibody. Conclusions: Chronic ethanol consumption attenuates the antifibrotic effects of NK/IFN-γ/STAT1 in the liver, representing new and different therapeutic targets with which to treat alcoholic liver fibrosis.

4.552 T Cell Development from Kit-Negative Progenitors in the Foxn1Δ/Δ Mutant Thymus
Xiao, S., Su, D-m. and Manley, N.R.
*J. Immunol., **180**, 914-921 (2008)*

*Foxn1*Δ is a hypomorphic allele of the nude gene that causes arrested thymic epithelial cell differentiation and abnormal thymic architecture lacking cortical and medullary domains. T cells develop in the *Foxn1*Δ/Δ adult thymus to the double- and single-positive stages, but in the apparent absence of double-negative 3 (DN3) cells; however, DN3 cells are present in the fetal thymus. To investigate the origin of this seemingly contradictory phenotype, we performed an analysis of fetal and adult DN cells in these mutants. Neither adult bone marrow-derived cells nor fetal liver cells from wild-type or *Rag*1$^{-/-}$ mice were able to differentiate to the DN2 or DN3 stage in the *Foxn1*Δ/Δ thymus. Our data suggest that thymopoiesis in the

Foxn1$\Delta^{/}\Delta$ adult thymus proceeds from CD117^{-} atypical progenitors, while CD117^{+} DN1a cells are absent or blocked in their ability to differentiate to the T lineage. Wild-type cells generated by this pathway in the postnatal thymus were exported to the periphery, demonstrating that these atypical cells contributed to the peripheral T cell pool. The *Foxn1*$\Delta^{/}\Delta$ adult (but not fetal) thymus also preferentially supports B cell development, specifically of the B-1 type, and this phenotype correlated with reduced Notch ligand expression in the adult stroma.

4.553 Red Blood Cells Are the Major Source of Alpha-Synuclein in Blood
Barbour, R. et al
*Neurodegenerative Dis., **5**, 55-59 (2008)*

Background: α-Synuclein has been directly linked to Parkinson's disease etiology by mutations in and multiplication of its gene that result in a familial form of Parkinson's disease. α-Synuclein has been detected in blood, and was found to be elevated in the blood of those individuals with the α-synuclein gene multiplication. *Objective:* A complete analysis of the level of α-synuclein in blood has not been performed. In this report, we determine the quantitative distribution of α-synuclein in the plasma and different cellular fractions of human blood. The levels of α-synuclein in human and mouse blood are compared. *Methods:* α-Synuclein levels in the different fractions of blood were quantified by a sandwich ELISA with purified recombinant α-synuclein as an assay standard. Samples were further characterized by Western immunoblot analysis. *Results:* More than 99% of the α-synuclein resides in the red blood cells (RBCs) with less than 1% of the total detected in the plasma, platelets and peripheral blood mononuclear cells. *Conclusions:* More than 99% of the α-synuclein in human blood is present in the peripheral blood cells, with the remainder in plasma. Fractionation of peripheral blood cells from human blood and quantification of α-synuclein revealed that only a very small amount of the total α-synuclein is present in peripheral blood mononuclear cells, and platelets, with the majority of α-synuclein in blood being present in RBCs. Considering the abundance and fragility of RBCs, α-synuclein levels in these other blood fractions or other bodily fluids such as cerebrospinal fluid may be artificially elevated by contamination with intact or lysed RBCs.

4.554 Effects of 30 min of aerobic exercise on gene expression in human neutrophils
Radom-Aizik, S., Zalvidar, Jr., F., Leu, S-Y., Galasetti, P. and Cooper, D.M.
*J. Appl. Physiol., **104**, 236-243 (2008)*

Relatively brief bouts of exercise alter gene expression in peripheral blood mononuclear cells (PBMCs), but whether exercise changes gene expression in circulating neutrophils (whose numbers, like PBMCs, increase) is not known. We hypothesized that exercise would activate neutrophil genes involved in apoptosis, inflammation, and cell growth and repair, since these functions in leukocytes are known to be influenced by exercise. Blood was sampled before and immediately after 30 min of constant, heavy (~80% peak O_2 uptake) cycle ergometer exercise in 12 healthy men (19–29 yr old) of average fitness. Neutrophils were isolated using density gradients; RNA was hybridized to Affymetrix U133+2 Genechip arrays. With false discovery rate (FDR) <0.05 with 95% confidence, a total of 526 genes were differentially expressed between before and after exercise. Three hundred and sixteen genes had higher expression after exercise. The Jak/STAT pathway, known to inhibit apoptosis, was significantly activated (EASE score, $P < 0.005$), but 14 genes were altered in a way likely to accelerate apoptosis as well. Similarly, both proinflammatory (e.g., IL-32, TNFSF8, and CCR5) and anti-inflammatory (e.g., ANXA1) were affected. Growth and repair genes like AREG and FGF2 receptor genes (involved in angiogenesis) were also activated. Finally, a number of neutrophil genes known to be involved in pathological conditions like asthma and arthritis were altered by exercise, suggesting novel links between physical activity and disease or its prevention. In summary, brief heavy exercise leads to a previously unknown substantial and significant alteration in neutrophil gene expression.

4.555 Pancreatic Islet Immunoreactivity to the Reg Protein INGAP
Taylor-Fishwick, D.A., Bowman, A., Korngiebel-Rosique. M.C. and Vinik, A.I.
*J. Histochem. Cytochem., **56(2)**, 183-191 (2008)*

The Reg-related protein family member INGAP (islet neogenesis-associated protein) is a pleiotropic factor enhancing islet neogenesis, neurite growth, β-cell protection, and β-cell function. Using an antibody to the N-termini of INGAP, we have identified that immunoreactivity to INGAP localized to the pancreatic endocrine cells in mouse. INGAP- and insulin-immunoreactive cells are mutually exclusive, with INGAP-

immunoreactive cells being preserved after streptozotocin-mediated destruction of β-cells. Glucagon- and INGAP-immunoreactive cells colocalize, although respective antigen expression occurs in different intracellular locations. These data suggest that INGAP-immunoreactive cells include α-cells; however, detection of single INGAP-immunoreactive/glucagon-negative cells indicates that this may not be exclusive. In addition to mouse, detection of islet endocrine cells that were INGAP immunoreactive/glucagon immunoreactive/insulin negative was also observed in islets from human, monkey, and rat. These findings reveal that INGAP and/or related group 3 Reg proteins have a conserved expression in the pancreatic islet.

4.556 Salmonella infection of afferent lymph dendritic cells
Chan, S.S.M., Mastroeni, P., McConnell, I. and Blacklaws, B.A.
J. Leukoc. Biol., ***88***, *272-279 (2008)*

The interactions of *Salmonella enterica* subspecies I serotype Abortusovis (*S.* Abortusovis) with ovine afferent lymph dendritic cells (ALDCs) were investigated for their ability to deliver Maedi visna virus (MVV) GAG p25 antigens to ALDCs purified from afferent lymph. *Salmonellae* were found to enter ALDC populations by a process of cell invasion, as confirmed by electron and confocal microscopy. This led to phenotypical changes in ALDC populations, as defined by CD1b and CD14 expression. No differences in the clearance kinetics of intracellular *aroA*-negative *Salmonella* from $CD1b^+$ $CD14^{lo}$ and $CD1b^+$ $CD14^-$ ALDC populations were noted over 72 h. ALDCs were also shown to present MVV GAG p25 expressed by *aroA*-negative *S.* Abortusovis to $CD4^+$ T lymphocytes. Thus, the poor immune responses that *Salmonella* vaccines elicited in large animal models compared with mice are neither a result of an inability of *Salmonella* to infect large animal DCs nor an inability of these DCs to present delivered antigens. However, the low efficiency of infection of ALDC compared with macrophages or monocyte-derived DCs may account for the poor immune responses induced in large animal models.

4.557 iPLA2β: front and center in human monocyte chemotaxis to MCP-1
Mishra, R.S., Carnevale, K.A. and Cathcart, M.K.
J. Exp. Med., ***205(2)***, *347-359 (2008)*

Monocyte chemoattractant protein-1 (MCP-1) directs migration of blood monocytes to inflamed tissues. Despite the central role of chemotaxis in immune responses, the regulation of chemotaxis by signal transduction pathways and their in vivo significance remain to be thoroughly deciphered. In this study, we examined the intracellular location and functions of two recently identified regulators of chemotaxis, Ca^{2+}-independent phospholipase ($iPLA_2\beta$) and cytosolic phospholipase ($cPLA_2\alpha$), and substantiate their in vivo importance. These enzymes are cytoplasmic in unstimulated monocytes. Upon MCP-1 stimulation, $iPLA_2\beta$ is recruited to the membrane-enriched pseudopod. In contrast, $cPLA_2\alpha$ is recruited to the endoplasmic reticulum. Although $iPLA_2\beta$ or $cPLA_2\alpha$ antisense oligodeoxyribonucleotide (ODN)–treated monocytes display reduced speed, $iPLA_2\beta$ also regulates directionality and actin polymerization. $iPLA_2\beta$ or $cPLA_2\alpha$ antisense ODN–treated adoptively transferred mouse monocytes display a profound defect in migration to the peritoneum in vivo. These converging observations reveal that $iPLA_2\beta$ and $cPLA_2\alpha$ regulate monocyte migration from different intracellular locations, with $iPLA_2\beta$ acting as a critical regulator of the cellular compass, and identify them as potential targets for antiinflammatory strategies.

4.558 SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells
Mossel, E.C. et al
Virology, ***372***, *127-135 (2008)*

Severe acute respiratory syndrome (SARS) is a disease characterized by diffuse alveolar damage. We isolated human alveolar type II cells and maintained them in a highly differentiated state. Type II cell cultures supported SARS-CoV replication as evidenced by RT-PCR detection of viral subgenomic RNA and an increase in virus titer. Virus titers were maximal by 24 h and peaked at approximately 10^5 pfu/mL. Two cell types within the cultures were infected. One cell type was type II cells, which were positive for SP-A, SP-C, cytokeratin, a type II cell-specific monoclonal antibody, and Ep-CAM. The other cell type was composed of spindle-shaped cells that were positive for vimentin and collagen III and likely fibroblasts. Viral replication was not detected in type I-like cells or macrophages. Hence, differentiated adult human alveolar type II cells were infectible but alveolar type I-like cells and alveolar macrophages did not support productive infection.

4.559 Phase I study of α-galactosylceramide-pulsed antigen presenting cells administration to the nasal

submucosa in unresectable or recurrent head and neck cancer
Uchida, T. et al
*Cancer Immunol. Immunother., **57**, 337-345 (2008)*

Background Human Vα24 natural killer T (NKT) cells are activated by the specific ligand, α-galactosylceramide (α-GalCer), in a CD1d-dependent manner. Potent anti-tumor activity of activated NKT cells has been previously demonstrated.
Methods We conducted a phase I study with α-GalCer-pulsed antigen presenting cells (APCs) administered in the nasal submucosa of patients with head and neck cancer, and evaluated the safety and feasibility of such a treatment. Nine patients with unresectable or recurrent head and neck cancer received two treatments 1 week apart, of 1×10^8 of α-GalCer-pulsed autologous APCs into the nasal submucosa.
Results During the clinical study period, no serious adverse events (Common Terminology Criteria for Adverse Events version 3.0 greater than grade 3) were observed. After the first and the second administration of α-GalCer-pulsed APCs, an increased number of NKT cells was observed in four patients and enhanced natural killer activity was detected in the peripheral blood of eight patients.
Conclusion The administration of α-GalCer-pulsed APCs into the nasal submucosa was found to be safe and induce anti-tumor activity in some patients.

4.560 Islet Cell Transplantation. *How Effective Is It?*
Liu, E.H. and Harlan, D.M.
Controversies in Treating Diabetes, 11-32 (2008), Humana Press

Islet cell transplantation as a treatment for diabetes has shown great promise, but has significant limitations. Since early animal studies in rats demonstrated its ability to restore euglycemia, islet transplantation has not proven to be a durable or practical therapy for type 1 diabetes. Here we review some of the history, technique, clinical outcomes, and potential alternatives of islet transplantation.

4.561 Galectin-3 Is an Amplifier of Inflammation in Atherosclerotic Plaque Progression Through Macrophage Activation And Monocyte Chemoattraction
Papaspyridonos, M. et al
*Arterioscler. Thromb. Vasc. Biol., **28**, 433-440 (2008)*

Objective— Galectin-3 (Gal-3) is a 26-kDa lectin known to regulate many aspects of inflammatory cell behavior. We assessed the hypothesis that increased levels of Gal-3 contribute to atherosclerotic plaque progression by enhancing monocyte chemoattraction through macrophage activation.
Methods and Results— Gal-3 was found to be upregulated in unstable plaque regions of carotid endarterectomy (CEA) specimens compared with stable regions from the same patient (3.2-fold, $P<0.05$) at the mRNA (n=12) and (2.3-fold, $P<0.01$) at the protein level (n=9). Analysis of aortic tissue from $ApoE^{-/-}$ mice on a high fat diet (n=14) and wild-type controls (n=9) showed that Gal-3 mRNA and protein levels are elevated by 16.3-fold ($P<0.001$) and 12.2-fold ($P<0.01$) and that Gal-3 staining colocalizes with macrophages. In vitro, conditioned media from Gal-3–treated human macrophages induced an up to 6-fold increase in human monocyte chemotaxis ($P<0.01$, ANOVA), an effect that was reduced by 66 and 60% by Pertussis Toxin (PTX) and the Vaccinia virus protein 35K, respectively. Microarray analysis of human macrophages and subsequent qPCR validation confirmed the upregulation of CC chemokines in response to Gal-3 treatment.
Conclusions— Our data suggest that Gal-3 is both a marker of atherosclerotic plaque progression and a central contributor to the pathology by amplification of key proinflammatory molecules.
Galectin-3 was upregulated in advanced human and murine $ApoE^{-/-}$ atherosclerotic plaques. Mediators released in response to Gal-3 treatment in macrophages increased monocyte chemotaxis, and microarray analysis confirmed the upregulation of several key chemoattractant molecules. Gal-3 is an amplifier of inflammation that could be used as a marker of atherosclerotic plaque progression or target for atherosclerosis.

4.562 PECAM-1 Polymorphism Affects Monocyte Adhesion to Endothelial Cells
Goodman, R.S. et al
*Transplantation, **85(3)**, 471-477 (2008)*

Background. Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) plays an important role in leukocyte-endothelial cell adhesion and transmigration. Single nucleotide polymorphisms of PECAM-1 encoding amino acid substitutions at positions 98 leucine/valine (L/V), 536 serine/asparagine (S/N), and 643 arginine/glycine (R/G) occur in strong genetic linkage resulting in two common haplotypes (LSR and

VNG). These PECAM-1 polymorphisms are associated with graft-versus-host disease after hematopoietic stem cell transplantation and with cardiovascular disease, but whether they influence PECAM-1 function is unknown.
Methods. We examined the effect of homozygous and heterozygous expression of the PECAM-1 LSR and VNG genotypes on the adhesive interactions of peripheral blood monocytes and activated endothelial cell monolayers under shear stress in a flow-based cell adhesion assay.
Results. There was no difference in monocyte adhesion between the two homozygous genotypes of PECAM-1 but when monocytes expressed both alleles in heterozygous form, firm adhesion of monocytes to endothelial cells was markedly increased. PECAM-1 polymorphism expressed in homozygous or heterozygous form by endothelial cells did not influence monocyte adhesion.
Conclusions. This is, to our knowledge, the first demonstration that PECAM-1 genotype can alter the level of monocyte binding to endothelial cells and a demonstration that heterozygous expression of a polymorphic protein may lead to altered function.

4.563 Acetylcholinesterase Expression in Muscle Is Specifically Controlled by a Promoter-Selective Enhancesome in the First Intron
Camp, S. et al
J. Neurosci., ***28(10)***, *2459-2470 (2008)*

Mammalian acetylcholinesterase (*AChE*) gene expression is exquisitely regulated in target tissues and cells during differentiation. An intron located between the first and second exons governs a ~100-fold increase in AChE expression during myoblast to myotube differentiation in C2C12 cells. Regulation is confined to 255 bp of evolutionarily conserved sequence containing functional transcription factor consensus motifs that indirectly interact with the endogenous promoter. To examine control *in vivo*, this region was deleted by homologous recombination. The knock-out mouse is virtually devoid of AChE activity and its encoding mRNA in skeletal muscle, yet activities in brain and spinal cord innervating skeletal muscle are unaltered. The transcription factors MyoD and myocyte enhancer factor-2 appear to be responsible for muscle regulation. Selective control of AChE expression by this region is also found in hematopoietic lineages. Expression patterns in muscle and CNS neurons establish that virtually all AChE activity at the mammalian neuromuscular junction arises from skeletal muscle rather than from biosynthesis in the motoneuron cell body and axoplasmic transport.

4.564 Paracrine Activation of Hepatic CB_1 Receptors by Stellate Cell-Derived Endocannabinoids Mediates Alcoholic Fatty Liver
Jeong, W-i. et al
Cell Metabolism, ***7***, *227-235 (2008)*

Alcohol-induced fatty liver, a major cause of morbidity, has been attributed to enhanced hepatic lipogenesis and decreased fat clearance of unknown mechanism. Here we report that the steatosis induced in mice by a low-fat, liquid ethanol diet is attenuated by concurrent blockade of cannabinoid CB_1 receptors. Global or hepatocyte-specific CB_1 knockout mice are resistant to ethanol-induced steatosis and increases in lipogenic gene expression and have increased carnitine palmitoyltransferase 1 activity, which, unlike in controls, is not reduced by ethanol treatment. Ethanol feeding increases the hepatic expression of CB_1 receptors and upregulates the endocannabinoid 2-arachidonoylglycerol (2-AG) and its biosynthetic enzyme diacylglycerol lipase β selectively in hepatic stellate cells. In control but not CB_1 receptor-deficient hepatocytes, coculture with stellate cells from ethanol-fed mice results in upregulation of CB_1 receptors and lipogenic gene expression. We conclude that paracrine activation of hepatic CB_1 receptors by stellate cell-derived 2-AG mediates ethanol-induced steatosis through increasing lipogenesis and decreasing fatty acid oxidation.

4.565 Increased intraneuronal resting [Ca^{2+}] in adult Alzheimer's disease mice
Lopez, J.R. et al
J. Neurochem., ***105***, *262-271 (2008)*

Neurodegeneration in Alzheimer's disease (AD) has been linked to intracellular accumulation of misfolded proteins and dysregulation of intracellular Ca^{2+}. In the current work, we determined the contribution of specific Ca^{2+} pathways to an alteration in Ca^{2+} homeostasis in primary cortical neurons from an adult triple transgenic (3xTg-AD) mouse model of AD that exhibits intraneuronal accumulation of β-amyloid proteins.

Resting free Ca^{2+} concentration ($[Ca^{2+}]_i$), as measured with Ca^{2+}-selective microelectrodes, was greatly elevated in neurons from 3xTg-AD and APP_{SWE} mouse strains when compared with their respective non-transgenic neurons, while there was no alteration in the resting membrane potential. In the absence of the extracellular Ca^{2+}, the $[Ca^{2+}]_i$ returned to near normal levels in 3xTg-AD neurons, demonstrating that extracellular Ca^{2+}contributed to elevated $[Ca^{2+}]_i$. Application of nifedipine, or a non-L-type channel blocker, SKF-96365, partially reduced $[Ca^{2+}]_i$. Blocking the ryanodine receptors, with ryanodine or FLA-365 had no effect, suggesting that these channels do not contribute to the elevated $[Ca^{2+}]_i$. Conversely, inhibition of inositol trisphosphate receptors with xestospongin C produced a partial reduction in $[Ca^{2+}]_i$. These results demonstrate that an elevation in resting $[Ca^{2+}]_i$, contributed by aberrant Ca^{2+}entry and release pathways, should be considered a major component of the abnormal Ca^{2+} homeostasis associated with AD.

4.566 Characterization and Application of a Glucose-Repressible Promoter in Francisella tularensis
Horzempa, J., Tarwacki, D.M., Carlson Jr., P.E., Robinson, C.M. and Nau, G.J.
*Appl. Envir. Microbiol., **74(7)**, 2161-2170 (2008)*

Francisella tularensis, the causative agent of tularemia, is a category A biodefense agent. The examination of gene function in this organism is limited due to the lack of available controllable promoters. Here, we identify a promoter element of *F. tularensis* LVS that is repressed by glucose (termed the *Francisella g*lucose-*r*epressible *p*romoter, or FGRp), allowing the management of downstream gene expression. In bacteria cultured in medium lacking glucose, this promoter induced the expression of a red fluorescent protein allele, *tdtomato*. FGRp activity was used to produce antisense RNA of *iglC*, an important virulence factor, which severely reduced IglC protein levels. Cultivation in glucose-containing medium restored IglC levels, indicating the usefulness of this promoter for controlling both exogenous and chromosomal gene expression. Moreover, FGRp was shown to be active during the infection of human macrophages by using the fluorescence reporter. In this environment, the FGRp-mediated expression of antisense *iglC* by *F. tularensis* LVS resulted in reduced bacterial fitness, demonstrating the applicability of this promoter. An analysis of the genomic sequence indicated that this promoter region controls a gene, FTL_0580, encoding a hypothetical protein. A deletion analysis determined the critical sites essential for FGRp activity to be located within a 44-bp region. This is the first report of a conditional promoter and the use of antisense constructs in *F. tularensis*, valuable genetic tools for studying gene function both in vitro and in vivo.

4.567 Morphological features and responses to AMPA receptor-mediated excitotoxicity of mouse motor neurons: comparison in purified, mixed anterior horn or motor neuron/glia cocultures
De Paola, M., Diana, V., Bigini, P. And Mennini, T.
*J. Neurosci. Methods, **170**, 85-95 (2008)*

Primary motor neuron cultures are widely used as in vitro model to study the early mechanisms involved in the aetiology of amyotrophic lateral sclerosis. In this study, we directly compared the morphological features and the responses to AMPA receptor (AMPAR) activation of mouse spinal cord motor neurons under different culture conditions (OptiPrep-purified, mixed anterior horn or motor neuron/glia cocultures). Motor neurons cocultured with a confluent glial layer had significant improvements in axonal length and in somata perimeter and area, compared both to mixed anterior horn cultures and to purified cultures, suggesting that the presence of more “mature” glial cells was determinant to obtain healthier motor neurons. By immuno-cytochemical assays we found that both in mixed anterior horn cultures and in cocultures, lower AMPA (0.3 μM) or kainate (5 μM) concentrations, but not the higher (1 or 15 μM, respectively), induced classical apoptotic events such as the nuclear fragmentation, the membrane externalization of phosphatidylserine residues and the activation of caspases-9 and -3. The morphological features and the different degenerative pathways induced by AMPAR agonist concentrations suggest that the experimental conditions used for in vitro studies are key factors that should be deeply considered to obtain more valid and reproducible results.

4.568 Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival
Van Damme, P. et al
*J. Cell Biolo., **181(1)**, 37-41 (2008)*

Recently, mutations in the *progranulin* (*PGRN*) gene were found to cause familial and apparently sporadic frontotemporal lobe dementia (FTLD). Moreover, missense changes in *PGRN* were identified in patients with motor neuron degeneration, a condition that is related to FTLD. Most mutations identified in patients

with FTLD until now have been null mutations. However, it remains unknown whether PGRN protein levels are reduced in the central nervous system from such patients. The effects of PGRN on neurons also remain to be established. We report that PGRN levels are reduced in the cerebrospinal fluid from FTLD patients carrying a PGRN mutation. We observe that PGRN and GRN E (one of the proteolytic fragments of PGRN) promote neuronal survival and enhance neurite outgrowth in cultured neurons. These results demonstrate that PGRN/GRN is a neurotrophic factor with activities that may be involved in the development of the nervous system and in neurodegeneration.

4.569 Costimulation of Dectin-1 and DC-SIGN Triggers the Arachidonic Acid Cascade in Human Monocyte-Derived Dendritic Cells
Valera, I. et al
*J. Immunol., **180**, 5727-5736 (2008)*

Inflammatory mediators derived from arachidonic acid (AA) alter the function of dendritic cells (DC), but data regarding their biosynthesis resulting from stimulation of opsonic and nonopsonic receptors are scarce. To address this issue, the production of eicosanoids by human monocyte-derived DC stimulated via receptors involved in Ag recognition was assessed. Activation of FcγR induced AA release, short-term, low-grade PG biosynthesis, and IL-10 production, whereas zymosan, which contains ligands of both the mannose receptor and the human β-glucan receptor dectin-1, induced a wider set of responses including cyclooxygenase 2 induction and biosynthesis of leukotriene C_4 and IL-12p70. The cytosolic phospholipase A_2 inhibitor pyrrolidine 1 completely inhibited AA release stimulated via all receptors, whereas the spleen tyrosine kinase (Syk) inhibitors piceatannol and R406 fully blocked AA release in response to immune complexes, but only partially blocked the effect of zymosan. Furthermore, anti-dectin-1 mAb partially inhibited the response to zymosan, and this inhibition was enhanced by mAb against DC-specific ICAM-3-grabbing nonintegrin (SIGN). Immunoprecipitation of DC lysates showed coimmunoprecipitation of DC-SIGN and dectin-1, which was confirmed using Myc-dectin-1 and DC-SIGN constructs in HEK293 cells. These data reveal a robust metabolism of AA in human DC stimulated through both opsonic and nonopsonic receptors. The FcγR route depends on the ITAM/Syk/cytosolic phospholipase A_2 axis, whereas the response to zymosan involves the interaction with the C-type lectin receptors dectin-1 and DC-SIGN. These findings help explain the distinct functional properties of DC matured by immune complexes vs those matured by β-glucans.

4.570 Adenoviral-Mediated Overexpression of Either Membrane-Bound Human FasL or Human Decoy Fas Can Prolong Pig Islet Xenograft Survival in a Rat Transplant Model
Kawamoto, K. et al
*Transplantation Proceedings, **40**, 477-479 (2008)*

The success of pancreatic islet transplantation is limited because of the severe shortage of allogeneic pancreas donors. Accordingly, pig islets are considered to be an attractive, promising alternative. However, cell-mediated immunity, especially $CD8^+$ cytotoxic T lymphocyte (CTL)-mediated cytotoxicity, remains a formidable barrier to prevent long-term islet survival in xenograft recipients. Therefore, it is particularly important to explore methods to specifically prevent cell-mediated immunity against pig islets. Our group previously demonstrated that the overexpression of either membrane-bound human FasL or human decoy Fas antigen in pig endothelial cells prevented CTL xenocytotoxicity. In this study, we assessed the cytoprotective effects of adenoviral-mediated overexpression of either membrane-bound human FasL or human decoy Fas antigen in pig islets to inhibit CTL xenocytotoxicity. The CTL-mediated killing of pig islets infected with an adenoviral vector carrying either membrane-bound human FasL or human decoy Fas was significantly reduced compares with that of control pig islets transfected with adenoviral vector encoding enhanced green fluorescent protein (EGFP). Moreover, we transfected pig islets with these molecules to confirm their cytoprotective effects in in vivo studies. The significant long-term survival of pig islets expressing these molecules was elicited through days 3 to 5 posttransplantation. Thus, these results demonstrated that the remodeling of either death receptor or death ligand on pig islets by adenoviral gene transfer prevented innate cellular immunity against xeno-islet grafts facilitating long-term xenograft survival.

4.571 Comparative methodologies of regulatory T cell depletion in a murine melanoma model
Matsushita, N., Pilon-Thomas, S.A., Martin, L.M. and Riker, A.I.
*J. Immunol. Methods, **333**, 167-179 (2008)*

There has been recent interest in the depletion of regulatory T cells (Tregs) as part of a multi-faceted

approach to the immunotherapy of melanoma patients. This is in part due recent findings that convincingly show that Tregs are an integral part of regulating and even suppressing an immune response to growing tumor cells. We therefore compared three methods of Treg depletion and/or elimination, utilizing low dose cyclophosphamide (CY), a specific antibody directed against the IL-2 receptor found on Tregs (PC61) and the use of denileukin diftitox (DD), which is a fusion protein designed to have a direct cytocidal action on cells which express the IL-2 receptor. We show that CY administration resulted in the highest reduction in Tregs among the three reagents. However, the reduction in Tregs with CY was also associated with the concomitant reduction of CD8(+) T cells and a lack of tumor antigen priming. Utilization of DD resulted in a > 50% Treg cell reduction without parallel cytocidal effects upon other T cell subsets but did not enhance anti-tumor immunity against B16 melanoma. Lastly, the PC61 showed a moderate reduction of Tregs that lasted longer than the other reagents, without a reduction in the total number of CD8(+) T cells. Furthermore, PC61 treatment did not abrogate tumor antigen-specific immunity elicited by dendritic cells (DC). We therefore conclude that PC61 administration was the most effective method of reducing Tregs in a murine melanoma model in addition to providing evidence of a synergistic effect when combined with DC-based immunotherapy.

4.572 Tonsilar NK Cells Restrict B Cell Transformation by the Epstein-Barr Virus via IFN-γ
Strowig, T. et al
*PLOS Pathogens, **4(2)**, e27 (2008)*

Cells of the innate immune system act in synergy to provide a first line of defense against pathogens. Here we describe that dendritic cells (DCs), matured with viral products or mimics thereof, including Epstein-Barr virus (EBV), activated natural killer (NK) cells more efficiently than other mature DC preparations. $CD56^{bright}CD16^{-}$ NK cells, which are enriched in human secondary lymphoid tissues, responded primarily to this DC activation. DCs elicited 50-fold stronger interferon-γ (IFN-γ) secretion from tonsilar NK cells than from peripheral blood NK cells, reaching levels that inhibited B cell transformation by EBV. In fact, 100- to 1,000-fold less tonsilar than peripheral blood NK cells were required to achieve the same protection in vitro, indicating that innate immune control of EBV by NK cells is most efficient at this primary site of EBV infection. The high IFN-γ concentrations, produced by tonsilar NK cells, delayed latent EBV antigen expression, resulting in decreased B cell proliferation during the first week after EBV infection in vitro. These results suggest that NK cell activation by DCs can limit primary EBV infection in tonsils until adaptive immunity establishes immune control of this persistent and oncogenic human pathogen.

4.573 Epigallocatechin-3-gallate Inhibits Growth of Activated Hepatic Stellate Cells by Enhancing the Capacity of Glutathione Synthesis
Fu, Y., Zheng, S., Lu, S.C. and Chen, A.
*Mol. Pharmacol., **73(5)**, 1465-1473 (2008)*

Activation of hepatic stellate cells (HSC), the key effectors in hepatic fibrogenesis, is characterized by enhanced cell proliferation and overproduction of extracellular matrix. Oxidative stress promotes HSC activation. Glutathione (GSH) is the most important intracellular antioxidant, whose synthesis is mainly regulated by glutamate-cysteine ligase (GCL). We reported previously that (-)-epigallocatechin-3-gallate (EGCG), the major and most active component in green tea extracts, inhibited HSC activation. The aim of this study is to elucidate the underlying mechanisms. We hypothesize that this inhibitory effect of EGCG might mainly result from its antioxidant capability by increasing de novo synthesis of GSH. In this report, we observe that EGCG enhances the levels of cytoplasmic and mitochondrial GSH and increases GCL activity by inducing gene expression of the catalytic subunit GCLc, leading to de novo synthesis of GSH. Real-time polymerase chain reaction and Western blotting analyses show that de novo synthesis of GSH is required for EGCG to regulate the expression of genes relevant to apoptosis and to cell proliferation. Additional experiments demonstrate that exogenous transforming growth factor (TGF)-β1 suppresses GCLc gene expression and reduces the level of GSH in cultured HSC. Transient transfection assays and Western blotting analyses further display that EGCG interrupts TGF-β signaling by reducing gene expression of TGF-β receptors and Smad4, leading to increased expression of GCLc. These results support our hypothesis and collectively demonstrate that EGCG increases the level of cellular GSH in HSC by stimulating gene expression of GCLc, leading to the inhibition of cell proliferation of activated HSC in vitro.

4.574 Isolation of chicken follicular dendritic cells
Del Cacho, E., Gallego, M., Lopez-Bernard, D.F., Sanchez-Acedo, C. and Liillehoj, H.S.

*J. Immunol. Methods, **334**, 59-69 (2008)*

The aim of the present study was to isolate chicken follicular dendritic cells (FDC). A combination of methods involving panning, iodixanol density gradient centrifugation, and magnetic cell separation technology made it possible to obtain functional FDC from the cecal tonsils from chickens, which had been infected with *Eimeria tenella*. $CD45^{-}$ dendritic cells were selected using the specific monoclonal antibody against chicken CD45, which is a marker for chicken leukocytes, but is not expressed on chicken FDC. Isolated FDC were characterized morphologically, phenotypically and functionally. The phenotype of the selected cells was consistent with FDC in that they expressed IgG, IgM, complement factors C3 and B, ICAM-1, and VCAM-1, but lacked cell surface markers characteristic of macrophages, T-, and B cells. Transmission electron microscopy confirmed their characteristic dendritic morphology. In addition, the identity of the FDC was further confirmed by their ability to trap chicken immune complexes (ICs) on their surface, whereas they did not trap naive antigen (ovalbumin) or ICs generated with mammalian immunoglobulins. Co-culturing allogeneic or autologous isolated FDC with B cells resulted in enhanced B cell proliferation and immunoglobulin production. The lack of MHC restriction, a functional characteristic feature of FDC, further reinforces the identity of the isolated cells as chicken FDC.

4.575 Activation of tonsil dendritic cells with immuno-adjuvants
Polak, M.E., Borthwick, N.J., Gabriel, F.G., Jager, M.J. and Cree, I.A.
*BMC Immunolog, **9**, 10-21 (2008)*

Background
Dendritic cells (DC) play the key role in directing antigen-specific immune responses and manipulating their function may be a useful tool for immunotherapy. The balance between immune stimulation and tolerance is particularly important at mucosal interfaces, where discrimination between dangerous pathogens and innocuous antigens takes place. In humans, although much is known about the responses of monocyte derived DC, relatively little is known about effect of immuno-stimulatory adjuvants on DC found in tonsil.
Results
To examine this, tonsil DC were isolated and cultured with potent DC activators; IFNγ, anti-CD40 antibody, LPS and Poly I:C either singly or in combination. To measure maturation and activation, DC were examined for changes in the expression of HLA-DR, HLA- class I, CD83, CD40, CD80 and CD86 and the release of IL12p70.
The DC isolated from tonsil were a mixed population containing both myeloid and plasmacytoid DC, but all showed similar responses. Tonsil DC released IL12p70 upon stimulation with IFNγ , anti-CD40 antibody, and LPS, but unlike monocyte-derived DC, they did not increase the expression of cell surface activation molecules above those induced by culture alone. Poly I:C, a potent stimulator of laboratory generated DC inhibited the activation of tonsil DC by other adjuvants.
Conclusion
As the response of this mixed population of DC does not mirror that of DC generated *in vitro*, this may have implications for other tissue residing DC and might be an important consideration for immunotherapy.

4.576 Plasmacytoid Dendritic Cells Migrate in Afferent Skin Lymph
Pascale, F. et al
*J. Immunol., **180**, 5963-5972 (2008)*

Conventional dendritic cells enter lymph nodes by migrating from peripheral tissues via the lymphatic route, whereas plasmacytoid dendritic cells (pDC), also called IFN-producing cells (IPC), are described to gain nodes from blood via the high endothelial venules. We demonstrate here that IPC/pDC migrate in the afferent lymph of two large mammals. In sheep, injection of type A CpG oligodinucleotide (ODN) induced lymph cells to produce type I IFN. Furthermore, low-density lymph cells collected at steady state produced type I IFN after stimulation with type A CpG ODN and enveloped viruses. Sheep lymph IPC were found within a minor $B^{neg}CD11c^{neg}$ subset expressing CD45RB. They presented a plasmacytoid morphology, expressed high levels of TLR-7, TLR-9, and IFN regulatory factor 7 mRNA, induced IFN-γ production in allogeneic $CD4^{pos}$ T cells, and differentiated into dendritic cell-like cells under viral stimulation, thus fulfilling criteria of bona fide pDC. In mini-pig, a $CD4^{pos}SIRP^{pos}$ subset in afferent lymph cells, corresponding to pDC homologs, produced type I IFN after type A CpG-ODN triggering. Thus, pDC can link innate and acquired immunity by migrating from tissue to draining node via lymph, similarly to conventional dendritic cells.

4.577 Activation of hepatic natural killer cells and control of liver-adapted lymphoma in the murine model of cytomegalovirus infection
Erlach, K.C. et al
*Med. Microbiol. Immunol., **197**, 167-178 (2008)*

Hematopoietic stem cell transplantation (HSCT) is a promising therapeutic option against hematopoietic malignancies. Infection with cytomegalovirus (CMV) and tumor relapse are complications that limit the success of HSCT. In theory, CMV infection can facilitate tumor relapse and growth by inhibiting "graft take" and reconstitution of the immune system or by inducing the secretion of tumor cell growth-promoting cytokines. Conversely, one can also envisage an anti-tumoral effect of CMV by cytopathic/oncolytic infection of tumor cells, by inducing the secretion of death ligands for tumor cell apoptosis, and by the activation of systemic innate and adaptive immunity. Here we will briefly review the current knowledge about tumor control in a murine model of CMV infection and liver-adapted B cell lymphoma, with a focus on a putative implication of $CD49^{+}NKG2D^{+}$ hepatic natural killer cells.

4.578 Ductal Injection of Preservation Solution Increases Islet Yields in Islet Isolation and Improves Islet Graft Function
Noguchi, H. et al
*Cell Transplantation, **17**, 69-81 (2008)*

For islet transplantation, it is important to obtain an available islet mass adequate for diabetes reversal from a single donor pancreas. A recent report demonstrated that the use of M-Kyoto solution instead of UW solution improved islet yields in the two-layer method for pancreas preservation. The present study investigated whether the ductal injection of a large volume of preservation solution (UW and M-Kyoto solution) before pancreas storage improves islet yields. Islet yield both before and after purification was significantly higher in the ductal injection (+) group compared with the ductal injection (−) group. TUNEL-positive cells in the ductal injection (+) group were significantly decreased in comparison to the ductal injection (−) group. The ductal injection of preservation solution increased the ATP level in the pancreas tissue and reduced trypsin activity during the digestion step. Annexin V and PI assays showed that the ductal injection prevents islet apoptosis. In a transplant model, the ductal injection improved islet graft function. These findings suggest that the ductal injection of preservation solution, especially the M-Kyoto solution, leads to improved outcomes for pancreatic islet transplantation. Based on these data, this technique is now used for clinical islet transplantation from non-heart-beating donor pancreata or living donor pancreas.

4.579 Secretory Unit of Islet in Transplantation (SUIT) and Engrafted Islet Rate (EIR) Indexes Are Useful for Evaluating Single Islet Transplantation
Noguchi, H. et al
*Cell Transplantation, **17,** 121-128 (2008)*

The evaluation of engraftment is important to assess the success of islet transplantation, but it is complex because islet transplantation usually requires two or more donors to achieve euglycemia. Islet transplantation from NHBDs was evaluated using new assessment forms for the secretory unit of islet in transplantation (SUIT) and engrafted islet rate (EIR) indexes. Insulin independence was obtained when the SUIT index was more than 28, which might indicate that 28% of the β-cell mass of a normal subject is required for insulin independence. Because the average EIR for a single transplantation is about 30, the percentage of engrafted islets following one transplantation is about 30%, assuming that a normal subject has 1 million islet equivalents. Although few cultured islet transplants have been performed, the increase of the SUIT and EIR indexes in patients who received cultured islets was significantly lower than in patients who received fresh islets, suggesting that fresh islets may be more effective than cultured islets. The SUIT and EIR indexes are thus considered to be useful values for evaluating islet transplantation, especially for single islet transplantation.

4.580 Nonmyeloablative Chemotherapy Followed by T-cell Adoptive Transfer and Dendritic Cell-based Vaccination Results in Rejection of Established Melanoma
Koike, N., Pilon-Thomas, S. and Mule, J.J.
*J. Immunother., **31(4)**, 402-412 (2008)*

We demonstrated previously that dendritic cell (DC)-based vaccines could mediate a specific and long-lasting antitumor immune response during early lymphoid reconstitution after lethal irradiation and bone marrow transplant. The purpose of this current study was to examine the potential therapeutic efficacy of DC-based vaccines in combination with sublethal lymphodepletion and T-cell transfer. In an aggressive model of melanoma, treatment with the combination of 200 mg/kg cyclophosphamide (Cy) and 100 mg/kg fludarabine (Flu) led to a lymphopenic state lasting approximately 14 days, but had no effect on the growth

of an established M05 melanoma. Addition of ovalbumin (OVA) peptide-pulsed DC-based immunization resulted in a delay in tumor growth but did not enhance overall survival in this model. To improve treatment, adoptively transferred naive T cells were added. After induction of lymphopenia with Cy and Flu, transferred T cells demonstrated an activated memory phenotype including high expression of CD44 and low expression of CD62L. Induction of lymphopenia with Cy and Flu in combination with adoptive transfer of naive T cells and OVA peptide-pulsed DCs immunization led to an enhancement in the number of OVA specific, CD8+ T cells that demonstrated specific cytotoxic activity, proliferation, and interferon-[gamma] production in response to the OVA expressing M05 melanoma. This combination therapy also led to tumor regression and enhanced survival in mice bearing M05 melanoma.

4.581 Uridine diphosphate (UDP) stimulates insulin secretion by activation of P2Y6 receptors
Parandeh, F., Abaraviciene, S.M., Amisten, S., erlinge, D. and Salehi, A.
*Biochem. Biophys. Res. Comm., **370**, 499-503 (2008)*

We examined the transcriptional expression and functional effects of receptors for the extracellular pyrimidines uridine triphosphate (UTP) and uridine diphosphate (UDP), on insulin and glucagon secretion in isolated mouse pancreatic islets and purified β-cells. Using real-time PCR, the UDP receptor $P2Y_6$ was found to be highly expressed in both whole islets and β-cells purified by repeated counter-flow elutriation, whereas no mRNA expression for UTP receptors $P2Y_4$ and $P2Y_2$ could be detected.
Functional *in vitro* experiments revealed that the $P2Y_6$ agonist UDPβS dose-dependently enhanced insulin and glucagon release during short-term incubation (1 h), while $P2Y_6$ activation during a longer period (24 h), selectively increased insulin release, especially at high glucose levels. The corresponding EC_{50} value for UDPβS ranged from 3.2×10^{-8} M to 1.6×10^{-8} M for both glucose concentrations. The $P2Y_6$ antagonist MRS2578 inhibited the effects of UDPβS, supporting a $P2Y_6$ specific effect. In addition to negative RT-PCR results, the lack of response to UTPγS a selective $P2Y_{2/4}$ agonist further rule out the involvement of $P2Y_{2/4}$ receptors in the islet hormone release. Our results suggest a modulatory role for UDP via a functional active $P2Y_6$ receptor in the regulation of islet hormone release.

4.582 Helicobacter pylori-Pulsed Dendritic Cells Induce H. pylori-Specific Immunity in Mice
Zhang, M. et al
*Helicobacter, **13**, 200-208 (2008)*

Background: The growing concern over the emergence of antibiotic-resistant *Helicobacter pylori* infection is propelling the development of an efficacious vaccine to control this highly adaptive organism.
Aim: We studied the use of a dendritic cell (DC)-based vaccine against *H. pylori* infection in mice.
Methods: The cellular immune responses to murine bone marrow-derived DCs pulsed with phosphate-buffered saline (PBS-DC) or live *H. pylori* SS1 (HP-DC) were assessed in vitro and in vivo. The protective immunity against *H. pylori* SS1 oral challenge was compared between HP-DC or PBS-DC immunized mice. The effect of regulatory T-cell (Treg) depletion by anti-CD25 antibody on HP-DC vaccine efficacy was also evaluated.
Results: HP-DC induced a Th1-dominant response in vitro. In vivo, HP-DC immunized mice were characterized by a mixed Th1/Th2 peripheral immune response. However, in the stomach, HP-DC immunized mice expressed a higher level of IFN-γ compared to PBS-DC immunized mice; no difference was found for interleukin-5 expressions in the stomach. A lower bacterial colonization post-*H. pylori* challenge was observed in HP-DC immunized mice compared to PBS-DC immunized mice with no significant difference in gastritis severity. *H. pylori*-specific Th1 response and protective immunity were further enhanced in vivo by depletion of Treg with anti-CD25 antibody.
Conclusion: DC-based anti-*H. pylori* vaccine induced *H. pylori*-specific helper T-cell responses capable of limiting bacterial colonization. Our data support the critical role of effector cellular immune response in the development of *H. pylori* vaccine.

4.583 Conversion Potential of Marrow Cells into Lung Cells Fluctuates with Cytokine-Induced Cell Cycle
Dooner, M.S. et al
*Stem Cells and Development, **17**, 207-219 (2008)*

Green fluorescent protein (GFP)-labeled marrow cells transplanted into lethally irradiated mice can be detected in the lungs of transplanted mice and have been shown to express lung-specific proteins while lacking the expression of hematopoietic markers. We have studied marrow cells induced to transit the cell cycle by exposure to interleukin-3 (IL-3), IL-6, IL-11, and Steel factor at different times of culture

corresponding to different phases of cell cycle. We have found that marrow cells at the G_1 /S interface of the cell cycle have a three-fold increase in cells that assume a nonhematopoietic or pulmonary epithelial cell phenotype and that this increase is no longer seen in late S/G_2. These cells have been characterized as GFP^+ CD45– and GFP^+ $cytokeratin^+$. Thus, marrow cells with the capacity to convert into cells with a lung phenotype after transplantation show a reversible increase with cytokine-induced cell cycle transit. Previous studies have shown that the phenotype of bone marrow stem cells fluctuates reversibly as these cells traverse the cell cycle, leading to a continuum model of stem cell regulation. The present study indicates that marrow stem cell production of nonhematopoietic cells also fluctuates on a continuum.

4.584 Fewer active motors per vesicle may explain slowed vesicle transport in chick motoneurons after three days in vitro
Macosko, J.C. et al
Brain Res., ***1211****, 6-12 (2008)*

Vesicle transport in cultured chick motoneurons was studied over a period of 3 days using motion-enhanced differential interference contrast (MEDIC) microscopy, an improved version of video-enhanced DIC. After 3 days in vitro (DIV), the average vesicle velocity was about 30% less than after 1 DIV. In observations at 1, 2 and 3 DIV, larger vesicles moved more slowly than small vesicles, and retrograde vesicles were larger than anterograde vesicles. The number of retrograde vesicles increased relative to anterograde vesicles after 3 DIV, but this fact alone could not explain the decrease in velocity, since the slowing of vesicle transport in maturing motoneurons was observed independently for both anterograde and retrograde vesicles. In order to better understand the slowing trend, the distance vs. time trajectories of individual vesicles were examined at a frame rate of 8.3/s. Qualitatively, these trajectories consisted of short (1–2 s) segments of constant velocity, and the changes in velocity between segments were abrupt (< 0.2 s). The trajectories were therefore fit to a series of connected straight lines. Surprisingly, the slopes of theses lines, i.e. the vesicle velocities, were often found to be multiples of ~ 0.6 μm/s. The velocity histogram showed multiple peaks, which, when fit with Gaussians using a least squares minimization, yielded an average spacing of 0.57 μm/s (taken as the slope of a fit to peak position vs. peak number, $R^2 = 0.994$). We propose that the abrupt velocity changes occur when 1 or 2 motors suddenly begin or cease actively participating in vesicle transport. Under this hypothesis, the decrease in average vesicle velocity observed for maturing motoneurons is due to a decrease in the average number of active motors per vesicle.

4.585 Translating innate response into long-lasting antibody response by the intrinsic antigen-adjuvant properties of papaya mosaic virus
Acosta-Ramirez, E. et al
Immunology, ***124****, 186-197 (2008)*

Identifying the properties of a molecule involved in the efficient activation of the innate and adaptive immune responses that lead to long-lasting immunity is crucial for vaccine and adjuvant development. Here we show that the papaya mosaic virus (PapMV) is recognized by the immune system as a pathogen-associated molecular pattern (PAMP) and as an antigen in mice (Pamptigen). A single immunization of PapMV without added adjuvant efficiently induced both cellular and specific long-lasting antibody responses. PapMV also efficiently activated innate immune responses, as shown by the induction of lipid raft aggregation, secretion of pro-inflammatory cytokines, up-regulation of co-stimulatory molecules on dendritic cells and macrophages, and long-lasting adjuvant effects upon the specific antibody responses to model antigens. PapMV mixed with *Salmonella enterica* serovar Typhi (*S. typhi*) outer membrane protein C increased its protective capacity against challenge with *S. typhi*, revealing the intrinsic adjuvant properties of PapMV in the induction of immunity. Antigen-presenting cells loaded with PapMV efficiently induced antibody responses *in vivo*, which may link the innate and adaptive responses observed. PapMV recognition as a Pamptigen might be translated into long-lasting antibody responses and protection observed. These properties could be used in the development of new vaccine platforms.

4.586 CD24a Expression Levels Discriminate Langerhans Cells from Dermal Dendritic Cells in Murine Skin and Lymph Nodes
Stutte, S., Jux, B., esser, C. and Förster, I.
J. Invest. Dermatol., ***128****, 1470-1475 (2008)*

Langerhans cells (LCs) and dermal dendritic cells (dDCs) are the professional antigen-presenting cells of the skin. Recently, their immunogenic versus tolerogenic role has come under re-investigation. LCs are

distinguished from dDCs by Langerin (CD207) staining or by detection of Birbeck granules. However, for in vitro experiments it is desirable to have a simple and robust flow cytometric demarcation of both cell types. We show here that CD24a is expressed on LCs but not on dDCs isolated directly from the skin. Moreover, in combination with major histocompatibility complex class II (MHCII), CD24a expression levels distinguish LCs from dDCs in skin-draining lymph nodes after antigen activation and migration. High expression of CD24a correlated strictly with CD207 expression. MHCIIhigh cells were unique for skin-draining lymph nodes and were shown to be the only cells carrying antigen after FITC painting of the skin. CD24a expression levels further differentiated LCs and dDCs in the MHCIIhigh population. As staining for CD24a does not require fixation of cells, CD24a-stained cells can be used for in vitro experiments to analyze and compare the functional roles and properties of dDCs and LCs.

4.587 The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues

Waskow, C. et al

Nature Immunol., ***9(6)***, *676-683 (2008)*

Dendritic cell (DC) development begins in the bone marrow but is not completed until after immature progenitors reach their sites of residence in lymphoid organs. The hematopoietic growth factors regulating these processes are poorly understood. Here we examined the effects of signaling by the receptor tyrosine kinase Flt3 on macrophage DC progenitors in the bone marrow and on peripheral DCs. We found that the macrophage DC progenitor compartment was responsive to superphysiological amounts of Flt3 ligand but was not dependent on Flt3 for its homeostatic maintenance *in vivo*. In contrast, Flt3 was essential to the regulation of homeostatic DC development in the spleen, where it was needed to maintain normal numbers of DCs by controlling their division in the periphery.

4.588 Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: an example using the Tardigrada

Sands, C.J., Convey, P., Linse, K. and McInnes, S.J.

BMC Ecology, ***8(7)***, *1-11 (2008)*

Background: Meiofauna – multicellular animals captured between sieve size 45 μm and 1000 μm – are a fundamental component of terrestrial, and marine benthic ecosystems, forming an integral element of food webs, and playing a critical roll in nutrient recycling. Most phyla have meiofaunal representatives and studies of these taxa impact on a wide variety of sub-disciplines as well as having social and economic implications. However, studies of variation in meiofauna are presented with several important challenges. Isolating individuals from a sample substrate is a time consuming process, and identification requires increasingly scarce taxonomic expertise. Finding suitable morphological characters in many of these organisms is often difficult even for experts. Molecular markers are extremely useful for identifying variation in morphologically conserved organisms. However, for many species markers need to be developed *de novo*, while DNA can often only be extracted from pooled samples in order to obtain sufficient quantity and quality. Importantly, multiple independent markers are required to reconcile gene evolution with species evolution. In this primarily methodological paper we provide a proof of principle of a novel and effective protocol for the isolation of meiofauna from an environmental sample. We also go on to illustrate examples of the implications arising from subsequent screening for genetic variation at the level of the individual using ribosomal, mitochondrial and single copy nuclear markers.
Results: To isolate individual tardigrades from their habitat substrate we used a non-toxic density gradient media that did not interfere with downstream biochemical processes. Using a simple DNA release technique and nested polymerase chain reaction with universal primers we were able amplify multi-copy and, to some extent, single copy genes from individual tardigrades. Maximum likelihood trees from ribosomal 18S, mitochondrial *cytochrome oxidase subunit* 1, and the single copy nuclear gene Wingless support a recent study indicating that the family Hypsibiidae is a non-monophyletic group. From these sequences we were able to detect variation between individuals at each locus that allowed us to identify the presence of cryptic taxa that would otherwise have been overlooked.
Conclusion: Molecular results obtained from individuals, rather than pooled samples, are a prerequisite to enable levels of variation to be placed into context. In this study we have provided a proof of principle of this approach for meiofaunal tardigrades, an important group of soil biota previously not considered amenable to such studies, thereby paving the way for more comprehensive phylogenetic studies using multiple nuclear markers, and population genetic studies.

4.589 Dendritic cell vaccine with mRNA targeted to the proteasome by polyubiquitination
Hosoi, A. et al
*Biochem. Biophys. Res. Comm., **371**, 242-246 (2008)*

Dendritic cells (DCs) transfected with mRNA encoding tumor-associated antigens (TAAs) can induce tumor-specific T-cell responses. To potentiate this, we transfected mature DCs (mDCs) with mRNA encoding TAA targeted to the proteasome. DCs were generated from bone marrow cells by culture with 20 ng/ml GM-CSF and maturation with 1 μg/ml LPS. These mDCs were then electroporated with 10 μg of mRNA. Antigen presentation after electroporation with *in vitro* transcribed mRNA was compared with mRNA from a construct of the TAA preceded by ubiquitin. Proteasomal targeting of mRNA encoding cotranslationally ubiquitinated antigen was found to enhance intracellular degradation of target protein, and result in more efficient priming and expansion of TAA-specific $CD8^+$ T-cells. We therefore suggest that RNA-transfected DC vaccine efficacy could be improved by the use of mRNA targeted to the proteasome.

4.590 The design of electrospun PLLA nanofiber scaffolds compatible with serum-free growth of primary motor and sensory neurons
Corey, J.M. et al
*Acta Biomaterialia, **4**, 863-875 (2008)*

Aligned electrospun nanofibers direct neurite growth and may prove effective for repair throughout the nervous system. Applying nanofiber scaffolds to different nervous system regions will require prior in vitro testing of scaffold designs with specific neuronal and glial cell types. This would be best accomplished using primary neurons in serum-free media; however, such growth on nanofiber substrates has not yet been achieved. Here we report the development of poly(l-lactic acid) (PLLA) nanofiber substrates that support serum-free growth of primary motor and sensory neurons at low plating densities. In our study, we first compared materials used to anchor fibers to glass to keep cells submerged and maintain fiber alignment. We found that poly(lactic-*co*-glycolic acid) (PLGA) anchors fibers to glass and is less toxic to primary neurons than bandage and glue used in other studies. We then designed a substrate produced by electrospinning PLLA nanofibers directly on cover slips pre-coated with PLGA. This substrate retains fiber alignment even when the fiber bundle detaches from the cover slip and keeps cells in the same focal plane. To see if increasing wettability improves motor neuron survival, some fibers were plasma etched before cell plating. Survival on etched fibers was reduced at the lower plating density. Finally, the alignment of neurons grown on this substrate was equal to nanofiber alignment and surpassed the alignment of neurites from explants tested in a previous study. This substrate should facilitate investigating the behavior of many neuronal types on electrospun fibers in serum-free conditions.

4.591 Culturing Adult Rat Hippocampal Neurons with Long-Interval Changing Media
Majd, S., Zarifkar, A., Rastegar, K. And Takhshid, M.A.
*Iranian Biomed. J., **12(2),** 101-107 (2008)*

Background: Primary cultures of embryonic neurons have been used to introduce a model of neurons in physiological and pathological conditions. However, age-related cellular events limit this method as an optimal model in adult neurodegenerative diseases studies. Besides, short-interval changing media in previous cultures decreases the effectiveness of this model. As an example of this matter, we can refer to the study on some special neuronal secreted factors or the influence of some experimental materials on neurons. Meanwhile, short-interval changing media could remove the effects of some released factors from the environment. In this study, the method for isolation and culturing adult rat hippocampal neurons with longintervals medium changing has been described. Methods: The hippocampal neurons of adult male rats were cultured. We used Neurobasal A/B27 culture medium, papain (2 mg/ml), trypsin 0.25% and collagenase (1mg/ml) for neuronal isolation, OptiPrep density gradient for separation of neurons from other cell types and also debris and FGF2 (10 ng/ml) for increasing neuronal survival and regeneration. Results: The neuronal sprouting and viability were increased by using papain and mild triturating ($P<0.05$). Adult neuronal culturing and their regeneration were impossible without FGF2. It was shown that adding new fresh medium every 4 days and exchanging half of it every 8 days had no detrimental effect on neuronal viability.
Conclusion: This investigation shows the possibility of culturing adult neuronal cells and their maintaining in long-interval media. It could be happened because of adult neurons rely significantly on the neighboring cells secreted factors for living and making synaptic connections. This model is very useful in physiological and pathological studies which need stable conditions of neuronal culture in a long period of

time.

4.592 Adenosine Triphosphate Production by Bovine Spermatozoa and Its Relationship to Semen Fertilizing Ability
Garrett, L.J.A., Revell, S.G. and Leese, H.J.
*J. Androl., **29**, 449-458 (2008)*

This article's objectives are to investigate the relationship between adenosine triphosphate (ATP) production (oxidative phosphorylation and glycolysis) and fertility of bovine spermatozoa, determine the proportion of oxygen consumption devoted to proton leak and that due to nonmitochondrial processes, and discover whether freeze/thawing affects sperm oxygen consumption. Oxygen consumption of bovine spermatozoa was measured using a standard Clark electrode and, for the first time, in an Oxygen Biosensor System (OBS). Total ATP formation by bovine spermatozoa was calculated from the oxygen consumption and lactate production (glycolysis) by the same spermatozoa sample. ATP production varied from 1.99 to 8.09 µmol ATP per 10^8 spermatozoa per hour; glycolysis accounted for 16% to 38% of ATP. Nonmitochondrial oxygen consumption could not be detected in bovine spermatozoa using these methods. A significant proportion (16%–43%) of oxygen consumption was insensitive to oligomycin and was due to "proton leak." There was no significant difference between oxygen consumption of frozen/thawed and fresh spermatozoa for 2 of the 3 bulls tested. However, oxygen consumption of frozen/thawed spermatozoa was significantly higher ($P < .05$) than fresh spermatozoa for the third bull. When ZO_2 of frozen/thawed spermatozoa from 20 bulls was compared with their 49 day nonreturn rates (NRRs), oxygen consumption was correlated positively with NRR (ie, fresh spermatozoa with a higher ZO_2 were more fertile). Moreover, total ATP production correlated with NNR better than ZO_2. Bulls with a lower NRR produce spermatozoa that are susceptible to damage during the freeze/thawing process, causing an increase in ZO_2, possibly due to mitochondrial membrane damage resulting in more energy being expended in maintaining the proton gradient, or capacitation-like changes causing hyperactivation. Oxygen consumption measured in the OBS may be useful in assessing bovine sperm fertility.

4.593 Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease
Kim, E.Y. et al
*Nature Med., **14(6)**, 633-640 (2008)*

To understand the pathogenesis of chronic inflammatory disease, we analyzed an experimental mouse model of chronic lung disease with pathology that resembles asthma and chronic obstructive pulmonary disease (COPD) in humans. In this model, chronic lung disease develops after an infection with a common type of respiratory virus is cleared to only trace levels of noninfectious virus. Chronic inflammatory disease is generally thought to depend on an altered adaptive immune response. However, here we find that this type of disease arises independently of an adaptive immune response and is driven instead by interleukin-13 produced by macrophages that have been stimulated by CD1d-dependent T cell receptor–invariant natural killer T (NKT) cells. This innate immune axis is also activated in the lungs of humans with chronic airway disease due to asthma or COPD. These findings provide new insight into the pathogenesis of chronic inflammatory disease with the discovery that the transition from respiratory viral infection into chronic lung disease requires persistent activation of a previously undescribed NKT cell–macrophage innate immune axis.

4.594 The Proinflammatory Mediator CD40 Ligand Is Increased in the Metabolic Syndrome and Modulated by Adiponectin
Natal, C. et al
*J. Clin. Endocrin. Metab., **93(6)**, 2319-2327 (2008)*

Objectives: We hypothesized that the CD40/CD40 ligand (CD40L) system is up-regulated in the metabolic syndrome (MS) and modulated by adiponectin (AN). The objectives were: 1) to compare plasma and monocyte CD40L in patients with MS and controls and its association with clinical and biochemical parameters, 2) to investigate platelets as a source of soluble CD40L (sCD40L), and 3) to analyze the effects of AN on CD40/CD40L.
Methods: Plasma sCD40L and AN were measured in 246 controls and 128 patients with MS by ELISA. Monocyte CD40/CD40L expression and platelet CD40L content and release were compared in patients with MS and controls. Monocytes and endothelial cells were cultured with AN and CD40/CD40L expression determined by real-time RT-PCR and Western blotting.

Results: Patients with MS had higher sCD40L and lower AN levels than controls (0.89 ± 0.1 *vs.* 0.76 ± 0.07 ng/ml and 10.10 ± 0.65 *vs.* 12.99 ± 0.80 µg/ml, $P < 0.05$). Monocyte CD40/CD40L expression was higher ($P < 0.05$) in patients than controls (CD40: 1.31 ± 0.31 *vs.* 0.80 ± 0.14 arbitrary units; CD40L: 1.24 ± 0.85 *vs.* 0.43 ± 0.14 pg/µg protein). No differences were observed on CD40L content between resting platelets from patients with MS and controls (7.7 ± 3.5 *vs.* 7.2 ± 2.2 pg/µg protein). Stimulated platelets from patients with the MS released more ($P < 0.05$) sCD40L than controls (582 ± 141 *vs.* 334 ± 60% change *vs.* nonstimulated platelets). AN reduced CD40L mRNA and protein expression in monocytes from MS patients and endothelial cells.
Conclusions: The enhanced sCD40L and cellular CD40L expression in the MS suggests that CD40L is of pathophysiological relevance in MS. Also, a new antiinflammatory effect of AN is described through the modulation of the CD40/CD40L system.

4.595 Islet transplantation at the Diabetes Research Institute Japan
Noguchi, H. and Matsumoto, S.
*J. Hepatobiliary Pancreat. Surg., **15**, 278-283 (2008)*

Since the Edmonton Protocol was announced, more than 600 patients with type 1 diabetes at more than 50 institutions have received islet transplantation to treat their disease. We recently established a new islet isolation protocol, called the Kyoto Islet Isolation Method, based on the Ricordi method. It includes an in-situ cooling system for pancreas procurement, pancreatic ductal protection, a modified two-layer (M-Kyoto /perfluorochemical [PFC]) method of pancreas preservation, and a new islet purification solution (Iodixanol-based solution). Using this islet isolation method, we isolated islets from 19 human pancreata of non-heart-beating donors and transplanted 16 preparations into seven patients with type 1 diabetes between April 7, 2004 and November 18, 2005. The percentage of those meeting the release criteria of the Edmonton Protocol was more than 80%. We also performed living-donor transplantation of islets for unstable diabetes on January 19, 2005. Establishment of this method enables us to make diabetic patients insulin-independent, using islets not only from two or three pancreata of non-heart-beating donors but also using islets from half a pancreas from a living donor.

4.596 Glycosylphosphatidylinositol-induced cardiac myocyte death might contribute to the fatal outcome of Plasmodium falciparum malaria
Wennicke, K. et al
*Apoptosis, **13**, 857-866 (2008)*

Background Glycosylphosphatidylinositol (GPI) purified from Plasmodium falciparum has been shown to play an important role as a toxin in the pathology of malaria. Previous studies demonstrated cardiac involvement in patients suffering from severe malaria due to P. falciparum. Therefore, we tested the hypothesis that GPI induces apoptosis in cardiomyocytes.
Methods and results By using TUNEL and caspase activity assays, we provided evidence for apoptosis induction in cardiomyocytes by P. falciparum GPI after 48 h of incubation. A similar result was obtained in heart cells of mice 48 h after in vivo injection of GPI. Gene expression analyses in GPI-treated cardiomyocytes showed an up-regulation of apoptotic genes (apaf-1, bax) and of a myocardial damage marker bnp (brain natriuretic peptide), while a down-regulation was observed for the anti-apoptotic gene bcl-2 and for the heat shock protein hsp70. In spite of inflammatory cytokine gene up-regulation by GPI, co-culture with peripheral mononuclear cells (PMNCs) did not change the results obtained with cardiomyocytes alone, indicating a direct effect of GPI on cardiac myocytes. Co-culture with non-myocytic cardiac cells (NMCCs) resulted in up-regulation of Hsp70 and Bcl-2 genes in GPI-treated cardiomyocytes but without repercussion on the apoptosis level. A malaria-infected patient, presenting fulminant heart failure showed typical signs of cardiac myocyte apoptosis demonstrating the clinical relevance of toxin induced heart damage for the lethality of malaria. Our studies performed in vitro and in mice suggest that the GPI could be responsible for cardiomyocyte apoptosis that occurred in this patient.
Conclusion Plasmodium falciparum GPI-induced apoptosis might participate in the lethality of malaria.

4.597 Different fibrillar Aβ 1–42 concentrations induce adult hippocampal neurons to reenter various phases of the cell cycle
Majd, S., Zarifkar, A., Rastegar, K. And Takhshid, M.A.
*Brain Res., **1218**, 224-229 (2008)*

In Alzheimer's disease (AD) cell cycle reentry precedes neuronal death, which could be induced by many

cytotoxic factors. It is believed that beta amyloid (Aβ), the major component of extracellular plaques in AD, is potent in inducing neurons to reenter cell cycle. In AD brains, neurons expressing cell cycle markers are reported in many brain regions without any plaque formation, although very low levels of Aβ may still be detected. In the other side, because cell cycle reentry is not an immediate cause of apoptosis, neurons may remain in cell cycle phases for some time prior to their final death. In this study we examined if very low concentrations of Aβ 1–42 (picomolar) can trigger the adult neurons to reenter the cell cycle, and the effect of different Aβ concentrations on neuronal progression through different cell cycle phases. Primary adult neurons were treated with Aβ 1–42 at 2×10^{-6}, 2×10^{-5}, 2×10^{-4}, 0.5 and 2.5 μM concentrations. Cyclin D1 and cyclin B1 (the markers for G1 and G2 phases of the cell cycle, respectively) and apoptosis were assessed. Treatment with Aβ at 2.5 μM induced apoptosis. At lower levels however, Aβ promoted neurons entering G1 and G2 phases without apoptosis, with 0.5 μM of Aβ inducing neurons into G2, and $2 \times 10^{-5,}$ 2×10^{-4} into G1 phases. Our results suggested that lower concentrations of Aβ induced neurons to reenter the cell cycle, and different concentrations had differential abilities to promote neurons into various cell cycle phases or trigger their death.

4.598 Quantitative nitric oxide production by rat, bovine and porcine macrophages
Zelnickowa, P. et al
*Nitric Oxide, **19**, 36-41 (2008)*

The aim of this work was to compare *in vitro* nitric oxide (NO) production by rat, bovine and porcine macrophages. NO production was induced by lipopolysaccharide (LPS) or by phorbol 12-myristate 13-acetate (PMA) with ionomycin or recombinant interferon gamma (rIFN-γ) and was assessed by Griess reaction. NO synthase type II (NOS II) expression was quantified by immunocytochemistry, Western blot and real-time polymerase chain reaction (RT-PCR). There were differences in NO production by pulmonary alveolar macrophages (PAM) in all species tested. The largest amounts of NO were produced by rat PAM. Less NO was produced by bovine PAM. Moreover, PAM in rats and cows differed in their abilities to respond to various stimulators. Neither porcine PAM nor Kupffer cells produced NO. Stimulation of porcine PAM with alternative concentrations of LPS did not lead to inducing NO production. Stimulation of porcine PAM with rIFN-γ together with LPS led to a significant increase in the expression of NOS II mRNA, albeit without detectable NO production or NOS II expression on the protein level.

4.599 Characterization of early and terminal complement proteins associated with polymorphonuclear leukocytes in vitro and in vivo after spinal cord injury
Nguyen, H.X., Galvan, M.D. and Anderson, A.J.
*J. Neuroinflammation, **5**, 26-38 (2008)*

Background
The complement system has been suggested to affect injury or disease of the central nervous system (CNS) by regulating numerous physiological events and pathways. The activation of complement following traumatic CNS injury can also result in the formation and deposition of C5b-9 membrane attack complex (C5b-9/MAC), causing cell lysis or sublytic effects on vital CNS cells. Although complement proteins derived from serum/blood-brain barrier breakdown can contribute to injury or disease, infiltrating immune cells may represent an important local source of complement after injury. As the first immune cells to infiltrate the CNS within hours post-injury, polymorphonuclear leukocytes (PMNs) may affect injury through mechanisms associated with complement-mediated events. However, the expression/association of both early and terminal complement proteins by PMNs has not been fully characterized in vitro, and has not observed previously in vivo after traumatic spinal cord injury (SCI).
Method
We investigated the expression of complement mRNAs using rt-PCR and the presence of complement proteins associated with PMNs using immunofluroescence and quantitative flow cytometry.
Results
Stimulated or unstimulated PMNs expressed mRNAs encoding for C1q, C3, and C4, but not C5, C6, C7 or C9 in culture. Complement protein C1q or C3 was also detected in less than 30% of cultured PMNs. In contrast, over 70% of PMNs that infiltrated the injured spinal cord were associated with C1q, C3, C7 and C5b-9/MAC 3 days post-SCI. The localization/association of C7 or C5b-9/MAC with infiltrating PMNs in the injured spinal cord suggests the incorporation or internalization of C7 or C5b-9/MAC bound cellular debris by infiltrating PMNs because C7 and C5b-9/MAC were mostly localized to granular vesicles within PMNs at the spinal cord epicenter region. Furthermore, PMN presence in the injured spinal cord was observed for many weeks post-SCI, suggesting that this infiltrating cell population could chronically affect

complement-mediated events and SCI pathogenesis after trauma.
Conclusion
Data presented here provide the first characterization of early and terminal complement proteins associated with PMNs in vitro and in vivo after SCI. Data also suggest a role for PMNs in the local internalization or deliverance of complement and complement activation in the post-SCI environment.

4.600 The Absence of Lymphoid CD8+ Dendritic Cell Maturation in L-Selectin–/– Respiratory Compartment Attenuates Antiviral Immunity

Pascual, D.W., Wang, X., Kochetkova, I., Callis, G. and Riccardi, C.
J. Immunol., ***181***, *1345-1356 (2008)*

Intratracheal instillation of L-selectin-deficient (L-Sel$^{-/-}$) mice with an adenovirus 2 (Ad2) vector resulted in the lack of respiratory Ad2- or β-galactosidase-specific CTLs with concomitant long-lived β-galactosidase transgene expression in the lungs. The absence of Ag-specific CTLs was attributed to a deficiency in lymphoid CD11c^{+}CD8^{+} dendritic cells (DCs) in the lower respiratory lymph nodes (LRLNs). To enable L-Sel$^{-/-}$ CTL activity, cell-sorted L-Sel$^{-/-}$CD8^{+} T cells were cocultured with cell-sorted L-Sel$^{+/+}$CD8^{+} or CD8^{-} DCs or L-Sel$^{-/-}$CD8^{-} DCs. Only the CD8^{+} DCs restored CTL activity; L-Sel$^{-/-}$CD8^{-} DCs failed to support L-Sel$^{+/+}$ CTLs because these remained immature, lacking the ability to express costimulatory molecules CD40, CD80, or CD86. Although no lung CD8^{+} DCs were detected, the DC environment remained suppressive in L-Sel$^{-/-}$ mice evident by the lack of CTL responses following adenoviral challenge with OVA in recipient L-Sel$^{-/-}$ adoptively transferred with OT-1 CD8^{+} T cells. To assess whether the L-Sel$^{-/-}$CD8^{-} DCs could be induced into maturity, microbial stimulation studies were performed showing the failure of L-Sel$^{-/-}$ LRLN to make matured DCs. When L-Sel$^{-/-}$ mice were subjected in vivo to microbial activation before Ad2 vector dosing, CTL activity was restored stimulating the renewed presence of LRLN CD8^{+} DCs in L-Sel$^{-/-}$ mice. These studies show that impairment of L-Sel$^{-/-}$ DC maturation results in insufficient mature DCs that require microbial activation to restore increases in respiratory CD8^{+} DCs to support CTL responses.

4.601 Proteomic Methodological Recommendations for Studies Involving Human Plasma, Platelets, and Peripheral Blood Mononuclear Cells

De Roos, B. et al
J. Proteome Res., ***7***, *2280-2290 (2008)*

This study was designed to develop, optimize and validate protocols for blood processing prior to proteomic analysis of plasma, platelets and peripheral blood mononuclear cells (PBMC) and to determine analytical variation of a single sample of depleted plasma, platelet and PBMC proteins within and between four laboratories each using their own standard operating protocols for 2D gel electrophoresis. Plasma depleted either using the Beckman Coulter IgY-12 proteome partitioning kit or the Amersham albumin and IgG depletion columns gave good quality gels, but reproducibility appeared better with the single-use immuno-affinity column. The use of the Millipore Filter Device for protein concentration gave a 16% ($p < 0.005$) higher recovery of protein in flow-through sample compared with acetone precipitation. The use of OptiPrep gave the lowest level of platelet contamination (1:0.8) during the isolation of PBMC from blood. Several proteins (among which are α-tropomyosin, fibrinogen and coagulation factor XIII A) were identified that may be used as biomarkers of platelet contamination in future studies. When identifying preselected spots, at least three out of the four centers found similar identities for 10 out of the 10 plasma proteins, 8 out of the 10 platelet proteins and 8 out of the 10 PBMC proteins. The discrepancy in spot identifications has been described before and may be explained by the mis-selection of spots due to laboratory-to-laboratory variation in gel formats, low scores on the peptide analysis leading to no or only tentative identifications, or incomplete resolution of different proteins in what appears as a single abundant spot. The average within-laboratory coefficient of variation (CV) for each of the matched spots after automatic matching using either PDQuest or ProteomWeaver software ranged between 18 and 69% for depleted plasma proteins, between 21 and 55% for platelet proteins, and between 22 and 38% for PBMC proteins. Subsequent manual matching improved the CV with on average between 1 and 16%. The average between laboratory CV for each of the matched spots after automatic matching ranged between 4 and 54% for depleted plasma proteins, between 5 and 60% for platelet proteins, and between 18 and 70% for PBMC proteins. This variation must be considered when designing sufficiently powered studies that use proteomics tools for biomarker discovery. The use of tricine in the running buffer for the second dimension appears to enhance the resolution of proteins especially in the high molecular weight range.

4.602 Cerebellar granule cells cultured from adolescent rats express functional NMDA receptors: an in

vitro model for studying the developing cerebellum
Popp, R.L., Reneau, J.C. and Dertien, J.S.
J. Neurochem., ***106***, *900-911 (2008)*

In the developing rat cerebellum functional NMDA receptors (NMDARs) expressing the NR2C subunit have been identified on or after postnatal day 19. We obtained primary cultured cells from 19- to 35-day-old rat cerebellum that expressed few oligodendrocytes or astrocytes. Cultured cells were immunoreactive for neuron-specific proteins thus indicating a neuronal population. The primary neuron present was the granule cell as indicated by immunofluorescence for the $GABA_A$ alpha 6 subunit. Whole-cell patch-clamp experiments indicated that functional NMDARs were present. Functional characteristics of NMDARs expressed in cerebellar granule cells (CGCs) obtained from adolescent animals were similar to those previously reported for NMDARs expressed in CGCs obtained from neonatal rats. Cultured CGCs obtained from older animals contained NMDARs that were inhibited by EtOH and were less sensitive to the NR2B subunit-specific antagonist Ro 25-6981. Furthermore, NMDA-induced currents were smaller than those observed in CGCs. Western blot analysis indicated the presence of the NMDA NR2A and NR2C subunits, but not the NR2B in cultures obtained from the adolescent rats. CGCs obtained from adolescent rats express functional NMDARs consistent with a developmental profile observed *in vivo*.

4.603 NO-synthase-/NO-independent regulation of human and murine platelet soluble guanylyl cyclase activity
Gambaryan, S. et al
J. Thromb. Haemostasis, ***6****, 1376-1384 (2008)*

Summary. *Objectives:* Platelets, specialized adhesive cells, play key roles in normal and pathological hemostasis through their ability to rapidly adhere to subendothelial matrix proteins (adhesion) and to other activated platelets (aggregation), functions which are inhibited by nitric oxide (NO). Platelets have been reported to be regulated not only by exogenous endothelium-derived NO, but also by two isoforms of NO synthase, endothelial (eNOS) and inducible (iNOS), endogenously expressed in platelets. However, data concerning expression, regulation and function of eNOS and iNOS in platelets remain controversial. *Methods and results:* Using important positive (endothelial cells, stimulated macrophages) and negative (eNOS/iNOS knock-out mouse) controls, as well as human platelets highly purified by a newly developed protocol, we now demonstrate that human and mouse platelets do not contain eNOS/iNOS proteins or mRNA. NOS substrate (L-arginine), NOS inhibitors (L-NAME, L-NMMA), and eNOS/iNOS deficiency did not produce detectable functional effects on human and mouse platelets. von Willebrand factor (VWF)/ristocetin treatment of platelets increased cGMP by NO-independent activation of soluble guanylyl cyclase (sGC) which correlated with Src kinase-dependent phosphorylation of sGC β_1-subunit-Tyr^{192}. *Conclusions:* Human and mouse platelets do not express eNOS/iNOS. VWF/ristocetin-mediated activation of the sGC/cGMP signaling pathway may contribute to feedback platelet inhibition.

4.604 Use of functional highly purified human platelets for the identification of new proteins of the IPP signaling pathway
Birschmann, I. et al
Thromb. Res., ***122***, *59-68 (2008)*

Introduction
Identification of the full content of platelet proteins and their mRNAs would be helpful for further studies of human platelet function. For this purpose, proteomic as well as transcriptomic methods (SAGE and qRT-PCR) can be utilized, but the purity of the platelet samples studied is crucial.
Here we report the development of a new, effective, and efficient technique for purification of human platelets from washed apheresis platelet concentrates and whole blood.
Materials and methods
Methods used are a combination of differential and gradient centrifugation steps. The level of purification was determined by nephelometry, FACS, and PCR.
Results
We could show that even the P2Y purinoceptor 12 ($P2Y_{12}$) receptor, which undergoes rapid homologous desensitization, was still functional after the purification procedure. The presence of PINCH (particularly interesting new Cys–His protein) and ∗-parvin, which constitute the IPP (ILK–PINCH–parvin) complex together with the integrin-linked kinase (ILK), has been predicted in platelets by proteomic analysis. We could confirm this observation with our purified platelets. Detection of these proteins is an example of the application of this purification protocol that can be used for the verification of proteins postulated by high-

throughput studies.
Conclusions
The procedure for obtaining purified platelets described here provides an essential, much-needed tool for the comprehensive investigation of platelet proteins and functions.

4.605 Factors impacting equine sperm recovery rate and quality following cushioned centrifugation
Waite, J.A. et al
Theriogenology, ***70(4)***, *704-714 (2008)*

Two experiments were conducted to investigate modifications in cushioned centrifugation of stallion semen. Specifically, the effects of tube type, centrifugation medium, cushion type, and centrifugation force on post-centrifugation sperm recovery rate and quality were evaluated. In Experiment 1, sperm recovery rate was higher ($P < 0.05$) in conventional plastic conical-bottom tubes (103%) than in newly developed glass nipple-bottom tubes (96%) following cushioned centrifugation; however, several measures of semen quality (i.e., % total motility [MOT], % progressive motility [PMOT], curvilinear velocity, and average-path velocity) yielded higher values following centrifugation in nipple-bottom tubes ($P < 0.05$). Sperm recovery rate following cushioned centrifugation was similar between semen previously diluted in optically clear centrifugation extender (100%) and semen diluted in opaque centrifugation extender (100%); however, MOT and PMOT were higher in semen subjected to cushioned centrifugation in opaque extender ($P < 0.05$). An extender by tube-type interaction was not detected for recovery rate or post-centrifugation semen quality. In Experiment 2, sperm recovery rate following cushioned centrifugation in nipple-bottom tubes was similar when forces of 400 × *g* or 600 × *g* were applied (90 and 90%, respectively; $P > 0.05$), and no resulting differences in semen quality were detected between these treatment groups ($P > 0.05$). The type of iodixanol cushion medium used (i.e., OptiPrep™, Eqcellsire® Component B, or Cushion Fluid™) did not impact post-centrifugation semen quality, based on the laboratory values measured ($P > 0.05$). In conclusion, cushioned centrifugation of stallion semen in either conical-bottom or nipple-bottom tubes yielded a high sperm harvest, while maintaining sperm function. An optically opaque extender, commonly used in the equine breeding industry, can be used to achieve this goal.

4.606 Expression of a Soluble TGF-β Receptor by Tumor Cells Enhances Dendritic Cell/Tumor Fusion Vaccine Efficacy
Zhang, M., Berndt, B.E., Chen, J-J. And Kao, J.Y.
J. Immunol., ***181***, *3690-3697 (2008)*

Dendritic cell (DC)-based antitumor immunotherapy is a promising cancer therapy. We have previously shown that tumor-derived TGF-β limits the efficacy of the DC/tumor fusion vaccine in mice. In the current study we investigated the effect of neutralizing tumor-derived TGF-β on the efficacy of the DC/tumor fusion vaccine. An adenovirus encoding human TGF-β receptor type II fused to the Fc region of human IgM (Adv-TGF-β-R) or a control adenovirus encoding LacZ (Adv-LacZ) was used to express a soluble form of the neutralizing TGF-β receptor (TGF-β-R). Murine breast carcinoma cells, 4T1, but not bone marrow-derived DCs, were successfully transfected with Adv-TGF-β-R (4T1+Adv-TGF-β-R) using a multiplicity of infection of 300. Immunization with irradiated 4T1+Adv-TGF-β-R tumor cells conferred enhanced antitumor immunity compared with immunization with irradiated 4T1+Adv-LacZ tumor cells. The DC/4T1+Adv-TGF-β-R fusion vaccine offered enhanced protective and therapeutic efficacy compared with the DC/4T1-Adv-LacZ fusion vaccine. Because TGF-β is known to induce regulatory T cells (Tregs), we further showed that the DC/4T1+Adv-TGF-β-R fusion vaccine induced fewer $CD4^+CD25^+Foxp3^+$ Tregs than the DC/4T1+Adv-LacZ fusion vaccine in vitro and in vivo. The suppressive role of splenic $CD4^+CD25^+$ Tregs isolated from mice immunized with DC/4T1+Adv-LacZ was demonstrated using a CTL killing assay. Similar enhanced therapeutic efficacy was observed in murine renal cell carcinoma, RenCa, which expresses a high level of TGF-β. We conclude that the blockade of tumor-derived TGF-β reduces Treg induction by the DC/tumor fusion vaccine and enhances antitumor immunity. This may be an effective strategy to enhance human DC-based antitumor vaccines.

4.607 Age-related differences in NFκB translocation and Bcl-2/Bax ratio caused by TNFα and Abeta42 promote survival in middle-age neurons and death in old neurons
Patel, J.R. and Brewer, G.J.
Exp. Neurol., ***213***, *93-100 (2008)*

Alzheimer's disease is associated with an age-related accumulation of Abeta and inflammation. The

inflammatory mediator, TNFα activates a signaling cascade involving NFκB translocation to the nucleus and a beneficial or detrimental transcriptional response, depending on the age of the neurons and the type of stress applied. Relative to treatment with Abeta42 alone, previously we found that TNFα plus Abeta42, applied to old rat neurons (24 month) is toxic, while the same treatment of middle-age neurons (10 month) is protective. In contrast to improved survival of middle-age rat cortical neurons, neurons from old rats are killed by TNFα plus Abeta42 despite greater p50 nuclear translocation. In middle-age neurons, blocking TNFR1 does not affect NFκB translocation, whereas blocking TNFR2 results in an increase in NFκB translocation. For old neurons, blocking either receptor, does not change NFκB translocation, but improves cell survival. To account for these effects on cell viability in response to TNF + Abeta, measures of the Bcl-2/Bax ratio positively correlate with survival. In the setting of old neurons, these results suggest that overactivated nuclear translocation of NFκB and lower Bcl-2 levels promote death that is reduced by inhibition of either TNFR1 or R2.

4.608 THE PURIFICATION METHOD USING IODIXANOL (OPTIPREP)-BASED DENSITY GRADIENT SIGNIFICANTLY REDUCE CYTOKINE/CHEMOKINE PRODUCTION FROM HUMAN ISLET PREPARATIONS, LEADING TO PROLONG ~ A-CELL SURVIVAL DURING CULTURE

Mita, A. et al

Transplantation. 86(2S) Supplement:570 (2008)

Background: Although Ficoll-based density gradient has been widely used for human islet purifi cation in most islet processing centers, OptiPrep-based density gradient was recently used in the limited centers. It has been reported that Islet preparations purifi ed using OptiPrep-based density gradient provided better clinical outcomes. It is well known that cytokine/chemokine production from islet preparations widely varies. Reducing cytokine/chemokine production from islet preparations may be a key to improve islet transplantation outcomes. The aim of current study is to investigate the variability of pro-infl ammatory cytokine/chemokine production from human islet preparations purifi ed using different density gradients.
Methods: Human islet isolations were performed using automated method. After digestion phase, pre-purification digests were divided into two groups andpurified using semi-automated cell processor with Ficoll-based or OptiPrepbased density gradient. Human Islet preparations were cultured for 2 days, and assessed regarding glucose stimulated insulin release, islet cell viability (FDA/PI), fractional *ƒ*À-cell viability and *ƒ*À-cell content. Cytokine/chemokine production from islet preparations was also examined.
Results: Between Ficoll-based and OptiPrep-based density gradient groups, islet purity (90.0 ¦ } 4.1% and 91.3 ¦ } 3.2%, respectively, p=0.718), postpurification IEQ (128,504.0 ¦ } 28,893.4 IEQ and 140,881.0 ¦ } 16,644.0 IEQ, respectively, p=0.726) and islet recovery rate (46.4 ¦ } 4.2% and 69.7 ¦ } 22.8%, respectively, p=0.390) were comparable. Although stimulated insulin release (stimulation index 0.5 ¦ } 0.2 and 0.8 ¦ } 0.3, respectively, p=0.123), FDA/PI (92.4 ¦ } 2.5% and 90.6 ¦ } 3.6%, respectively, p=0.550) and fractional *ƒ*À-cell viability showed no signifi cant differences (88.2 ¦ } 7.7% relative to OptiPrep-based density gradient group, p=0.178), *ƒ*À-cell survival during culture signifi cantly improved in OptiPrep-based density gradient group when compared to Ficoll-based density gradient group (130.6 ¦ } 1.8% relative, p<0.05). TNF-*ƒ¿*, IL-1*ƒ*À, IFN-*ƒ*Á, IL-6 and MIP-1*ƒ*À production from OptiPrep-based density gradient group signifi cantly decreased when compared to Ficoll-based density gradient group (22.8 ¦ } 11.8% relative, p=0.003, 27.7 ¦ } 14.0% relative, p=0.002, 37.3 ¦ } 11.2% relative, p=0.001, 45.3 ¦ } 16.2% relative, p=0.015, and 38.5 ¦ } 18.6% relative, p=0.016, respectively).
Conclusions: The purifi cation method using OptiPrep-based density gradient can signifi cantly reduce cytokine/chemokine production from islet preparationswhen compared to Ficoll-based density gradient, leading to the improvement of quantity of *ƒ*À-cell mass. Our results suggest that the purifi cation method using OptiPrep-based density gradient may be of assistance in improving clinical outcomes, and that Cytokine/chemokine profi ling from islet preparations could
be helpful to develop better isolation methods.

4.609 Microfluidic-Based Cell Sorting of *Francisella tularensis* Infected Macrophages Using Optical Forces

Perroud, T.D. et al

*Anal. Chem., **80**, 6365-6372 (2008)*

We have extended the principle of optical tweezers as a noninvasive technique to actively sort hydrodynamically focused cells based on their fluorescence signal in a microfluidic device. This micro fluorescence-activated cell sorter (μFACS) uses an infrared laser to laterally deflect cells into a collection channel. Green-labeled macrophages were sorted from a 40/60 ratio mixture at a throughput of 22 cells/s

over 30 min achieving a 93% sorting purity and a 60% recovery yield. To rule out potential photoinduced cell damage during optical deflection, we investigated the response of mouse macrophage to brief exposures (<4 ms) of focused 1064-nm laser light (9.6 W at the sample). We found no significant difference in viability, cell proliferation, activation state, and functionality between infrared-exposed and unexposed cells. Activation state was measured by the phosphorylation of ERK and nuclear translocation of NF-κB, while functionality was assessed in a similar manner, but after a lipopolysaccharide challenge. To demonstrate the selective nature of optical sorting, we isolated a subpopulation of macrophages highly infected with the fluorescently labeled pathogen *Francisella tularensis* subsp. *novicida*. A total of 10 738 infected cells were sorted at a throughput of 11 cells/s with 93% purity and 39% recovery.

4.610 An improved method for the extraction of nematodes using iodixanol (OptiPrep ™)
Deng, D. et al
*African J. Microbiol. Res., **2**, 167-170 (2008)*

A new nematode extraction technique was established, which is based on an iso-osmotic densitygradient medium (OptiPrep™). This technique resulted in significantly higher numbers of clean eggs and vermiform nematodes that retain higher viability (48.6%) than samples processed with the sucrose method (28.7%). Nematodes survived exposure to OptiPrep™ for 22 hours without significant mortality whereas all nematodes died in the sucrose medium. OptiPrep™ provided a suitable, non-toxic alternative to the traditional density gradient material for the isolation of nematodes. This technique is convenient and relatively simple, with the added benefit of yielding cleaner samples compared to traditional isolation techniques.

4.611 Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans
Jaensson, E. et al
*J. Exp. Med., **205(9)**, 2139-2149 (2008)*

A functionally distinct subset of $CD103^+$ dendritic cells (DCs) has recently been identified in murine mesenteric lymph nodes (MLN) that induces enhanced $FoxP3^+$ T cell differentiation, retinoic acid receptor signaling, and gut-homing receptor (CCR9 and $\alpha4\beta7$) expression in responding T cells. We show that this function is specific to small intestinal lamina propria (SI-LP) and MLN $CD103^+$ DCs. $CD103^+$ SI-LP DCs appeared to derive from circulating DC precursors that continually seed the SI-LP. BrdU pulse-chase experiments suggested that most $CD103^+$ DCs do not derive from a $CD103^-$ SI-LP DC intermediate. The majority of $CD103^+$ MLN DCs appear to represent a tissue-derived migratory population that plays a central role in presenting orally derived soluble antigen to $CD8^+$ and $CD4^+$ T cells. In contrast, most $CD103^-$ MLN DCs appear to derive from blood precursors, and these cells could proliferate within the MLN and present systemic soluble antigen. Critically, $CD103^+$ DCs with similar phenotype and functional properties were present in human MLN, and their selective ability to induce CCR9 was maintained by $CD103^+$ MLN DCs isolated from SB Crohn's patients. Thus, small intestinal $CD103^+$ DCs represent a potential novel target for regulating human intestinal inflammatory responses.

4.612 Chromatin-bound mitogen-activated protein kinases transmit dynamic signals in transcription complexes in β-cells
Lawrence, M.C. et al
*PNAS, **105(36)**, 13315-13320 (2008)*

MAPK pathways regulate transcription through phosphorylation of transcription factors and other DNA-binding proteins. In pancreatic β-cells, ERK1/2 are required for transcription of the insulin gene and several other genes in response to glucose. We show that binding of glucose-sensitive transcription activators and repressors to the insulin gene promoter depends on ERK1/2 activity. We also find that glucose and NGF stimulate the binding of ERK1/2 to the insulin gene and other promoters. An ERK1/2 cascade module, including MEK1/2 and Rsk, are found in complexes bound to these promoters. These findings imply that MAPK-containing signaling complexes are positioned on sensitive promoters with their protein substrates to modulate transcription *in situ* in response to incoming signals.

4.613 Adenovirus-Mediated VEGF Gene Therapy Enhances Venous Thrombus Recanalization and

Resolution
Modarai, B. et al
*Arterioscler. Thromb. Vasc. Biol., **28**, 1753-1759 (2008)*

***Objective*—** Rapid thrombus recanalization reduces the incidence of post–thrombotic complications. This study aimed to discover whether adenovirus-mediated transfection of the vascular endothelial growth factor gene (ad.VEGF) enhanced thrombus recanalization and resolution.
***Methods and Results*—** In rats, thrombi were directly injected with either ad.VEGF (n=40) or ad.GFP (n=37). Thrombi in SCID mice (n=12) were injected with human macrophages transfected with ad.VEGF or ad.GFP. Thrombi were analyzed at 1 to 14 days. GFP was found mainly in the vein wall and adventitia by 3 days, but was predominantly found in cells within the body of thrombus by day 7. VEGF levels peaked at 4 days (376±299 pg/mg protein). Ad.VEGF treatment reduced thrombus size by >50% (47.7±5.1 mm^2 to 22.0±4.0 mm^2, *P*=0.0003) and increased recanalization by >3-fold (3.9±0.69% to 13.6±4.1%, *P*=0.024) compared with controls. Ad.VEGF treatment increased macrophage recruitment into the thrombus by more than 50% (*P*=0.002). Ad.VEGF-transfected macrophages reduced thrombus size by 30% compared with controls (12.3±0.89 mm^2 to 8.7±1.4 mm^2, *P*=0.04) and enhanced vein lumen recanalization (3.39±0.34% to 5.07±0.57%, *P*=0.02).
***Conclusion*—** Treatment with ad.VEGF enhanced thrombus recanalization and resolution, probably as a consequence of an increase in macrophage recruitment.

4.614 Microenvironment of the feto–maternal interface protects the semiallogenic fetus through its immunomodulatory activity on dendritic cells
Zarnani, A.H. et al
*Fertility and Stwerility, **90(3)**, 781-788 (2008)*

Objective
To investigate the immunomodulatory activity of decidual culture supernatant on dendritic cell (DC) functions.
Design
In vivo and in vitro experimental study using mice.
Setting
Academic research laboratory.
Animal(s)
C57BL/6-mated female Balb/c mice.
Intervention(s)
Culture supernatants of decidual cells obtained from the uteri of allogenic pregnant mice (Balb/c × C57BL/6) were collected. Dendritic cells were purified from Balb/c mice spleens and pulsed with antigen during overnight culture. In some cultures, decidual supernatant was added at 5%, 10%, or 20% final concentration. Endometrial culture supernatant-treated DCs served as a control. Antigen-pulsed DCs were injected into the front footpads of syngeneic mice.
Main Outcome Measure(s)
Lymph nodes of primed mice were removed 5 days after DC injection. Antigen-specific proliferation and interleukin-10 and interferon gamma production by lymphocytes were measured by ^{3}H-Thymidine incorporation and ELISA, respectively.
Result(s)
The results showed that decidual culture supernatant markedly blocked in vivo antigen presentation by DCs and inhibited their capacity to induce interferon gamma (but not interleukin-10) production by primed lymphocytes.
Conclusion(s)
It seems that soluble factors produced by decidual cells are important mediators of immunoregulation at the feto–maternal interface, which provide the two fundamental requirements for protection of the semiallogenic fetus, namely immunologic tolerance and predominance of T helper 2 immunity, through modulation of DCs function.

4.615 Carbon Monoxide and Nitric Oxide Mediate Cytoskeletal Reorganization in Microvascular Cells via Vasodilator-Stimulated Phosphoprotein Phosphorylation: Evidence for Blunted Responsiveness in Diabetes
Calzi, S.U. et al
*Diabetes, **57**, 2488-2494 (2008)*

OBJECTIVE— We examined the effect of the vasoactive agents carbon monoxide (CO) and nitric oxide (NO) on the phosphorylation and intracellular redistribution of vasodilator-stimulated phosphoprotein (VASP), a critical actin motor protein required for cell migration that also controls vasodilation and platelet aggregation.
RESEARCH DESIGN AND METHODS— We examined the effect of donor-released CO and NO in endothelial progenitor cells (EPCs) and platelets from nondiabetic and diabetic subjects and in human microvascular endothelial cells (HMECs) cultured under low (5.5 mmol/l) or high (25 mmol/l) glucose conditions. VASP phosphorylation was evaluated using phosphorylation site-specific antibodies.
RESULTS— In control platelets, CO selectively promotes phosphorylation at VASP Ser-157, whereas NO promotes phosphorylation primarily at Ser-157 and also at Ser-239, with maximal responses at 1 min with both agents on Ser-157 and at 15 min on Ser-239 with NO treatment. In diabetic platelets, neither agent resulted in VASP phosphorylation. In nondiabetic EPCs, NO and CO increased phosphorylation at Ser-239 and Ser-157, respectively, but this response was markedly reduced in diabetic EPCs. In endothelial cells cultured under low glucose conditions, both CO and NO induced phosphorylation at Ser-157 and Ser-239; however, this response was completely lost when cells were cultured under high glucose conditions. In control EPCs and in HMECs exposed to low glucose, VASP was redistributed to filopodia-like structures following CO or NO exposure; however, redistribution was dramatically attenuated under high glucose conditions.
CONCLUSIONS— Vasoactive gases CO and NO promote cytoskeletal changes through site- and cell type–specific VASP phosphorylation, and in diabetes, blunted responses to these agents may lead to reduced vascular repair and tissue perfusion.

4.616 Activation of peroxisome proliferator-activated receptor-γ by curcumin blocks the signaling pathways for PDGF and EGF in hepatic stellate cells
Lin, J. and Chen, A.
Lab. Invest., ***88***, *529-540 (2008)*

During hepatic fibrogenesis, reduction in the abundance of peroxisome proliferator-activated receptor-γ (PPARγ) is accompanied by activation of mitogenic signaling for platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) in hepatic stellate cells (HSCs), the major effector cells. We previously reported that curcumin, the yellow pigment in curry, interrupted PDGF and EGF signaling, stimulated PPARγ gene expression, and enhanced its activity, leading to inhibition of cell proliferation of activated HSC in vitro and in vivo. The aim of this study was to elucidate the underlying mechanisms. We hypothesized that the enhancement of PPARγ activity by curcumin might result in the interruption of PDGF and EGF signaling. Our experiments demonstrated that curcumin, with different treatment strategies, showed different efficiencies in the inhibition of PDGF- or EGF-stimulated HSC proliferation. Further experiments observed that curcumin dose dependently reduced gene expression of PDGF and EGF receptors (ie, PDGF-βR and EGFR), which required PPARγ activation. The activation of PPARγ by its agonist suppressed pdgf-βr and egfr expression in HSC. In addition, curcumin reduced the phosphorylation levels of PDGF-βR and EGFR, as well as their downstream signaling cascades, including ERK1/2 and JNK1/2. Moreover, activation of PPARγ induced gene expression of glutamate–cysteine ligase, the rate-limiting enzyme in de novo synthesis of the major intracellular antioxidant, glutathione. De novo synthesis of glutathione was required for curcumin to suppress pdgf-βr and egfr expression in activated HSCs. Our results collectively demonstrated that enhancement of PPARγ activity by curcumin interrupted PDGF and EGF signaling in activated HSCs by reducing the phosphorylation levels of PDGF-βR and EGFR, and by suppressing the receptor gene expression. These results provide novel insights into the mechanisms of curcumin in the inhibition of HSC activation and the suppression of hepatic fibrogenesis.

4.617 GM-CSF mediates autoimmunity by enhancing IL-6–dependent Th17 cell development and survival
Sonderegger, I. et al
J. Exp. Med., ***205(10)***, *2281-2294 (2008)*

Granulocyte macrophage–colony stimulating factor (GM-CSF) is critically involved in development of organ-related autoimmune inflammatory diseases including experimental allergic encephalitis and collagen-induced arthritis. Roles of GM-CSF in the initiation and in the effector phase of the autoimmune response have been proposed. Our study was designed to investigate the mechanisms of GM-CSF in autoimmunity using a model of autoimmune heart inflammatory disease (myocarditis). The pathological sequel after immunization with heart myosin has been shown previously to depend on IL-1, IL-6, IL-23, and IL-17. We found that innate GM-CSF was critical for IL-6 and IL-23 responses by dendritic cells and

generation of pathological Th17 cells in vivo. Moreover, GM-CSF promoted autoimmunity by enhancing IL-6–dependent survival of antigen specific $CD4^+$ T cells. These results suggest a novel role for GM-CSF in promoting generation and maintenance of Th17 cells by regulation of IL-6 and IL-23 in vivo.

4.618 IFN-γ-Dependent Recruitment of Mature CD27high NK Cells to Lymph Nodes Primed by Dendritic Cells
Watt, S.V., Andrews, D.M., Takeda, K., Smyth, M.J. and Hayakawa, Y.
*J. Immunol., **181**, 5323-5330 (2008)*

NK cells have been proposed to be an initial source of IFN-γ that supports either Th1 or CTL priming. Although NK cells reside in naive lymph nodes (LN) at a very low frequency, they can be recruited into LN draining sites of infection, inflammation, or immunization where they potentially influence adaptive immunity. In this study, we report that mature $CD27^{high}$ NK cells are predominantly recruited into the draining LN following dendritic cell (DC) challenge. Importantly, the recruitment of the $CD27^{high}$ NK cell subset in the draining LN was dependent on host IFN-γ and the activation status of NK cells. Endogenous epidermal DC migration induced by hapten challenge also triggers NK cell recruitment to the draining LN in an IFN-γ-dependent mechanism. Thus, our results identify that $CD27^{high}$ NK cells are the dominant population recruited to the draining LN and NK cell recruitment requires endogenous IFN-γ in coordinating with DC migration.

4.619 CD11c identifies a subset of murine liver natural killer cells that responds to adenoviral hepatitis
Burt, B.M. et al
*J. Leukoc. Biol., **84(4)**, 1039-1046 (2008)*

The liver contains a unique repertoire of immune cells and a particular abundance of NK cells. We have found that CD11c defines a distinct subset of NK cells ($NK1.1^+CD3^-$) in the murine liver whose function was currently unknown. In naïve animals, $CD11c^+$ liver NK cells displayed an activated phenotype and possessed enhanced effector functions when compared with $CD11c^-$ liver NK cells. During the innate response to adenovirus infection, $CD11c^+$ NK cells were the more common IFN-γ-producing NK cells in the liver, demonstrated enhanced lytic capability, and gained a modest degree of APC function. The mechanism of IFN-γ production in vivo depended on TLR9 ligation as well as IL-12 and -18. Taken together, our findings demonstrate that $CD11c^+$ NK cells are a unique subset of NK cells in the murine liver that contribute to the defense against adenoviral hepatitis.

4.620 Combined functional and molecular analysis of tumor cell signaling defines 2 distinct myeloma subgroups: Akt-dependent and Akt-independent multiple myeloma
Zöllinger, A. et al
*Blood, **112(8)**, 3403-3411 (2008)*

Although the phosphatidylinositide 3-kinase (PI3K)/Akt pathway has been reported to contribute to the malignant growth of multiple myeloma (MM), the true relevance of Akt kinases for this disease is still unclear. In particular, functional analyses in primary tumor cells and genetic target validation experiments are missing. Here, we used combined functional and molecular analyses to determine the importance of Akt activity in a large panel of primary MM samples and in MM cell lines. Akt down-regulation with isoform-specific siRNA constructs or with an Akt1/2-specific pharmacologic inhibitor strongly induced apoptosis in approximately half of the primary MM samples analyzed. Sensitivity to Akt inhibition strongly correlated with the activation status of Akt as determined by immunohistochemistry, phospho-Akt–specific flow cytometry, and Western analysis. Additional blockade of the MAPK and the IL-6R/STAT3 pathways was often not sufficient to decrease the viability of MM cells resilient to Akt inhibition. Taken together, these experiments led to the identification of 2 myeloma subgroups: Akt-dependent and Akt-independent MM.

4.621 Novel Role for Vascular Endothelial Growth Factor (VEGF) Receptor-1 and Its Ligand VEGF-B in Motor Neuron Degeneration
Poesen, K. et al
*J. Neurosci., **28(42)**, 10451-10459 (2008)*

Although vascular endothelial growth factor-B (VEGF-B) is a homolog of the angiogenic factor VEGF, it has only minimal angiogenic activity, raising the question of whether this factor has other (more relevant) biological properties. Intrigued by the possibility that VEGF family members affect neuronal cells, we

explored whether VEGF-B might have a role in the nervous system. Here, we document that the 60 kDa VEGF-B isoform, VEGF-B^{186}, is a neuroprotective factor. VEGF-B^{186} protected cultured primary motor neurons against degeneration. Mice lacking VEGF-B also developed a more severe form of motor neuron degeneration when intercrossed with mutant SOD1 mice. The *in vitro* and *in vivo* effects of VEGF-B^{186} were dependent on the tyrosine kinase activities of its receptor, Flt1, in motor neurons. When delivered intracerebroventricularly, VEGF-B^{186} prolonged the survival of mutant SOD1 rats. Compared with a similar dose of VEGF, VEGF-B^{186} was safer and did not cause vessel growth or blood–brain barrier leakiness. The neuroprotective activity of VEGF-B, in combination with its negligible angiogenic/permeability activity, offers attractive opportunities for the treatment of neurodegenerative diseases.

4.622 Analysis of Donor- and Isolation-Related Variables From Non-Heart-Beating Donors (NHBDs) Using the Kyoto Islet Isolation Method
Liu, X. et al
*Cel Transplantation, **17**, 649-656 (2008)*

Recently, we demonstrated that islet transplantation from non-heart-beating donors (NHBDs) using the Kyoto islet isolation method (KIIM) successfully reversed patients' diabetes state. In this study, we evaluated the effects of donor- and isolation-related variables on islet isolation results from NHBDs by KIIM. Twenty-one islet preparations from the pancreata of NHBDs were isolated by KIIM. Islet preparations that met transplantation criteria and achieved improved patient diabetes control after transplantation were defined as successful isolations. Potential risk factors deemed to affect islet isolation results, such as age, gender, body mass index, hospital stay, donors' blood biochemical tests, a modified pancreata procurement method, and isolation and purification procedure-related variables, were analyzed. Seventeen out of 21 islet isolations (81%) were successful isolations. Postpurification islet yield was 447,639 ± 39,902 islet equivalents (IE) in the successful isolation group and 108,007 ± 31,532 IE in the failure group. Donor age was significantly younger in the success group (41.9 ± 4.0 years old in the success group vs. 57.5 ± 2.2 years old in the failure group, $p = 0.003$). Chronic pancreatitis significantly decreased islet yields ($p = 0.006$). Phase I time was significantly shorter ($p = 0.010$) and undigested tissue volume was significantly smaller ($p = 0.020$) in the success group. Purity was in positive correlation to postpurification islet yield, while donor age was in reverse correlation to postpurification islet yield. KIIM enables us to perform islet transplantation from NHBDs; however, the decision to use pancreata from older donors or those with chronic pancreatitis requires careful consideration.

4.623 Ikaros Regulates Notch Target Gene Expression in Developing Thymocytes
Chari, S. and Winandy, S.
*J. Immunol., **181**, 6265-6274 (2008)*

Both Ikaros and Notch are essential for normal T cell development. Collaborative mutations causing a reduction in Ikaros activity and an increase in Notch activation promote T cell leukemogenesis. Although the molecular mechanisms of this cooperation have been studied, its consequences in thymocyte development remain unexplored. In this study, we show that Ikaros regulates expression of a subset of Notch target genes, including *Hes1*, *Deltex1*, *pTa*, *Gata3*, and *Runx1*, in both Ikaros null T cell leukemia lines and Ikaros null primary thymocytes. In Ikaros null leukemia cells, Notch deregulation occurs at both the level of Notch receptor cleavage and expression of Notch target genes, because re-expression of Ikaros in these cells down-regulates Notch target gene expression without affecting levels of intracellular cleaved Notch. In addition, abnormal expression of Notch target genes is observed in Ikaros null double-positive thymocytes, in the absence of detectable intracellular cleaved Notch. Finally, we show that this role of Ikaros is specific to double-positive and single-positive thymocytes because derepression of Notch target gene expression is not observed in Ikaros null double-negative thymocytes or lineage-depleted bone marrow. Thus, in this study, we provide evidence that Ikaros and Notch play opposing roles in regulation of a subset of Notch target genes and that this role is restricted to developing thymocytes where Ikaros is required to appropriately regulate the Notch program as they progress through T cell development.

4.624 Effect of Interferon-Alpha, Ribavirin, Pentoxifylline, and Interleukin-18 Antibody on Hepatitis C Sera-Stimulated Hepatic Stellate Cell Proliferation
Khan, F., Peltekian, K.M. and Peterson, T.C.
*J. Interferon & Cytokine Res., **28**, 643-652 (2008)*

Chronic hepatitis C virus (HCV) infection is a major cause of liver fibrosis ultimately leading to cirrhosis.

Hepatic stellate cell (HSC) proliferation is crucial in fibrosis development. Current antiviral treatment for HCV involves interferon-alpha (IFN-α) and Ribavirin combination therapy. IL-18, a novel cytokine of the IL-1 family of cytokines, is involved in inflammation and may be important in HCV-related inflammation. We hypothesize that block of one of the crucial events will block fibrosis due to HCV. The effect of HCV patient sera with and without IFN-α, ribavirin, and IL-18 antibody on HSC proliferation was assessed by [^{3}H]-thymidine incorporation assays. Western analysis was used to assess the effect of pentoxifylline (PTX) on c-Jun immediate early gene phosphorylation (p-c-Jun formation). We demonstrate that HCV patient sera-stimulated HSC proliferation. Ribavirin with or without IFN-α significantly decreased HCV sera-stimulated HSC proliferation by 50%. Western analysis revealed that HCV serum increased p-c-Jun levels, which were decreased with Ribavirin and PTX. ELISA results showed an elevation of IL-18 levels in HCV sera when compared to normal sera. IL-18 did not stimulate HSC proliferation. However, IL-18 antibody significantly decreased patient sera-stimulated HSC proliferation. In conclusion, Ribavirin decreased HSC proliferation and may act by decreasing p-c-Jun levels in HSCs. IL-18 alone did not stimulate HSC proliferation but IL-18 antibody decreased stimulation, suggesting that IL-18 may work in conjunction with some other factor to increase HSC proliferation.

4.625 Global transcriptional response to mammalian temperature provides new insight into Francisella tularensis pathogenesis

Horzempa, J., Carlson, P.E., O'Dee, D.N., Shank, R.M.Q. and Nau, G.J.
*BMC Microbiol., **8**, 172-188 (2008)*

Background
After infecting a mammalian host, the facultative intracellular bacterium, *Francisella tularensis*, encounters an elevated environmental temperature. We hypothesized that this temperature change may regulate genes essential for infection.
Results
Microarray analysis of *F. tularensis* LVS shifted from 26°C (environmental) to 37°C (mammalian) showed ~11% of this bacterium's genes were differentially-regulated. Importantly, 40% of the protein-coding genes that were induced at 37°C have been previously implicated in virulence or intracellular growth of *Francisella* in other studies, associating the bacterial response to this temperature shift with pathogenesis. Forty-four percent of the genes induced at 37°C encode proteins of unknown function, suggesting novel *Francisella* virulence traits are regulated by mammalian temperature. To explore this possibility, we generated two mutants of loci induced at 37°C [FTL_1581 and FTL_1664 (*deoB*)]. The FTL_1581 mutant was attenuated in a chicken embryo infection model, which was likely attributable to a defect in survival within macrophages. FTL_1581 encodes a novel hypothetical protein that we suggest naming *t*emperature-*i*nduced, *v*irulence-associated locus *A*, *tivA*. Interestingly, the *deoB* mutant showed diminished entry into mammalian cells compared to wild-type LVS, including primary human macrophages and dendritic cells, the macrophage-like RAW 264.7 line, and non-phagocytic HEK-293 cells. This is the first study identifying a *Francisella* gene that contributes to uptake into both phagocytic and non-phagocytic host cells.
Conclusion
Our results provide new insight into mechanisms of *Francisella* virulence regulation and pathogenesis. *F. tularensis* LVS undergoes considerable gene expression changes in response to mammalian body temperature. This temperature shift is important for the regulation of genes that are critical for the pathogenesis of *Francisella*. Importantly, the compilation of temperature-regulated genes also defines a rich collection of novel candidate virulence determinants, including *tivA* (FTL_1581). An analysis of *tivA* and *deoB* (FTL_1664) revealed that these genes contribute to intracellular survival and entry into mammalian cells, respectively.

4.626 Improved Islet Yields After Purification Following the Novel Endogenous Trypsin Inhibitor and Histidine-Tryptophan-Ketoglutarate Treatment in Pigs

Wee, Y.M. et al
*Transplant. Proceedings, **40**, 2585-2587 (2008)*

Background
Adult porcine islet xenotransplantation into humans is greatly diminished by the difficulty to isolate islets because of their fragility. The goal of this study was to improve the efficacy of islet yields using endogenous trypsin inhibitor and histidine-tryptophan-ketoglutarate (HTK) perfusate.
Method
We compared two porcine islet isolation protocols: Eurocollins solution for in situ pancreas perfusion

without use of an endogenous trypsin inhibitor versus HTK solution including endogenous trypsin inhibitor for pancreas perfusion.
Results
Endogenous trypsin inhibitor and HTK strategies significantly improved total islet yield, recovery, and islet index after purification ($P < .05$), whereas unpurified islet yield did not increase. An average of 228,000 ± 95,000 islet equivalents (IEQ) ($n = 20$) purified islets were obtained in the first group compared with 115,000 ± 56,000 IEQ ($n = 18$) in the second group. The average islet index was significantly increased in the first group compared with the second group before and after purification: before: 0.28 versus 0.49 versus after: 0.25 versus 0.4 ($P < .05$). At this time, islet purity, viability, and stimulation index did not show a significant difference between groups.
Conclusion
Our study showed that endogenous trypsin inhibitor and HTK strategies significantly improved purified islet isolation efficacy because of reduction of islet fragility.

4.627 Essential roles of SHPS-1 in induction of contact hypersensitivity of skin
Motegi, S-I. et al
Immunol. Lett., ***121***, *52-60 (2008)*

SHPS-1 is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 and is abundant on the surface of $CD11c^+$ dendritic cells (DCs). We recently showed that SHPS-1 is essential for priming by DCs of $CD4^+$ T cells and for development of Th17 cell-mediated experimental autoimmunity. We have now further evaluated the importance of SHPS-1 and that of its ligand CD47 in contact hypersensitivity (CHS) to 2,4-dinitro-1-fluorobenzene (DNFB). Whereas the DNFB-induced CHS response was impaired in mice that express a mutant form of SHPS-1 lacking most of the cytoplasmic region, it was unaffected in CD47-deficient mice. Moreover, treatment of wild-type mice with mAbs to SHPS-1 that either block or do not block the binding of SHPS-1 to CD47 inhibited the CHS response. A mAb to CD47 had no such effect. The 2,4-dinitro-benzenesulfonic acid-induced proliferation of, and production of IFN-γ or IL-17 by, T cells from DNFB-sensitized wild-type mice were inhibited by either mAb to SHPS-1 but not by that to CD47. In contrast, the blocking mAbs to SHPS-1, but not that to CD47, inhibited an allogeneic mixed leukocyte reaction. Both mAbs to SHPS-1, but not that to CD47, also inhibited the lipopolysaccharide- or polyinosinic–polycytidylic acid-induced production of TNF-α by DCs. These results suggest that SHPS-1 is essential for development of CHS, likely as a result of its positive regulation of the priming by DCs of $CD4^+$ T cells. However, such regulation by SHPS-1 does not appear to require its interaction with CD47.

4.628 Nitric oxide and MCP-1 regulation in LPS activated rat Kupffer cells
Kolios, G. et al
Mol. Cell. Biochem., ***319***, *91-98 (2008)*

Nitric oxide (NO) and Monocyte Chemoattractant Protein (MCP)-1 co-regulation has been found in endotoxin-activated macrophages. Kupffer cells (KC) are a main source of soluble-mediators production in liver abnormalities. We investigated in vitro similar co-regulation of NO and MCP-1 production in rat activated KC. Isolated rat KC were cultured in the presence of 1 μg/ml LPS and various concentrations of Wortmannin (0–300 nM), L-NAME (0–500 μM) or MCP-1 (0–100 ng/ml). Production of MCP-1 and NO were measured in supernatants, by ELISA and a modification of the Griess reaction, respectively. Growth arrested KC, stimulated with vehicle, produced a basal amount of NO and MCP-1. In the presence of LPS, cultured KC secreted significantly ($P < 0.01$) increased amounts of MCP-1 and NO. Pre-treatment of KC with various concentrations of L-NAME significantly ($P < 0.05$) reduced the LPS-induced secretion of NO in a concentration dependent manner, but the MCP-1 production remained unaffected. Pre-treatment with Wortmannin significantly ($P < 0.05$) inhibited LPS-induced secretion of MCP-1 and NO in a concentration dependent manner. Linear regression analysis revealed a positive correlation between MCP-1 and NO in the LPS ($r = 0.59171$, $P < 0.0001$) and Wortmannin ($r = 0.9215$, $P = 0.009$) treated groups, but not in the L-NAME ($r = -0.08513$, $P = 0.873$). Incubation of KC with various concentrations of MCP-1 did not increase the NO production. These results indicate that KC might be the main source of NO and MCP-1 production in liver disorders, probably through the induction of PI3-kinase(s) and without any co-regulation between these molecules, which might represent two independent immunoregulatory pathways in the role of KC in hepatic disorders.

4.629 Regulation of CCN2 mRNA expression and promoter activity in activated hepatic stellate cells
Leask, A., Chen, S., Pala, D and Brigstock, D.R.

*J. Cell Commun. Signal., **2**, 49-56 (2008)*

The matricellular protein connective tissue growth factor (CCN2) is considered a faithful marker of fibroblast activation in wound healing and in fibrosis. CCN2 is induced during activation of hepatic stellate cells (HSC). Here, we investigate the molecular basis of CCN2 gene expression in HSC. Fluoroscence activated cell sorting was used to investigate CCN2 expression in HSC *in vivo* in mice treated with CCl_4. CCN2 and TGF-β mRNA expression were assessed by polymerase chain reaction as a function of culture-induced activation of HSC. CCN2 promoter/reporter constructs were used to map cis-acting elements required for basal and TGFβ-induced CCN2 promoter activity. Real-time polymerase chain reaction analysis was used to further clarify signaling pathways required for CCN2 expression in HSC. CCl_4 administration *in vivo* increased CCN2 production by HSC. *In vitro*, expression of CCN2 and TGF-β mRNA were concommitantly increased in mouse HSC between days 0 and 14 of culture. TGFβ-induced CCN2 promoter activity required the Smad and Ets-1 elements in the CCN2 promoter and was reduced by TGFβ type I receptor (ALK4/5/7) inhibition. CCN2 overexpression in activated HSC was ALK4/5/7-dependent. As CCN2 overexpression is a faithful marker of fibrogenesis, our data are consistent with the notion that signaling through TGFβ type I receptors such as ALK5 contributes to the activation of HSC and hence ALK4/5/7 inhibition would be expected to be an appropriate treatment for liver fibrosis.

4.630 Hydrogen Peroxide-Induced VCAM-1 Expression in Pancreatic Islets and [beta]-Cells Through Extracellular Ca2+ Influx
Lee, S. et al
*Transplantation, **86(9)**, 1257-1266 (2008)*

Background. The use of porcine islets as alternatives to transplantable human islets is hampered by xenotransplant rejection. To identify molecular mechanisms that would allow subversion of xenoislet rejection, we investigated the role of H2O2 in vascular cell adhesion molecule-1 (VCAM-1) expression by porcine and mouse islets and [beta]-cell lines.
Methods. Porcine islets were treated with H2O2, tumor necrosis factor alpha, interferon-[gamma], interleukin-1[beta], and lipopolysaccharide, to assess the effects of inflammatory stimulators on VCAM-1 expression using flow cytometry. The role of Ca2+ in H2O2-induced VCAM-1 expression was investigated in [beta]-cell lines using an extracellular Ca2+ chelator and Ca2+-depleted media. Furthermore, H2O2-induced VCAM-1 expression was measured in [beta]-cells, pretreated with inhibitors of protein kinase C, phospholipase D, and phosphatidylinositol-3 kinase/Akt. Finally, H2O2-induced VCAM-1 expression was evaluated in porcine islets and rodent [beta]-cell lines infected with an adenovirus encoding catalase, a H2O2-removing enzyme.
Results. H2O2 was most potent inflammatory stimulator of VCAM-1 expression in porcine islets and had the greatest effect on VCAM-1 expression by [beta]-cells. Signaling pathway analysis demonstrated that extracellular Ca2+ influx was critical to H2O2-mediated VCAM-1 expression; however, protein kinase C, phospholipase D, and phosphatidylinositol-3 kinase/Akt activation were not required for VCAM-1 expression. Finally, catalase overexpression inhibited H2O2-induced VCAM-1 expression by islets and [beta]-cell lines.
Conclusion. An extracellular calcium-dependent H2O2 pathway is the critical mediator of VCAM-1 expression by pancreatic islets and [beta]-cells. Inhibition of this pathway by catalase overexpression in donor islets can be exploited to protect against xenoislet immune responses.

4.631 Ex vivo priming of CD4 T cells converts immunological tolerance into effective antitumor immunity in a murine model of acute lymphoblastic leukemia
Hegazy, A.N. and Klein, C.
*Leukemia, **22**, 2070-2079 (2008)*

Tumor escape mechanisms in leukemia are not well defined. To dissect immunological mechanisms responsible for immune tolerance toward leukemia, we established a murine model system allowing clonotypic analysis of leukemia-specific CD4 T cells recognizing ovalbumin (OVA). Upon i.v. injection of genetically engineered leukemia cells, dendritic cells (DCs) engulfed, processed and presented OVA to OVA-specific CD4 T cells. Consequently, leukemia-specific T cells were primed *in vivo* as shown by expression of activation markers and proliferative responses. However, in spite of detectable CD4 T cell responses *in vitro* and *in vivo*, no effective anti-leukemia immunity was established. In contrast, adoptively transferred DO11.10 T cells that were primed *ex vivo* mediated effective antitumor immunity. Furthermore, *ex vivo* primed DO11.10 T cells showed high expression of Th1 cytokines (interferon-ϒ, tumor necrosis factor-α and interleukin-2) whereas *in vivo* primed OVA-specific CD4 T cells showed incomplete

differentiation (proliferation without cytokine production). We conclude that activated T cells lacking effector function develop through incomplete differentiation in leukemia-bearing mice. Thus, priming conditions of leukemia-specific CD4 T cells critically determines the balance between immunity or tolerance toward leukemia.

4.632 Expression of Neurexin, Neuroligin, and Their Cytoplasmic Binding Partners in the Pancreatic β-Cells and the Involvement of Neuroligin in Insulin Secretion
Suckow, A.T. et al
*Endocrinology, **149(12)**, 6006-6017 (2008)*

The composition of the β-cell exocytic machinery is very similar to that of neuronal synapses, and the developmental pathway of β-cells and neurons substantially overlap. β-Cells secrete γ-aminobutyric acid and express proteins that, in the brain, are specific markers of inhibitory synapses. Recently, neuronal coculture experiments have identified three families of synaptic cell-surface molecules (neurexins, neuroligins, and SynCAM) that drive synapse formation *in vitro* and that control the differentiation of nascent synapses into either excitatory or inhibitory fully mature nerve terminals. The inhibitory synapse-like character of the β-cells led us to hypothesize that members of these families of synapse-inducing adhesion molecules would be expressed in β-cells and that the pattern of expression would resemble that associated with neuronal inhibitory synaptogenesis. Here, we describe β-cell expression of the neuroligins, neurexins, and SynCAM, and show that neuroligin expression affects insulin secretion in INS-1 β-cells and rat islet cells. Our findings demonstrate that neuroligins and neurexins are expressed outside the central nervous system and help confer an inhibitory synaptic-like phenotype onto the β-cell surface. Analogous to their role in synaptic neurotransmission, neurexin-neuroligin interactions may play a role in the formation of the submembrane insulin secretory apparatus.

4.633 Semen Processing for the Subfertile Stallion
Varner, D.D. et al
*J. Equine Vet. Sci., **28(11)**, 677-685 (2008)*

Stallions become sires based on three qualities: pedigree, performance record, and conformation. Conspicuously absent from this formula for sire status is that of reproductive health. Stallions represent 50% of the breeding equation, and the horse industry is replete with stallions whose level of fertility is undesirable. In natural-cover programs, physical, mental, or environmental aberrations can result in disruption of efficient semen transfer from the stallion to the reproductive tract of the mare. Some forms of subfertility may have a genetic basis, but subfertility is often associated with aging in stallions, and the attendant effects of age on testicular function. Effects of long-term medications, such as progestogens or anabolic steroids, on testicular health and fertility of younger sires that have recently retired from a performance career, must also be considered. Other environmental effects, such as hot environmental temperature, fever, and genital trauma can also induce a subfertile state in an otherwise fertile stallion. Taken together, these scenarios rationalize the need for veterinary intervention as a means to maximize the fertility of subfertile stallions. This communication contains an assortment of stallion cases in which various semen-processing methods were used in an effort to improve the fertility of subfertile stallions.

4.634 The Effect of Isolation Methods and the Use of Different Enzymes on Islet Yield and In Vivo Function
Sabek, O.M., Cowan, P., Fraga, D.W. and Gaber, A.O.
*Cell Transplantation, **17**, 785-792 (2008)*

The ability to isolate high-yield pure and viable islets from human cadaver pancreas donors is dependent on donor factor as well as isolation factors. The aim of this study was to examine factors influencing islets recovery and in vivo function with an emphasis on donor and isolation methods as well as to compare the effectiveness of Liberase, widely used in clinical islet isolation, with Serva for the isolation of pure functional islets. The results of 123 islet isolations using Liberase for digestion were compared with those of 113 isolations with Serva. Islet equivalents per gram of tissue were similar between Liberase and Serva (3620 ± 1858 vs. 4132 ± 2104, $p < 0.2$) as well as the percent purity (75 ± 16 vs. 74 ± 15, $p < 0.9$). In vivo function of islets from 71 isolations (Liberase = 45, Serva = 26) were further tested by transplantation into NOD-SCID mice following short-term culture (<6 days, $n = 71$). Our data show that both Liberase- and Serva-isolated islets showed similar function results following short-term culture. These data demonstrate

that there is no difference in islet yield, purity, and function between the two enzymes. However, when these 71 isolations were analyzed for in vivo function with emphasis on donor factors, cold ischemia time (12.0 ± 5.3 vs. 15.0 ± 5.7, $p < 0.04$), islet integrity (1.6 ± 0.7 vs. 1.3 ± 0.5, $p < 0.05$), and female gender were the only factors that correlated with in vivo function. We also compared the mechanical-shaking method for islets isolation with hand-shaking methods. Our results show that although there is no different in islet yield, purity, and integrity between different enzymes using the same method, hand-shaking method yields more islets with better integrity than mechanical-shaking method.

4.635 Non-Cell-Autonomous Effect of Human SOD1G37R Astrocytes on Motor Neurons Derived from Human Embryonic Stem Cells
Marchetto, M.C.N. et al
*Cell Stem Cell, **3**, 649-657 (2008)*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron death. ALS can be induced by mutations in the superoxide dismutase 1 gene (SOD1). Evidence for the non-cell-autonomous nature of ALS emerged from the observation that wild-type glial cells extended the survival of SOD1 mutant motor neurons in chimeric mice. To uncover the contribution of astrocytes to human motor neuron degeneration, we cocultured hESC-derived motor neurons with human primary astrocytes expressing mutated SOD1. We detected a selective motor neuron toxicity that was correlated with increased inflammatory response in SOD1-mutated astrocytes. Furthermore, we present evidence that astrocytes can activate NOX2 to produce superoxide and that effect can be reversed by antioxidants. We show that NOX2 inhibitor, apocynin, can prevent the loss of motor neurons caused by SOD1-mutated astrocytes. These results provide an assay for drug screening using a human ALS in vitro astrocyte-based cell model.

4.636 FUSION OF THE TETANUS TOXIN C FRAGMENT BINDING DOMAIN AND BCL-XL FOR PROTECTION OF PERIPHERAL NERVE NEURONS
Carlton, E. et al
*Neurosurgery, **63(6)**, 1175-1184 (2008)*

OBJECTIVE: Apoptosis has been shown to play an important role in motor neuron (MN) degeneration in both neurodegenerative disease and peripheral neuropathy. Bcl-xL, an antiapoptotic protein, is down-regulated in these origins. The carboxyl-terminal domain of the tetanus toxin heavy chain (Hc) has high affinity for axon terminal binding and uptake into motor and dorsal root ganglion (DRG) neurons. We report the development of a fusion protein between Hc and Bcl-xL to enhance uptake of Bcl-xL by MNs as a strategy for inhibiting peripheral neuronal apoptosis.
METHODS: The genes for Hc, Bcl-xL, and green fluorescent protein were cloned into an Escherichia coli expression system in 2 different arrangements. Fusion proteins were purified through chromatography. Cultured E15 rat spinal cord MNs and DRG cells were used to demonstrate neuron-specific uptake and retrograde transport of the fusion proteins mediated by Hc. Finally, glutamate-induced apoptosis was used as an in vitro model to measure the antiapoptotic effects of the fusion proteins.
RESULTS: Bcl-xL fusion proteins were found to bind specifically and undergo uptake into cultured rat spinal MNs. The fusion proteins were also taken up by DRG axonal terminals and transported back to the cell bodies in Campenot compartmentalized chambers (Tyler Research Corp., Edmonton, Canada). Finally, fusion protein application improved cell survival and decreased apoptosis in glutamate-mediated excitotoxicity of the SH-SY5Y neuronal cells.
CONCLUSION: Hc can be applied as a universal carrier for therapeutic cargo delivery specifically to MNs or DRGs. The fusion proteins between Bcl-xL and Hc constructed in this study might bear applications to the treatment of MN disease, neuropathy, or nerve injury through nerve or intramuscular injection.

4.637 Elastin peptide receptor-directed monocyte chemotactic polysaccharides derived from seaweed sporophyll and from infectious fungus
Li,Y. et al
*Microbial Pathogenesis, **45**, 423-434 (2008)*

We discovered that a seaweed sporophyll-derived polysaccharide of brown alga, *Wakame* (*Undaria pinnatifida*) bound to monocytes and attracted them *in vitro* and *in vivo*. Physicochemical properties, affinity to a lectin-bead column and sugar composition of the chemotactic polysaccharide indicated this molecule to be a highly sulfated fucogalactan. We then identified the monocyte receptor of the sulfated fucogalactan as the elastin peptide receptor by prophylactic inhibition of the binding and the

chemoattraction with lactose and the synthetic elastin peptide, Val-Gly-Val-Ala-Pro-Gly. We assume that the galactose-binding lectin, which is a component of the elastin peptide receptor complex, would recognize a Gal residue of the sulfated fucogalactan. We also observed a similar chemoattracting polysaccharide in a pathogenic fungus, *Candida albicans*, although the content of it was much lower than in the case of seaweed sporophyll. We speculate that the chemotactic response of monocytes to the sulfated fucogalactan is part of the innate immune system to fungal infection.

4.638 Nrf2 Activation in Astrocytes Protects against Neurodegeneration in Mouse Models of Familial Amyotrophic Lateral Sclerosis

Vargas, M.R., Johnson, D.A., Sirkis, D.W., Messing, A. and Johnson, J.A.
J. Neurosci., ***28(50)***, *13574-13581 (2008)*

Activation of the transcription factor Nrf2 in astrocytes coordinates the upregulation of antioxidant defenses and confers protection to neighboring neurons. Dominant mutations in Cu/Zn-superoxide dismutase (SOD1) cause familial forms of amyotrophic lateral sclerosis (ALS), a fatal disorder characterized by the progressive loss of motor neurons. Non-neuronal cells, including astrocytes, shape motor neuron survival in ALS and are a potential target to prevent motor neuron degeneration. The protective effect of Nrf2 activation in astrocytes has never been examined in a chronic model of neurodegeneration. We generated transgenic mice over-expressing Nrf2 selectively in astrocytes using the glial fibrillary acidic protein (GFAP) promoter. The toxicity of astrocytes expressing ALS-linked mutant hSOD1 to cocultured motor neurons was reversed by Nrf2 over-expression. Motor neuron protection depended on increased glutathione secretion from astrocytes. This protective effect was also observed by crossing the GFAP-Nrf2 mice with two ALS-mouse models. Over-expression of Nrf2 in astrocytes significantly delayed onset and extended survival. These findings demonstrate that Nrf2 activation in astrocytes is a viable therapeutic target to prevent chronic neurodegeneration.

4.639 Deficiency in Complement C1q Improves Histological and Functional Locomotor Outcome after Spinal Cord Injury

Galvan, M.D., Luchetti, S., Burgos, A.M., Nguyen, H.X., Hooshmand, M.J., Hamers, F.P.T. and Anderson, A.J.
J. Neurosci., ***28(51)***, *13876-13888 (2008)*

Although studies have suggested a role for the complement system in the pathophysiology of spinal cord injury (SCI), that role remains poorly defined. Additionally, the relative contribution of individual complement pathways in SCI is unknown. Our initial studies revealed that systemic complement activation was strongly influenced by genetic background and gender. Thus, to investigate the role of the classical complement pathway in contusion-induced SCI, male C1q knock-out (KO) and wild-type (WT) mice on a complement sufficient background (BUB) received a mild-moderate T9 contusion injury with the Infinite Horizon impactor. BUB C1q KO mice exhibited greater locomotor recovery compared with BUB WT mice ($p < 0.05$). Improved recovery observed in BUB C1q KO mice was also associated with decreased threshold for withdrawal from a mild stimulus using von Frey filament testing. Surprisingly, quantification of microglia/macrophages (F4/80) by FACS analysis showed that BUB C1q KO mice exhibited a significantly greater percentage of macrophages in the spinal cord compared with BUB WT mice 3 d post-injury ($p < 0.05$). However, this increased macrophage response appeared to be transient as stereological assessment of spinal cord tissue obtained 28 d post-injury revealed no difference in F4/80-positive cells between groups. Stereological assessment of spinal cord tissue showed that BUB C1q KO mice had reduced lesion volume and an increase in tissue sparing compared with BUB WT mice ($p < 0.05$). Together, these data suggest that initiation of the classical complement pathway via C1q is detrimental to recovery after SCI.

4.640 Innate and Adaptive Interleukin-22 Protects Mice from Inflammatory Bowel Disease

Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Stevens, S. and Flavell, R.A.
Immunity, ***29***, *947-957 (2008)*

Inflammatory bowel disease (IBD) is a chronic inflammatory disease thought to be mediated by dysfunctional innate and/or adaptive immunity. This aberrant immune response leads to the secretion of harmful cytokines that destroy the epithelium of the gastrointestinal tract and thus cause further inflammation. Interleukin-22 (IL-22) is a T helper 17 (Th17) T cell-associated cytokine that is bifunctional in that it has both proinflammatory and protective effects on tissues depending on the inflammatory context. We show herein that IL-22 protected mice from IBD. Interestingly, not only was this protection

mediated by $CD4^+$ T cells, but IL-22-expressing natural killer (NK) cells also conferred protection. In addition, IL-22 expression was differentially regulated between NK cell subsets. Thus, both the innate and adaptive immune responses have developed protective mechanisms to counteract the damaging effects of inflammation on tissues.

4.641 Efficient tumour formation by single human melanoma cells
Quintana, E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M. and Morrison, S.J.
*Nature, **456**, 593-599 (2008)*

A fundamental question in cancer biology is whether cells with tumorigenic potential are common or rare within human cancers. Studies on diverse cancers, including melanoma, have indicated that only rare human cancer cells (0.1–0.0001%) form tumours when transplanted into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. However, the extent to which NOD/SCID mice underestimate the frequency of tumorigenic human cancer cells has been uncertain. Here we show that modified xenotransplantation assay conditions, including the use of more highly immunocompromised NOD/SCID interleukin-2 receptor gamma chain null (*Il2rg* $^{-/-}$) mice, can increase the detection of tumorigenic melanoma cells by several orders of magnitude. In limiting dilution assays, approximately 25% of unselected melanoma cells from 12 different patients, including cells from primary and metastatic melanomas obtained directly from patients, formed tumours under these more permissive conditions. In single-cell transplants, an average of 27% of unselected melanoma cells from four different patients formed tumours. Modifications to xenotransplantation assays can therefore dramatically increase the detectable frequency of tumorigenic cells, demonstrating that they are common in some human cancers.

4.642 Identification of adult hepatic progenitor cells capable of repopulating injured rat liver
Yovchev, M:I., Grozdanov, P.N., Zhou, H., Racherla, H., Guha, C. and Dabeva, M.D.
*Hepatology, **47(2)**, 636-647 (2008)*

Oval cells appear and expand in the liver when hepatocyte proliferation is compromised. Many different markers have been attributed to these cells, but their nature still remains obscure. This study is a detailed gene expression analysis aimed at revealing their identity and repopulating in vivo capacity. Oval cells were activated in 2-acetylaminofluorene–treated rats subjected to partial hepatectomy or in D-galactosamine–treated rats. Two surface markers [epithelial cell adhesion molecule (EpCAM) and thymus cell antigen 1 (Thy-1)] were used for purification of freshly isolated cells. Their gene expression analysis was studied with Affymetrix Rat Expression Array 230 2.0, reverse-transcriptase polymerase chain reaction, and immunofluorescent microscopy. We found that $EpCAM^+$ and $Thy\text{-}1^+$ cells represent two different populations of cells in the oval cell niche. $EpCAM^+$ cells express the classical oval cell markers (alpha-fetoprotein, cytokeratin-19, OV-1 antigen, a6 integrin, and connexin 43), cell surface markers recently identified by us (CD44, CD24, EpCAM, aquaporin 5, claudin-4, secretin receptor, claudin-7, V-ros sarcoma virus oncogene homolog 1, cadherin 22, mucin-1, and CD133), and liver-enriched transcription factors (forkhead box q, forkhead box a2, onecut 1, and transcription factor 2). Oval cells do not express previously reported hematopoietic stem cell markers Thy-1, c-kit, and CD34 or the neuroepithelial marker neural cell adhesion molecule 1. However, oval cells express a number of mesenchymal markers including vimentin, mesothelin, bone morphogenetic protein 7, and Tweak receptor (tumor necrosis factor receptor superfamily, member 12A). A group of novel differentially expressed oval cell genes is also presented. It is shown that $Thy\text{-}1^+$ cells are mesenchymal cells with characteristics of myofibroblasts/activated stellate cells. Transplantation experiments reveal that $EpCAM^+$ cells are true progenitors capable of repopulating injured rat liver. *Conclusion:* We have shown that $EpCAM^+$ oval cells are bipotential adult hepatic epithelial progenitors. These cells display a mixed epithelial/mesenchymal phenotype that has not been recognized previously. They are valuable candidates for liver cell therapy.

4.643 Dendritic cells are required for effective cross-presentation in the murine liver
Plitas, G., Burt, B.M., Stableford, J.A., Nguyen, H.M., Welles, A.P. and DeMatteo, R.P.
*Hepatology, **47(4)**, 1343-1351 (2008)*

The liver harbors a diversity of cell types that have been reported to stimulate T cells. Although most hepatic dendritic cells are immature, a small population of CD11chigh conventional dendritic cells (cDCs) exists that expresses high levels of costimulatory molecules. We sought to determine the relative contribution of cDCs to cross-presentation by the liver. In vitro, liver nonparenchymal cells (NPCs) depleted of cDCs induced only minimal proliferation and activation of antigen-specific CD8+ T cells when

loaded with soluble protein antigen. Using a transgenic mouse with the CD11c promoter driving expression of the human diphtheria toxin receptor, we found that selective depletion of cDCs in vivo reduced the number and activation of antigen-specific CD8+ T cells in the liver after intravenous administration of soluble protein antigen. Adoptive transfer of DCs, but not CD40 stimulation, restored the hepatic T-cell response. Conclusion: Our findings indicate that the ability of the liver to effectively cross-present soluble protein to antigen-specific CD8+ T cells depends primarily on cDCs. Despite costimulation, other resident liver antigen-presenting cells cannot compensate for the absence of cDCs.

4.644 Systemic transmigration of allosensitizing donor dendritic cells to host secondary lymphoid organs after rat liver transplantation
Ueta, H., Shi, C., Miyanari, N., Xu, X-D., Zhou, S., Yamashita, M., Ezaki, T. and Matsuno, K.
Hepatology, ***47(4)***, *1352-1362 (2008)*

Donor dendritic cell (DC) migration and allosensitization in host secondary lymphoid organs after liver transplantation are ill defined. We used rat models to investigate graft-derived cells and intrahost allosensitization. Liver transplantation induced diffuse blood-borne migration of donor major histocompatibility class II antigen–positive (MHCII+) cells and MHCI+ cells from the graft to host secondary lymphoid organs, not only the spleen, but also lymph nodes and Peyer's patches. The migrated MHCII+ cells included DCs and some T cells and B cells. The DCs formed clusters with host BrdU+ cells where they up-regulated CD86+, and a CD8+ T cell proliferative response originated within 24 hours after liver transplantation, demonstrating that these DCs can quickly mature and trigger direct allosensitization in host lymphoid organs. Transfer of allogeneic bone marrow cells also induced DC transmigration and a similar host response. In contrast, allogeneic thoracic duct lymph cells contained many fewer transmigrating DCs, and their transfer induced a comparable T cell response but significantly weaker CD8+ T cell proliferation. Thus, there is a different outcome via the indirect pathway by host DCs that have captured donor alloantigens. Conclusion: The rat liver as well as bone marrow contains an immature DC population that can systemically transmigrate through blood vessel walls of the host secondary lymphoid organs, quickly mature, and induce diffuse intrahost CD8+ T cell responses, which may promote graft rejection.

4.645 Sinusoidal endothelial cells prevent rat stellate cell activation and promote reversion to quiescence
DeLeve, L.D., Wang, X. and Guo, Y.
Hepatology, ***48(3)***, *920-930 (2008)*

Capillarization precedes hepatic fibrosis. We hypothesize that capillarization of sinusoidal endothelial cells (SEC) is permissive for hepatic stellate cell (HSC) activation and therefore permissive for fibrosis. We examined whether freshly isolated SECs prevent activation of HSCs and promote reversion to quiescence, and whether this effect was lost in capillarization. HSCs were cultured alone or co-cultured with differentiated or capillarized SECs. Results: Co-culture with freshly isolated SECs markedly decreased HSC activation after 3 days in culture, but co-culture with capillarized SEC had no effect. Inhibition of nitric oxide (NO) synthesis abolished SEC suppression of HSC activation. Activated HSCs reverted to quiescence when co-cultured with SEC plus vascular endothelial growth factor (VEGF) (that is, with SECs that maintained differentiation), but co-culture with capillarized SECs did not. Reversion of activated HSCs to quiescence in the presence of SECs plus VEGF was abolished by inhibition of NO synthesis. To establish whether there was indeed reversion, activated and quiescent HSCs were counted before and 3 days after adding freshly isolated SECs plus VEGF to activated HSCs, and proliferation was quantified in quiescent HSCs; the stoichiometry demonstrated reversion. Conclusion: Differentiated SECs prevent HSC activation and promote reversion of activated HSCs to quiescence through VEGF-stimulated NO production. Capillarized SECs do not promote HSC quiescence, because of loss of VEGF-stimulated NO production.

4.646 Age-related changes to tumor necrosis factor receptors affect neuron survival in the presence of beta-amyloid
Patel, J.R. and Brewer, G.J.
J. Neurosci. Res., ***86(10)***, *2303-2313 (2008)*

Inflammation including local accumulations of tumor necrosis factor alpha (TNF-α) is a part of Alzheimer's disease pathology and may exacerbate age-related neurodegeneration. Most studies on TNF-α and TNF neuronal receptors are conducted by using embryonic neurons. Few studies consider age-related deficits that may occur in neurons. Age-related changes in susceptibility to TNF-α through TNF receptor 1

(TNFR1) and receptor 2 (TNFR2) expression could increase susceptibility to β-amyloid (1–42, Aβ42). Evidence is conflicting about which receptor mediates survival and/or apoptosis. We determined how aging affects receptor expression in cultured adult rat cortical neurons. Old neurons were more susceptible to Aβ42 toxicity than middle-aged neurons, and the addition of TNF-α was neuroprotective in middle-aged neurons, but exacerbated the toxicity from Aβ42 in old neurons. These pathologic and protective responses in old and middle-aged neurons, respectively, correlated with higher starting TNFR1 and TNFR2 mRNA levels in old vs. middle-aged neurons. Middle-aged neurons treated with TNF-α plus Aβ42 did not show an increase in either TNFR1 or TNFR2 mRNA, but old neurons showed an up-regulation in TNFR2 mRNA and not TNFR1 mRNA. Despite these mRNA changes, surface immunoreactivity of both TNFR1 and TNFR2 increased with the dose of TNF-α in middle-aged neurons. However, middle-aged neurons treated with TNF-α plus Aβ42 showed an up-regulation in both TNFR1 and TNFR2 surface expression, whereas old neurons failed to up-regulate surface expression of either receptor. These findings support the hypothesis that age-related changes in TNF-α surface receptor expression contribute to the neuronal loss associated with inflammation in Alzheimer's disease.

4.647 Age-related decreases in NAD(P)H and glutathione cause redox declines before ATP loss during glutamate treatment of hippocampal neurons
Parihar, M.S., Kunz, E.A. and Brewer, G.J.
J. Neurosci. Res., ***86(10)***, *2339-2352 (2008)*

Age-related glutamate excitotoxicity depends in an unknown manner on active mitochondria, which are key determinants of the cellular redox potential. Compared with embryonic and middle-aged neurons, old-aged rat hippocampal neurons have a lower resting reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and a lower redox ratio (NAD(P)H/flavin adenine nucleotide). Glutamate treatment resulted in an initial increase in NAD(P)H concentrations in all ages, followed by a profound calcium-dependent, age-related decline in NAD(P)H concentration and redox ratio. With complex I of the electron transport chain inhibited by rotenone, treatment with glutamate or ionomycin only resulted in the increase in NAD(P)H fluorescence. High-performance liquid chromatography analysis of adenine nucleotides in brain extracts showed 50% less nicotinamide adenine dinucleotide (NADH) and almost twice as much oxidized nicotinamide adenine dinucleotide, demonstrating a more oxidized ratio in old than middle-aged brain. Resting glutathione content also declined with age and further decreased with glutamate treatment without accompanying changes in adenosine triphosphate levels. We conclude that age does not affect production of NADH by dehydrogenases but that old-aged neurons consume more NADH and glutathione, leading to a catastrophic decline in redox ratio.

4.648 Phylum Tardigrada: an “individual” approach
Sands, C.J., McInnes, S.J., Marley, N.J., Goodall-Copestake, W.P., Convey, P. and Linse, K.
Cladistics, ***24(6)***, *861-871 (2008)*

Phylum Tardigrada consists of ~ 1000 tiny, hardy metazoan species distributed throughout terrestrial, limno-terrestrial and oceanic habitats. Their phylogenetic status has been debated, with current evidence placing them in the Ecdysozoa. Although there have been efforts to explore tardigrade phylogeny using both morphological and molecular data, limitations such as their few morphological characters and low genomic DNA concentrations have resulted in restricted taxonomic coverage. Using a protocol that allows us to identify and extract DNA from individuals, we have sequenced 18S rDNA from 343 tardigrades from across the globe. Using maximum parsimony and Bayesian analyses we have found support for dividing Order Parachela into three super-families and further evidence that indicates the traditional taxonomic perspective of families in the class Eutardigrada are nonmonophyletic and require re-working. It appears that conserved morphology within Tardigrada has resulted in conservative taxonomy as we have found cases of several discrete lineages grouped into single genera. Although this work substantially adds to the understanding of the evolution and taxonomy of the phylum, we highlight that inferences gained from this work are likely to be refined with the inclusion of further taxa—specifically representatives of the nine families yet to be sampled.

4.649 Exogenous Hsc70, but not thermal preconditioning, confers protection to motoneurons subjected to oxidative stress
Robinson, M.B., Taylor, A.R., Gifondorwa, D.J., Tytell, M. and Milligan, C.E.
Develop. Neurobiol., ***68(1)***, *1-17 (2008)*

Proper sensing of stress and the initiation of the stress response are critical to maintaining cell viability in

response to noxious stimuli. Induction of the stress response prior to the exposure of a lethal stress (preconditioning) can be protective. Heat shock proteins (Hsps), the main products of the stress response, are considered to be responsible for this protective effect. Most cells readily initiate a stress response, but some neuronal phenotypes, including motoneurons (MNs), have a diminished capacity to do so. We have found that, given a proper stimulus, MNs can execute a heat stress response; but, it does not protect them from death caused by hydrogen peroxide (H_2O_2) induced oxidative stress, despite inhibiting H_2O_2-induced caspase activation. Conversely, we demonstrate that incubation with the heat shock cognate 70 (Hsc70) protein prior to oxidative insult can protect MNs from oxidative stress. This survival promoting effect may be mediated through the substrate binding domain (SBD) of Hsc70. Our data suggest that stress preconditioning may not be beneficial to MNs, but that pharmacological interventions and alternative means of acquiring components of the stress response are an effective means of ameliorating lethal stress in MNs and may be potentially useful therapeutically in preventing pathological MN loss.

4.650 Gene expression fluctuations in murine hematopoietic stem cells with cell cycle progression
Dooner, G.J., Colvin, G.A., Dooner, M.S., Johnson, K.W. and Quesenberry, P.J.
J. Cell. Physiol., ***214(3)***, *786-795 (2008)*

Evolving data suggest that marrow hematopoietic stem cells show reversible changes in homing, engraftment, and differentiation phenotype with cell cycle progression. Furthermore, marrow stem cells are a cycling population. Traditional concepts hold that the system is hierarchical, but the information on the lability of phenotype with cycle progression suggests a model in which stem cells are on a reversible continuum. Here we have investigated mRNA expression in murine lineage negative stem cell antigen-1 positive stem cells of a variety of cell surface epitopes and transcription regulators associated with stem cell identity or regulation. At isolation these stem cells expressed almost all cell surface markers, and transcription factors studied, including receptors for G-CSF, GM-CSF, and IL-7. When these stem cells were induced to transit cell cycle in vitro by exposure to interleukin-3 (IL-3), Il-6, IL-11, and steel factor some (CD34, CD45R c-kit, Gata-1, Gata-2, Ikaros, and Fog) showed stable expression over time, despite previously documented alterations in phenotype, while others showed variation of expression between and within experiments. These latter included Sca-1, Mac-1, c-fms, and c-mpl. Tal-1, endoglin, and CD4. These studies indicate that defined marrow stem cells express a wide variety of genes at isolation and with cytokine induced cell cycle transit show marked and reversible phenotype lability. Altogether, the phenotypic plasticity of gene expression for murine stem cells indicates a continuum model of stem cell regulation and extends the model to reversible expression with cell cycle transit of mRNA for cytokine receptors and stem cell markers.

4.651 Prolonged Insulin Independence After Islet Allotransplants in Recipients with Type 1 Diabetes
Bellin, M.D., Kandaswamy, R., Parkey, J., Zhang, H-J., Liu, B., Ihm, S.H., Ansite, J.D., Witson, J., Bansal-Pakala, P., Balamurugan, A.N., Papas, K., Sutherland, D.E.R., Moran, A. and Hering, B.J.
Am. J. Transplant., ***8(11)***, *2463-2470 (2008)*

We sought to determine the long-term outcomes in type 1 diabetic recipients of intraportal alloislet transplants on a modified immunosuppressive protocol. Six recipients with hypoglycemia unawareness received one to two islet infusions. Induction therapy was with antithymocyte globulin (ATG) plus etanercept for tumor necrosis factor-α blockade. Recipients received cyclosporine and everolimus for maintenance immunosuppression for the first year posttransplant, with mycophenolic acid or mycophenolate mofetil subsequently substituted for everolimus. Recipients have been followed for 1173 ± 270 days since their last infusion for islet graft function (insulin independence, hemoglobin A_{1c} levels and C-peptide production) and for adverse events associated with the study protocol. Of the six recipients, five were insulin-independent at 1 year, and four continue to be insulin-independent at a mean of 3.4 ± 0.4 years posttransplant. None of the six recipients experienced recurrence of severe hypoglycemia. Measured glomerular filtration rate decreased from 110.5 ± 21.2 mL/min/1.73 m^2 pretransplant to 82.6 ±19.1 mL/min/1.73 m^2 at 1 year posttransplant. In conclusion, islet transplants restored insulin independence for a mean of >3 years in four of six recipients treated with ATG and etanercept induction therapy and with cyclosporine and, initially, everolimus for maintenance. Our results suggest this immunosuppressive protocol may allow long-term graft survival.

4.652 CD40L-expressing CD8 T cells prime CD8α^+ DC for IL-12p70 production
Wong, K.L., Lew, F.C., MacAry, P.A. and Kemeny, D.M.
Eur. J. Immunol., ***38(8)***, *2251-2262 (2008)*

CD8α⁺ DC are implicated as the principle DC subset for cross-presentation and cross-priming of cytotoxic CD8 T cell responses. In this study, we demonstrate another unique facet of the CD8α⁺ DC and CD8 T cell relationship, by showing that CD8 T cells reciprocally activate CD8α⁺ DC, but not CD8α⁻ DC, for IL-12p70 production, the key Th1-promoting cytokine. This effect was observed during an antigen-specific interaction between DC and activated CD8 T cells, along with secondary TLR stimulation of DC by LPS. Activated CD8 T cells use a combination of IFN-γ and CD40L, which is rapidly up-regulated post-stimulation, to prime DC for IL-12p70 production during an antigen-specific response. Our results suggest that the interaction between CD8α⁺ DC and antigen-primed CD8 T cells may form an important component of Th1-mediated immunity through the induction of IL-12p70.

4.653 **A new *in vitro* model of the glial scar inhibits axon growth**
Wanner, I.B., Deik, A., Torres, M., Rosendahl, A., Neary, J.T., Lemmon, V.P. and Bixby, J.L.
GLIA, ***56(15)***, *1691-1709 (2008)*

Astrocytes respond to central nervous system (CNS) injury with reactive astrogliosis and participate in the formation of the glial scar, an inhibitory barrier for axonal regeneration. Little is known about the injury-induced mechanisms underlying astrocyte reactivity and subsequent development of an axon-inhibitory scar. We combined two key aspects of CNS injury, mechanical trauma and co-culture with meningeal cells, to produce an *in vitro* model of the scar from cultures of highly differentiated astrocytes. Our model displayed widespread morphological signs of astrocyte reactivity, increases in expression of glial fibrillary acidic protein (GFAP), and accumulation of GFAP in astrocytic processes. Expression levels of scar-associated markers, phosphacan, neurocan, and tenascins, were also increased. Importantly, neurite growth from various CNS neuronal populations was significantly reduced when neurons were seeded on the scar-like cultures, compared with growth on cultures of mature astrocytes. Quantification of neurite growth parameters on the scar model demonstrated significant reductions in neuronal adhesion and neurite lengths. Interestingly, neurite outgrowth of postnatal neurons was reduced to a greater extent than that of embryonic neurons, and outgrowth inhibition varied among neuronal populations. Scar-like reactive sites and neurite-inhibitory patches were found throughout these cultures, creating a patchwork of growth-inhibitory areas mimicking a CNS injury site. Thus, our model showed relevant aspects of scar formation and produced widespread inhibition of axonal regeneration; it should be useful both for examining mechanisms underlying scar formation and to assess various treatments for their potential to improve regeneration after CNS injury

4.654 **Differential regulation of sphingomyelin synthesis and catabolism in oligodendrocytes and neurons**
Kilkus, J.P., Goswami, R., Dawson, S.A., Testai, F.D., Berdyshev, E.V., Han, X. and dawson, G.
J. Neurochem., ***106(4)***, *1745-1757 (2008)*

Neurons (both primary cultures of 3-day rat hippocampal neurons and embryonic chick neurons) rapidly converted exogenous NBD-sphingomyelin (SM) to NBD-Cer but only slowly converted NBD-Cer to NBD-SM. This was confirmed by demonstrating low *in vitro* sphingomyelin synthase (SMS) and high sphingomyelinase (SMase) activity in neurons. Similar results were observed in a human neuroblastoma cell line (LA-N-5). In contrast, primary cultures of 3-day-old rat oligodendrocytes only slowly converted NBD-SM to NBD-Cer but rapidly converted NBD-Cer to NBD-SM. This difference was confirmed by high *in vitro* SMS and low SMase activity in neonatal rat oligodendrocytes. Similar results were observed in a human oligodendroglioma cell line. Mass-Spectrometric analyses confirmed that neurons had a low SM/Cer ratio of (1.5 : 1) whereas oligodendroglia had a high SM/Cer ratio (9 : 1). Differences were also confirmed by [^{3}H]palmitate-labeling of ceramide, which was higher in neurons compared with oligodendrocytes. Stable transfection of human oligodendroglioma cells with neutral SMase, which enhanced the conversion of NBD-SM to NBD-Cer and increased cell death, whereas transfection with SMS1 or SMS2 enhanced conversion of NBD-Cer to NBD-SM and was somewhat protective against cell death. Thus, SMS rather than SMases may be more important for sphingolipid homeostasis in oligodendrocytes, whereas the reverse may be true for neurons.

4.655 **Graft-versus-Host Disease Prevents the Maturation of Plasmacytoid Dendritic Cells**
Banovic, T., Markey, K.A., Kuns, R.D., Olver, S.D., Raffelt, N.C., Don, A.L., Degli-Esposti, M.A., Engwerda, C.R., MacDonald, K.P.A. and Hill, G.R.
J. Immunol., ***182***, *912-920 (2009)*

The role of Ag presenting cell subsets in graft-versus-host disease (GVHD) remains unclear. We have thus examined the ability of plasmacytoid dendritic cells (pDC) to modulate transplant outcome. Surprisingly,

host pDC were exquisitely sensitive to total body irradiation and were depleted before transplantation, thus allowing us to focus on donor pDC. The depletion of all pDC from bone marrow grafts resulted in an acceleration of GVHD mortality while the depletion of mature pDC from G-CSF mobilized splenic grafts had no effect. Thus, donor bone marrow pDC, but not mature pDC contained within stem cell grafts attenuate acute GVHD. In the presence of GVHD, donor pDC completely failed to reconstitute although a $CD11c^{low}120G8^{+}$ precursor DC reconstituted in an exaggerated and transient manner. These cells expressed Flt-3, the macrophage colony stimulating factor receptor and, consistent with a common dendritic cell (DC) precursor, were capable of differentiation into pDC and conventional DC in vivo in the absence of GVHD. These precursors were MHC class II^{+} and $CD80/86^{+}$ but lacked CD40, were actively presenting host Ag and inhibited GVHD and T cell proliferation in a contact-dependent fashion. These data demonstrate that GVHD prevents the maturation of pDC and instead promotes the generation of a suppressive precursor DC, further contributing to the state of immune paralysis after transplantation.

4.656 Type I IFN-Mediated Protection of Macrophages and Dendritic Cells Secures Control of Murine Coronavirus Infection

Cervantes-Barragan, L., Kalinke, U., Züst, R., König, M., Reizis, B., Lopez-Macias, C., Thiel, V. And Ludewig, B.

J. Immunol., ***182****, 1099-1106 (2009)*

The swift production of type I IFNs is one of the fundamental aspects of innate immune responses against viruses. Plasmacytoid dendritic cell-derived type I IFNs are of prime importance for the initial control of highly cytopathic viruses such as the mouse hepatitis virus (MHV). The aim of this study was to determine the major target cell populations of this first wave of type I IFNs. Generation of bone marrow-chimeric mice expressing the type I IFN receptor (IFNAR) on either hemopoietic or non-bone marrow-derived cells revealed that the early control of MHV depended mainly on IFNAR expression on hemopoietic cells. To establish which cell population responds most efficiently to type I IFNs, mice conditionally deficient for the IFNAR on different leukocyte subsets were infected with MHV. This genetic analysis revealed that IFNAR expression on $LysM^{+}$ macrophages and $CD11c^{+}$ dendritic cells was most important for the early containment of MHV within secondary lymphoid organs and to prevent lethal liver disease. This study identifies type I IFN-mediated cross-talk between plasmacytoid dendritic cells on one side and macrophages and conventional dendritic cells on the other, as an essential cellular pathway for the control of fatal cytopathic virus infection.

4.657 A fluorescence-based assay for measuring the viable cell concentration of mixed microbial communities in soil

Pascaud, A., Amellal, S., Soulas, M-L. and Soulas, G.

J. Microbiol. Methods, ***76(1)****, 81-87 (2009)*

Microbial cell concentration is a particularly important bioindicator of soil health and a yardstick for determining biological quotients which are likely to gain in ecological significance if they are calculated in relation to the viable, rather than total, microbial density. A dual-staining technique with fluorescent dyes was used for the spectrofluorimetric quantitative determination of the concentration of viable microbial cells present in three different soil types. This is a novel and substantially modified application of the dual-staining procedure implemented in the LIVE/DEAD™ *Bac*Light® viability kit which has never been successfully applied to the quantification of naturally occurring soil microbial communities. Indigenous microbial cell concentrations were quantified using an internal standard, i.e. spiking environmental samples with suspensions containing different concentrations of live *E. coli* cells, and external calibration, by comparing fluorescence emission by indigenous bacteria and known concentrations of *E. coli* in nutrient saline. Two types of environmental samples were tested: bacterial preparations obtained by density gradient centrifugation and soil suspensions. In both cases, prior dilution of the sample was necessary to minimise fluorescence quenching by soil particulate matter. Spectrofluorimetric measurements of indigenous cell concentration in bacterial preparations were in close agreement with those found using epifluorescence microscopy. Limits of detection of 5×10^{6} for the soil bacterial preparations and 8×10^{7} for the soil suspensions were estimated. Deviations observed when soil suspensions are dealt with are likely due to the selection of a unique bacterial strain for standardisation and calibration. Thorough testing of a variety of reference bacteria and fungi is suggested to determine a more accurate average fluorescence enhancement per microbial cell or mass unit.

4.658 Differential expression of Prnp and Sprn in scrapie infected sheep also reveals Prnp genotype specific differences
Gossner, A.G., Bennet, N., Hunter, N. And Hopkins, J.
*Biochem. Biophys. Res. Comm., **378(4)**, 862-866 (2009)*

The central role for PrP in the pathogenesis of the transmissible spongiform encephalopathies (TSEs) is illustrated by the resistance of $Prnp^{0/0}$ mice to disease and by the inverse association of Prnp gene dosage with incubation period. Understanding the role of PrP^{C} in TSEs necessitates knowledge of expression levels of the Prnp gene during the development of disease. SSBP/1 scrapie shows a defined pattern of disease progression and here we show that Prnp and shadow of PrP (Sprn) are differentially expressed in different brain areas and lymphoid tissues. Counter-intuitively we found that there is no positive correlation between expression of Prnp or Sprn and patterns of disease progression. Prnp and Sprn expression levels are both influenced by Prnp genotype; although the scrapie-sensitive VRQ/VRQ sheep did not express the highest level of either. In addition, infection with SSBP/1 scrapie seems to have little effect on either PrP or Shadoo expression levels.

4.659 Regeneration and characterization of adult mouse hippocampal neurons in a defined in vitro system
Varghese, K., Das, M., Bhargava, N., Stancescu, M., Molnar, P., Kindy, M.S. and Hickman, J.J.
*J. Neurosci. Methods, **177**, 51-59 (2009)*

Although the majority of human illnesses occur during adulthood, most of the available *in vitro* disease models are based upon cells obtained from embryonic/fetal tissues because of the difficulties involved with culturing adult cells. Development of adult mouse neuronal cultures has a special significance because of the abundance of transgenic disease models that use this species. In this study a novel cell culture method has been developed that supports the long-term survival and physiological regeneration of adult mouse hippocampal cells in a serum-free defined environment. In this well-defined, controlled system, adult mouse hippocampal cells survived for up to 21 days in culture. The cultured cells exhibited typical hippocampal neuronal morphology and electrophysiological properties after recovery from the trauma of dissociation, and stained positive for the expected neuronal markers. This system has great potential as an investigative tool for *in vitro* studies of adult diseases, the aging brain or transgenic models of age-associated disorders.

4.660 Calumenin but not reticulocalbin forms a Ca2+-dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis
Westegaard Hansen, G.A., Vorum, H., Jacobsen, C. and Honore, B.
*Mol. Cell Biochem., **320**, 25-33 (2009)*

Thrombocytes express thrombospondin-1 (TSP1), as well as the CREC proteins, calumenin and reticulocalbin. TSP1 and calumenin are released upon stimulation with thrombin. Calumenin has recently been shown to influence the synthesis of certain coagulation factors. Calumenin is present in atherosclerotic lesions but not in normal vasculature [Coppinger et al. (Blood 103:2096–2104, 2004)] and is able to modulate the protein expression pattern as well as the cell cycle of fibroblasts [Østergaard et al. (Proteomics 6:3509–3519, 2006)]. We here show that calumenin in the presence of Ca^{2+} binds to TSP1 with a dissociation constant K_d around 0.4 μM. This interaction is specific with respect to the secreted calumenin as the closest relative among the CREC family members, the non-secreted reticulocalbin, does not form a similar complex. This further indicates that calumenin may be broadly involved in haemostasis and in the pathophysiology of thrombosis.

4.661 Critical age-related loss of cofactors of neuron cytochrome *C* oxidase reversed by estrogen
Jones, T.T. and Brewer, G.J.
*Exp. Neurol., **215**, 212-219 (2009)*

The mechanistic basis for the correlation between mitochondrial dysfunction and neurodegenerative disease is unclear, but evidence supports involvement of cytochrome *C* oxidase (CCO) deficits with age. Neurons isolated from the brains of 24 month and 9 month rats and cultured in common conditions provide a model of intrinsic neuronal aging. *In situ* CCO activity was decreased in 24 month neurons relative to 9 month neurons. Possible CCO-related deficits include holoenzyme activity, cofactor, and substrate. No difference was found between neurons from 24 month and 9 month rats in mitochondrial counts per neuron, CCO activity in submitochondrial particles, or basal respiration. Immunostaining for cytochrome *C* in

individual mitochondria revealed an age-related deficit of this electron donor. 24 month neurons did not have adequate respiratory capacity to upregulate respiration after a glutamate stimulus, in spite of a two-fold upregulation of respiration seen in 9 month neurons. Respiration in 24 month neurons was inhibited by lower concentrations of potassium cyanide, suggesting a 50% deficit in functional enzyme in 24 month compared to 9 month neurons. In addition to cytochrome *C*, CCO requires cardiolipin to function. Staining with nonylacridine orange revealed an age-related deficit in cardiolipin. Treatment of 24 month neurons with 17-β-estradiol restored cardiolipin levels (10 ng/mL) and upregulated respiration under glutamate stress (1 pg/mL). Attempts to induce mitochondrial turnover by neuronal multiplication also rejuvenated CCO activity in 24 month neurons. These data suggest cytochrome *C* and cardiolipin levels are deficient in 24 month neurons, preventing normal upregulation of respiration needed for oxidative phosphorylation in response to stress. Furthermore, the data suggest this deficit can be corrected with estrogen treatment.

4.662 Impaired calcium homeostasis in aged hippocampal neurons
Hajieva, P., Kuhlmann, C., Luhmann, H.J. and Behl, C.
Neurosci. Lett., ***451***, *119-123 (2009)*

Development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease is strongly age-associated. The impairment of calcium homeostasis is considered to be a key pathological event leading to neuronal dysfunction and cell death. However, the exact impact of aging on calcium homeostasis in neurons remains largely unknown. In the present work we have investigated intracellular calcium levels in cultured primary hippocampal neurons from young (2 months) and aged (24 months) rat brains. Upon stimulation with glutamate or hydrogen peroxide aged neurons in comparison to young neurons demonstrated an increased vulnerability to these disease-related toxins. Measurement of calpain activity using Western blot analysis showed a significant increase in basal activity of calpains in aged neurons. The observed increase of calpain activity was correlated with elevated protein levels of μ-calpain. Ca^{2+}-imaging experiments performed on living individual neurons using the dye calcium green demonstrated a twofold increase in intracellular calcium concentration in aged neurons as compared to young neurons. The observed changes of intracellular calcium in aged neurons might play a role in their increased vulnerability to neurodegeneration.

4.663 Genomic Survey of the Non-Cultivatable Opportunistic Human Pathogen, Enterocytozoon bieneusi
Akiyoshi, D.E., Morrison, H.G., Kei, S., Feng, X., Zhang, Q., Corradi, N., Mayanja, H., Tumwine, J.K., Keeling, P.J., Weiss, L.M. and Tzipori,.S.
PloSPathogens, ***5(1)****, e100261 (2009)*

Enterocytozoon bieneusi is the most common microsporidian associated with human disease, particularly in the immunocompromised population. In the setting of HIV infection, it is associated with diarrhea and wasting syndrome. Like all microsporidia, *E. bieneusi* is an obligate, intracellular parasite, but unlike others, it is in direct contact with the host cell cytoplasm. Studies of *E. bieneusi* have been greatly limited due to the absence of genomic data and lack of a robust cultivation system. Here, we present the first large-scale genomic dataset for *E. bieneusi*. Approximately 3.86 Mb of unique sequence was generated by paired end Sanger sequencing, representing about 64% of the estimated 6 Mb genome. A total of 3,804 genes were identified in *E. bieneusi*, of which 1,702 encode proteins with assigned functions. Of these, 653 are homologs of *Encephalitozoon cuniculi* proteins. Only one *E. bieneusi* protein with assigned function had no *E. cuniculi* homolog. The shared proteins were, in general, evenly distributed among the functional categories, with the exception of a dearth of genes encoding proteins associated with pathways for fatty acid and core carbon metabolism. Short intergenic regions, high gene density, and shortened protein-coding sequences were observed in the *E. bieneusi* genome, all traits consistent with genomic compaction. Our findings suggest that *E. bieneusi* is a likely model for extreme genome reduction and host dependence.

4.664 Increased expression of urokinase plasminogen activator in Quebec platelet disorder is linked to megakaryocyte differentiation
Veljkovic, D.K., Rivard, G.E., Diamandis, M., Blavignac, J., Cramer-Borde, E.M. and Hayward, C.P.M.
Blood, ***113(7)****, 1535-1542 (2009)*

Quebec platelet disorder (QPD) is an inherited bleeding disorder associated with increased urokinase plasminogen activator (uPA) in platelets but not in plasma, intraplatelet plasmin generation, and α-granule protein degradation. These abnormalities led us to investigate uPA expression by QPD $CD34^+$ progenitors,

cultured megakaryocytes, and platelets, and whether uPA was stored in QPD α-granules. Although QPD $CD34^+$ progenitors expressed normal amounts of uPA, their differentiation into megakaryocytes abnormally increased expression of the uPA gene but not the flanking genes for vinculin or calcium/calmodulin-dependent protein kinase IIγ on chromosome 10. The increased uPA production by cultured QPD megakaryocytes mirrored their production of α-granule proteins, which was normal. uPA was localized to QPD α-granules and it showed extensive colocalization with α-granule proteins in both cultured QPD megakaryocytes and platelets, and with plasminogen in QPD platelets. In QPD megakaryocytes, cultured without or with plasma as a source of plasminogen, α-granule proteins were stored undegraded and this was associated with much less uPA-plasminogen colocalization than in QPD platelets. Our studies indicate that the overexpression of uPA in QPD emerges with megakaryocyte differentiation, without altering the expression of flanking genes, and that uPA is costored with α-granule proteins prior to their proteolysis in QPD.

4.665 Discovery of a functional protein complex of netrin-4, laminin γ1 chain, and integrin α6β1 in mouse neural stem cells
Staquicini, F.I., Dias-neto, E., Li, J., Snyder, E.Y., Sidman, R.L., Pasqualini, R. and Arap, W.
*PNAS, **106(8)**, 2903-2908 (2009)*

Molecular and cellular interactions coordinating the origin and fate of neural stem cells (NSCs) in the adult brain are far from being understood. We present a protein complex that controls proliferation and migration of adult NSCs destined for the mouse olfactory bulb (OB). Combinatorial selection based on phage display technology revealed a previously unrecognized complex between the soluble protein netrin-4 and laminin γ1 subunit that in turn activates an α6β1 integrin-mediated signaling pathway in NSCs. Differentiation of NSCs is accompanied by a decrease in netrin-4 receptors, indicating that netrin-4 participates in the continual propagation of this stem cell population. Notably, the stem cells themselves do not synthesize netrin-4. Further, we show that netrin-4 is produced by selected GFAP-positive astrocytes positioned close to newborn neurons migrating in the anterior part of the rostral migratory stream (RMS) and within the OB. Our findings present a unique molecular mechanism mediating astrocytic/neuronal crosstalk that regulates ongoing neurogenesis in the adult olfactory system.

4.666 Distinct Subtypes of Cholecystokinin (CCK)-Containing Interneurons of the Basolateral Amygdala Identified Using a CCK Promoter-Specific Lentivirus
Jasnow, A.M., Ressler, K.J., Hammack, S.E., Chhatwal, J.P. and Rainnie, D.G.
*J. Neurophysiol., **101**, 1494-1506 (2009)*

The basolateral amygdala (BLA) is critical for the formation of emotional memories. Little is known about the physiological properties of BLA interneurons, which can be divided into four subtypes based on their immunocytochemical profiles. Cholecystokinin (CCK) interneurons play critical roles in feedforward inhibition and behavioral fear responses. Evidence suggests that interneurons within a subgroup can display heterogeneous physiological properties. However, little is known about the physiological properties of CCK interneurons in the BLA and/or whether they represent a homogeneous or heterogeneous population. To address this question, we generated a lentivirus-expressing GFP under the control of the CCK promoter to identify CCK neurons in vivo. We combined this with whole cell patch-clamp recording techniques to examine the physiological properties of CCK-containing interneurons of the rat BLA. Here, we describe the physiological properties of 57 cells recorded in current-clamp mode; we used hierarchical cluster and discriminant function analysis to demonstrate that CCK interneurons can be segregated into three distinct subtypes (I, II, III) based on their passive and active membrane properties. Additionally, Type II neurons could be further separated into adapting and nonadapting types based on their rates of spike frequency adaptation. These data suggest that CCK interneurons of the BLA are a heterogeneous population and may be functionally distinct subpopulations that differentially contribute to the processing of emotionally salient stimuli.

4.667 A Phase I-II Study of α-Galactosylceramide-Pulsed IL-2/GM-CSF-Cultured Peripheral Blood Mononuclear Cells in Patients with Advanced and Recurrent Non-Small Cell Lung Cancer
Motohashi, S., Nagato, K., Kunii, N., Yamamoto, H., Yamasaki, K., Okita, K., Hanaoka, H., Shimizu, N., Suzuki, M., Yoshino, I., Taniguchi, M., Fujisawa, T. and Nakayama, T.,
*J. Immunol., **182**, 2492-2501 (2009)*

To evaluate the safety, immune responses, and antitumor responses after the administration of α-galactosylceramide (αGalCer) KRN7000-pulsed PBMC cultured with IL-2 and GM-CSF (IL-2/GM-CSF-cultured PBMCs), a phase I-II study in patients with non-small cell lung cancer was conducted. Patients with advanced non-small cell lung cancer or recurrent lung cancer refractory to the standard therapy were eligible. αGalCer-pulsed IL-2/GM-CSF-cultured PBMCs ($1 \times 10^9/m^2$) were i.v. administered four times. Immune responses were monitored weekly. Twenty-three patients were enrolled in this study and 17 cases (73.9%) completed. No severe adverse event related to the treatment was observed. After the injection of α GalCer-pulsed IL-2/GM-CSF-cultured PBMCs, an increased number of IFN-γ-producing cells in the peripheral blood were detected in 10 patients (58.8%). Five cases remained as stable disease, and the remaining 12 cases were evaluated as progressive disease. The estimated median survival time (MST) of the 17 cases was 18.6 mo (range, 3.8 to 36.3 mo). Ten patients who displayed increased IFN-γ-producing cells (≥2-fold) showed prolonged MST (31.9 mo; range, 14.5 to 36.3 mo) as compared with poor-responder patients ($n = 7$) MST (9.7 mo; range, 3.8 to 25.0 mo) (log-rank test, $p = 0.0015$). The administration of αGalCer-pulsed IL-2/GM-CSF-cultured PBMCs was well tolerated and was accompanied by the successful induction of NKT cell-dependent immune responses. The increased IFN-γ-producing cells that result from αGalCer stimulation in PBMCs were significantly associated with prolonged MST. These results are encouraging and warrant further evaluation for survival benefit of this immunotherapy.

4.668 Blood Glucose Levels Regulate Pancreatic β-Cell Proliferation during Experimentally-Induced and Spontaneous Autoimmune Diabetes in Mice
Pechhold, K., Koczwara, K., Zhu, X., Harrison, V.S., Walker, G., Lee, J.and Harlan, D.M.

Background
Type 1 diabetes mellitus is caused by immune-mediated destruction of pancreatic β-cells leading to insulin deficiency, impaired intermediary metabolism, and elevated blood glucose concentrations. While at autoimmune diabetes onset a limited number of β-cells persist, the cells' regenerative potential and its regulation have remained largely unexplored. Using two mouse autoimmune diabetes models, this study examined the proliferation of pancreatic islet ß-cells and other endocrine and non-endocrine subsets, and the factors regulating that proliferation.
Methodology and Principal Findings
We adapted multi-parameter flow cytometry techniques (including DNA-content measurements and 5′-bromo-2′-deoxyuridine [BrdU] incorporation) to study pancreatic islet single cell suspensions. These studies demonstrate that β-cell proliferation rapidly increases at diabetes onset, and that this proliferation is closely correlated with the diabetic animals' elevated blood glucose levels. For instance, we show that when normoglycemia is restored by exogenous insulin or islet transplantation, the β-cell proliferation rate returns towards low levels found in control animals, yet surges when hyperglycemia recurs. In contrast, other-than-ß endocrine islet cells did not exhibit the same glucose-dependent proliferative responses. Rather, disease-associated alterations of BrdU-incorporation rates of δ-cells (minor decrease), and non-endocrine islet cells (slight increase) were not affected by blood glucose levels, or were inversely related to glycemia control after diabetes onset (α-cells).
Conclusion
We conclude that murine β-cells' ability to proliferate in response to metabolic need (i.e. rising blood glucose concentrations) is remarkably well preserved during severe, chronic β-cell autoimmunity. These data suggest that timely control of the destructive immune response after disease manifestation could allow spontaneous regeneration of sufficient β-cell mass to restore normal glucose homeostasis.

4.669 Superoxide dismutase, copper and zinc concentrations in platelet-rich plasma of pneumonia patients
Laskaj, R., Dodig, S., Cepelak, I. and Kuzman, I.
Ann. Clin. Biochem., ***46****, 123-128 (2009)*

Background: The aim of this study was to analyse platelet superoxide dismutase (SOD) activities (total SOD, manganese SOD and copper zinc SOD) and copper (Cu) and zinc (Zn) concentrations during the course of community-acquired pneumonia (CAP), and to compare them between patients with normal platelet count and those who have developed reactive thrombocytosis (RT).
Methods: Platelet count, SOD activities and Cu and Zn concentrations in platelet-rich plasma were measured in patients with CAP on admission and at discharge.
Results: Post-therapeutic platelet count increased significantly from the value recorded on admission. By the end of treatment, 42% of patients developed RT. All platelet SOD activities as well as Cu concentration were significantly lower in CAP patients than in control subjects. The initial Zn concentration was greater

in CAP patients compared with controls and showed a decrease at discharge. On admission, there was no difference in all SOD activities between either subgroup with normal platelet count or subgroup with RT. At discharge all SOD activities were significantly lower in patients with RT. Also, catalytic activities of those enzymes were significantly lower in both subgroups in comparison with the initial values. Post-therapeutic Cu value was lower in patients with RT in comparison with patients having normal platelet count. Zn concentration decreased significantly at discharge when compared with the initial values only in patients with RT.
Conclusion: The pattern of changes might be indicative of a certain role of platelets in antioxidant response during treatment in CAP patients.

4.670 Differentiated Human Alveolar Type II Cells Secrete Antiviral IL-29 (IFN-λ1) in Response to Influenza A Infection
Wang, J., Oberley-deegan, R., Wang, S., Nikrad, M., Funk, C.J., Hartshorn, K.L. and Mason, R.J.
J. Immunol., ***182***, *1296-1304 (2009)*

Alveolar type II epithelial cells (ATIIs) are one of the primary targets for influenza A pneumonia. The lack of a culture system for maintaining differentiated ATIIs hinders our understanding of pulmonary innate immunity during viral infection. We studied influenza A virus (IAV)-induced innate immune responses in differentiated primary human ATIIs and alveolar macrophages (AMs). Our results indicate that ATIIs, but not AMs, support productive IAV infection. Viral infection elicited strong inflammatory chemokine and cytokine responses in ATIIs, including secretion of IL-8, IL-6, MCP-1, RANTES, and MIP-1β, but not TNF-α, whereas AMs secreted TNF-α as well as other cytokines in response to infection. Wild-type virus A/PR/8/34 induced a greater cytokine response than reassortant PR/8 virus, A/Phil/82, despite similar levels of replication. IAV infection increased mRNA expression of IFN genes IFN-β, IL-29 (IFN-λ1), and IL-28A (IFN-λ2). The major IFN protein secreted by type II cells was IL-29 and ATIIs appear to be a major resource for production of IL-29. Administration of IL-29 and IFN-β before infection significantly reduced the release of infectious viral particles and CXC and CC chemokines. IL-29 treatment of type II cells induced mRNA expression of antiviral genes MX1, OAS, and ISG56 but not IFN-β. IL-29 induced a dose-dependent decrease of viral nucleoprotein and an increase of antiviral genes but not IFN-β. These results suggest that IL-29 exerts IFN-β-independent protection in type II cells through direct activation of antiviral genes during IAV infection.

4.671 Protective role of angiotensin II type 2 receptor signaling in a mouse model of pancreatic fibrosis
Ulmasov, B., Xu, Z., Tetri, L.H., Inagami, T. and Neuschwander-tetri, B.A.
Am. J. Physiol. Gastrointest. Liver Physiol., ***296***, *G282-G294 (2009)*

The renin-angiotensin system contributes to pathological processes in a variety of organs. In the pancreas, blocking the angiotensin II (AII) type 1 receptor (AT1) attenuates pancreatic fibrogenesis in animal models of pancreatitis. Because the role of the AII type 2 receptor (AT2) in modulating pancreatic injury is unknown we investigated the role of AT2 in pancreatic injury and fibrosis. Pancreatic fibrosis was induced by repetitive cerulein administration in C57BL/6 wild-type (WT) or AT2-deficient (AT2–/–) mice and assessed by morphology and gene expression at 10 days. There was no difference between WT and AT2–/– mice in the degree of acute pancreatic injury as assessed by amylase release at 9 and 12 h and by histological examination of the pancreas at 12 h. In contrast, parenchymal atrophy and fibrosis were more pronounced in AT2–/– mice compared with WT mice at 10 days. Fibrosis was accompanied by activation of pancreatic stellate cells (PSC) evaluated by Western blot analysis for α-smooth muscle actin and by immunocytochemistry; PSC activation was further increased in AT2–/– mice compared with WT mice. The level of pancreatic transforming growth factor-β1 mRNA and protein after repetitive cerulein treatment was higher in AT2–/– mice than in WT mice. Our results demonstrate that, in contrast to AT1 receptor signaling, AT2 receptor signaling modulates protective antifibrogenic effects in a mouse model of cerulein-induced pancreatic fibrogenesis. We propose that the effects of AII on injury-induced pancreatic fibrosis may be determined by the balance between AT1 and AT2 receptor signaling.

4.672 The ataxia3 Mutation in the N-Terminal Cytoplasmic Domain of Sodium Channel Nav1.6 Disrupts Intracellular Trafficking
Sharkey, L.M., Cheng, X., Drews, V., Buchner, D.A., Jones, J.M., Justice, M.J., Waxman, S.G., Dib-Hajj, S.D. and Meisler, M.H.
J. Neurosci., ***29(9)***, *2733-2741 (2009)*

ENU-induced neurological mutant *ataxia3* was mapped to distal mouse chromosome 15. Sequencing of the

positional candidate gene *Scn8a* encoding the sodium channel $Na_v1.6$ identified a T>C transition in exon 1 resulting in the amino acid substitution p.S21P near the N terminus of the channel. The cytoplasmic N-terminal region is evolutionarily conserved but its function has not been well characterized. *ataxia3* homozygotes exhibit a severe disorder that includes ataxia, tremor, and juvenile lethality. Unlike *Scn8a* null mice, they retain partial hindlimb function. The mutant transcript is stable but protein abundance is reduced and the mutant channel is not detected in its usual site of concentration at nodes of Ranvier. In whole-cell patch-clamp studies of transfected ND7/23 cells that were maintained at 37°C, the mutant channel did not produce sodium current, and function was not restored by coexpression of β1 and β2 subunits. However, when transfected cells were maintained at 30°C, the mutant channel generated voltage-dependent inward sodium currents with an average peak current density comparable with wild type, demonstrating recovery of channel activity. Immunohistochemistry of primary cerebellar granule cells from *ataxia3* mice demonstrated that the mutant protein is retained in the *cis*-Golgi. This trafficking defect can account for the low level of $Na_v1.6$-S21P at nodes of Ranvier *in vivo* and at the surface of transfected cells. The data demonstrate that the cytoplasmic N-terminal domain of the sodium channel is required for anterograde transport from the Golgi complex to the plasma membrane.

4.673 Effect of Probiotics Lactobacillus acidophilus on Citrobacter rodentium Colitis: The Role of Dendritic Cells

Chen, C-C., Chiu, C-H., Lin, T-Y., Shi, H.N. and Walker, W.
Pediatric Res., ***65(2)***, *169-175 (2009)*

Modulation of the intestinal immune response early in life by administration of probiotic bacteria may be an effective strategy for preventing or attenuating infectious diarrhea. We preinoculated the mice early in life with the probiotic bacteria *Lactobacillus acidophilus* NCFM (*La*) at age 2 wk. Dendritic cells (DCs) were collected and purified from mesenteric lymph nodes (MLN) and spleens of the BalbC/ByJ mice. DC isolation and adoptive transfer was used to examine the function of probiotics. We demonstrated that when mice were adoptively transferred with *La*-primed DCs (t-*La*DC) instead of oral consumption with *La*, there was a similar effect on fecal bacteria counts, IgA levels, and colonic histopathology, as well as cytokine levels in MLN when there was intestinal bacterial infection. The above findings suggest that DCs play a key role in probiotics attenuating *Citrobacter rodentium* (*Cr*) colitis. Moreover, the location of *La*-primed DC hints that there is interaction of DCs and T cells in the digestive system of the host. Up-regulated expression of a surface marker on DCs indicated that inoculation with probiotics will stimulate the function of DCs, thereby further increasing immune response triggered by DC.

4.674 ATP Measurement Predicts Porcine Islet Transplantation Outcome in Nude Mice

Kim, J.H., Park, S.G., Lee, H.N., Lee, Y.Y., Park, H.S., Kim, H-I., Yu, J.E., Kim, S.H., Park, C-G., Ha, J., Kim, S.J. and Park, K.S.
Transplantation, ***87(2)***, *166-169 (2009)*

Current nude mice islet transplantation studies cannot be used prospectively. Therefore, to predict transplantation outcomes, reliable and rapid assays for islet quality assessment are warranted. This study evaluated the predictive power of the porcine islet ATP content on the outcomes of islet transplantation in nude mice. Here, we report that the ATP measurement using a small number of handpicked islets with a diameter of 100 to 150 μm is a good predictor of islet graft efficacy in nude mice. Using receiver-operator characteristic analysis, the area under the curve of the ATP content using a small number of handpicked islets was 0.867 (95% confidence interval 0.744-0.989, $P<0.001$). The sensitivity and the specificity measured were 83.3% and 73.3%, respectively. In conclusion, a simple and a rapid measurement of intraislet ATP content could be a promising substitute for current nude mice islet transplantation studies.

4.675 Mitigation of peroxynitrite-mediated nitric oxide (NO) toxicity as a mechanism of induced adaptive NO resistance in the CNS

Bishop, A., Gooch, R., Eguchi, A., Jeffrey, S., Smallwood, L., Anderson, J. and Estevez, A.G.
J. Neurochem., ***109***, *74-84 (2009)*

During CNS injury and diseases, nitric oxide (NO) is released at a high flux rate leading to formation of peroxynitrite ($ONOO^{\bullet}$) and other reactive nitrogenous species, which nitrate tyrosines of proteins to form 3-nitrotyrosine (3NY), leading to cell death. Previously, we have found that motor neurons exposed to low levels of NO become resistant to subsequent cytotoxic NO challenge; an effect dubbed induced adaptive resistance (IAR). Here, we report IAR mitigates, not only cell death, but 3NY formation in response to cytotoxic NO. Addition of an NO scavenger before NO challenge duplicates IAR, implicating reactive

nitrogenous species in cell death. Addition of uric acid (a peroxynitrite scavenger) before cytotoxic NO challenge, duplicates IAR, implicating peroxynitrite, with subsequent 3NY formation, in cell death, and abrogation of this pathway as a mechanism of IAR. IAR is dependent on the heme-metabolizing enzyme, heme oxygenase-1 (HO1), as indicated by the elimination of IAR by a specific HO1 inhibitor, and by the finding that neurons isolated from HO1 null mice have increased NO sensitivity with concomitant increased 3NY formation. This data indicate that IAR is an HO1-dependent mechanism that prevents peroxynitrite-mediated NO toxicity in motor neurons, thereby elucidating therapeutic targets for the mitigation of CNS disease and injury.

4.676 Differential sensitivity of oligodendrocytes and motor neurons to reactive nitrogen species: implications for multiple sclerosis

Bishop, A., Green Hobbs, K., Eguchi, A., Jeffrey, S., Smallwood, L., Pennie, C., Anderson, J. and estevez, A.G.

J. Neurochem., ***109***, *93-104 (2009)*

Depending on its concentration, nitric oxide (NO) has beneficial or toxic effects. In pathological conditions, NO reacts with superoxide to form peroxynitrite, which nitrates proteins forming nitrotyrosine residues (3NY), leading to loss of protein function, perturbation of signal transduction, and cell death. 3NY immunoreactivity is present in many CNS diseases, particularly multiple sclerosis. Here, using the high flux NO donor, spermine-NONOate, we report that oligodendrocytes are resistant to NO, while motor neurons are NO sensitive. Motor neuron sensitivity correlates with the NO-dependent formation of 3NY, which is significantly more pronounced in motor neurons when compared with oligodendrocytes, suggesting peroxynitrite as the toxic molecule. The heme-metabolizing enzyme, heme-oxygenase-1 (HO1), is necessary for oligodendrocyte NO resistance, as demonstrated by loss of resistance after HO1 inhibition. Resistance is reinstated by peroxynitrite scavenging with uric acid further implicating peroxynitrite as responsible for NO sensitivity. Most importantly, differential sensitivity to NO is also present in cultures of primary oligodendrocytes and motor neurons. Finally, motor neurons cocultured with oligodendrocytes, or oligodendrocyte-conditioned media, become resistant to NO toxicity. Preliminary studies suggest oligodendrocytes release a soluble factor that protects motor neurons. Our findings challenge the current paradigm that oligodendrocytes are the exclusive target of multiple sclerosis pathology.

4.677 Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus

Li, J., Park, J., Foss, D. and Goldschneider, I.

J. Exp. Med., ***206(3)***, *607-622 (2009)*

Many dendritic cells (DCs) in the normal mouse thymus are generated intrathymically from common T cell/DC progenitors. However, our previous work suggested that at least 50% of thymic DCs originate independently of these progenitors. We now formally demonstrate by parabiotic, adoptive transfer, and developmental studies that two of the three major subsets of thymic DCs originate extrathymically and continually migrate to the thymus, where they occupy a finite number of microenvironmental niches. The thymus-homing DCs consisted of immature plasmacytoid DCs (pDCs) and the signal regulatory protein α–positive (Sirpα^+) CD11b$^+$ CD8α^- subset of conventional DCs (cDCs), both of which could take up and transport circulating antigen to the thymus. The cDCs of intrathymic origin were mostly Sirpα^- CD11b$^-$ CD8α^{hi} cells. Upon arrival in the thymus, the migrant pDCs enlarged and up-regulated CD11c, major histocompatibility complex II (MHC II), and CD8α, but maintained their plasmacytoid morphology. In contrast, the migrant cDCs proliferated extensively, up-regulated CD11c, MHC II, and CD86, and expressed dendritic processes. The possible functional implications of these findings are discussed.

4.678 Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Inhibits Experimental Autoimmune Thyroiditis by the Expansion of CD4+CD25+ Regulatory T Cells

Wang, S.H., Chen. G-H., Fan, Y., Van Antwerp, M. and Baker Jr., J.R.

Endocrinology, ***150(4)***, *2000-2007 (2009)*

There have been several reports that TNF-related apoptosis-inducing ligand (TRAIL) has the ability to suppress the development of experimental autoimmune diseases, including a mouse model of experimental autoimmune encephalomyelitis, a rabbit model of rheumatoid arthritis, type 1 diabetes mellitus, in mice and experimental autoimmune thyroiditis (EAT) in mice. However, the mechanism underlying TRAIL effect is not well defined. In the present study, we specifically examined TRAIL effects on CD4$^+$CD25$^+$

regulatory T cells. $CD4^+CD25^+$ T cells prepared from mouse thyroglobulin (mTg)-immunized CBA/J mice proliferate in the presence of TRAIL and dendritic cells *in vitro*. These $CD4^+CD25^+$ T cells included both $CD4^+CD25^+CD45RB^{Low}$ (regulatory) and $CD4^+CD25^+CD45RB^{High}$ (effector) T cells. Our results demonstrated that mTg-immunized mice treated with TRAIL showed significant increases in the number of $CD4^+CD25^+CD45RB^{Low}$ T cells compared with mice immunized with mTg alone. $CD4^+CD25^+CD45RB^{Low}$ T cells expressed much higher levels of the forkhead family transcription factor, IL-10, and TGFβ1 than $CD4^+CD25^+CD45RB^{High}$ T cells, and these cells can completely suppress the proliferation of the mTg-primed splenocytes in lower concentrations than the unfractionated $CD4^+CD25^+$ T cells. Furthermore, transfer of these cells into CBA/J mice prior to mTg-primed splenocyte injection could markedly reduce the frequency and severity of EAT development. $CD4^+CD25^+CD45RB^{Low}$ T cells were more effective at suppressing histological thyroiditis than unfractionated cells. These results indicated that TRAIL can increase the number of mTg-specific $CD4^+CD25^+CD45RB^{Low}$ T cells, inhibiting autoimmune responses and preventing the progression of EAT. These findings reveal a novel mechanism by which TRAIL could inhibit autoimmune disease.

4.679 Brief Bout of Exercise Alters Gene Expression in Peripheral Blood Mononuclear Cells of Early- and Late-Pubertal Males

Radom-Aizik, S., Zaldivar Jr., F., Leu, S-Y. and Cooper, D.M.
Pediatric Res., ***65(4)***, *447-452 (2009)*

Peripheral blood mononuclear cells (PBMCs) are stimulated by exercise and contribute not only to host defense, but also to growth, repair, and disease pathogenesis. Whether PBMC gene expression is altered by exercise in children is not known. Ten early pubertal boys (8-12 y) and 10 late pubertal boys (15-18 y) performed ten 2-min bouts of strenuous, constant work rate exercise with 1-min rest intervals. PBMCs were isolated before and after exercise and microarray (Affymetrix U133 + 2 chips) analyzed. Statistical criterion to identify gene expression changes was less than 5% false discovery rate (FDR) with 95% confidence interval. One thousand two hundred forty-six genes were altered in older boys (517 up, 729 down), but only 109 were altered in the younger group (79 up, 30 down). In older boys, 13 gene pathways (using Expression Analysis Systematic Explorer, $p < 0.05$) were found (*e.g.* natural killer cell cytotoxicity, apoptosis). Epiregulin gene expression (EREG, a growth factor involved in wound healing) increased in older boys. In older boys exercise altered genes such as TBX21, GZMA, PGTDR, and CCL5 also play roles in pediatric inflammatory diseases like asthma. Sixty-six genes were changed significantly in both groups. The pattern of PBMC gene expression suggests the initiation of an immunologic danger signal associated with a sudden change in energy expenditure.

4.680 Elevated plasma prostaglandins and acetylated histone in monocytes in Type 1 diabetes patients

Chen, S.S.H., jenkins, A.J. and Majewski, H.
Diabet. Med., ***26***, *182-186 (2009)*

Aims/hypothesis: Inflammation is implicated in diabetes and cyclooxygenase (COX) is involved in vascular inflammatory processes, participating in both atherosclerosis and thrombosis. The aims were to determine whether levels of monocyte COX and plasma COX metabolites are increased in Type 1 diabetic patients and to determine whether these could be linked to histone hyperacetylation.
Materials and methods: Monocytes from 19 Type 1 diabetic and 39 non-diabetic control subjects were probed for COX and acetylated histone H4 proteins by immunoblotting. Plasma COX metabolite levels [thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2)] were determined by enzyme immunoassay.
Results: Monocyte COX-2 expression was significantly up-regulated (1.3-fold) in diabetic relative to the non-diabetic control subjects and plasma PGE_2 was markedly elevated (2.7-fold). In diabetic subjects, monocyte acetylated histone H4 levels were significantly elevated; sub-group analysis indicated that the increased histone acetylation was found only in the complication-free group.
Conclusions: Results support increased inflammatory activity in Type 1 diabetes that involves COX-2 and increased prostaglandin production, which may predispose patients to cardiovascular events. The observation of elevated histone acetylation only in complication-free diabetic subjects suggests that this may be a protective mechanism. This merits further investigation as histone hyperacetylation has been associated with reduced expression of factors involved in vascular injury and remodelling.

4.681 Ethanol-mediated expression of connective tissue growth factor (CCN2) in mouse pancreatic stellate cells

Lawrencia, C., Charrier, A., Huang, g. and Brigstock, D.R.
Growth Factors, ***27(2)***, *91-99 (2009)*

Activated pancreatic stellate cells (PSC) play a central role in the pathogenesis of pancreatic fibrosis, a common feature of chronic pancreatitis which is often caused by excessive alcohol consumption. In view of the central role of connective tissue growth factor (CCN2) in fibrosis, we investigated the mechanisms by which CCN2 is regulated in PSC following their exposure to ethanol or acetaldehyde. Primary cultures of PSC from Balb/c mice were treated with 0-50 mM ethanol or 0-200 μM acetaldehyde in the presence or absence of 4-methylpyrazole (4MP; an inhibitor of alcohol dehydrogenase), diallyl sulfide (DAS; an inhibitor of cytochrome P4502E1) or anti-oxidant catalase or vitamin D. CCN2 production, assessed by reverse-transcriptase polymerase chain reaction to measure CCN2 mRNA levels or by fluorescence activated cell sorting to assess CCN2 protein, was enhanced in a dose-dependent manner by ethanol or acetaldehyde. In the presence of 4MP, DAS, or the anti-oxidants vitamin D or catalase, there was a substantial decrease in the ability of ethanol to stimulate CCN2 mRNA expression and a concomitant decrease in CCN2-positive PSC. Accumulation of reactive oxygen species in PSC after exposure to ethanol was verified by loading the cells with dichlorofluorescin diacetate and showing that there was a stimulation of its oxidized fluorescent product, the latter of which was diminished in the presence of catalase or vitamin D. These results show the production of acetaldehyde and oxidant stress in mouse PSC are the cause of increased CCN2 mRNA and protein production after exposure of the cells to ethanol. The potential therapeutic effects of inhibitors of ethanol metabolism or anti-oxidants in alcoholic pancreatitis may arise in part through their ability to attenuate CCN2 production by PSC.

4.682 Deciphering von Hippel-Lindau (VHL/Vhl)-Associated Pancreatic Manifestations by Inactivating Vhl in Specific Pancreatic Cell Populations

Shen, H-C.J., Adem, A., Ylaya, K., Wilson, A., He, M., Lorang, D., Hewitt, S.M., Pechhold, K., harlan, D.M., Lubensky, I.A., Schmidt, L.S., Linehan, W.M. and Libutti, S.K.
*PloSOne, **4(4)**, e4897 (2009)*

The von Hippel-Lindau (VHL) syndrome is a pleomorphic familial disease characterized by the development of highly vascularized tumors, such as hemangioblastomas of the central nervous system, pheochromocytomas, renal cell carcinomas, cysts and neuroendocrine tumors of the pancreas. Up to 75% of VHL patients are affected by VHL-associated pancreatic lesions; however, very few reports in the published literature have described the cellular origins and biological roles of VHL in the pancreas. Since homozygous loss of *Vhl* in mice resulted in embryonic lethality, this study aimed to characterize the functional significance of VHL in the pancreas by conditionally inactivating *Vhl* utilizing the Cre/LoxP system. Specifically, *Vhl* was inactivated in different pancreatic cell populations distinguished by their roles during embryonic organ development and their endocrine lineage commitment. With Cre recombinase expression directed by a glucagon promoter in α-cells or an insulin promoter in β-cells, we showed that deletion of *Vhl* is dispensable for normal functions of the endocrine pancreas. In addition, deficiency of VHL protein (pVHL) in terminally differentiated α-cells or β-cells is insufficient to induce pancreatic neuroendocrine tumorigenesis. Most significantly, we presented the first mouse model of VHL-associated pancreatic disease in mice lacking pVHL utilizing Pdx1-Cre transgenic mice to inactivate *Vhl* in pancreatic progenitor cells. The highly vascularized microcystic adenomas and hyperplastic islets that developed in Pdx1-Cre;Vhl f/f homozygous mice exhibited clinical features similar to VHL patients. Establishment of three different, cell-specific *Vhl* knockouts in the pancreas have allowed us to provide evidence suggesting that VHL is functionally important for postnatal ductal and exocrine pancreas, and that VHL-associated pancreatic lesions are likely to originate from progenitor cells, not mature endocrine cells. The novel model systems reported here will provide the basis for further functional and genetic studies to define molecular mechanisms involved in VHL-associated pancreatic diseases.

4.683 The Toll-Like Receptor Signaling Molecule *Myd88* Contributes to Pancreatic Beta-Cell Homeostasis in Response to Injury

Bollyky, P.L., Bice, J.B., Sweet, I.R., Falk, B.A., Gebe, J.A., Clark, A.E., Gersuk, V.H., Aderem, A., Hawn, T.R. and Nepom, G.T.
*PloSOne, **4(4)**, e5063 (2009)*

Commensal flora and pathogenic microbes influence the incidence of diabetes in animal models yet little is known about the mechanistic basis of these interactions. We hypothesized that *Myd88*, an adaptor molecule in the Toll-like-receptor (TLR) pathway, regulates pancreatic β-cell function and homeostasis. We first examined β-cells histologically and found that *Myd88*−/− mice have smaller islets in comparison to C57Bl/6 controls. *Myd88*−/− mice were nonetheless normoglycemic both at rest and after an intra-peritoneal glucose tolerance test (IPGTT). In contrast, after low-dose streptozotocin (STZ) challenge,

Myd88−/− mice had an abnormal IPGTT relative to WT controls. Furthermore, *Myd88*−/− mice suffer enhanced β-cell apoptosis and have enhanced hepatic damage with delayed recovery upon low-dose STZ treatment. Finally, we treated WT mice with broad-spectrum oral antibiotics to deplete their commensal flora. In WT mice, low dose oral lipopolysaccharide, but not lipotichoic acid or antibiotics alone, strongly promoted enhanced glycemic control. These data suggest that *Myd88* signaling and certain TLR ligands mediate a homeostatic effect on β-cells primarily in the setting of injury.

4.684 Islet cell transplantation for the treatment of type 1 diabetes in the USA
Ikemoto, T., Noguchi, H., Shimoda, M., Naziruddin, B., Jackson, A., Tamura, Y., Fujita, Y., Onaca, N., Levy, M.F. and Matsumoto, S.
J. Hepatobiliary Pancreat. Surg., ***16***, *118-123 (2009)*

Islet cell transplantation (ICTx) is one of the most effective treatments for type 1 diabetes and is less invasive compared to whole organ transplantation. The US has been the leader in the research and clinical applications of ICTx for the last 40 years. ICTx requires complex procedures, including pancreas procurement and preservation; pancreas digestion; islet purification; and transplantation. Even with the dramatic progresses in each of the procedures listed above, there are still challenges to make ICTx the standard therapy. These challenges are: (1) obtaining enough islets from a single donor and (2) preventing graft loss due to allogenic rejection and recurrence of autoimmune islet destruction. A new preservation strategy for pancreata and pancreatic ducts using ET-Kyoto solution as well as a new islet purification method using iodixanol has substantially improved islet yields. Continuous research to improve the efficacy of islet isolation will solve the issue of obtaining enough islets from a single donor. Immunological tolerance is an ideal solution for the issue of rejection and autoimmune recurrence and a regulatory T cell strategy seems promising. Moreover, the SUITO index is a simple and powerful tool to assess engrafted islet mass and is, therefore, useful for evaluating the efficacy of new immunosuppressant strategies. Once ICTx becomes a standard treatment, the donor shortage will become the next challenge. Marginal or living donor islet transplantations could help alleviate this issue; however, bio-artificial islet transplantation with animal islets could be the ultimate solution.

4.685 An in vitro screening cascade to identify neuroprotective antioxidants in ALS
Barber, S.C., Higginbottom, A., Mead, R.J., Barber, S. and Shaw, P.J.
Free Radical Biology & Medicine, ***46***, *1127-1138 (2009)*

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, characterized by progressive dysfunction and death of motor neurons. Although evidence for oxidative stress in ALS pathogenesis is well described, antioxidants have generally shown poor efficacy in animal models and human clinical trials. We have developed an in vitro screening cascade to identify antioxidant molecules capable of rescuing NSC34 motor neuron cells expressing an ALS-associated mutation of superoxide dismutase 1. We have tested known antioxidants and screened a library of 2000 small molecules. The library screen identified 164 antioxidant molecules, which were refined to the 9 most promising molecules in subsequent experiments. Analysis of the in silico properties of hit compounds and a review of published literature on their in vivo effectiveness have enabled us to systematically identify molecules with antioxidant activity combined with chemical properties necessary to penetrate the central nervous system. The top-performing molecules identified include caffeic acid phenethyl ester, esculetin, and resveratrol. These compounds were tested for their ability to rescue primary motor neuron cultures after trophic factor withdrawal, and the mechanisms of action of their antioxidant effects were investigated. Subsequent in vivo studies can be targeted using molecules with the greatest probability of success.

4.686 Sperm sex-sorting in the Asian elephant (*Elephas maximus*)
Hermes, R., Behr, B., Hildebrandt, T.B., Blottner, S., Sieg, B., Frenzel, A., Knieriem, A., Saragusty, J. and Rath, D.
Animal Reprod. Sci., ***112***, *390-396 (2009)*

In captive Asian elephants, there is a strong need for production of female offspring to enhance reproduction, counter premature aging processes in female animals and reduce challenging management situations derived from husbandry of several bulls in one institution. Artificial insemination of flow cytometrically sex-sorted spermatozoa offers the possibility to predetermine the sex of offspring with high accuracy. The aims of this study were to determine a suitable semen extender and basic parameters for flow cytometrical sex-sorting of Asian elephant spermatozoa. In total 18 semen samples were collected by manual rectal stimulation from one bull. Sperm quality parameters and sex sortability of spermatozoa were

evaluated after dilution in three semen extenders (MES-HEPES-skim milk, MES-HEPES, TRIS–citric acid) and DNA staining. MES-HEPES-skim milk was the only semen extender found suitable to sex Asian elephant spermatozoa. From 18 ejaculates collected, 12 were successfully sorted with a purity of 94.5 ± 0.7% at an average sort rate of 1945.5 ± 187.5 spermatozoa per second. Sperm integrity, progressive and total motility were 42.6 ± 3.9%, 48.1 ± 3.3%, 59.4 ± 3.8% after DNA labelling, and 64.8 ± 3.2%, 58.0 ± 5.0%, 70.8 ± 4.4% after sorting, respectively. After liquid storage of sorted spermatozoa for 12 h at 4 °C, sperm integrity, progressive and total motility were 46.4 ± 5.2%, 32.2 ± 4.2% and 58.2 ± 3.9%, respectively. The obtained results provide a promising base to inseminate Asian elephants with sexed semen.

4.687 Flotillins Interact with PSGL-1 in Neutrophils and, upon Stimulation, Rapidly Organize into Membrane Domains Subsequently Accumulating in the Uropod
Rossy, J., Schlicht, D., engelhardt, B. and Niggli, V.
*PloSOne, **4**, e5403 (2009)*

Background
Neutrophils polarize and migrate in response to chemokines. Different types of membrane microdomains (rafts) have been postulated to be present in rear and front of polarized leukocytes and disruption of rafts by cholesterol sequestration prevents leukocyte polarization. Reggie/flotillin-1 and -2 are two highly homologous proteins that are ubiquitously enriched in detergent resistant membranes and are thought to shape membrane microdomains by forming homo- and hetero-oligomers. It was the goal of this study to investigate dynamic membrane microdomain reorganization during neutrophil activation.
Methodology/Principal Findings
We show now, using immunofluorescence staining and co-immunoprecipitation, that endogenous flotillin-1 and -2 colocalize and associate in resting spherical and polarized primary neutrophils. Flotillins redistribute very early after chemoattractant stimulation, and form distinct caps in more than 90% of the neutrophils. At later time points flotillins accumulate in the uropod of polarized cells. Chemotactic peptide-induced redistribution and capping of flotillins requires integrity and dynamics of the actin cytoskeleton, but does not involve Rho-kinase dependent signaling related to formation of the uropod. Both flotillin isoforms are involved in the formation of this membrane domain, as uropod location of exogenously expressed flotillins is dramatically enhanced by co-overexpression of tagged flotillin-1 and -2 in differentiated HL-60 cells as compared to cells expressing only one tagged isoform.
Flotillin-1 and -2 associate with P-selectin glycoprotein ligand 1 (PSGL-1) in resting and in stimulated neutrophils as shown by colocalization and co-immunoprecipitation. Neutrophils isolated from PSGL-1-deficient mice exhibit flotillin caps to the same extent as cells isolated from wild type animals, implying that PSGL-1 is not required for the formation of the flotillin caps. Finally we show that stimulus-dependent redistribution of other uropod-located proteins, CD43 and ezrin/radixin/moesin, occurs much slower than that of flotillins and PSGL-1.
Conclusions/Significance
These results suggest that flotillin-rich actin-dependent membrane microdomains are importantly involved in neutrophil uropod formation and/or stabilization and organize uropod localization of PSGL-1.

4.688 Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation
Nakayama, M., Akiba, H., Takeda, K., Kojima, Y., Hashiguchi, M., Azuma, M., Yagita, H. and Okumura, K.
*Blood, **113(16)**, 3821-3830 (2009)*

Phagocytes such as macrophages and dendritic cells (DCs) engulf apoptotic cells to maintain peripheral immune tolerance. However, the mechanism for the recognition of dying cells by phagocytes is not fully understood. Here, we demonstrate that T-cell immunoglobulin mucin-3 (Tim-3) recognizes apoptotic cells through the FG loop in the IgV domain, and is crucial for clearance of apoptotic cells by phagocytes. Whereas Tim-4 is highly expressed on peritoneal resident macrophages, Tim-3 is expressed on peritoneal exudate macrophages, monocytes, and splenic DCs, indicating distinct Tim-mediated phagocytic pathways used by different phagocytes. Furthermore, phagocytosis of apoptotic cells by $CD8^+$ DCs is inhibited by anti–Tim-3 mAb, resulting in a reduced cross-presentation of dying cell-associated antigens in vitro and in vivo. Administration of anti–Tim-3 as well as anti–Tim-4 mAb induces autoantibody production. These results indicate a crucial role for Tim-3 in phagocytosis of apoptotic cells and cross-presentation, which may be linked to peripheral tolerance.

4.689 T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a–

specific, HLA-DRB3*0101–restricted CD4+ T cells
Ahlen, M.T., Husebekk, A., Killie, M.K., Skogen, B. and Stuge, T.B.
*Blood, **113(16)**, 3838-3844 (2009)*

T-cell responses have been implicated in the development of HPA-1a–induced neonatal alloimmune thrombocytopenia (NAIT). However, HPA-1a–specific T cells have neither been isolated nor characterized. Here, we aimed to determine whether HPA-1a–specific T cells could be isolated from HPA-1a–immunized women. In the present study, peripheral blood mononuclear cells (PBMCs) from an HPA-1a–alloimmunized woman were cultured for weeks in the presence of HPA-1a peptide, labeled with CFSE, and assayed for antigen-specific proliferation. Individual proliferating cells were isolated by fluorescence-activated cell sorting and expanded in culture. Antigen specificity and HLA restriction were determined by cytokine secretion (enzyme-linked immunospot [ELISPOT]) and proliferation assays. Several $CD3^+CD4^+$ T-cell clones were isolated that proliferated and secreted cytokines in response to HPA-1a peptide. Two of these clones have been established in long-term culture in our laboratory. Both of these recognize synthetic as well as naturally processed HPA-1a antigen, and the recognition is restricted by the MHC molecule HLA-DRB3*0101 that is strongly associated with NAIT. These HPA-1a–specific T-cell clones represent unambiguous evidence for the association of T-cell responses with NAIT, and they will serve as unique tools to elucidate the cellular immune response that may result in NAIT.

4.690 Differential contribution of the Na+-K+-2Cl– cotransporter NKCC1 to chloride handling in rat embryonic dorsal root ganglion neurons and motor neurons
Chabwine, J.N., Talavwera, K., Verbert, L., Eggermont, J., Vanderwinden, J-M., De Smedt, H., Van Den Bosch, L., Robberecht, W. and Callewaert, G.
*FASEB J., **23**, 1168-1176 (2009)*

Plasma membrane chloride (Cl^-) pathways play an important role in neuronal physiology. Here, we investigated the role of NKCC1 cotransporters (a secondary active Cl^- uptake mechanism) in Cl^- handling in cultured rat dorsal root ganglion neurons (DRGNs) and motor neurons (MNs) derived from fetal stage embryonic day 14. Gramicidin-perforated patch-clamp recordings revealed that DRGNs accumulate intracellular Cl^- through a bumetanide- and Na^+-sensitive mechanism, indicative of the functional expression of NKCC1. Western blotting confirmed the expression of NKCC1 in both DRGNs and MNs, but immunocytochemistry experiments showed a restricted expression in dendrites of MNs, which contrasts with a homogeneous expression in DRGNs. Both MNs and DRGNs could be readily loaded with or depleted of Cl^- during $GABA_A$ receptor activation at depolarizing or hyperpolarizing membrane potentials. After loading, the rate of recovery to the resting Cl^- concentration (*i.e.*, $[Cl^-]_i$ decrease) was similar in both cell types and was unaffected by lowering the extracellular Na^+ concentration. In contrast, the recovery on depletion (*i.e.*, $[Cl^-]_i$ increase) was significantly faster in DRGNs in control conditions but not in low extracellular Na^+. The experimental observations could be reproduced by a mathematical model for intracellular Cl^- kinetics, in which DRGNs show higher NKCC1 activity and smaller Cl^--handling volume than MNs. On the basis of these results, we conclude that embryonic DRGNs show a higher somatic functional expression of NKCC1 than embryonic MNs. The high NKCC1 activity in DRGNs is important for maintaining high $[Cl^-]_i$, whereas lower NKCC1 activity in MNs allows large $[Cl^-]_i$ variations during neuronal activity.—Chabwine, J. N., Talavera, K., Verbert, L., Eggermont, J., Vanderwinden, J.-M., De Smedt, H., Van Den Bosch, L., Robberecht, W., Callewaert, G. Differential contribution of the Na^+-K^+-$2Cl^-$ cotransporter NKCC1 to chloride handling in rat embryonic dorsal root ganglion neurons and motor neurons.

4.691 Immunostimulatory cancer chemotherapy using local ingenol-3-angelate and synergy with immunotherapies
Le, T.T.T., Gardner, J., Hoang-Le, D., Schmidt, C.W., MacDonald, K.P., Lambley, e., Schroder, W.A., Ogbourne, S.M. and Suhrbier, A.
*Vaccine, **27**, 3053-3062 (2009)*

Ingenol-3-angelate is a new local chemotherapeutic agent in clinical trails that induces primary necrosis of tumour cells and transient local inflammation. Here we show that cure of subcutaneous tumours with ingenol-3-angelate (PEP005) resulted in the generation of anti-cancer CD8 T cells that could regress metastases. Furthermore, PEP005-mediated cure synergized with several CD8 T cell-based immunotherapies to regress further distant metastases. PEP005 was shown to have adjuvant properties, being able to upregulate CD80 and CD86 expression on dendritic cells in vivo, and to promote CD8 T cell induction when co-delivered with a protein antigen. PEP005 thus emerges as a unique local

chemotherapeutic immunostimulatory debulking agent that could be used in conjunction with immunotherapies to promote regression of metastases.

4.692 Mutual Helper Effect in Copulsing of Dendritic Cells With 2 Antigens: A Novel Approach for Improvement of Dendritic-based Vaccine Efficacy Against Tumors and Infectious Diseases Simultaneously
Shojaeian, J. et al
*J. Immunother., **32(4)**, 325-332 (2009)*

To develop an efficient dendritic cell (DC)-based immunotherapy protocol, we examined whether simultaneous pulsing of DCs with a given antigen and a third-party antigen could enhance their antigen presentation capacity. Purified splenic DCs of Balb/c mice were pulsed separately with immunoglobulin G, ovalbumin, conalbumin, P15 peptide of Mycobacterium tuberculosis, and prostate-specific antigen or double combinations of the aforementioned antigens. In some settings, DCs pulsed with 1 antigen were mixed equally with those pulsed with another antigen. Antigen-pulsed DCs were injected into the footpad of syngeneic mice and proliferation of whole, CD4- and CD8- depleted lymph node cells was measured after restimulation with cognate antigen. Antigen-specific production of interferon-gamma (IFN[gamma]) was tested in culture supernatants. Frequency of responding lymph node cells was determined by IFN[gamma] enzyme-linked immunosorbent spot assay. Our results showed that copulsing of DCs with 2 unrelated antigens increased the capacity of DCs to induce antigen-specific T-cell proliferation against both antigens up to 16-fold. Injection of 2 populations of DCs each pulsed with a different antigen, increased proliferation of primed T cells significantly as well. Both CD4- and CD8- depleted populations showed vigorous proliferative response in copulsing system. In addition, copulsing of DCs with 2 antigens resulted in higher frequency of antigen-specific responding cells and significantly more IFN[gamma] production. Our results clearly showed that unrelated peptides and proteins could be used to enhance efficacy of DC-based vaccines

4.693 Superiority of Iodixanol (OptiPrep) over Ficoll in Human Islet Purification
Mita, A. et al
*Am. J. Transplant., **9**, Suppl.2, 406 (2009)*

Continuous Ficoll-based density gradient purifi cation with top-loading using semiautomated computerized COBE-2991 cell processor is considered the gold-standard method in islet transplantation at the present time. The purifi cation methods using OptiPrep-based density gradient have been recently reported to provide more successful clinical outcomes. The aim of current study was to investigate the effects of purifi cation method using OptiPrep-based density gradients.
Human islet isolations were performed using a modifi ed automated method. After digestion phase, pancreatic digests were equally separated into two groups and purifi ed using OptiPrep-based density gradient (OptiPrep group) or Ficollbased density gradient (Ficoll group). The quantity, purity, viability and cellular composition of islet preparations from each group were assessed. Cytokine/
chemokine and tissue factor production from islet preparations after 48h culture were also measured. Although islet purity, post-purifi cation IEQ, islet recovery rate, FDA/PI and fractional ®-cell viability were comparable, absolute ®-cell mass after 48h culture signifi cantly improved in OptiPrep group when compared to Ficoll group. TNF-⟨, IL-1®, IFN-©, IL-6, IL-8, MIP-1®, MCP-1 and RANTES production except for tissue factor in OptiPrep group were signifi cantly lower. Each preparation contained the similar number of ductal cell and macrophage, which are one of cytokine/chemokine producers in human islet preparation. Endotoxin level in both gradient medium was also comparable.
The purifi cation method using OptiPrep gradient media signifi cantly reduced cytokine/chemokine production but not tissue factor from human islet preparations and improved beta cell survival during pre-transplant culture. Our results suggest that the purifi cation method using OptiPrep gradient media may be of assistance in increasing successful islet transplantation.

4.694 Identification of Critical Factors Leading to Successful Islet Isolations and Transplantation
Avila, J.G. et al
*Am. J. Transplant., **9**, Suppl. 2, 403-404 (2009)*

Islet transplantation remains a feasible therapy for the treatment of a specifi c group of patients with type I diabetes. However, islet isolation remains inconsistent due to many factors that infl uence fi nal islet yield. New products are leading to improved outcome, even in younger donor pancreata. The purpose of this

study was to identify a group of variables with a critical role in obtaining successful outcomes in islet isolations, leading to single donor islet transplants with insulin independence in our clinical trial.
Methods: Thirty-nine human islet isolations where studied. Final islet yields in islet equivalents (IEQ) were compared from pancreata procured from our surgical group versus or surgeons from non-islet centers. In addition, age and body mass index (BMI) of the donor, enzyme type and purifi cation methods were compared between successful (transplanted) isolations and non-transplanted ones.
Results: Final islet yields were signifi cantly higher when an islet experienced surgeon performed the procurement compared to a surgeon from another institution (401,787±177,894 IEQ vs. 237,825±124,987, mean±SD, p<0.01) respectively. A new generation of collagenase (NB1) and neutral protease (NB) available enabled us to obtain higher islet yields from younger pancreata. The mean age of donor pancreata resulting in an islet transplant was signifi cantly younger than in unsuccessful isolations using the same enzyme (32±4 years vs. 44±10 respectively, p=0.04).
Regression analysis shows a negative correlation between age and islet yield using this enzyme (Spearman R=-0.58, p=0.039). As in previous studies, isolations leading to a transplant resulted from donors with signifi cantly higher body mass index (BMI) than the unsuccessful isolations (38.4±4.9 vs. 30±6.4 respectively, p=0.04).
An improved method for islet purifi cation using Penta starch and Iodixanol resulted in a significantly higher percent recovery of islets than the Ficoll-based method (86%±17 vs. 64%±25 respectively, p=0.01).
Conclusion: Results suggest that surgeons with interest in islets may have a positive impact in the outcome of the isolation. In addition, islets from younger and heavier donors can be successfully isolated with the new enzyme combination NB1-NB. Moreover, the combination of a careful procurement, an appropriate enzyme and an effi cient purifi cation method can result in improved outcomes of islet isolations.

4.695 **Protective effects of iodixanol during bovine sperm cryopreservation**
Saragusty, J., Gacitua, H., Rozenboim, I. and Arav, A.
Theriogenology, ***71***, *1425-1432 (2009)*

The aim of cryopreservation is to maintain cellular integrity, thereby enabling resumption of proper biological functioning after thawing. Here we propose OptiPrep™ (60% iodixanol in water) as a protectant during sperm cryopreservation using pooled bull semen as the model. We evaluated OptiPrep concentration effect and its relation to cryopreservation by comparing frozen-thawed and chilled samples. Semen, extended in Andromed® with 0 (control), 1.25%, 2.5%, and 5% OptiPrep™, was compared after either chilling or freezing in large volume by directional freezing. Sample evaluation included sperm motility upon thawing and after 3 h incubation at 37 °C for frozen-thawed samples and after 3 h and 6 h of chilling for chilled samples; viability, acrosomal integrity, and hypoosmotic swelling were also tested for frozen-thawed and chilled samples. Chilled samples with 5% OptiPrep™ showed inferior viability (P = 0.047) and 3 h motility (P = 0.017) relative to that for chilled samples with 2.5% OptiPrep and inferior viability (P = 0.042), acrosomal integrity (P = 0.045), and 0 h motility (P = 0.024) relative to that for chilled samples with 1.25% OptiPrep. The 1.25%, 2.5%, and control samples did not differ. In frozen-thawed samples, 2.5% OptiPrep was superior to all other concentrations for 3 h motility (control, P = 0.007; 5% OptiPrep, P = 0.005; 1.25% OptiPrep, P = 0.004) and to 1.25% OptiPrep for acrosomal integrity (P = 0.001). In a search for a protection mechanism, we measured glass transition temperature (T_g) of Andromed® and of Andromed® with 1.25%, 2.5%, and 5% OptiPrep™. Andromed® (–58.78 °C) and 1.25% OptiPrep™ (–58.75 °C) groups had lower mean T_g than that of the 2.5% (–57.67 °C) and the 5% (–57.10 °C) groups. Directional cryomicroscopy revealed that the presence of iodixanol alters ice crystal formation into an intricate net of dendrites. Thus, iodixanol appears to possess cryoprotective properties by helping spermatozoa maintain motility and membrane integrity, possibly through altering ice crystals formation into a more hospitable environment and increasing the glass transition temperature.

4.696 **Foxn1 is required to maintain the postnatal thymic microenvironment in a dosage-sensitive manner**
Chen, L., Xiao, S. and Manley, N.R.
Blood, ***113(3)***, *567-574 (2009)*

The postnatal thymus is the primary source of T cells in vertebrates, and many if not all stages of thymocyte development require interactions with thymic epithelial cells (TECs). The *Foxn1* gene is a key

regulator of TEC differentiation, and is required for multiple aspects of fetal TEC differentiation. *Foxn1* is also expressed in the postnatal thymus, but its function after birth is unknown. We generated a *Foxn1* allele with normal fetal expression and thymus development, but decreased expression in the postnatal thymus. This down-regulation causes rapid thymic compartment degeneration and reduced T-cell production. TEC subsets that express higher *Foxn1* levels are most sensitive to its down-regulation, in particular $MHCII^{hi}UEA\text{-}1^{hi}$ medullary TECs. The requirement for Foxn1 is extremely dosage sensitive, with small changes in *Foxn1* levels having large effects on thymus phenotypes. Our results provide the first evidence that *Foxn1* is required to maintain the postnatal thymus. Furthermore, the similarities of this phenotype to accelerated aging-related thymic involution support the possibility that changes in Foxn1 expression in TECs during aging contribute to the mechanism of involution.

4.697 Motor neuronal protection by l-arginine prolongs survival of mutant SOD1 (G93A) ALS mice
Lee, J., Ryu, H. and Kowall, N.W.
Biochem. Biophys. Res. Comm., ***384***, *524-529 (2009)*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive paralysis due to motor neuron degeneration. Despite the fact that many different therapeutic strategies have been applied to prevent disease progression, no cure or effective therapy is currently available for ALS. We found that l-arginine protects cultured motor neurons from excitotoxic injury. We also found that l-arginine supplementation both prior to and after the onset of motor neuron degeneration in mtSOD1 (G93A) transgenic ALS mice significantly slowed the progression of neuropathology in lumbar spinal cord, delayed onset of motor dysfunction, and prolonged life span. Moreover, l-arginine treatment was associated with preservation of arginase I activity and neuroprotective polyamines in spinal cord motor neurons. Our findings show that l-arginine has potent *in vitro* and *in vivo* neuroprotective properties and may be a candidate for therapeutic trials in ALS.

4.698 Up-regulation of uPARAP/Endo180 during culture activation of rat hepatic stellate cells and its presence in hepatic stellate cell lines from different species
Mousavi, S.A., Fønhus, M.S. and berg, T.
BMC Cell Biol., ***10***, *39-49 (2009)*

Background
The urokinase plasminogen activator receptor associated protein (uPARAP)/Endo180 is a novel endocytic receptor that mediates collagen uptake and is implicated to play a role in physiological and pathological tissue-remodelling processes by mediating intracellular collagen degradation.
Result
This study investigates the expression of uPARAP/Endo180 protein and messenger RNA in primary rat hepatic stellate cell (HSC) cultures. The results show that uPARAP/Endo180 protein is not expressed in freshly isolated HSCs or during the first few days of culture while the cells still display quiescent features. In contrast, uPARAP/Endo180 protein is expressed early during HSC activation when cells are transdifferentiated into myofibroblast-like cells. Very low levels of uPARAP/Endo180 mRNA are detectable during the first days of culture but uPARAP/Endo180 mRNA is strongly up-regulated with increasing time in culture. Moreover, endocytic uptake of denatured collagen increases as transdifferentiation proceeds over time and correlates with increased expression of uPARAP/Endo180. Finally, analysis of uPARAP/Endo180 expression in four hepatic stellate cell lines from three different species showed that all these cell lines express uPARAP/Endo180 and are able to take up denatured collagen efficiently.
Conclusion
These results demonstrate that uPARAP/Endo180 expression by rat HSCs is strongly up-regulated during culture activation and identify this receptor as a feature common to culture-activated HSCs.

4.699 Langerhans Cell Maturation and Contact Hypersensitivity Are Impaired in Aryl Hydrocarbon Receptor-Null Mice
Jux, B., Kadow, S. and Esser, C.
J. Immunol., ***182***, *6709-6717 (2009)*

Langerhans cells (LC) are professional APCs of the epidermis. Recently, it was suggested that they are tolerogenic and control adverse immune reactions, including against low molecular mass chemicals. The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is involved in low molecular mass

chemical metabolism and cell differentiation. Growing evidence suggests a role for the AhR in the immune system, for example, by influencing dendritic cell and T cell differentiation. We found that the AhR and its repressor AhRR are expressed in LC of C57BL/6 mice. LC, unexpectedly, did not respond to a strong AhR agonist with induction of transcripts of xenobiotic metabolizing enzymes. To test for a physiological role of the AhR in LC, we investigated how AhR deficiency affects LC. We found that AhR-deficient LC were impaired in maturation; they remained smaller and less granular, did not up-regulate expression of costimulatory molecules CD40, CD80, and CD24a during in vitro maturation, and their phagocytic capacity was higher. Interestingly, the mRNA expression of tolerogenic *Ido* was severely decreased in AhR-deficient LC, and enzyme activity could not be induced in AhR-deficient bone marrow-derived dendritic cells. GM-CSF, needed for LC maturation, was secreted in significantly lower amounts by AhR-deficient epidermal cells. Congruent with this impaired maturity and capacity to mature, mice mounted significantly weaker contact hypersensitivity against FITC. Our data suggest that the AhR is involved in LC maturation, both cell autonomously and through bystander cells. At the same time, the AhR might be part of the risk strategy of LC against unwanted immune activation by potential skin allergens.

4.700 SP-A Preserves Airway Homeostasis During Mycoplasma pneumoniae Infection in Mice
Ledford, J.G., Goto, H., Potts, E.N., Degan, S., Chu, H.W., Voelker, D.R., Sunday, M.E., Cianciolo, G.J., Foster, W.M., Kraft, M. and Wright, J.R.
J. Immunol., ***182***, *7818-7827 (2009)*

The lung is constantly challenged during normal breathing by a myriad of environmental irritants and infectious insults. Pulmonary host defense mechanisms maintain homeostasis between inhibition/clearance of pathogens and regulation of inflammatory responses that could injure the airway epithelium. One component of this defense mechanism, surfactant protein-A (SP-A), exerts multifunctional roles in mediating host responses to inflammatory and infectious agents. SP-A has a bacteriostatic effect on *Mycoplasma pneumoniae* (Mp), which occurs by binding surface disaturated phosphatidylglycerols. SP-A can also bind the Mp membrane protein, MPN372. In this study, we investigated the role of SP-A during acute phase pulmonary infection with Mp using mice deficient in SP-A. Biologic responses, inflammation, and cellular infiltration, were much greater in Mp infected SP-$A^{-/-}$ mice than wild-type mice. Likewise, physiologic responses (airway hyperresponsiveness and lung compliance) to Mp infection were more severely affected in SP-$A^{-/-}$ mice. Both Mp-induced biologic and physiologic changes were attenuated by pharmacologic inhibition of TNF-α. Our findings demonstrate that SP-A is vital to preserving lung homeostasis and host defense to this clinically relevant strain of Mp by curtailing inflammatory cell recruitment and limiting an overzealous TNF-α response.

4.701 Cyclooxygenase-2 Induced by Zymosan in Human Monocyte-Derived Dendritic Cells Shows High Stability, and Its Expression Is Enhanced by Atorvastatin
Alvarez, Y., Municio, C., Alonso, S., Roman, J.A.S., Sanchez Crespo, M and Fernandez, N.*Infect. Immun.*, *J. Pharmacol. Exp. Ther.*, ***329(3)***, *987-994 (2009)*

Cyclooxygenase (COX)-2 is a central enzyme of arachidonic acid metabolism, and its modulation by statins may explain some of the myocardial protective effects of these drugs. Dendritic cells (DCs) play a central role in microbial defense and in atherogenesis, and COX-2 expression in DCs is important for their migration to lymph nodes and antibody response, thus explaining why prostaglandin E_2 is a main component of the cocktails used to prepare DCs for clinical applications. On this basis, we addressed the effect of atorvastatin (ATV) on the release of arachidonic acid and on the expression of COX-2 in human monocyte-derived DCs. Although ATV on its own lacked any effect on COX-2 protein induction expression, it enhanced the release of arachidonic acid, the expression of COX-2 protein, and the production of prostaglandin E_2 induced by the fungal wall extract zymosan, and to a lower extent the effect of peptidoglycan. The effect on COX-2 protein was observed mainly 24 h after stimulation by zymosan and was not reverted by mevalonate, thus pointing to an effect unrelated to cholesterol metabolism. It is noteworthy that COX-2 protein showed a great stability, with a $t^{1}/_{2}$ of approximately 12 h, which was enhanced in the presence of ATV. In view of the important role played by COX-2 on DC function, these data indicate that ATV, by enhancing COX-2 stability, may increase DC function after infectious bouts and also counteract some of the risks associated with sustained inhibition of COX-2.

4.702 Interruption of β-Catenin Signaling Reduces Neurogenesis in Alzheimer's Disease
He, P. and Shen, Y.
J. Neurosci., ***29(20)***, *6545-6557 (2009)*

Although recent studies have shown that new neurons can be generated from progenitor cells in the neocortices of healthy adults, the neurogenic potential of the stem/progenitor cells of AD patients is not known. To answer this question, we compared the properties of glial progenitor cells (GPCs) from the cortices of healthy control (HC) and AD subjects. The GPCs from AD brain samples displayed reduced renewal capability and reduced neurogenesis compared with GPCs from HC brains. To investigate the mechanisms underlying this difference, we compared β-catenin signaling proteins in GPCs from AD versus HC subjects and studied the effect of amyloid β peptide (Aβ, a hallmark of AD pathology) on GPCs. Interestingly, GPCs from AD patients exhibited elevated levels of glycogen synthase kinase 3β (GSK-3β, an enzyme known to phosphorylate β-catenin), accompanied by an increase in phosphorylated β-catenin and a decrease in nonphosphorylated β-catenin compared with HC counterparts. Furthermore. we found that Aβ treatment impaired the ability of GPCs from HC subjects to generate new neurons and caused changes in β-catenin signaling proteins similar to those observed in GPCs from AD patients. Similar results were observed in GPCs isolated from AD transgenic mice. These results suggest that Aβ-induced interruption of β-catenin signaling may contribute to the impairment of neurogenesis in AD progenitor cells.

4.703 Nonhematopoietic Cells Control the Outcome of Infection with Listeria monocytogenes in a Nucleotide Oligomerization Domain 1-Dependent Manner
Mosa, A., Trumstedt, C., Eriksson, E., Soehnlein, O., Heuts, F., Janik, K., Klos, A., Dittrich-Breiholz, O., Kracht, M., Hidmark, Å., Wigzell, H. and Rottenberg, M.E.
Infect. Immun., ***77(7)***, *2908-2918 (2009)*

We analyzed the defensive role of the cytosolic innate recognition receptor nucleotide oligomerization domain 1 (NOD1) during infection with *Listeria monocytogenes*. Mice lacking NOD1 showed increased susceptibility to systemic intraperitoneal and intravenous infection with high or low doses of *L. monocytogenes*, as measured by the bacterial load and survival. NOD1 also controlled dissemination of *L. monocytogenes* into the brain. The increased susceptibility to reinfection of $NOD1^{-/-}$ mice was not associated with impaired triggering of listeria-specific T cells, and similar levels of costimulatory molecules or activation of dendritic cells was observed. Higher numbers of $F480^{+}$ $Gr1^{+}$ inflammatory monocytes and lower numbers of $F480^{-}$ $Gr1^{+}$ neutrophils were recruited into the peritoneum of infected WT mice than into the peritoneum of infected $NOD1^{-/-}$ mice. We determined that nonhematopoietic cells accounted for NOD1-mediated resistance to *L. monocytogenes* in bone marrow radiation chimeras. The levels of NOD1 mRNA in fibroblasts and bone marrow-derived macrophages (BMM) were upregulated after infection with *L. monocytogenes* or stimulation with different Toll-like receptor ligands. $NOD1^{-/-}$ BMM, astrocytes, and fibroblasts all showed enhanced intracellular growth of *L monocytogenes* compared to WT controls. Gamma interferon-mediated nitric oxide production and inhibition of *L. monocytogenes* growth were hampered in $NOD1^{-/-}$ BMM. Thus, NOD1 confers nonhematopoietic cell-mediated resistance to infection with *L. monocytogenes* and controls intracellular bacterial growth in different cell populations in vitro.

4.704 Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3
Gamerdinger, M., Hajieva, P., Kaya, A.M., Wolfrum, U., Hart, F.U. and Behl, C.
EMBO J., ***28***, *889-901 (2009)*

The Hsc/Hsp70 co-chaperones of the BAG (Bcl-2-associated athanogene) protein family are modulators of protein quality control. We examined the specific roles of BAG1 and BAG3 in protein degradation during the aging process. We show that BAG1 and BAG3 regulate proteasomal and macroautophagic pathways, respectively, for the degradation of polyubiquitinated proteins. Moreover, using models of cellular aging, we find that a switch from BAG1 to BAG3 determines that aged cells use more intensively the macroautophagic system for turnover of polyubiquitinated proteins. This increased macroautophagic flux is regulated by BAG3 in concert with the ubiquitin-binding protein p62/SQSTM1. The BAG3/BAG1 ratio is also elevated in neurons during aging of the rodent brain, where, consistent with a higher macroautophagy activity, we find increased levels of the autophagosomal marker LC3-II as well as a higher cathepsin activity. We conclude that the BAG3-mediated recruitment of the macroautophagy pathway is an important adaptation of the protein quality control system to maintain protein homeostasis in the presence of an enhanced pro-oxidant and aggregation-prone milieu characteristic of aging.

4.705 Ontogeny and phagocytic function of baboon lung dendritic cells
Awasthi, S., Wolf, R. and White, G.
Immunol. Cell Biol., ***87***, *419-427 (2009)*

Dendritic cells (DCs) are the most potent antigen-presenting cells, but the ontogeny and functions of lung DCs are not known during prenatal period. Here, we isolated lung DC population from fetal (125–175 days of gestation age) and adult baboons. The cells were stained with fluorochrome-conjugated-HLA-DP, DQ, DR, CD1a, CD11c, CD14, CD40, CD80, CD86, CD209, CMKLR1, ILT7-specific antibodies, and staining was analyzed by flow cytometry. The phagocytic function was investigated by incubating the cells with fluorescent-labeled *Escherichia coli* bioparticles and analyzed by flow cytometry and fluorescence microscopy. The fetal baboon lung DCs expressed low levels of HLA-DP, DQ, DR, CD11c and CD86 as compared to adult baboon lung DCs and showed distinct DC morphology. The fetal lung DCs were also less capable of phagocytosing *E. coli* as compared to the adult lung DCs ($P<0.05$). In conclusion, the fetal lung DCs are not only phenotypically immature, but also less efficient in phagocytosing *E. coli*.

4.706 Dynamic Changes in Pancreatic Endocrine Cell Abundance, Distribution, and Function in Antigen-Induced and Spontaneous Autoimmune Diabetes

Pechhold, K., Zhu, X., Harison, V.S., Lee, J., Chakabarty, S., Kocwara, K., Gavrilova, O. and Harlan, D.M.
*Diabetes, **58(5)**, 1175-1184 (2009)*

OBJECTIVE Insulin deficiency in type 1 diabetes and in rodent autoimmune diabetes models is caused by β-cell–specific killing by autoreactive T-cells. Less is known about β-cell numbers and phenotype remaining at diabetes onset and the fate of other pancreatic endocrine cellular constituents.
RESEARCH DESIGN AND METHODS We applied multicolor flow cytometry, confocal microscopy, and immunohistochemistry, supported by quantitative RT-PCR, to simultaneously track pancreatic endocrine cell frequencies and phenotypes during a T-cell–mediated β-cell–destructive process using two independent autoimmune diabetes models, an inducible autoantigen-specific model and the spontaneously diabetic NOD mouse.
RESULTS The proportion of pancreatic insulin-positive β-cells to glucagon-positive α-cells was about 4:1 in nondiabetic mice. Islets isolated from newly diabetic mice exhibited the expected severe β-cell depletion accompanied by phenotypic β-cell changes (i.e., hypertrophy and degranulation), but they also revealed a substantial loss of α-cells, which was further confirmed by quantitative immunohistochemisty. While maintaining normal randomly timed serum glucagon levels, newly diabetic mice displayed an impaired glucagon secretory response to non–insulin-induced hypoglycemia.
CONCLUSIONS Systematically applying multicolor flow cytometry and immunohistochemistry to track declining β-cell numbers in recently diabetic mice revealed an altered endocrine cell composition that is consistent with a prominent and unexpected islet α-cell loss. These alterations were observed in induced and spontaneous autoimmune diabetes models, became apparent at diabetes onset, and differed markedly within islets compared with sub–islet-sized endocrine cell clusters and among pancreatic lobes. We propose that these changes are adaptive in nature, possibly fueled by worsening glycemia and regenerative processes.

4.707 Iodixanol-Controlled Density Gradient During Islet Purification Improves Recovery Rate in Human Islet Isolation

Noguchi, H., Ikemoto, T., Naziruddin, B., Jackson, A., Shimoda, M., Fujita, Y., Chujo, D., Takita, M., Kobayashi, N., Onaca, N., Levy, M.F. and Matsumoto, S.
*Transplantation, **87(11)**, 1629-1635 (2009)*

Background. For pancreatic islet transplantation, islet purification minimizes the risks associated with islet infusion through the portal vein by reducing the amount of transplanted tissue. However, the purification step may result in decreased numbers of islets recovered from digested tissue and be traumatic to the islets. In this study, we evaluated the effectiveness of iodixanol-controlled density gradients on the islet purification step.
Methods. For 14.3% of the isolations, the density was 1.085 g/cm3, 32.1% were 1.090 g/cm3, 46.4% were 1.095 g/cm3, 3.6% were 1.100 g/cm3, and 3.6% were 1.105 g/cm3, indicating that the density varies with each isolation. This has profound implications for the difficulty of islet purification. According to the density of digested tissue before purification, the density of the purification solutions was controlled by changing the volumetric ratio of iodixanol and the purification solutions (iodixanol-Kyoto [IK] solutions).
Results. Islet yield after purification and rate of postpurification recovery were significantly higher in the IK group than with standard continuous gradient purification by Ficoll solutions (islet yield=Ficoll group: 377,230+/-50,207 islet equivalents, IK group: 594,136+/-50,570 islet equivalents, P less than 0.01; percentage of recovery=Ficoll group: 55.6%+/-5.8%, IK group: 84.9%+/-4.2%, P less than 0.01). In vitro

and in vivo assays suggest that the quality of islets was similar between the two groups.
Conclusion. Our data suggest that using an iodixanol-controlled density gradient improves the islet recovery rate in human islet isolation. On the basis of these data, we now use this purification method for clinical islet transplantation.

4.708 A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females
Radom-Aizik, S., Zaldivar Jr, F., Leu, S-Y. and Cooper, D.M.
*J. Appl. Physiol., **107**, 168-175 (2009)*

Recent studies show that brief exercise alters circulating neutrophil and peripheral blood mononuclear cell (PBMC) gene expression, ranging from cell growth to both pro-and anti-inflammatory processes. These initial observations were made solely in males, but whether PBMC gene expression is altered by exercise in females is not known. Ten early-pubertal girls (8–11 yr old) and 10 late-pubertal girls (15–17 yr old) performed ten 2-min bouts of cycle ergometry (~90% peak heart rate) interspersed with 1-min rest intervals. Blood was obtained at rest and after exercise, and microarrays were performed in each individual subject. RNA was hybridized to Affymetrix U133+2.0 Arrays. Exercise induced significant changes in PBMC gene expression in early (1,320 genes)- and late (877 genes)-pubertal girls. The expression of 622 genes changed similarly in both groups. Exercise influenced a variety of established gene pathways (EASE < 0.04) in both older (6 pathways) and younger girls (11 pathways). Five pathways were the same in both groups and were functionally related to inflammation, stress, and apoptosis, such as natural killer cell-mediated cytotoxicity, antigen processing and presentation, B cell receptor signaling, and apoptosis. In summary, brief exercise alters PBMC gene expression in early- and late-pubertal girls. The pattern of change involves diverse genetic pathways, consistent with a global danger-type response, perhaps readying PBMCs for a range of physiological functions from inflammation to tissue repair that would be useful following a bout of physical activity.

4.709 Gut Homing Receptors on CD8 T Cells Are Retinoic Acid Dependent and Not Maintained by Liver Dendritic or Stellate Cells
Eksteen, B., Rodrigo Mora, J., Haughton, E.L., henderson, N.C., Lee-Turner, L., Villablanca, E.J., Curbishley, S.M., Aspinall, A.I., von Andrian, U.H. and Adams, D.H.
*Gastroenterology, **137(1)**, 320-329 (2009)*

Background & Aims
Lymphocytes primed by intestinal dendritic cells (DC) express the gut-homing receptors CCR9 and α4β7, which recognize CCL25 and mucosal addressin cell-adhesion molecule-1 in the intestine promoting the development of regional immunity. In mice, imprinting of CCR9 and α4β7 is dependent on retinoic acid during T-cell activation. Tissue specificity is lost in primary sclerosing cholangitis (PSC), an extraintestinal manifestation of inflammatory bowel disease, when ectopic expression of mucosal addressin cell-adhesion molecule-1 and CCL25 in the liver promotes recruitment of CCR9+α4β7+ T cells to the liver. We investigated the processes that control enterohepatic T-cell migration and whether the ability to imprint CCR9 and α4β7 is restricted to intestinal DCs or can under some circumstances be acquired by hepatic DCs in diseases such as PSC.
Methods
Human and murine DCs from gut, liver, or portal lymph nodes and hepatic stellate cells were used to activate CD8 T cells. Imprinting of CCR9 and α4β7 and functional migration responses were determined. Crossover activation protocols assessed plasticity of gut homing.
Results
Activation by gut DCs imprinted high levels of functional CCR9 and α4β7 on naïve CD8 T cells, whereas hepatic DCs and stellate cells proved inferior. Imprinting was RA dependent and demonstrated plasticity.
Conclusions
Imprinting and plasticity of gut-homing human CD8 T cells requires primary activation or reactivation by gut DCs and is retinoic acid dependent. The inability of liver DCs to imprint gut tropism implies that α4β7+CCR9+ T cell that infiltrate the liver in PSC are primed in the gut.

4.710 CD44high Memory CD8 T Cells Synergize with CpG DNA to Activate Dendritic Cell IL-12p70 Production
Wong, K.L., Tang, L.F.M., Lew, F.C., Wong, H.S.K., Chua, Y.L., MacAry, P.A. and Kemeny, D.M.
*J. Immunol., **183**, 41-50 (2009)*

Protective memory CD8 T cell responses are generally associated with the rapid and efficient acquisition of CTL function. However, the ability of memory CD8 T cells to modulate immune responses through interactions with dendritic cells (DCs) during the early states of secondary Ag exposure is poorly understood. In this study, we show that murine Ag-specific $CD44^{high}$ CD8 T cells, representing CD8 T cells of the memory phenotype, potently activate DCs to produce high levels of IL-12p70 in conjunction with stimulation of DCs with the TLR 9 ligand, unmethylated CpG DNA. IL-12p70 production was produced predominantly by $CD8\alpha^+$ DCs and plasmacytoid DCs, and mediated by CD8 T cell-derived cytokines IFN-γ, GM-CSF, TNF-α, and surface CD40L. We also find that $CD44^{high}$ memory phenotype CD8 T cells were better DC IL-12p70 stimulators than $CD44^{low}$ naive phenotype CD8 T cells, and this was attributed to higher levels of IFN-γ and GM-CSF produced by $CD44^{high}$ memory phenotype CD8 T cells during their Ag specific interaction with DCs. Our study identifies CpG DNA as the most effective TLR ligand that cooperates with CD8 T cells for DC IL-12p70 production, and suggests that effectiveness of memory CD8 T cells could be attributed to their ability to rapidly and effectively induce protective Th1 immunity during early stages of pathogen reinfection.

4.711 The Induction of IL-10 by Zymosan in Dendritic Cells Depends on CREB Activation by the Coactivators CREB-Binding Protein and TORC2 and Autocrine PGE2
Alvarez, Y., Municio, C., Alonso, S., Sanchez Crespo, M. and Fernandez, N.
J. Immunol., ***183***, *1471-1479 (2009)*

Stimulation of human monocyte-derived dendritic cells with the yeast extract zymosan is characterized by a predominant production of IL-10 and a strong induction of cyclooxygenase-2, but the molecular mechanisms underlying this response are only partially understood. To address this issue, the activation of transcription factors that may bind to the *il10* proximal promoter was studied. Binding activity to Sp1, Sp3, NF-Y, and cAMP response element (CRE) sites was detected in the nuclear extracts of dendritic cells; however these binding activities were not influenced by zymosan. No binding activity to Stat1, Stat3, and c/EBP sites was detected. Notably, zymosan activated κB-binding activity, but inhibition of NF-κB was associated with enhanced IL-10 production. In sharp contrast, treatments acting on CREB (CRE binding protein), including 8-Br-cAMP, PGE_2, and inhibitors of PKA, COX, and glycogen-synthase kinase-3β showed a direct correlation between CREB activation and IL-10 production. Zymosan induced binding of both P-CREB and CREB-binding protein (CBP) to the *il10* promoter as judged from chromatin immunoprecipitation assays, whereas negative results were obtained with Ab reactive to Sp1, Sp3, c-Maf, and NF-Y. Zymosan also induced nuclear translocation of the CREB coactivator transducer of regulated CREB activity 2 (TORC2) and interaction of TORC2 with P-CREB coincidental with the association of CREB to the *il10* promoter. Altogether, our data show that zymosan induces *il10* transcription by a CRE-dependent mechanism that involves autocrine secretion of PGE_2 and a network of interactions of PKA, MAP/ERK, glycogen-synthase kinase-3β, and calcineurin, which regulate CREB transcriptional activity by binding the coactivators CBP and TORC2 and inhibiting CBP interaction with other transcription factors.

4.712 Carbon Monoxide Rescues Heme Oxygenase-1-Deficient Mice from Arterial Thrombosis in Allogeneic Aortic Transplantation
Chen, B., Guo, L., Fan, C., Bolisetty, S., Joseph, R., Wright, M.M., Agarwal, A. and George, J.F.
Am. J. Pathol., ***175(1)***, *422-429 (2009)*

Heme oxygenase-1 (HO-1) catalyzes the conversion of heme into carbon monoxide (CO), iron, and biliverdin. In preliminary studies, we observed that the absence of HO-1 in aortic allograft recipients resulted in 100% mortality within 4 days due to arterial thrombosis. In contrast, recipients normally expressing HO-1 showed 100% graft patency and survival for more than 56 days. Abdominal aortic transplants were performed using Balb/cJ mice as donors and either ***HO-1***$^{+/+}$ or ***HO-1***$^{-/-}$ (C57BL/6xFVB) mice as recipients. Light and electron microscopy revealed extensive platelet-rich thrombi along the entire length of the graft in ***HO-1***$^{-/-}$ recipients at 24 hours. Treatment of recipients with CORM-2, a CO-releasing molecule (10 mg/kg of body weight intravenously), 1 hour prior and 1, 3, and 6 days after transplantation, significantly improved survival (62% at >56 days, $P < 0.001$) compared with ***HO-1***$^{-/-}$ recipients treated with inactive CORM-2 (median survival 1 day). Histological analyses revealed that CO treatment markedly reduced platelet aggregation within the graft. Adoptive transfer of wild-type platelets to ***HO-1***$^{-/-}$ recipients also conferred protection and increased survival. Aortic transplants from either ***HO-1***$^{-/-}$ or ***HO-1***$^{+/+}$ C57BL/6 donors into ***HO-1***$^{+/+}$ (Balb/cJ) mice did not develop arterial thrombosis, surviving more than 56 days. These studies demonstrate an important role for systemic HO-1/CO for protection against vascular arterial thrombosis in murine aortic allotransplantation.

4.713 Role of neuroglobin in regulating reactive oxygen species in the brain of the anoxia-tolerant turtle Trachemys scripta

Nayak, G., Prentice, H.M. and Milton, S.L.
J. Neurochem., ***110(2)***, *603-612 (2009)*

Neuroglobin (Ngb) is an oxygen binding heme protein found in nervous tissue with a yet unclear physiological and protective role in the hypoxia-sensitive mammalian brain. Here we utilized *in vivo* and *in vitro* studies to examine the role of Ngb in anoxic and post-anoxic neuronal survival in the freshwater turtle. We employed semiquantitative RT-PCR and western blotting to analyze Ngb mRNA and protein levels in turtle brain and neuronally enriched cultures. Ngb expression is strongly up-regulated by hypoxia and post-anoxia reoxygenation but increases only modestly in anoxia. The potential neuroprotective role of Ngb in this species was analyzed by knocking down Ngb using specific small interfering RNA. Ngb knockdown in neuronally enriched cell cultures resulted in significant increases in H_2O_2 release compared to controls but no change in cell death. Cell survival may be linked to activation of other protective responses such as the extracellular regulated kinase transduction pathway, as phosphorylated extracellular regulated kinase levels in anoxia were significantly higher in Ngb knockdown cultures compared to controls. The greater expression of Ngb when reactive oxygen species are likely to be high, and the increased susceptibility of neurons to H_2O_2 release and external oxidative stress in knockdown cultures, suggests a role for Ngb in reducing reactive oxygen species production or in detoxification, though it does not appear to be of primary importance in the anoxia tolerant turtle in the presence of compensatory survival mechanisms.

4.714 Successful cryopreservation of Asian elephant (*Elephas maximus*) spermatozoa

Saragusty, J., Hildebrandt, T.B., Behr, B., Knieriem, A., Kruse, J. and Hermes, R.
Animal Reprod. Sci., ***115***, *255-266 (2009)*

Reproduction in captive elephants is low and infant mortality is high, collectively leading to possible population extinction. Artificial insemination was developed a decade ago; however, it relies on fresh-chilled semen from just a handful of bulls with inconsistent sperm quality. Artificial insemination with frozen–thawed sperm has never been described, probably, in part, due to low semen quality after cryopreservation. The present study was designed with the aim of finding a reliable semen freezing protocol. Screening tests included freezing semen with varying concentrations of ethylene glycol, propylene glycol, trehalose, dimethyl sulfoxide and glycerol as cryoprotectants and assessing cushioned centrifugation, rapid chilling to suprazero temperatures, freezing extender osmolarity, egg yolk concentration, post-thaw dilution with cryoprotectant-free BC solution and the addition of 10% (v/v) of autologous seminal plasma. The resulting optimal freezing protocol uses cushioned centrifugation, two-step dilution with isothermal 285 m Osm/kg Berliner Cryomedium (BC) with final glycerol concentration of 7% and 16% egg yolk, and freezing in large volume by the directional freezing technique. After thawing, samples are diluted 1:1 with BC solution. Using this protocol, post-thaw evaluations results were: motility upon thawing: 57.2 ± 5.4%, motility following 30 min incubation at 37 °C: 58.5 ± 6.0% and following 3 h incubation: 21.7 ± 7.6%, intact acrosome: 57.1 ± 5.2%, normal morphology: 52.0 ± 5.8% and viability: 67.3 ± 6.1%. With this protocol, good quality semen can be accumulated for future use in artificial inseminations when and where needed.

4.715 Melatonin protects against alcoholic liver injury by attenuating oxidative stress, inflammatory response, and apoptosis

Hu, S., Yin, S., Jiang, X., Huang, D. and Shen, G.
Eur. J. Pharmacol., ***616***, *287-292 (2009)*

Melatonin is reported to exhibit a wide variety of biological effects, including antioxidant and anti-inflammatory. Previous studies show that melatonin has a protective role in different types of liver injury and fibrosis. But its role in the pathogenesis of alcoholic liver injury remains obscure. The present investigation was designed to determine the effects of melatonin on alcohol-induced hepatic injury in mice. The degree of alcoholic liver injury was evaluated by measuring serum markers and pathological examination. Treatment with melatonin significantly attenuated the increased level of serum aminotransferase, reduced the severe extent of hepatic cell damage, steatosis and the immigration of inflammatory cells, but had no effects on hepatic expression of lipogenic genes. Furthermore, melatonin

decreased serum and tissue inflammatory cytokines levels, tissue lipid peroxidation, neutrophil infiltration and inhibited the apoptosis of hepatocytes. Kupffer cells isolated from ethanol-fed mice produced high amounts of reactive oxygen species and tumor necrosis factor alpha, whereas Kupffer cells from melatonin treatment mice produced less reactive oxygen species and tumor necrosis factor alpha compared with model alcohol-feeding mice. These findings suggest that melatonin may represent a novel, protective strategy against alcoholic liver injury by attenuating oxidative stress, inflammatory response and apoptosis.

4.716 Tumor necrosis factor alpha and interferon gamma cooperatively induce oxidative stress and motoneuron death in rat spinal cord embryonic explants
Mir, M., Asensio, V.J., Tolosa, L., Gou-Fabregas, M., Soler, R.M., llado, J. And Olmos, G.
*Neuroscience, **162**, 959-971 (2009)*

The accumulation of reactive microglia in the degenerating areas of amyotrophic lateral sclerosis (ALS) tissue is a key cellular event creating a chronic inflammatory environment that results in motoneuron death. We have developed a new culture system that consists in rat spinal cord embryonic explants in which motoneurons migrate outside the explant, growing as a monolayer in the presence of glial cells. The proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) have been proposed to be involved in ALS-linked microglial activation. In our explants, the combined exposure to these cytokines resulted in an increased expression of the pro-oxidative enzymes inducible nitric oxide synthase (iNOS), the catalytic subunit of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, $gp91^{phox}$ and cyclooxygenase-2 (COX-2), as compared to each cytokine alone. This effect was related to their cooperation in the activation of the transcription factor nuclear factor kappa B (NF-κB). TNF-α and IFN-γ also cooperated to promote protein oxidation and nitration, thus increasing the percentage of motoneurons immunoreactive for nitrotyrosine. Apoptotic motoneuron death, measured through annexin V-Cy3 and active caspase-3 immunoreactivities, was also found cooperatively induced by TNF-α and IFN-γ. Interestingly, these cytokines did not affect the viability of purified spinal cord motoneurons in the absence of glial cells. It is proposed that the proinflammatory cytokines TNF-α and IFN-γ have cooperative/complementary roles in inflammation-induced motoneuron death.

4.717 Bluetongue Virus Targets Conventional Dendritic Cells in Skin Lymph
Hemati, B., Contreras, V., Urien, C., Bonneau, M., Takamatsu, H-H., Mertens, P.P., Breard, E., Sailleau, C., Zientara, S. and Schwartz-Cornil, I.
*J. Virol., **83(17)**, 8789-8799 (2009)*

Bluetongue virus (BTV) is the etiological agent of bluetongue, a hemorrhagic disease of ruminants (particularly sheep), which causes important economic losses around the world. BTV is transmitted primarily via the bites of infected midges, which inject the virus into the ruminant's skin during blood feeding. The virus initially replicates in the draining lymph node and then disseminates to secondary organs where it induces edema, hemorrhages, and necrosis. In this study, we show that ovine conventional dendritic cells (cDCs) are the primary targets of BTV that contribute to the primary dissemination of BTV from the skin to draining lymph nodes. Lymph cDCs support BTV RNA and protein synthesis, as well as the production of infectious virus belonging to several different BTV serotypes, regardless of their level of attenuation. Afferent lymph cell subsets, other than cDCs, showed only marginal levels of BTV protein expression. BTV infection provoked a massive recruitment of cDCs to the sheep skin and afferent lymph, providing cellular targets for infection. Although BTV productively infects cDCs, no negative impact on their physiology was detected. Indeed, BTV infection and protein expression in cDCs enhanced their survival rate. Several serotypes of BTV stimulated the surface expression of the CD80 and CD86 costimulatory molecules on cDCs as well as the mRNA synthesis of cytokines involved in inflammation and immunity, i.e., interleukin-12 (IL-12), IL-1β, and IL-6. BTV-infected cDCs stimulated antigen-specific CD4 and CD8 proliferation as well as gamma interferon production. BTV initially targets cDCs while preserving their functional properties, reflecting the optimal adaptation of the virus to its host cells for its first spread.

4.718 Liver Sinusoidal Endothelial Cells Are a Site of Murine Cytomegalovirus Latency and Reactivation
Seckert, C.K., Renzaho, A., Tervo, H-M., Krause, C., Deegen, P., Kühnapfel, B.,. Reddehase, M.J. and Grzimek, K.A.
*J. Virol., **83(17)**, 8869-8884 (2009)*

Latent cytomegalovirus (CMV) is frequently transmitted by organ transplantation, and its reactivation under conditions of immunosuppressive prophylaxis against graft rejection by host-versus-graft disease

bears a risk of graft failure due to viral pathogenesis. CMV is the most common cause of infection following liver transplantation. Although hematopoietic cells of the myeloid lineage are a recognized source of latent CMV, the cellular sites of latency in the liver are not comprehensively typed. Here we have used the BALB/c mouse model of murine CMV infection to identify latently infected hepatic cell types. We performed sex-mismatched bone marrow transplantation with male donors and female recipients to generate latently infected sex chromosome chimeras, allowing us to distinguish between Y-chromosome (gene *sry* or *tdy*)-positive donor-derived hematopoietic descendants and Y-chromosome-negative cells of recipients' tissues. The viral genome was found to localize primarily to *sry*-negative $CD11b^-$ $CD11c^-$ $CD31^+$ $CD146^+$ cells lacking major histocompatibility complex class II antigen (MHC-II) but expressing murine L-SIGN. This cell surface phenotype is typical of liver sinusoidal endothelial cells (LSECs). Notably, *sry*-positive $CD146^+$ cells were distinguished by the expression of MHC-II and did not harbor latent viral DNA. In this model, the frequency of latently infected cells was found to be 1 to 2 per 10^4 LSECs, with an average copy number of 9 (range, 4 to 17) viral genomes. Ex vivo-isolated, latently infected LSECs expressed the viral genes *m123/ie1* and *M122/ie3* but not *M112-M113/e1*, *M55/gB*, or *M86/MCP*. Importantly, in an LSEC transfer model, infectious virus reactivated from recipients' tissue explants with an incidence of one reactivation per 1,000 viral-genome-carrying LSECs. These findings identified LSECs as the main cellular site of murine CMV latency and reactivation in the liver.

4.719 An In Vitro Model of Cell Transplantation for Evaluation of Cell Engraftment Enhancers
Alfaro, F.J., Grau, M., Ramirez, E., Cevey, M., Mellado, M., Castro, M.J., Meneu, J.C., Abradelo, M., Camanas, C., Moreno, E., Morales, P., Paz-Artal, E. and Serrano, A.
Transplant. Proceedings, ***41***, *2487-2490 (2009)*

The limited availability of organs for liver transplantation has focused interest on the use of cell transplants to restore hepatic function. Advances have been made in rodent models, but efficacy is limited in humans due to low engraftment efficiency. In rodents, pretransplantation treatment of the liver with engraftment enhancers (EE) shows that repopulation is feasible, although the toxicity of the substances impedes their application in humans. Evaluation of low-toxicity engraftment enhancers for human use requires testing in animal models, a time-consuming, expensive process that also raises ethical issues. To reduce animal use in the preliminary evaluation of a new EE, we designed an easily quantitated in vitro method that mimics an intraportal cell transplant. It is based on EE-mediated disruption of intercellular adhesion in confluent endothelial cell cultures.

4.720 Aminophospholipid translocase and phospholipid scramblase activities in sickle erythrocyte subpopulations
Barber, L.A., Palascak, M.B., Joiner, C.H. and Franco, R.S.
Br. J. Hematol., ***146***, *447-455 (2009)*

Phosphatidylserine (PS) externalization may contribute to Sickle Cell Disease (SCD) characteristics including thrombogenesis, endothelial adhesion and shortened red blood cell (RBC) lifespan. Aminophospholipid translocase (APLT) returns externalized PS to the inner membrane, and phospholipid scramblase (PLSCR) equilibrates phospholipids (PL) across the membrane. APLT inhibition and PLSCR activation appear to be important for PS externalization. We examined relationships between APLT, PLSCR and external PS in mature sickle RBC and reticulocytes. Normally-hydrated sickle RBC without external PS had active APLT and inactive PLSCR. PS-exposing sickle RBC had inhibited APLT and active PLSCR. Sickle reticulocytes had active APLT and active PLSCR independent of external PS. Sickle RBC dehydrated in vivo had the highest proportion of PS-exposing RBC and markedly inhibited APLT. Normal and sickle RBC dehydrated in vitro had moderately decreased APLT. Rehydration resulted in significant recovery of APLT in RBC previously dehydrated in vitro, but not in sickle RBC dehydrated in vivo. These findings indicate that (i) PS externalization in mature sickle RBC depends on the balance between APLT and PLSCR activities, (ii) PS externalization in sickle reticulocytes depends primarily on PLSCR activation and (iii) APLT inhibition in sickle RBC dehydrated in vivo is due to dehydration itself and other factors.

4.721 A central role for monocytes in Toll-like receptor-mediated activation of the vasculature
Ward, J.R., Francis, S.E., Marsden, L., Sudddddddddason, T., Lord, G.M., Dower, S.K., Crossman, D.C. and Sabroe, I.
Immunology, ***128***, *58-68 (2009)*

There is increasing evidence that activation of inflammatory responses in a variety of tissues is mediated

co-operatively by the actions of more than one cell type. In particular, the monocyte has been implicated as a potentially important cell in the initiation of inflammatory responses to Toll-like receptor (TLR)-activating signals. To determine the potential for monocyte-regulated activation of tissue cells to underpin inflammatory responses in the vasculature, we established cocultures of primary human endothelial cells and monocytes and dissected the inflammatory responses of these systems following activation with TLR agonists. We observed that effective activation of inflammatory responses required bidirectional signalling between the monocyte and the tissue cell. Activation of cocultures was dependent on interleukin-1 (IL-1). Although monocyte-mediated IL-1[beta] production was crucial to the activation of cocultures, TLR specificity to these responses was also provided by the endothelial cells, which served to regulate the signalling of the monocytes. TLR4-induced IL-1[beta] production by monocytes was increased by TLR4-dependent endothelial activation in coculture, and was associated with increased monocyte CD14 expression. Activation of this inflammatory network also supported the potential for downstream monocyte-dependent T helper type 17 activation. These data define co-operative networks regulating inflammatory responses to TLR agonists, identify points amenable to targeting for the amelioration of vascular inflammation, and offer the potential to modify atherosclerotic plaque instability after a severe infection.

4.722 Coadministration of the fungal immunomodulatory protein FIP-Fve and a tumour-associated antigen enhanced antitumour immunity

Ding, Y., Seow, S.V., Huang, C.H., Liew, L.M., Lim, T.C., Kuo, J.C. and Chua, K.Y.
*Immunology, **128**, e881-e894 (2009)*

Fve is a fungal protein isolated from the golden needle mushroom Flammulina velutipes and has previously been reported to trigger immunological responses in both mouse and human lymphocytes. In this study, we evaluated the potential application of Fve as an adjuvant for tumour immunotherapy and examined the underlying mechanism(s). When the human papillomavirus (HPV)-16 E7 oncoprotein was used as a model antigen, mice coimmunized with HPV-16 E7 and Fve showed enhanced production of HPV-16 E7-specific antibodies as well as expansion of HPV-16 E7-specific interferon (IFN)-[gamma]-producing CD4+ and CD8+ T cells as compared with mice immunized with HPV-16 E7 alone. Tumour protection assays showed that 60% of mice coimmunized with HPV-16 E7 plus Fve, as compared with 20% of those immunized only with HPV-16 E7, remained tumour-free for up to 167 days after challenge with the tumour cells. Tumour therapeutic assays showed that HPV-16 E7 plus Fve treatment significantly prolonged the survival of tumour-bearing mice as compared with those treated only with HPV-16 E7. In vivo cell depletion and adoptive T-cell transfer assays showed that CD4+ and CD8+ T cells and IFN-[gamma] played critical roles in conferring the antitumour effects. Interestingly, Fve could stimulate the maturation of splenic dendritic cells in vivo and induce antigen-specific CD8+ T-cell immune responses. In summary, Fve has potent adjuvant properties that enhance T helper type 1 antigen-specific humoral and cellular immune responses which confer strong antitumour effects. The use of Fve as an adjuvant could be an attractive alternative to the current vaccination strategy for cancer immunotherapy.

4.723 Probiotic Preparation VSL#3 Alters the Distribution and Phenotypes of Dendritic Cells within the Intestinal Mucosa in C57BL/10J Mice

Wang, X., O'Gorman, M.R.G., Bu, H-F., Koti, V., Zuo, X-L. and Tan, X-D.
*J. Nutr., **139**, 1595-1602 (2009)*

Probiotic nutrients have shown promise in therapy for the treatment of gastrointestinal inflammation, infection, and atopic disease. Intestinal dendritic cells (DC) play a critical role in shaping the intestinal immune response. In this study, we tested the effect of a probiotic preparation (VSL#3) on DC distribution and phenotypes within the intestinal mucosa using a lineage depletion-based flow cytometric analysis. In naïve C57BL/10J mice, intestinal mucosal DC were composed of plasmacytoid DC (pDC) and myeloid DC (mDC). The pDC were the dominant form in lamina propria and Peyer's patches, whereas mDC were the prevailing type in the mesenteric lymph nodes. Additional characterization of pDC and mDC with flow cytometry revealed that they expressed heterogeneous phenotypes in the intestinal mucosa. In mice gavaged with the probiotic VSL#3 for 7 d, the proportion of pDC within the lamina propria was >60% lower, whereas the pDC subset in the mesenteric lymph nodes was more than 200% greater than in sham-treated controls ($P < 0.01$). Within pDC, the proportion of functionally unique $CX3CR1^{+}$ DC was greater than in controls in both the lamina propria and the Peyer's patches ($P < 0.01$). In contrast to pDC, the mDC number was greater than in controls in all intestinal lymphoid tissue compartments in VSL#3-treated mice ($P < 0.01$). In conclusion, this study suggests that phenotypically and functionally distinct DC subsets are localized to specific lymphoid tissues within the intestinal mucosa and that the VSL#3 probiotic nutritional

supplement alters the distribution of the DC subsets within the intestinal mucosa. These changes may be important in the alteration of mucosal immunity following probiotic VSL#3 therapy.

4.724 A highly energetic process couples calcium influx through L-type calcium channels to insulin secretion in pancreatic β-cells

Jung, S-R., Reed, B.J. and Sweet, I.R.

Am. J. Physiol. Endocrinol. Metab., ***297***, *E717-E727 (2009)*

Calcium (Ca^{2+}) influx is required for the sustained secretion of insulin and is accompanied by a large rate of energy usage. We hypothesize that the energy usage reflects a process [Ca^{2+}/metabolic coupling process (CMCP)] that couples Ca^{2+} to insulin secretion by pancreatic islets. The aim of the study was to test this hypothesis by testing the effect of inhibiting candidate Ca^{2+}-sensitive proteins proposed to play a critical role in the CMCP. The effects of the inhibitors on oxygen consumption rate (OCR), a reflection of ATP usage, and insulin secretion rate (ISR) were compared with those seen when L-type Ca^{2+} channels were blocked with nimodipine. We reasoned that if a downstream Ca^{2+}-regulated site was responsible for the OCR associated with the CMCP, then its inhibition should mimic the effect of nimodipine. Consistent with previous findings, nimodipine decreased glucose-stimulated OCR by 36% and cytosolic Ca^{2+} by 46% and completely suppressed ISR in rat pancreatic islets. Inhibitors of three calmodulin-sensitive proteins (myosin light-chain kinase, calcineurin, and Ca^{2+}/calmodulin-dependent protein kinase II) did not meet the criteria. In contrast, KN-62 severed the connection between Ca^{2+} influx, OCR, and ISR without interfering with Ca^{2+} influx. In the presence of nimodipine or KN-62, potentiators of ISR, acetylcholine, GLP-1, and arginine had little effect on insulin secretion, suggesting that the CMCP is also essential for the amplification of ISR. In conclusion, a KN-62-sensitive process directly mediates the effects of Ca^{2+} influx via L-type Ca^{2+} channels on OCR and ISR, supporting the essential role of the CMCP in mediating ISR.

4.725 *Salmonella* Induces Flagellin- and MyD88-Dependent Migration of Bacteria-Capturing Dendritic Cells Into the Gut Lumen

Arques, J.L., Hautefort, I., Ivory, K., Bertelli, E., Regoli, M., Clare, S., Hinton, J.C.D. and Nicoletti, C.

Gastroenterology, ***137***, *579-587 (2009)*

Background & Aims
Intestinal dendritic cells (DCs) sample bacteria, such as *Salmonella*, by extending cellular processes into the lumen to capture bacteria and shuttle them across the epithelium; however, direct evidence of bacteria-loaded DCs travelling back into the tissue is lacking. We hypothesized that sampling is paralleled by migration of DCs into the lumen prior to or following the internalization of *Salmonella*.
Methods
The small intestine and the colon of BALB/c and C57BL/6 mice were challenged with noninvasive *Salmonella enterica* serovar Typhimurium SL1344-Δ*Salmonella* pathogenicity island (SPI) 1 or *Escherichia coli* DH5α by using isolated loops or oral administration by gavage. Transepithelial migration of DCs was documented by immunohistochemistry, microscopy, and flow cytometry. The role of flagellin was determined by using flagellin (ΔfliC ΔfljB)- and SPI1-SPI2 (ΔSPI1 ΔssrA)-deficient *Salmonella*, flagellated *E coli* K12, and MyD88 mice.
Results
Salmonella ΔSPI1 induced migration of $CD11c^{+}CX_3CR1^{+}MHCII^{+}CD11b^{-}CD8\alpha^{-}$ DCs into the small intestine, whereas flagellin- and SPI1-SPI2-deficient *Salmonella*, soluble flagellin, and *E coli* DH5α or flagellated K12, failed to do so. DC migration did not occur in the colon; it was not observed in MyD88 mice, and intraluminal DCs internalized *Salmonella* but did not cross the epithelium to return into tissues. Finally, DC migration was not linked to *Salmonella*-induced damage of the epithelium.
Conclusions
DC-mediated sampling of *Salmonella* is accompanied by flagellin- and MyD88-dependent migration of *Salmonella*-capturing DCs into the intestinal lumen. We suggest that the rapid intraluminal migration of *Salmonella*-capturing DCs may play a role in the protection of the intestinal mucosa against bacterial infection.

4.726 Opposing Effects of TGF-β and IL-15 Cytokines Control the Number of Short-Lived Effector $CD8^{+}$ T Cells

Sanjabi, S., Mosaheb, M.M. and Flavell, R.A.

Immunity, ***31***, *131-144 (2009)*

An effective immune response against infectious agents involves massive expansion of $CD8^{+}$ T cells. Once

the infection is cleared, the majority of these effector cells die through unknown mechanisms. How is expansion controlled to maximize pathogen clearance and minimize immunopathology? We found, after *Listeria* infection, plasma transforming growth factor β (TGF-β) titers increased concomitant with the expansion of effector $CD8^+$ T cells. Blocking TGF-β signaling did not affect effector function of $CD8^+$ T cells. However, TGF-β controlled effector cell number by lowering Bcl-2 amounts and selectively promoting the apoptosis of short-lived effector cells. TGF-β-mediated apoptosis of this effector subpopulation occurred during clonal expansion and contraction, whereas interleukin-15 (IL-15) promoted their survival only during contraction. We demonstrate that the number of effector $CD8^+$ T cells is tightly controlled by multiple extrinsic signals throughout effector differentiation; this plasticity should be exploited during vaccine design and immunotherapy against tumors and autoimmune diseases.

4.727 Differential regulation of neuronal and inducible nitric oxide synthase (NOS) in the spinal cord of mutant SOD1 (G93A) ALS mice
Lee, J., Ryu, H. and Kowall, N.W.
Biochem. Biophys. Res. Comm., ***387***, *202-206 (2009)*

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder characterized by degeneration of motor neurons throughout the central nervous system. Mutations of the free radical scavenging enzyme superoxide dismutase-1 (SOD1) are a cause of familial ALS but it is not known how mutations lead to cell death. Free radicals such as nitric oxide (NO) are thought to play a key pathogenic role. NO is synthesized by NO synthases (NOSs) from arginine, which is a rate-limiting factor for NO production. We found that neuronal NOS (nNOS)-positive motor neurons were depleted while inducible NOS (iNOS)-positive activated glial cells were increased in transgenic mtSOD1 (G93A) ALS mice. iNOS expression was up regulated consistent with the increases of motor neuron loss and glial activation and citrulline and NO levels while nNOS expression was decreased in G93A ALS mice. Administration of l-arginine to G93A mice reduced the severity of motor neuron depletion and glial activation. In treated animals, nNOS expression was preserved while citrulline and NO were reduced, possibly due to reduced activation of glia expressing iNOS. Our findings show that high concentrations of NO correlate with iNOS expression rather than nNOS expression in G93A ALS mice. This suggests that therapy focused on iNOS inhibition might be a fruitful direction for future ALS therapeutic trials.

4.728 Purification Method Using Iodixanol (OptiPrep)-Based Density Gradient Significantly Reduces Cytokine Chemokine Production From Human Islet Preparations, Leading to Prolonged β-Cell Survival During Pretransplantation Culture
Mita, A., Ricordi, C., Miki, A. Barker, S., Khan, A., Alvarez, A., Hashikura, Y., Miyagawa, S. and Ichii, H.
Transplant. Proc., ***41***, *314-315 (2009)*

Purification is one of the most important steps in human islet isolation. Although Ficoll-based density gradients are widely used, OptiPrep-based density gradients are used in few centers. Cytokine/chemokine production from human islet preparations varies widely. Some cytokines/chemokines have been reported to have adverse effects on human islet preparations. Control of cytokine/chemokine production may be a key to improve islet quality and quantity, leading to better transplantation outcomes. The aim of the present study was to investigate the effects on islet preparations of purification methods using various density gradients on viability, cellular composition, and proinflammatory cytokine/chemokine production. After the digestion phase, the extracts were divided into 2 groups for purification using a semiautomated cell processor with Ficoll-based or OptiPrep-based density gradients. Islet preparations cultured for 2 days were assessed regarding islet cell viability (fluorescein diacetate/propidium iodide [FDA/PI]), fractional β-cell viability by FACS, and β-cell content using iCys. Cytokine/chemokine production from islet preparations was also measured by Bio-plex.
After purification, the purity, islet equivalents (IEQ), and islet recovery rates were comparable between the 2 groups. Although FDA/PI and fractional β-cell viability showed no significant difference, survival of β cells during culture was significantly higher in the OptiPrep compared with the Ficoll-based density gradient group. There were significantly lower tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ, IL-6, and MIP-1β productions from the OptiPrep-based density gradient group. OptiPrep-based density gradients reduced cytokine/chemokine production by islet preparations. In addition, OptiPrep-based density gradient purification significantly reduced the loss of β-cell mass during pretransplantation culture.

4.729 Node of Ranvier formation on motoneurons *in vitro*
Rumsey, J.W., Das, M., Stancescu, M., Bott, M., Fernandez-Valle, C. And Hickman, J.J.

*Biomaterials, **30**, 3567-3572 (2009)*

One of the most significant interactions between Schwann cells and neurons is myelin sheath formation. Myelination is a vertebrate adaptation that enables rapid conduction of action potentials without a commensurate increase in axon diameter. *In vitro* neuronal systems provide a unique modality to study both factors influencing myelination and diseases associated with myelination. Currently, no *in vitro* system for motoneuron myelination by Schwann cells has been demonstrated. This work details the myelination of motoneuron axons by Schwann cells, with complete Node of Ranvier formation, in a defined *in vitro* culture system. This defined system utilizes a novel serum-free medium in combination with the non-biological substrate, N-1[3 (trimethoxysilyl) propyl] diethylenetriamine (DETA). The myelinated segments and nodal proteins were visualized and quantified using confocal microscopy. This defined system provides a highly controlled, reproducible model for studying Schwann cell interactions with motoneurons as well as the myelination process and its effect on neuronal plasticity. Furthermore, an *in vitro* system that would allow studies of motoneuron myelination would be beneficial for understanding peripheral demyelinating neuropathies such as diabetes induced peripheral neuropathy and could lead to a better understanding of CNS demyelinating diseases like multiple sclerosis, as well as neuromuscular junction maturation and maintenance.

4.730 Effect of Centrifugation Technique on Post-storage Characteristics of Stallion Spermatozoa
Webb, G.W. and Dean, M.M.
*J. Equine Vet. Sci., **29(9)**, 675-680 (2009)*

Three ejaculates were collected from four stallions and used to compare the effects of three centrifugation methods on post-storage motility and recovery of available sperm. Two aliquots per ejaculate were diluted with skim milk-glucose (SKMG) extender to 50×10^6 sperm/mL, placed in 50-mL conical bottom tubes, and centrifuged at either 700*g* for 15 minutes (700*g*) or 600*g* for 12 minutes (600*g*). A third aliquot was diluted 1:1 with SKMG, placed in 15-mL conical tubes, and centrifuged at 400*g* for 7 minutes (400*g*). Subsamples from each pre-treated diluted ejaculate were held at room temperature and evaluated for motility at the same time as the post-centrifugation pre-storage motility evaluation was made for treated aliquots. After centrifugation, samples from each aliquot were stored at 5°C for evaluation after 24 and 48 hours or frozen in liquid nitrogen. Percentage of available sperm harvested was higher ($P \leq .05$) for aliquots centrifuged in 15-mL tubes at 400*g* versus 600*g* in 50-mL tubes. After centrifugation, total but not progressive motility of aliquots centrifuged at 700*g* was lower than that for noncentrifuged controls and sperm from aliquots centrifuged at 400*g* in 15-mL tubes. After cold storage, values for total but not progressive motility or velocity were higher ($P \leq .05$) for aliquots centrifuged in 15-mL tubes at 400*g* compared with those centrifuged in 50-mL tubes at both 600*g* and 700*g*. Postthaw motility of frozen sperm was not different between centrifugation treatments. Poststorage percentages of intact acrosomes and detached heads did not differ because of centrifugation treatment.

4.731 Determinants of Leukocyte Margination in Rectangular Microchannels
Jain, A. and Munn, L.L.
*PloSOne, **4(9)**, e7104 (2009)*

Microfabrication of polydimethylsiloxane (PDMS) devices has provided a new set of tools for studying fluid dynamics of blood at the scale of real microvessels. However, we are only starting to understand the power and limitations of this technology. To determine the applicability of PDMS microchannels for blood flow analysis, we studied white blood cell (WBC) margination in channels of various geometries and blood compositions. We found that WBCs prefer to marginate downstream of sudden expansions, and that red blood cell (RBC) aggregation facilitates the process. In contrast to tubes, WBC margination was restricted to the sidewalls in our low aspect ratio, pseudo-2D rectangular channels and consequently, margination efficiencies of more than 95% were achieved in a variety of channel geometries. In these pseudo-2D channels blood rheology and cell integrity were preserved over a range of flow rates, with the upper range limited by the shear in the vertical direction. We conclude that, with certain limitations, rectangular PDMS microfluidic channels are useful tools for quantitative studies of blood rheology.

4.732 Comparison of Trypsin Inhibitors in Preservation Solution for Islet Isolation
Noguchi, H., Ueda, M., Hayashi, S., Kobayashi, N., Okitsu, T., Iwanaga, Y., Nagata, Y., Nagata, H., Liu, X., Kamiya, H., Levy, M.F. and Matsumoto, S.
*Cell Transplant., **18(5-6)**, 541-547 (2009)*

Islet transplantation has recently emerged as an effective therapy and potential cure for type 1 diabetes mellitus. Recent reports show that the two-layer method (TLM), which employs oxygenated perfluorochemical (PFC) and University of Wisconsin (UW) solution, is superior to simple cold storage in UW for pancreas preservation in islet transplantation. Moreover, we recently reported that islet yield was significantly higher in the ET-Kyoto solution with ulinastatin (MK)/PFC preservation solution compared with the UW/PFC preservation solution in the porcine model and that the advantages of MK solution are trypsin inhibition and less collagenase inhibition. In this study, we compared ulinastatin with another trypsin inhibitor, Pefabloc, in preservation solution for islet isolation. Islet yield before purification was higher in the MK/PFC group compared with the ET-Kyoto with Pefabloc (PK)/PFC group. The stimulation index was higher for the MK/PFC group than for the PK/PFC group. These data suggest that ET-Kyoto with ulinastatin was the better combination for pancreas preservation than ET-Kyoto with Pefabloc. Based on these data, we now use ET-Kyoto solution with ulinastatin for clinical islet transplantation.

4.733 Estimation of Donor Usability for Islet Transplantation in the United States With the Kyoto Islet Isolation Method

Matsumoto, S., Noguchi, H., Hatanaka, N., Shimoda, M., Kobayashi, N., jackson, A., Onaca, N., Naziruddin, B. and Levy, M.F.
*Cell Transplant, **18**, 549-556 (2009)*

The quality of donor pancreata is important for successful islet isolation. However, in some countries like Japan, the number of donor pancreata is very low; therefore, marginal donors have been used with less restrictive donor criteria. In order to use marginal donor pancreata, we established the Kyoto islet isolation method (KIIM). According to United Network for Organ Sharing (UNOS) in 2005, more than 6,000 pancreata were not clinically used in the US. In this study, we applied the KIIM for brain-dead donors and reevaluated donor usability based on the Japanese islet donor criteria. Islets were isolated with the Ricordi method using pancreata stored in University of Wisconsin (UW) solution (UW group) or by the two-layer method (TLM group) or the TLM combined with ductal injection (DI group). We implemented the KIIM (KIIM group) to confirm the effect of the KIIM on brain-dead donors. Donor charts in Texas from 2005 to 2006 were reviewed. If pancreata were not used clinically, the reason was reviewed and donors were reevaluated based on Japanese criteria. There were no significant differences of islet yield, viability, and purity between the UW and TLM groups. The DI group significantly improved islet yields and isolations were further improved in the KIIM group [UW: 251,663 ± 60,217 islet equivalent (IE); TLM: 243,738 ± 54,170 IE; DI: 498,639 ± 28,853 IE; KIIM: 678,286 ± 55,853]. The KIIM provided high-quality islets in high numbers from islet isolations from brain-dead donors. A total of 236 donor charts were reviewed and 194 pancreata (82%) were not used. Of these, 185 cases identified the reasons that the pancreata were not used. When we applied the Japanese criteria, an additional 82 cases out of 185 (44%) seem to be suitable for islet isolations. With the KIIM, more than 2,500 additional donor pancreata can be used for islet isolation in the US every year when the Japanese criteria are applied.

4.734 Human Islet Isolation for Autologous Transplantation: Comparison of Yield and Function Using SERVA/Nordmark Versus Roche Enzymes

Anazawa, T., Balamurugan, A.N., Bellin, M., Zhang, H.J., Matsumoto,, S., Yonekawa, Y., Tanaka, T., Loganathan, G., Papas, K.K., Beilman, G:J., hering, B.J. and Sutherland, E.R.
*Am. J. Transplant., **9**, 2383-2391 (2009)*

Islet autotransplantation (IAT) is used to preserve as much insulin-secretory capacity as possible in patients undergoing total pancreatectomy for painful chronic pancreatitis. The enzyme used to dissociate the pancreas is a critical determinant of islet yield, which is correlated with posttransplant function. Here, we present our experience with IAT procedures to compare islet product data using the new enzyme SERVA/Nordmark (SN group; n = 46) with the standard enzyme Liberase-HI (LH group; n = 40). Total islet yields (mean +/- standard deviation; 216 417 +/- 79 278 islet equivalent [IEQ] in the LH group; 227 958 +/- 58 544 IEQ in the SN group; p = 0.67) were similar. However, the percentage of embedded islets is higher in the SN group compared to the LH group. Significant differences were found in pancreas digestion time, dilution time, and digested pancreas weight between the two groups. Multivariate linear regression analysis showed the two groups differed in portal venous pressure changes. The incidence of graft function and insulin independence was not different between the two groups. The SN and LH enzymes are associated with similar outcomes for IAT. Further optimization of the collagenase/neutral protease ratio is necessary to reduce the number of embedded islets obtained when using the SN enzyme.

4.735 Successful Clinical Islet Isolation Using a GMP-Manufactured Collagenase and Neutral Protease
Szot, G.L., Lee, M.R., Tavakol., M.M., Lang, J., Derkovic, F., Kerlan, R.K., Stock, P.G. and Posselt, A.M.
*Transplantation, **88(6)**, 753-756 (2009)*

In 2007, the islet community was notified that the collagenase product most commonly used for human islet isolations contained bovine neural tissue contaminants. To minimize this potential hazard, we adapted our human islet processing procedure to use a GMP-manufactured, bovine neural tissue-free collagenase blend. Here, we describe the factors that we consider most important for achieving reproducible and clinically useable islet isolations using this product.

4.736 Specific vulnerability of mouse spinal cord motoneurons to membrane depolarization
Grou-Fabregas, M., Garcera, A., Mincheva, S., Perez-Garcia, M.J., Comella, J.X. and Soler, R.M.
*J. Neurochem., **110(6)**, 1842-1854 (2009)*

Intracellular calcium (Ca^{2+}) concentration determines neuronal dependence on neurotrophic factors (NTFs) and susceptibility to cell death. Ca^{2+} overload induces neuronal death and the consequences are thought to be a probable cause of motoneuron (MN) degeneration in neurodegenerative diseases. In the present study, we show that membrane depolarization with elevated extracellular potassium (K^{+}) was toxic to cultured embryonic mouse spinal cord MNs even in the presence of NTFs. Membrane depolarization induced an intracellular Ca^{2+} increase. Depolarization-induced toxicity and increased intracellular Ca^{2+} were blocked by treatment with antagonists to some of the voltage-gated Ca^{2+} channels (VGCCs), indicating that Ca^{2+} influx through these channels contributed to the toxic effect of depolarization. Ca^{2+} activates the calpains, cysteine proteases that degrade a variety of substrates, causing cell death. We investigated the functional involvement of calpain using a calpain inhibitor and calpain gene silencing. Pre-treatment of MNs with calpeptin (a cell-permeable calpain inhibitor) rescued MNs survival; calpain RNA interference had the same protective effect, indicating that endogenous calpain contributes to the cell death caused by membrane depolarization. These findings suggest that MNs are especially vulnerable to extracellular K^{+} concentration, which induces cell death by causing both intracellular Ca^{2+} increase and calpain activation.

4.737 Human telomerase activity, telomerase and telomeric template expression in hepatic stem cells and in livers from fetal and postnatal donors
Schmelzer, E. and Reid, L.M.
*Eur. J. Gastroenterol. Hepatol., **21(10)**, 1191-1198 (2009)*

Background: Although telomerase activity has been analyzed in various normal and malignant tissues, including liver, it is still unknown to what extent telomerase can be associated with specific maturational lineage stages.
Methods: We assessed human telomerase activity, protein and gene expression for the telomerase reverse transcriptase, as well as expression of the telomeric template RNA hTER in hepatic stem cells and in various developmental stages of the liver from fetal to adult. In addition, the effect of growth factors on telomerase activity was analyzed in hepatic stem cells *in vitro*.
Results: Telomerase was found to be highly active in fetal liver cells and was significantly higher than in hepatic stem cells, correlating with gene and protein expression levels. Activity in postnatal livers from all donor ages varied considerably and did not correlate with age or gene expression levels. The hter expression could be detected throughout the development. A short stimulation by growth factors of cultured hepatic stem cells did not increase telomerase activity.
Conclusion: Telomerase is considerably active in fetal liver and variably in postnatal livers. Although telomerase protein is present at varying levels in liver cells of all donor ages, gene expression is solely associated with fetal liver cells.

4.738 Molecular and immunocytochemical characterization of primary neuronal cultures from adult rat brain: Differential expression of neuronal and glial protein markers
Ray, B., Bailey, J.A., Sarkar, S. and Lahiri, D.K.
*J. Neurosci. Methods, **184**, 294-302 (2009)*

Neurobiological studies using primary neuronal cultures commonly employ fetal-derived neurons, but much less often adult brain-derived neurons. Our goal is to perform morphological and molecular characterization of primary neuronal cultures from adult rat brain, including the relative expression of neuronal and glial cell markers at different time points. We tested the hypothesis that long-term neuronal viability is compatible with glial proliferation in adult neuron culture. We examined neuron culture from

adult rat brain, which was maintained at steady state up to 24 days, and characterized them on the basis of cellular, molecular and biochemical properties at different time points of the culture. We identified neuronal and glial cells by both immunocytochemical and western immunoblotting techniques using NSE and Tau as neuronal markers and GFAP as glial protein marker, which revealed the presence of predominantly neuronal cells in the initial phase of the culture and a rise in glial cells from day 12 onwards. Notably, neuronal cells were preserved in the culture along with the glial cells even at day 24. Transfection of the cultured cells with a GFP expression vector and plasmids containing a luciferase reporter gene under the control of two different gene promoters demonstrated DNA transfectability. Taken together, these results suggest a differential expression of neuronal and glial cells at different time points and long-term neuronal viability in the presence of glial proliferation. Such adult neurons serve as a suitable system for the application of neurodegeneration models and for drug target discovery in various brain disorders including Alzheimer's disease.

4.739 Proinflammatory and immunoregulatory mechanisms in periapical lesions
Colic, M., Gazivoda, D., Vucevic, D., Vasilijic, S., Rudolf, R. and Lukic, A.
Mol. Immunol., ***47***, *101-113 (2009)*

Proinflammatory and immunoregulatory cytokines are important for the pathogenesis of periapical lesions. However, little is known about how their functions are balanced and controlled at different phases of lesion development. The aim of this study was to examine the relationship between the production of Th1, Th2, Th17 and T regulatory cell (T reg) cytokines by human periapical lesion mononuclear cells (PL-MNC) in culture and their correlation with cellular composition and clinical presentation of the lesions. We show that symptomatic lesions are characterized by the infiltration of neutrophils, high production of IL-17, positive correlation between IL-17 and IFN-γ, but not between IL-17 and IL-23 production. Most IL-17$^+$ cells coexpressed IFN-γ. Asymptomatic lesions were phenotypically heterogeneous. The lesions with the predominance of T cells over B cells/plasma cells expressed higher levels of IFN-γ which correlated with higher production of IL-12 and the frequency of macrophages. In contrast, in most B-type lesions higher levels of IL-5 and TGF-β were observed, as well as positive correlation between the production of TGF-β and IL-10. The addition of Th cytokines in PL-MNC cultures confirmed that Th1, Th2 and Th17 cytokines are mutually antagonistic, except that IL-17, unexpectedly, augmented the production of IFN-γ. IL-10 and TGF-β inhibited the production of both Th1 and Th17 cytokines. Dendritic cells (DCs) from periapical lesions, composed of immature (CD83$^-$), and mature (CD83$^+$) myeloid type DCs and plasmacytoid (BDCA2$^+$) DCs produced higher levels of IL-12 and IL-23 but lower levels of IL-10 and TNF-α than monocyte (Mo) -derived DCs. IL-23 stimulated the production of IL-17 by PL-MNC, whereas the secretion of IFN-γ was enhanced by both IL-12 and IL-23. Cumulatively, these results suggest that: (1) Th1 immune response is most probably important for all stages of periapical lesion development; (2) Th2 and immunoregulatory cytokines are more significant for advanced types of lesions with the predominance of B cells/plasma cells; (3) Th17 immune response seems to play a dominant role in exacerbating inflammation.

4.740 Mitochondrial DNA content in peripheral blood monocytes: relationship with age of diabetes onsetand diabetic complications
Wong, J., McLennan, S.V., Molyneaux, L., Min, D., Twigg, S.M. and Yue, D.K.
Diabetologia, ***52***, *1953-1961 (2009)*

Aims/hypothesis We examined whether age of type 2 diabetes onset is related to mitochondrial DNA content in peripheral blood monocytes (PBMCs).
Methods PBMCs were isolated from 65 patients with type 2 diabetes. To minimise age as a confounder, only patients aged ≥50 years were studied. Sample mitochondrial DNA (mtDNA) content was determined by amplification of the mitochondrial gene *CYT-B* (also known as *MT-CYB*) and adjusted for single-copy nuclear control genes (*36B4* [also known as *RPLPO*] and *GAPDH*).
Results Age of diabetes onset ranged from 25 to 69 years. There was a significant positive relationship between age of diabetes onset in quartiles and mtDNA content for the whole group ($p = 0.02$ for trend). When stratified by the presence of diabetes complications, a strong positive relationship was observed between age of diagnosis and mtDNA content for participants without diabetic complications ($r = 0.7$; $p = 0.0002$), but not for those with complications ($r = -0.04$; $p = 0.8$). Multivariate analysis confirmed age of onset and complication status as independent determinants. There was co-linearity between age of onset and disease duration, with similar relationships also seen between duration and mtDNA content.
Conclusions/interpretation An earlier age of type 2 diabetes onset is associated with a lower PBMC mtDNA content, but only in patients without diabetes complications. This may reflect a differing biology

of PBMC mtDNA in those with early-onset diabetes and those who are prone to complications. PBMC mtDNA depletion may accelerate diabetes onset; however the independent effect of diabetes duration remains to be evaluated.

4.741 Analysis of Neuroprotective Effects of Valproic Acid on Primary Motor Neurons in Monoculture or Co-cultures with Astrocytes or Schwann Cells
Ragancokova, D., Jahn, K., Kotsiari, A., Schlesinger, F., Haastert, K., Stangel, M., petri, S. and Krampfl, K.
Cell. Mol. Neurobiol., ***29****, 1037-1043 (2009)*

Chronic dysregulation of the intracellular Ca^{2+} homeostasis (excitotoxicity) is thought to contribute to the development of motor neuron diseases. Valproic acid (VPA) is widely used as an antiepileptic drug and acts mainly by inhibition of sodium channels and by enhancing the level of the inhibitory neurotransmitter γ-aminobutyric acid. Neuroprotective capacities of VPA are supposed to arise also from the inhibition of histone deacetylases. We investigated the viability of highly purified rat embryonic motor neurons cultured on glial feeder layers, composed of either astrocytes or Schwann cells, or in the absence of glia, monoculture in presence of VPA and/or kainate (KA) using immunocytochemistry and calcium imaging. A significant effect of the culture and co-culture conditions on the viability of motor neurons in our in vitro model of excitotoxicity was detected. The neuroprotective effect of VPA on primary embryonic motor neuron cultures was not proven. A functional interaction between VPA and KA occurred during the first 10 days in culture.

4.742 Transcriptional analysis of intracytoplasmically stained, FACS-purified cells by high-throughput, quantitative nuclease protection
Pechhold, S., Stouffer, M., Walker, G., Martel, R., Seligmann, B., Hang, Y., Stein, R., harlan, D.M. and Pechhold, K.
Nature Biotechnol., ***27(11)****, 1038-1042 (2009)*

Analyzing specialized cells in heterogeneous tissues is crucial for understanding organ function in health and disease. Thus far, however, there has been no convenient method for studying gene expression in cells purified by fluorescence-activated cell sorting (FACS) using intracellular markers. Here we show that the quantitative nuclease protection assay (qNPA) enables transcriptional analysis of intracytoplasmically stained cells sorted by FACS. Applying the method to mouse pancreatic islet–cell subsets, we detected both expected and unknown lineage-specific gene expression patterns. Some beta cells from pregnant animals were found to express *Mafb*, previously observed only in immature beta cells during embryonic development. The four 'housekeeping' genes tested were expressed in purified islet-cell subpopulations with a notable variability, dependent on both cell lineage and developmental stage. Application of qNPA to intracellularly stained, FACS-sorted cells should be broadly applicable to the analysis of gene expression in subpopulations of any heterogeneous tissue, including tumors.

4.743 Galactosylated LDL Nanoparticles: A Novel Targeting Delivery System To Deliver Antigen to Macrophages and Enhance Antigen Specific T Cell Responses
Wu, F., Wuensch, S.A., Azadniv, M., Ebrahimkhani, M.R. and Crispe, I.N.
Mol. Pharmaceutics, ***6(5)****, 1506-1517 (2009)*

We aim to define the role of Kupffer cells in intrahepatic antigen presentation, using the selective delivery of antigen to Kupffer cells rather than other populations of liver antigen-presenting cells. To achieve this we developed a novel antigen delivery system that can target antigens to macrophages, based on a galactosylated low-density lipoprotein nanoscale platform. Antigen was delivered via the galactose particle receptor (GPr), internalized, degraded and presented to T cells. The conjugation of fluoresceinated ovalbumin (FLUO-OVA) and lactobionic acid with LDL resulted in a substantially increased uptake of FLUO-OVA by murine macrophage-like ANA1 cells in preference to NIH3T3 cells, and by primary peritoneal macrophages in preference to primary hepatic stellate cells. Such preferential uptake led to enhanced proliferation of OVA specific T cells, showing that the galactosylated LDL nanoscale platform is a successful antigen carrier, targeting antigen to macrophages but not to all categories of antigen presenting cells. This system will allow targeted delivery of antigen to macrophages in the liver and elsewhere, addressing the question of the role of Kupffer cells in liver immunology. It may also be an effective way of delivering drugs or vaccines directly at macrophages.

4.744 Th1/Th17 Immune Response Is Induced by Mesenteric Lymph Node Dendritic Cells in Crohn's

Disease
Sakuraba, A., Sato, T., Kamada, N., Kitazume, M., Sugita, A. And Hibi, T.
Gastroenterol., ***137***, *1736-1745 (2009)*

Background & Aims
Dendritic cells (DCs) possess the most potent ability to induce acquired immunity. However, their involvement in the pathogenesis of Crohn's disease (CD) has not yet been determined. We aimed to establish the immune status of mesenteric lymph nodes, the major gut-associated lymphoid tissue, and isolated DCs and determine their involvement in the pathogenesis of CD.
Methods
$CD4^+$ T cells and DCs were isolated from mesenteric lymph nodes of CD, ulcerative colitis, and normal control. The immune status of $CD4^+$ T cells was analyzed by cytokine production and transcriptional profile. Surface phenotype of DCs was analyzed by flow cytometry. Cytokine production by myeloid DCs was analyzed by real-time polymerase chain reaction and exogenous bacterial stimulation. Immune stimulating activity of DCs was determined by mixed lymphocyte reaction.
Results
In CD, mesenteric lymph node $CD4^+$ T cells produced higher amounts of interferon-γ and interleukin (IL)-17 compared with ulcerative colitis and normal control, and this was dictated by increased T-bet and retinoic acid-related orphan receptor-γ expression. Three subtypes of DCs, myeloid DC, plasmacytoid DC, and mature DC, were identified in all groups. When stimulated with exogenous bacterial derivative, myeloid DCs from CD produced a higher amount of IL-23 and a lower amount of IL-10. Myeloid DCs from CD induced stronger T helper cell (Th)1 immune response in mixed lymphocyte reaction compared with those from ulcerative colitis and normal control.
Conclusions
Our findings revealed that mesenteric lymph node is the key pathogenic location of CD elicited by the unique cytokine milieu produced by DCs leading to a dysregulated Th1/Th17 immune response.

4.745 Factor VIII C1 domain residues Lys 2092 and Phe 2093 contribute to membrane binding and cofactor activity
Meems, H., Meijer, A.B., Cullinan, D.B., Mertens, K. and Gilbert, G.E.
Blood, ***114(18)***, *3938-3946 (2009)*

Binding of factor VIII to membranes containing phosphatidyl-L-serine (Ptd-L-Ser) is mediated, in part, by a motif localized to the C2 domain. We evaluated a putative membrane-binding role of the C1 domain using an anti-C1 antibody fragment, $KM33_{scFv}$, and factor VIII mutants with an altered KM33 epitope. We prepared a dual mutant Lys2092/Phe2093 →Ala/Ala ($fVIII_{YFP\ 2092/93}$) and 2 single mutants Lys2092 →Ala and Phe2093 →Ala. $KM33_{scFv}$ inhibited binding of fluorescein-labeled factor VIII to synthetic membranes and inhibited at least 95% of factor Xase activity. $fVIII_{YFP\ 2092/93}$ had 3-fold lower affinity for membranes containing 15% Ptd-L-Ser but more than 10-fold reduction in affinity for membranes with 4% Ptd-L-Ser. In a microtiter plate, $KM33_{scFv}$ was additive with an anti-C2 antibody for blocking binding to vesicles of 15% Ptd-L-Ser, whereas either antibody blocked binding to vesicles of 4% Ptd-L-Ser. $KM33_{scFv}$ inhibited binding to platelets and $fVIII_{YFP\ 2092/93}$ had reduced binding to A23187 [GenBank] -stimulated platelets. $fVIII_{YFP\ 2092}$ exhibited normal activity at various Ptd-L-Ser concentrations, whereas $fVIII_{YFP\ 2093}$ showed a reduction of activity with Ptd-L-Ser less than 12%. $fVIII_{YFP\ 2092/93}$ had a greater reduction of activity than either single mutant. These results indicate that Lys 2092 and Phe 2093 are elements of a membrane-binding motif on the factor VIII C1 domain.

4.746 Esterase 22 and beta-glucuronidase hydrolyze retinoids in mouse liver
Schreiber, R., Taschler, U., Wolinski, H., Seper, A., Tamegger, S.N., Graf, M., Kohlwein, S.D., Haemmerle, G., Zimmermann, R., Zechner, R. and Lass, A.
J. Lipid Res., ***50***, *2514-2523 (2009)*

Excess dietary vitamin A is esterified with fatty acids and stored in the form of retinyl ester (RE) predominantly in the liver. According to the requirements of the body, liver RE stores are hydrolyzed and retinol is delivered to peripheral tissues. The controlled mobilization of retinol ensures a constant supply of the body with the vitamin. Currently, the enzymes catalyzing liver RE hydrolysis are unknown. In this study, we identified mouse esterase 22 (Es22) as potent RE hydrolase highly expressed in the liver, particularly in hepatocytes. The enzyme is located exclusively at the endoplasmic reticulum (ER), implying that it is not involved in the mobilization of RE present in cytosolic lipid droplets. Nevertheless, cell culture experiments revealed that overexpression of Es22 attenuated the formation of cellular RE stores,

presumably by counteracting retinol esterification at the ER. Es22 was previously shown to form a complex with β-glucuronidase (Gus). Our studies revealed that Gus colocalizes with Es22 at the ER but does not affect its RE hydrolase activity. Interestingly, however, Gus was capable of hydrolyzing the naturally occurring vitamin A metabolite retinoyl β-glucuronide. In conclusion, our observations implicate that both Es22 and Gus play a role in liver retinoid metabolism.

4.747 AIRE activated tissue specific genes have histone modifications associated with inactive chromatin
Org, T., Rebane, A., Kisand, K., Laan, M., Haljasorg, U., Andreson, R. And Peterson, P.
Hum. Mol. Genet., ***18(24)***, *4699-4710 (2009)*

The Autoimmune Regulator (AIRE) protein is expressed in thymic medullary epithelial cells, where it promotes the ectopic expression of tissue-restricted antigens needed for efficient negative selection of developing thymocytes. Mutations in AIRE cause APECED syndrome, which is characterized by a breakdown of self-tolerance. The molecular mechanism by which AIRE increases the expression of a variety of different genes remains unknown. Here, we studied AIRE-regulated genes using whole genome expression analysis and chromatin immunoprecipitation. We show that AIRE preferentially activates genes that are tissue-specific and characterized by low levels of initial expression in stably transfected HEK293 cell model and mouse thymic medullary epithelial cells. In addition, the AIRE-regulated genes lack active chromatin marks, such as histone H3 trimethylation (H3K4me3) and acetylation (AcH3), on their promoters. We also show that during activation by AIRE, the target genes acquire histone H3 modifications associated with transcription and RNA polymerase II. In conclusion, our data show that AIRE is able to promote ectopic gene expression from chromatin associated with histone modifications characteristic to inactive genes.

4.748 Monocyte urokinase-type plasminogen activator up-regulation reduces thrombus size in a model of venous thrombosis
Humphries, J., Gossage, J.A., Modarai, B., Burnand, K.G., Sisson, T.H., Murdoch, C. and Smith, A.
J. Vasc. Surg., ***50***, *1127-1134 (2009)*

Background Our previous studies showed that the direct injection of an adenovirus construct expressing urokinase-type plasminogen activator (uPA) into experimental venous thrombi significantly reduces thrombus weight. The systemic use of adenovirus vectors is limited by inherent hepatic tropism and inflammatory response. As macrophages are recruited into venous thrombi, it is reasonable to speculate that these cells could be used to target the adenovirus uPA (ad-uPA) gene construct to the thrombus. The aims of this study were to determine whether macrophages transduced with ad-uPA have increased fibrinolytic activity and whether systemic injection of transduced cells could be used to target uPA expression to the thrombus and reduce its size.
Methods The effect of up-regulating uPA was examined in an immortalized macrophage cell line (MM6) and macrophages differentiated from human blood monocyte-derived macrophages (HBMMs). Cells were infected with ad-uPA or blank control virus (ad-blank). Fibrinolytic mediator expression, cell viability, and cytokine expression were measured by activity assays and enzyme-linked immunosorbent assays. Monocyte migration was measured using a modified Boyden chamber assay. A model of venous thrombosis was developed and characterized in mice with severe combined immunodeficiency (SCID). This model was used to study whether systemically administered macrophages over-expressing uPA reduced thrombus size. Uptake of HBMMs into the thrombus induced in these mice was confirmed by a combination of PKH2-labeled cell tracking and colocalization with human leukocyte antigen (HLA) by immunohistology.
Results Compared with ad-blank, treated HBMMs transduction with ad-uPA increased uPA production by >1000-fold (P = .003), uPA activity by 150-fold (P = .0001), and soluble uPA receptor (uPAR) by almost twofold (P = .043). Expression of plasminogen activator inhibitor (PAI-1) and PAI-2 was decreased by about twofold (P = .011) and threefold (P = .005), respectively. Up-regulation of uPA had no effect on cell viability or inflammatory cytokine production compared with ad-blank or untreated cells. Ad-uPA transduction increased the migration rate of HBMMs (about 20%, P = .03) and MM6 cells (>twofold, P = .005) compared with ad-blank treated controls. Human macrophage recruitment into the mouse thrombus was confirmed by the colocalization of HLA with the PKH2-marked cells. Systemic injection of uPA-up-regulated HBMMs reduced thrombus weight by approximately 20% compared with ad-blank (P = .038) or sham-treated controls (P = .0028).
Conclusion Transduction of HBBM with ad-uPA increases their fibrinolytic activity. Systemic administration of uPA up-regulated HBBMs reduced thrombus size in an experimental model of venous thrombosis. Alternative methods of delivering fibrinolytic agents are worth exploring.

Clinical Relevance
The use of thrombolysis in the treatment of acute iliofemoral deep vein thrombosis is not suitable for all patients. Our previous studies have shown that direct injection of an adenovirus construct expressing urokinase plasminogen activator (uPA) into experimental venous thrombi significantly reduced thrombus weight. The systemic use of adenovirus vectors is, however, limited by both their inherent hepatic tropism, which precludes targeted delivery to disease sites, and by the associated host inflammatory response. As macrophages are recruited into venous thrombi, these cells could be used to target uPA gene constructs to the thrombus after systemic administration.

4.749 Muscle-conditioned media and cAMP promote survival and neurite outgrowth of adult spinal cord motor neurons

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Exp. Neurol., ***220***, *303-315 (2009)*

Embryonic spinal cord motor neurons (MNs) can be maintained *in vitro* for weeks with a cocktail of trophic factors and muscle-derived factors under serum-containing conditions. Here we investigated the beneficial effects of muscle-derived factors in the form of muscle-conditioned medium (MCM) on the survival and neurite outgrowth of adult rat spinal cord MNs under serum-free conditions. Ventral horn dissociated cell cultures from the cervical enlargement were maintained in the presence of one or more of the following factors: brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), a cell permeant cyclic adenosine-3′,5′-monophosphate (cAMP) analog and MCM. The cell cultures were immunostained with several antibodies recognizing a general neuronal marker the microtubule-associated protein 2 (MAP2) and either one or more motor neuronal markers: the non-phosphorylated neurofilament heavy isoform (SMI32), the transcription factors HB9 and Islet-1 and the choline acetyl transferase. We found that treatment with MCM together with the cAMP analog was sufficient to promote selective survival and neurite outgrowth of adult spinal cord MNs. These conditions can be used to maintain adult spinal cord MNs in dissociated cultures for several weeks and may have therapeutic potential following spinal cord injury or motor neuropathies. More studies are necessary to evaluate how MCM and the cAMP analog act in synergy to promote the survival and neurite outgrowth of adult MNs.

4.750 Phosphodiesterase 3 Inhibition Reduces Platelet Activation and Monocyte Tissue Factor Expression in Knee Arthroplasty Patients

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Anesthesiology, ***111***, *1227-1237 (2009)*

Background: Tissue damage during surgery activates platelets and provokes a prothrombic state. The current study attempted to determine the impact of phosphodiesterase 3 inhibitors on platelet activation, platelet-leukocyte aggregate formation, and monocyte tissue factor expression during and after total knee arthroplasty.
Methods: Thirty-four patients undergoing scheduled total knee arthroplasty were randomly assigned to receive either the phosphodiesterase 3 inhibitor milrinone or the same amount of saline perioperatively. The effects of milrinone on platelet and leukocyte function in vitro were then assessed in healthy volunteers.
Results: Perioperative infusion of milrinone significantly attenuated platelet activation; phosphorylation of intraplatelet p38 mitogen-activated protein kinase, extracellular signal-regulated kinase 1/2, and Akt; and platelet-leukocyte aggregation. Furthermore, perioperative tissue factor expression on monocytes and fibrin monomer complex production were reduced by milrinone infusion in patients undergoing total knee arthroplasty. In vitro studies using adenosine diphosphate- and collagen-stimulated blood samples from healthy volunteers confirmed the antiplatelet effects and reduced monocyte tissue factor expression by milrinone. These studies further showed that platelet aggregation and integrin [alpha]IIb[beta]3 activation were modified by intraplatelet phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways, and that P-selectin expression on platelets and platelet-leukocyte aggregation were modulated by intraplatelet p38 mitogen-activated protein kinase pathway.
Conclusion: Continuous milrinone infusion has the potential to reduce platelet activation and monocyte tissue factor expression during the perioperative period in total knee arthroplasty. These events may be mediated in part by the ability of milrinone to reduce activation of intraplatelet mitogen-activated protein kinases and phosphatidylinositol 3-kinase. The clinical impact of phosphodiesterase 3 inhibition on perioperative hemostasis remains to be elucidated.

4.751 Improved, Low-Cost Methods for Pancreatic Islet Purification in Rats

Zhang, X-L., Yang, J-K., Yu, M. And Xue, G-F.
*Transplant. Proceedings, **41**, 4297-4301 (2009)*

Objective
Density gradient separation of islets from exocrine tissue is usually performed using Ficoll. However, this reagent significantly increases the cost of isolation. The aim of the present study was to investigate the effects on islet preparations of purification methods using Lymphoprep and Iodixanol (OptiPrep) density gradients.
Methods
Pancreata were procured from 46 Wistar rats, loaded with collagenase V (Sigma), and mechanically dissociated using standard procedures. After the digestion phase, the islets purified by 3 methods—Ficoll, Lymphoprep, and Iodixanol (OptiPrep)—were assessed for yields, purity, morphology, and in vitro function.
Result
We expressed the yields as islet equivalents (IEQ, diameter standardizing to 150 μm), showing no significant differences. Compared with the Ficoll group, the purity was significantly higher in the Lymphoprep (P = .005) and Iodixanol (OptiPrep) groups (P = .011). While the viability was >90% in all 3 groups, the viability in the Lymphoprep Group and OptiPrep groups was significantly higher than that of the Ficoll group (P < .001). In vitro islet function did not differ among the 3 experimental groups.
Conclusion
Lymphoprep and Iodixanol were as effective as Ficoll in terms of islet yield and in vitro function. High-purity and high-viability islet cells were obtained using improved Lymphoprep-based or Iodixanol (OptiPrep)-based density gradient methods, potential low-cost substitutes for Ficoll.

4.752 Stimulation of stellate cells by injured acinar cells: a model of acute pancreatitis induced by alcohol and fat (VLDL)

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*Am. J. Physiol. Gastrintest. Liver Physiol., **297**, G1163-G1171 (2009)*

Mechanisms leading to acute pancreatitis after a fat-enriched meal combined with excess alcohol are incompletely understood. We have studied the effects of alcohol and fat (VLDL) on pancreatic acinar cell (PAC) function, oxidative stress, and repair mechanisms by pancreatic stellate cells (PSC) leading to fibrogenesis. To do so, PAC (rat) were isolated and cultured up to 24 h. Ethanol and/or VLDL were added to PAC. We measured PAC function (amylase, lipase), injury (lactic dehydrogenase), apoptosis (TUNEL, Apo2.7, annexin V binding), oxidative stress, and lipid peroxidation (conjugated dienes, malondialdehyde, chemoluminescence); we also measured PSC proliferation (bromodeoxyuridine incorporation), matrix synthesis (immunofluorescence of collagens and fibronectin, fibronectin immunoassay), and fatty acids in PAC supernatants (gas chromatography). Within 6 h, cultured PAC degraded and hydrolyzed VLDL completely. VLDL alone (50 μg/ml) and in combination with alcohol (0.2, 0.5, and 1% vol/vol) induced PAC injury (LDL, amylase, and lipase release) within 2 h through generation of oxidative stress. Depending on the dose of VLDL and alcohol, apoptosis and/or necrosis were induced. Antioxidants (Trolox, Probucol) reduced the cytotoxic effect of alcohol and VLDL. Supernatants of alcohol/VLDL-treated PAC stimulated stellate cell proliferation and extracellular matrix synthesis. We concluded that, in the presence of lipoproteins, alcohol induces acinar cell injury. Our results provide a biochemical pathway for the clinical observation that a fat-enriched meal combined with excess alcohol consumption can induce acinar cell injury (acute pancreatitis) followed by repair mechanisms (proliferation and increased matrix synthesis in PSC).

4.753 Toll-Like Receptor Triggering and T-Cell Costimulation Induce Potent Antitumor Immunity in Mice

Westwood, J.A., Haynes, N.M., Sharkey, J., McLaughlin, N., Pegram, H.J., Schwendener, R.A., Smyth, M.J., Darcy, P.K. and Kershaw, M.H.
*Clin. Cancer Res., **15(24)**, 7624-7633 (2009)*

Purpose: To determine the antitumor activity of a novel combination of two immunomodulatory agents that simultaneously direct multiple components of immunity against cancer.
Experimental Design: We combined the Toll-like receptor agonist CpG 1826 with a T-cell costimulatory

antibody specific for CD137 in an optimal treatment route and dosing schedule against established tumors in two mouse models. Mechanistic insight was gained using gene-deficient mice and cell-depleting antibodies.
Results: The combination was shown to eradicate tumors in a large proportion of mice. Crucial roles for $CD8^+$ T cells, natural killer cells, and IFNs were shown. CpG and anti-CD137 injection led to activation of dendritic cells and optimal expansion of activated T cells in the blood. Macrophages were not necessary for therapeutic effect, and indeed depletion of macrophages *in vivo* enhanced therapy leading to tumor rejection in 100% of mice, which has not been previously reported in the immunotherapeutic setting. Long-term surviving mice were resistant to tumor rechallenge, demonstrating immunologic memory. In addition, we show, for the first time, that mice lacking B cells have a total loss of a recall response against tumor, suggesting a role for B cells in the induction of antitumor immunologic memory.
Conclusion: This study provides support for the use of a novel combination of immunomodulatory agents stimulating multiple facets of immunity for the effective immunotherapy of cancer.

4.754 Neuron survival in vitro is more influenced by the developmental age of the cells than by glucose condition

Sepehr, A., Ruud, J. and Mohseni, S.
Cytotechnology, ***61,*** *73-79 (2009)*

The objective of this study was to determine whether the sensitivity to varying glucose conditions differs for the peripheral and central nervous system neurons at different developmental stages. Ventral horn neurons (VHN) and dorsal root ganglion neurons (DRG) from rats of different postnatal ages were exposed to glucose-free or glucose-rich culture conditions. Following 24 h at those conditions, the number of protein gene product 9.5 positive (PGP+) DRG neurons and choline acetyltransferase positive (ChAT+) VHN were counted and their neurite lengths and soma diameters were measured. For both DRG and VHN, the highest number of cells with and without neurite outgrowth was seen when cells from postnatal day 4 donors were cultured, while the lowest cell numbers were when neurons were from donors early after birth and grown under glucose-free conditions. The length of the neurites and the soma diameter for VHN were not affected by either glucose level or age. DRG neurons, however, exhibited the shortest neurites and smallest soma diameter when neurons were obtained and cultured early after birth. Our results indicate that survival of neurons in vitro is more influenced by the developmental stage than by glucose concentrations.

4.755 Hoechst 33342 Side Population Identification Is a Conserved and Unified Mechanism in Urological Cancers

Oates, J.E., Grey, B.R., Addla, S.K., Samuel, J.D., Hart, C.A., Ramani, V.A.C., Brown, M.D. and Clarke, N.W.
Stem Cells and Development, ***18(10)****, 1515-1521 (2009)*
Mutation within the adult human stem cell (SC) compartment has been proposed as a factor in the initiation and promotion of carcinogenesis. Isolation of these cancer stem cells (CSCs) has proven difficult, limiting their subsequent phenotypic, functional, and genetic characterization. We have used the Hoechst 33342 dye efflux technique to isolate an epithelial side population (SP) from genitourinary (GU) cancers, which is enriched for cells with SC traits. With informed consent, samples were taken from patients with primary tumors and undergoing surgery for prostatic (CaP), invasive bladder transitional cell (TCC), and renal cell carcinomas (RCC). Single cell epithelial suspensions were extracted from these and incubated with Hoechst 33342. Hoechst SP/non-SP profiles were then generated by flow cytometry using standardized protocols. SP/non-SP cell cycle status was established by Hoechst 33342 and Pyronin Y staining. Immunocytochemistry staining was performed for markers suggested as stem markers as well as lineage-specific markers. Functionality was determined using colony-forming assays and long-term monolayer culture. A characteristic verapamil-sensitive SP was isolated from all 3 urological malignancies and represented 0.57% ± 0.11% (CaP), 0.52% ± 0.49% (TCC), and 5.9% ± 0.9% (RCC) of the total epithelial population. Cell cycle analysis showed that the SP had enhanced numbers of cells in G_0 as compared to the total cell population (CaP 12.4% ± 3.2 vs. 3.8% ± 1.0, RCC 23.2% ± 3.4 vs. 1.8% ± 0.9, and TCC 28.5% ± 4.9 vs. 4% ± 1.3). Immunocytochemistry demonstrated an increased expression of proliferative and putative stem markers within the SP fraction. Cultures confirmed significant enhancement of colony-forming ability and proliferative capacity of the SP fraction. A characteristic SP enriched for stem-like cells has been isolated from the 3 most common urological malignancies. This provides strong evidence that Hoechst 33342 efflux is a conserved and unified mechanism in GU cancer.

4.756 Indirect action of tumor necrosis factor-alpha in liver injury during the CD8+ T cell response to an adeno-associated virus vector in mice
Ginnandrea, M., Pierce, R.H. and Crispe, I.N.
Hepatology, ***49(6)***, *2010-2020 (2009)*

CD8+ T cells can cause hepatocellular injury by two distinct mechanisms. In addition to their direct cytotoxic effect, there is also collateral liver injury, which occurs when cells are killed in an antigen-independent manner. Whereas immune effector cytokines interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) have both been implicated in various forms of hepatitis, their respective roles in direct and/or collateral liver damage remains unclear. In order to investigate these elements of liver injury, we developed a new experimental model of CD8+ T-cell–mediated hepatitis based on an adeno-associated virus-based gene therapy vector. This vector is used to deliver antigen to hepatocytes, and CD8+ T cells specific for the vector-encoded transgene are adoptively transferred to produce liver immunopathology. In this experimental model, CD8+ T-cell IFNγ acts on Kupffer cells, inducing TNFα secretion and liver injury. Both IFNγ and TNFα are important in this injury process, but TNFα acts as an autocrine amplifier of Kupffer cell function, rather than as a direct effector of hepatocellular damage. Conclusions: TNFα indirectly promotes liver damage and is not a direct hepatotoxic agent. IFNγ also indirectly contributes to liver injury through Kupffer cell activation while, in parallel, directly promoting hepatitis through induction of hepatocyte major histocompatability complex class I. In principle, it may be possible to ameliorate this immunopathologic indirect mechanism by developing therapies that target Kupffer cells, without impairing CD8+ T-cell-mediated antiviral immunity. This would have great therapeutic potential in chronic viral hepatitis.

4.757 Thymus cell antigen-1-expressing cells in the oval cell compartment
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Hepatology, ***50(2)***, *601-611 (2009)*

Thymus cell antigen-1 (Thy-1)-expressing cells proliferate in the liver during oval cell (OC)-mediated liver regeneration. We characterized these cells in normal liver, in carbon tetrachloride-injured liver, and in several models of OC activation. The gene expression analyses were performed using reverse-transcriptase polymerase chain reaction (RT-PCR), quantitative RT-PCR (Q-RT-PCR) of cells isolated by fluorescence-activated cell sorting (FACS), and by immunofluorescent microscopy of tissue sections and isolated cells. In normal liver, Thy-1+ cells are a heterogeneous population: those located in the periportal region do not coexpress desmin or alpha smooth muscle actin (α-SMA). The majority of Thy-1+ cells located at the lobular interface and in the parenchyma coexpress desmin but not α-SMA, i.e., they are not resident myofibroblasts. Although Thy-1+ cells proliferate moderately after carbon tetrachloride injury, in all models of OC-mediated liver regeneration they proliferate quickly and expand significantly and disappear from the liver when the OC response subsides. Activated Thy-1+ cells do not express OC genes but they express genes known to be expressed in mesenchymal stem cells (CD105, CD73, CD29), genes considered specific for activated stellate cells (desmin, collagen I-a2, Mmp2, Mmp14) and myofibroblasts (α-SMA, fibulin-2), as well as growth factors and cytokines (Hgf, Tweak, IL-1b, IL-6, IL-15) that can affect OC growth. Activated in vitro stellate cells do not express Thy-1. Subcloning of Thy-1+ cells from OC-activated livers yield Thy-1+ fibroblastic cells and a population of E-cadherin+ mesenchymal cells that gradually discontinue expression of Thy-1 and begin to express cytokeratins. However, upon transplantation these cells do not differentiate into hepatocytes or cholangiocytes. Activated Thy-1+ cells produce predominantly latent transforming growth factor beta. Conclusion: Thy-1+ cells in the OC niche are activated mesenchymal-epithelial cells that are distinct from resident stellate cells, myofibroblasts, and oval cells.

4.758 Porcine liver sinusoidal endothelial cells contribute significantly to intrahepatic ammonia metabolism
Nedredal, G.I., Elvevold, K., Ytrebø, L.M., Fuskevåg, O-M., Pettersen, I., McCourt, P.A., Bertheussen, K., Smedsrød, b. and Revhaug, A.
Hepatology, ***50(3)***, *900-908 (2009)*

Ammonia metabolism in the liver has been largely credited to hepatocytes (HCs). We have shown that liver nonparenchymal cells that include liver sinusoidal endothelial cells (LSECs) produce ammonia. To address the limited knowledge regarding a role for LSECs in ammonia metabolism, we investigated the ammonia metabolism of isolated LSECs and HCs under three different conditions: (1) bioreactors containing LSECs (LSEC-bioreactors), (2) bioreactors containing HCs (HC-bioreactors), and (3) separate

bioreactors containing LSECs and HCs connected in sequence (Seq-bioreactors). Our results showed that LSEC-bioreactors released six-fold more ammonia (22.2 nM/hour/106 cells) into the growth media than HC-bioreactors (3.3 nM/hour/106 cells) and Seq-bioreactors (3.8 nM/hour/106 cells). The glutamate released by LSEC-bioreactors (32.0 nM/hour/106 cells) was over four-fold larger than that released by HC-bioreactors and Seq-bioreactors (<7 nM/hour/106 cells). LSEC-bioreactors and HC-bioreactors consumed large amounts of glutamine (>25 nM/hour/106 cells). Glutaminase is known for catalyzing glutamine into glutamate and ammonia. To determine if this mechanism may be responsible for the large levels of glutamate and ammonia found in LSEC-bioreactors, immunolabeling of glutaminase and messenger RNA expression were tested. Our results demonstrated that glutaminase was present with colocalization of an LSEC-specific functional probe in lysosomes of LSECs. Furthermore, using a nucleotide sequence specific for kidney-type glutaminase, reverse-transcription polymerase chain reaction revealed that this isoform of glutaminase was expressed in porcine LSECs. Conclusion: LSECs released large amounts of ammonia, perhaps due to the presence of glutaminase in lysosomes. The ammonia and glutamate released by LSECs in Seq-bioreactors were used by hepatocytes, suggesting an intrahepatic collaboration between these two cell types.

4.759 Qualitative and quantitative comparison of *N*-glycans between pig endothelial and islet cells by high-performance liquid chromatography and mass spectrometry-based strategy
Kim, Y-G., Gil, G-C., Jang, K-S., Lee, S., Kim, H-i., Kim, J-S., Chung, J., Park, C-G., Harvey, D.J. and Kim, B-G.
*J. Mass. Spectrom., **44(7)**, 1087-1104 (2009)*

N-glycan structures released from miniature pig endothelial and islet cells were determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), negative ion electrospray ionization (ESI) MS/MS and normal-phase high performance liquid chromatography (NP-HPLC) combined with exoglycosidase digestion. Totally, the identified structures were 181 N-glycans including 129 sialylated and 18 α-galactosylated glycans from pig endothelial cells and 80 N-glycans including 41 sialylated and one α-galactosylated glycans from pig islet cells. The quantity of the α-galactosylated glycans from pig islet cells was certainly neglectable compared to pig endothelial cells. A number of NeuGc-terminated N-glycans (80 from pig endothelial cells and 13 from pig islet cells) are newly detected by our mass spectrometric strategies. The detailed structural information will be a matter of great interest in organ or cell xenotransplantation using α1,3-galactosyltransferase gene-knockout (GalT-KO) pig.

4.760 Mass spectrometric analysis of the glycosphingolipid-derived glycans from miniature pig endothelial cells and islets: identification of NeuGc epitope in pig islets
Kim, Y-G., Harvey, D.J., Yang, Y-H., Park, C-G. and Kim, B-G.
*J. Mass. Spectrom., **44(10)**, 1489-1499 (2009)*

Glycosphingolipid (GSL) is a major component of the plasma membrane in eukaryotic cells that is involved directly in a variety of immunological events via cell-to-cell or cell-to-protein interactions. In this study, qualitative and quantitative analyses of GSL-derived glycans on endothelial cells and islets from a miniature pig were performed and their glycosylation patterns were compared. A total of 60 and 47 sialylated and neutral GSL-derived glycans from the endothelial cells and islets, respectively, were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and collision-induced fragmentation using positive-ion electrospray ionization (ESI) ion-trap tandem mass spectrometry (MS/MS). In accordance with previous immunohistochemistry studies, the α-Gal-terminated GSL was not detected but NeuGc-terminated GSLs were newly detected from miniature pig islets. In addition, the neutral GSL-derived glycans were relatively quantified by derivatization with carboxymethyl trimethylammonium hydrazide (so called Girard's T reagent) and MALDI-TOF MS. The structural information of the GSL-derived glycans from pig endothelial cells and islets suggests that special attention should be paid to all types of glycoconjugates expressed on pig tissues or cells for successful clinical xenotransplantation.

4.761 Rapamycin inhibits hepatic fibrosis in rats by attenuating multiple profibrogenic pathways
Bridle, K.R., Popa, C., Morgan, M.L., Sobbe, A.L., Clouston, A.D., Fletcher, L.M. aand Crawford, D.H.G.
*Liver Transplant, **15(10)**, 1315-1324 (2009)*

Hepatic stellate cell transdifferentiation, epithelial-mesenchymal cell transition, and the ductular reaction each contribute to the development of hepatic fibrosis in cholestatic liver diseases. Inhibitors of mammalian target of rapamycin have antifibrotic properties. We evaluated the hypothesis that the

antifibrotic action of rapamycin is due to attenuated myofibroblast proliferation in addition to an inhibitory effect on epithelial-mesenchymal transition and the ductular reaction. Hepatic fibrosis was induced by bile duct ligation, and rodents received 1.5 mg/kg/day rapamycin by subcutaneous infusion for 21 days. The expression of various markers of hepatic fibrosis, stellate cell transactivation, epithelial-mesenchymal transition, and the ductular reaction was compared between treated and untreated animals. Hepatic fibrosis, hepatic procollagen type 1 messenger RNA, and alpha-smooth muscle actin expression were significantly reduced in treated animals. Hepatic stellate cell procollagen expression and proliferation were also reduced by rapamycin. The following markers of epithelial-mesenchymal transition—vimentin protein expression, S100 calcium binding protein A4 and transforming growth factor beta 1 messenger RNA, and the mothers against decapentaplegic homolog signaling pathway—were all reduced after rapamycin treatment. The intensity of the ductular reaction was reduced by rapamycin as assessed by histopathological scoring and by reduced cytokeratin 19 expression. Rapamycin caused a reduction in hepatic progenitor cell proliferation. Together, these data show that multiple profibrogenic pathways are activated in an animal model of cholestasis and that rapamycin attenuates epithelial-mesenchymal transition and the ductular reaction as well as hepatic stellate cell activation.

4.762 Cholesterol-promoted synaptogenesis requires the conversion of chelosterol to estradiol in the Hippocampus

Fester, L., Zhou, L., Bütow, A., Huber, C., von Lossow, R., Prange-Kiel, J., Jarry, H. and Rune, G.M.
*Hippocampus, **19(8)**, 692-705 (2009)*

Cholesterol of glial origin promotes synaptogenesis (Mauch et al., (2001) Science 294:1354–1357). Because in the hippocampus local estradiol synthesis is essential for synaptogenesis, we addressed the question of whether cholesterol-promoted synapse formation results from the function of cholesterol as a precursor of estradiol synthesis in this brain area. To this end, we treated hippocampal cultures with cholesterol, estradiol, or with letrozole, a potent aromatase inhibitor. Cholesterol increased neuronal estradiol release into the medium, the number of spine synapses in hippocampal slice cultures, and immunoreactivity of synaptic proteins in dispersed cultures. Simultaneous application of cholesterol and letrozole or blockade of estrogen receptors by ICI 182 780 abolished cholesterol-induced synapse formation. As a further approach, we inhibited the access of cholesterol to the first enzyme of steroidogenesis by knock-down of steroidogenic acute regulatory protein, the rate-limiting step in steroidogenesis. A rescue of reduced synaptic protein expression in transfected cells was achieved by estradiol but not by cholesterol. Our data indicate that in the hippocampus cholesterol-promoted synapse formation requires the conversion of cholesterol to estradiol.

4.763 Androgen ablation augments prostate cancer vaccine immunogenicity only when applied after immunization

Koh, Y.T., gray, A., Higgins, S.A., Hubby, B. and Kast, W.M.
*The Prostate, **69(6)**, 571-584 (2009)*

BACKGROUND
Androgen ablation (AA) causes apoptosis of normal and neoplastic prostate cells. It is a standard treatment for advanced prostate cancer. Androgen ablation-mediated immunological effects include bone marrow hyperplasia, thymic regeneration, T and B cell lymphopoeisis and restoration of age-related peripheral T cell dysfunction. Androgens also regulate the transcription of several cytokines. Dendritic cells (DC) are the most potent antigen presenting cells that can activate antigen-specific naïve T cells. Despite myriad clinical trials involving DC-based prostate cancer immunotherapies, the effects of AA on DC function remain largely uncharacterized. Therefore, we investigated the effects of AA on DC and whether it could improve the efficacy of prostate cancer immunotherapy.
METHODS
Cytokine expression changes due to AA were quantified by multiplex ELISA. Flow cytometry was used to assess AA-mediated effects on DC maturation and expression of costimulatory markers. Mixed leukocyte reactions and cell-mediated lysis assays elucidated the role of androgens in DC function. The effect of AA on the efficacy of vaccination against a prostate tumor-associated antigen was tested using Elispot assays.
RESULTS
Androgen ablation increased dendritic cell maturation and costimulatory marker expression, but had no effect on DC costimulatory function. However, DC isolated from castrated mice increased the expression of key cytokines by antigen-experienced T cells while decreasing their expression in naïve cells. Finally, androgen ablation improved immune responses to vaccination only when applied after immunization.
CONCLUSION

Androgen ablation causes differential effects of DC on primary and secondary T cell responses, thus augmenting vaccine immunogenicity only when applied after immunization.

4.764 Parameters for successful pig islet isolation as determined using 68 specific-pathogen-free miniature pigs

Kim, H-I., Lee, S-Y., Jin, S.M., Kim, K.S., YU, J.E., Yeom, S-C., Yoon, T.W., Kim, J.H., Ha, J., Park, C-G. and Kim, S-J.
*Xenotransplant.,**16(1)**, 11-18 (2009)*

Abstract: Background: Islet cell transplantation is a novel therapeutic modality for the cure of diabetes. Pig islet cells are an attractive substitute for human islet cells; however, they are known to be particularly difficult to isolate because of a weak islet capsule and a tendency to be fragmented during enzymatic digestion. Therefore, parameters favoring successful pig islet isolation were investigated using specific-pathogen-free (SPF) miniature pigs.
Methods: Sixty-eight SPF miniature pigs were used for islet isolation. Birth weight, body weight, age, sex, pregnancy history, and the fasting blood glucose levels of each pig were determined. Each pig's general condition was assessed with regard to feeding status and physical activity. Pancreas procurement was performed by one surgical team. Anesthesia duration, operation duration, procedure quality, and perfusate type were recorded. After pancreatectomy, a biopsy was performed for islet density analysis. Decapsulation, cannulation duration, degree of distension, and cold ischemic time were assessed. During islet isolation, pancreas weight, digestion time, and digested tissue proportion were recorded. Isolation results were evaluated by total islet equivalents (IEQ), islet equivalents per gram of pancreas (IEQ/g), isolation index, islet recovery rate, purity, and visual grade. To identify the predictors of higher islet isolation yield, we performed binary logistic regression analysis with significant ($P < 0.05$) variables from the univariate analysis.
Results: The pigs were categorized into high (n = 34) and low yield (n = 34) groups according to the median IEQ/g or total IEQ values. Body weight and age were significantly different between the two groups. Being male or a positive history of pregnancy in females was factors favoring successful islet isolation. General condition assessments failed to estimate islet isolation results. Long anesthesia duration, which might have caused ischemic injury to the pancreas, negatively affected islet isolation results. Decapsulation, cannulation duration, and subsequent pancreas distension were significantly important in successful islet isolation. Inter-lot variability of Liberase was not observed because of screening processes performed before purchase. Isolation index and islet recovery rate correlated well with islet yields.
Conclusions: Multivariate analysis using total IEQ and IEQ/g as outcome variables indicated that age older than 2, being male and moderate distension by Liberase injection are major determinants influencing successful islet isolation.

4.765 Tissue targeting of anti-RNP autoimmunity: Effects of T cells and myeloid dendritic cells in a murine model

Greidinger, E.L., Zang, Y., Fernandez, I., Berho, M., Nassiri, M., Martinez, L. and Hoffmann, R.W.
*Arthritis & Rheumatism, **60(2)**, 534-542 (2009)*

Objective
To explore the role of immune cells in anti-RNP autoimmunity in a murine model of pneumonitis or glomerulonephritis, using adoptive transfer techniques.
Methods
Donor mice were immunized with 50 μg of U1–70-kd small nuclear RNP fusion protein and 50 μg of U1 RNA adjuvant. Whole splenocytes as well as CD4+ cell and dendritic cell (DC) subsets from the immunized mice were infused into naive syngeneic recipients. Anti-RNP and T cell responses were assessed by immunoblotting, enzyme-linked immunosorbent assay, and flow cytometry. Development of renal or lung disease was assessed by histology and urinalysis.
Results
Unfractionated splenocytes from donor mice without proteinuria induced predominantly lung disease in recipients (8 [57%] of 14 versus 2 [14%] of 14 developing renal disease; $P = 0.046$). However, infusion of CD4+ cells from donors without proteinuria induced renal disease more frequently than lung disease (7 [70%] of 10 versus 2 [20%] of 10; $P = 0.01$); adoptive transfer of RNP+CD4+ T cells from short-term culture yielded similar results (renal disease in 8 [73%] of 11 recipients versus lung disease in 3 [27%] of 11). Cotransfer of splenic myeloid DCs and CD4+ T cells from immunized donors prevented induction of renal disease in all 5 recipients ($P = 0.026$ versus recipients of fresh CD4+ cells alone), although lung disease was still observed in 1 of 5 mice. Transfer of myeloid DCs alone from immunized donors induced

lung disease in 3 (60%) of 5 recipients, without evidence of nephritis. Cotransfer of splenocytes from mice with and those without nephritis led to renal disease in 4 of 5 recipients, without evidence of lung disease. Conclusion
These findings indicate that RNP+CD4+ T cells are sufficient to induce anti-RNP autoimmunity, tissue targeting in anti-RNP autoimmunity can be deviated to either a renal or pulmonary phenotype depending on the presence of accessory cells such as myeloid DCs, and DC subsets can play a role in both propagation of autoimmunity and end-organ targeting.

4.766 Effect of short-term culture on functional and stress-related parameters in isolated human islets
Ihm, S-H., Matsumoto, I., Zhang, H.J., Ansite, J.D. and hering, B.J.
*Transplant Int., **22(2)**, 207-216 (2009)*

The Edmonton protocol for islet transplantation utilizes fresh islet grafts but other protocols increasingly transplant short-term cultured grafts mainly for practical reasons. To improve our understanding of the impact of culture pretreatment of human islets, we assessed post-transplant function by nude mouse bioassay, islet ATP, activity of stress-activated MAP kinases, and expression of stress-related genes by focused cDNA array in freshly isolated and cultured islets. Mean blood glucose levels over 4 weeks after transplantation (2000 IE) of (i) freshly isolated, (ii) cultured and preculture counted (recovery rate; 78 ± 6%), and (iii) cultured and postculture counted islets in diabetic mice were 330 ± 40, 277 ± 65, and 256 ± 52 mg/dl (i versus ii, $P = 0.004$; i versus iii, $P = 0.002$). During culture, islet ATP/DNA and ATP/ADP increased; JNK and p38 MAPK activities decreased. Among 96 genes studied, mRNA expression of heat shock protein 70 genes decreased >twofold during culture in all four pairs; expression of cyclooxygenase-2, superoxide dismutase-2, interleukin-6 and cytochromes P450 1A1 genes increased. Our results show that culturing human islets before transplantation is not disadvantageous in regard of functional recovery from changes induced by nonphysiologic stimuli during islet isolation. The increase in expression of several stress-related genes during culture also shows that improving culture conditions may further enhance post-transplant islet function.

4.767 Vascular endothelial growth factor prevents G93A-SOD1-induced motor neuron degeneration
Lunn, J.S., Sakowski, S.A., Kim, B., Rosenberg, A.A. and Feldman, E.L.
*Develop. Neurobiol., **69(13)**, 871-884 (2009)*

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by selective loss of motor neurons (MNs). Twenty percent of familial ALS cases are associated with mutations in Cu2+/Zn2+ superoxide dismutase (SOD1). To specifically understand the cellular mechanisms underlying mutant SOD1 toxicity, we have established an in vitro model of ALS using rat primary MN cultures transfected with an adenoviral vector encoding a mutant SOD1, G93A-SOD1. Transfected cells undergo axonal degeneration and alterations in biochemical responses characteristic of cell death such as activation of caspase-3. Vascular endothelial growth factor (VEGF) is an angiogenic and neuroprotective growth factor that can increase axonal outgrowth, block neuronal apoptosis, and promote neurogenesis. DecreasedVEGF gene expression in mice results in a phenotype similar to that seen in patients with ALS, thus linking loss of VEGF to the pathogenesis of MN degeneration. Decreased neurotrophic signals prior to and during disease progression may increase MN susceptibility to mutant SOD1-induced toxicity. In this study, we demonstrate a decrease in VEGF and VEGFR2 levels in the spinal cord of G93A-SOD1 ALS mice. Furthermore, in isolated MN cultures, VEGF alleviates the effects of G93A-SOD1 toxicity and neuroprotection involves phosphatidylinositol 3-kinase/protein kinase B (PI3K/ Akt) signaling. Overall, these studies validate the usefulness of VEGF as a potential therapeutic factor for the treatment of ALS and give valuable insight into the responsible signaling pathways and mechanisms involved.

4.768 A Re-evaluation of the Mechanisms Leading to Dengue Hemorrhagic Fever
Noisakran, S., Chokephaibulkit, K., Songprakhon, P., Onlamoon, N., Hsiao, H-M., Villinger, F., Ansari, A. and Perng, G.C.
Ann. N.Y. Acad. Sc., 1171, E24-E35 (2009)

Viremia is one of the features of dengue virus infection among the flaviviruses. Dengue virus infection results in a spectrum of clinical symptoms, ranging from undifferentiated flu-like illness, mild dengue fever, to dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS), a life-threatening illness. Several mechanisms have been hypothesized based primarily on data collected from post-acute clinical phase to account for DHF/DSS. Lack of a suitable animal model for DHF/DSS has hindered progress in

defining the etiology of DHF/DSS. Levels of circulating dengue virus have been well-correlated to severe dengue disease. However, the cell lineage(s) serving as a primary target for the source of viremia are largely unknown. Results from *in vivo* and *in vitro* pilot studies using molecular and more advanced technologies reveal that dengue virus appears to be associated with platelets and the megakaryocytic lineage. The observation may partially explain the dysfunction of platelets observed in dengue affected patients.

4.769 Short-term exposure to human cytomegalovirus–infected fibroblasts induces a proportional increase of active CD94/NKG2A$^+$ natural killer cells
Petersen, L., Petersen, C.C., Møller-Larsen, A. and Hokland, M.E.
Human Immunol., ***71***, *29-35 (2010)*

Natural killer (NK) cells are essential components of the immune response against human cytomegalovirus (HCMV). As NK cells are part of the innate immune system providing an immediate defense against pathogens, short-term exposure to HCMV-infected cells may induce changes in the phenotype and function of these cells. To identify immediate reactions of NK cells to HCMV, we co-cultured peripheral blood mononuclear cells with HCMV-infected fibroblasts for 24 and 72 hours. A distinct, HCMV-mediated, proportional enlargement of a subset of NK cells expressing CD94/NKG2A was sustained throughout the period of incubation. As preceding studies have shown that HCMV can cause an increase in CD94/NKG2C$^+$ NK cells, our results were surprising. The NK cells showed intense upregulation of the early activation marker CD69 in response to HCMV. The CD94/NKG2A$^+$ NK cells demonstrated the highest expression of CD69. Studies of HCMV-induced interferon-γ expression after 24 hours of co-culture showed that this cytokine was almost exclusively produced by the CD94/NKG2A$^+$ subset of NK cells. In summary, our data demonstrate that HCMV induces an immediate proportional enlargement of a functionally active CD94/NKG2A expressing subset of NK cells.

4.770 The quest for liver progenitor cells: A practical point of view
Dolle, L., Best, J., Mei, J., Al Battah, F., Reynaert, H., van Grunsven, L.A. and Geerts, A.
J. Hepatol., ***52***, *117-129 (2010)*

Many chronic liver diseases can lead to hepatic dysfunction with organ failure. At present, orthotopic liver transplantation represents the benchmark therapy of terminal liver disease. However this practice is limited by shortage of donor grafts, the need for lifelong immunosuppression and very demanding state-of-the-art surgery. For this reason, new therapies have been developed to restore liver function, primarily in the form of hepatocyte transplantation and artificial liver support devices. While already offered in very specialized centers, both of these modalities still remain experimental. Recently, liver progenitor cells have shown great promise for cell therapy, and consequently they have attracted a lot of attention as an alternative or supportive tool for liver transplantation. These liver progenitor cells are quiescent in the healthy liver and become activated in certain liver diseases in which the regenerative capacity of mature hepatocytes and/or cholangiocytes is impaired. Although reports describing liver progenitor cells are numerous, they have not led to a consensus on the identity of the liver progenitor cell. In this review, we will discuss some of the characteristics of these cells and the different ways that have been used to obtain these from rodents. We will also highlight the challenges that researchers are facing in their quest to identify and use liver progenitor cells.

4.771 Cytokines Involved in Interferon-γ Production by Human Macrophages
Robinson, C.M., O'Dee, D., Hamilton, T. and Nau, G.J.
J. Innat. Immun., ***2***, *56-65 (2010)*

Interferon (IFN)-γ is important to the immune defense against intracellular pathogens and specifically the ability of macrophages to control *Mycobacterium tuberculosis* (MTB). Increasing evidence has accumulated to support the idea that macrophages produce IFN-γ. We describe here the cytokine interactions that determine IFN-γ expression and secretion during MTB infection of human macrophages. Detection of biologically important IFN-γ levels in culture supernatants of MTB-infected human macrophages requires the addition of interleukin (IL)-12. IL-18 augmented IFN-γ production from human macrophages in response to the combination of MTB and supplemental IL-12. Although IL-18 gene expression was generally unchanged, IL-18 protein secretion was enhanced by the combination of MTB and IL-12, and functioned primarily to stimulate IFN-γ release. Importantly, IL-27 induced by MTB infection opposed IFN-γ production by antagonizing IL-18 activity in human macrophages. Neutralization of IL-27 increased the expression of the IL-18 receptor β-chain. Additionally, IL-27 blocked NF-κB

activation in response to IL-18. These results define the signals required for IFN-γ production by human macrophages and highlight the interactions between cytokines produced during MTB infection. Together, they identify a novel role for IL-27 in regulating macrophage function by disrupting IL-18 activity.

4.772 Age-related deficiencies in complex I endogenous substrate availability and reserve capacity of complex IV in cortical neuron electron transport
Jones, T.T. and Brewer, G.J.
*Biochim. Biophys. Acta, **1797**, 167-176 (2010)*

Respiratory enzyme complex dysfunction is mechanistically involved in mitochondrial failure leading to neurodegenerative disease, but the pathway is unclear. Here, age-related differences in mitochondrial respiration were measured in both whole and permeabilized neurons from 9-month and 24-month adult rat cortex cultured in common conditions. After permeabilization, respiration increased in both ages of neurons with excess substrates. To dissect specific deficiencies in the respiratory chain, inhibitors for each respiratory chain complex were used to isolate their contributions. Relative to neurons from 9-month rats, in neurons isolated from 24-month rats, complexes I, III, and IV were more sensitive to selective inhibition. Flux control point analysis identified complex I in neurons isolated from 24-month rats as the most sensitive to endogenous substrate availability. The greatest age-related deficit in flux capacity occurred at complex IV with a 29% decrease in neurons isolated from 24-month rats relative to those from 9-month rats. The deficits in complexes I and III may contribute to a redox shift in the quinone pool within the electron transport chain, further extending these age-related deficits. Together these changes could lead to an age-related catastrophic decline in energy production and neuronal death.

4.773 A Large-Scale Chemical Screen for Regulators of the Arginase 1 Promoter Identifies the Soy Isoflavone Daidzeinas a Clinically Approved Small Molecule That Can Promote Neuronal Protection or Regeneration via a cAMP-Independent Pathway
Ma, T.C., Campana, A., Lange, P.S., Lee, H-H., Banerjee, K., Bryson, J.B., Mahishi, L., Alam, S., Giger, R.J., Barnes, S., Morris jr, S.M., Willis, D.E., Rwiss, J.L., Filbin, M.T. and Ratan, R.R.
*J. Neurosci., **30(2)**, 739-748 (2010)*

An ideal therapeutic for stroke or spinal cord injury should promote survival and regeneration in the CNS. Arginase 1 (Arg1) has been shown to protect motor neurons from trophic factor deprivation and allow sensory neurons to overcome neurite outgrowth inhibition by myelin proteins. To identify small molecules that capture Arg1's protective and regenerative properties, we screened a hippocampal cell line stably expressing the proximal promoter region of the *arginase 1* gene fused to a reporter gene against a library of compounds containing clinically approved drugs. This screen identified daidzein as a transcriptional inducer of Arg1. Both CNS and PNS neurons primed *in vitro* with daidzein overcame neurite outgrowth inhibition from myelin-associated glycoprotein, which was mirrored by acutely dissociated and cultured sensory neurons primed *in vivo* by intrathecal or subcutaneous daidzein infusion. Further, daidzein was effective in promoting axonal regeneration *in vivo* in an optic nerve crush model when given intraocularly without lens damage, or most importantly, when given subcutaneously after injury. Mechanistically, daidzein requires transcription and induction of Arg1 activity for its ability to overcome myelin inhibition. In contrast to canonical Arg1 activators, daidzein increases Arg1 without increasing CREB phosphorylation, suggesting its effects are cAMP-independent. Accordingly, it may circumvent known CNS side effects of some cAMP modulators. Indeed, daidzein appears to be safe as it has been widely consumed in soy products, crosses the blood–brain barrier, and is effective without pretreatment, making it an ideal candidate for development as a therapeutic for spinal cord injury or stroke.

4.774 Rotavirus Infection Activates Dendritic Cells from Peyer's Patches in Adult Mice
Lopez-Guerrero, D.V., Meza-Perez, S., Ramirez-Pliego, O., Santana-Calderon, M.A., Espino-Solis, P., Gutierrez-Xicotencatl, L., Flores-Romo, L. and Eszuivel-Guadarrama, F.R.
*J. Virol., **84(4)**, 1856-1866 (2010)*

This study used an in vivo mouse model to analyze the response of dendritic cells (DCs) in Peyer's patches (PPs) within the first 48 h of infection with the wild-type murine rotavirus EDIM (EDIMwt). After the infection, the absolute number of DCs was increased by 2-fold in the PPs without a modification of their relative percentage of the total cell number. Also, the DCs from PPs of infected mice showed a time-dependent migration to the subepithelial dome (SED) and an increase of the surface activation markers CD40, CD80, and CD86. This response was more evident at 48 h postinfection (p.i.) and depended on viral replication, since DCs from PPs of mice inoculated with UV-treated virus did not show this phenotype. As

a result of the activation, the DCs showed an increase in the expression of mRNA for the proinflammatory cytokines interleukin-12/23p40 (IL-12/23p40), tumor necrosis factor alpha (TNF-α), and beta interferon (IFN-β), as well as for the regulatory cytokine IL-10. These results suggest that, a short time after rotavirus infection, the DCs from PPs play a critical role in controlling the infection and, at the same time, avoiding an excessive inflammatory immune response.

4.775 Low-Temperature Preservation of Isolated Islets is Superior to Conventional Islet Culture Before Islet Transplantation

Noguchi, H., Naziruddin, B., Jackson, A., Shimoda, M., Ikemoto, T., Fujita, Y., Chujo, D., Takota, M., Kobayashi, N., Onaca, N., Levy, M.F. and Matsumoto, S.
Transplantation, ***89(1)****, 47-54 (2010)*

Background. Although culturing islets before transplantation provides flexibility for evaluation of isolated islets and pretreatment of patients, it is well-known that isolated islets deteriorate rapidly in culture. In this study, we evaluated optimal temperature for culture/preservation of isolated human islets before transplantation.
Methods. Isolated islets were cultured or preserved for 48 hr in the following culture/preservation conditions: preservation at 4[degrees]C in University of Wisconsin solution and culture at 22[degrees]C or 37[degrees]C in culture medium.
Results. Islet morphology after 4[degrees]C preservation was similar to that of fresh islets, whereas islet diameter after 37[degrees]C or 22[degrees]C culture was smaller than that of fresh islets. Islet yield significantly decreased at higher temperatures (24% loss in 37[degrees]C culture and 19% loss in 22[degrees]C culture, but <5% loss in 4[degrees]C preservation). Cultured/preserved islets were transplanted into diabetic nude mice. The attainability of posttransplantation normoglycemia was significantly higher in the 4[degrees]C preservation group than in 22[degrees]C and 37[degrees]C culture groups.
Conclusion. Preservation of isolated islets at 4[degrees]C improves the outcome of islet transplantation more efficiently than preservation at 22[degrees]C or 37[degrees]C. Based on these data, we have performed short-time cold storage of isolated islets instead of culturing for current clinical islet transplantation.

4.776 Paeonol, the main active principles of *Paeonia moutan*, ameliorates alcoholic steatohepatitis in mice

Hu, A., Shwn, G., Zhao, W., Wang, F., Jiang, X. and Huang, D.
J. Ethnopharmacol., ***128****, 100-106 (2010)*

Aim of study
Paeonol, a major phenolic component of Moutan Cortex, is traditionally used as a Chinese herbal medicine in various diseases including hepatitis. Evidence shows that paeonol has anti-inflammatory, anti-tumor, and anti-atherosclerosis effects. However, the effect of paeonol on alcoholic liver injury remains obscure. The present investigation was designed to determine the effects of paeonol on alcohol-induced hepatic injury in mice.
Materials and methods
The degree of alcoholic liver injury was evaluated biochemically by measuring serum markers and pathological examination. Real-time PCR and ELISA methods were used to check the expression of cytokines. Western blotting was used to check CYP 450 expression.
Results
Treatment with paeonol significantly attenuated the level of serum aminotransferase, reduced the severe extent of hepatic cell damage, steatosis, and the infiltration of inflammatory cells in a model of alcoholic liver injury ($P < 0.05$). Interestingly, paeonol markedly decreased hepatic mRNA expression of lipogenic genes ($P < 0.05$) while had no effect on protein expression of hepatic CYP2E1. Furthermore, paeonol significantly decreased serum and tissue inflammatory cytokine levels, tissue lipid peroxidation, neutrophil infiltration and inhibited the apoptosis of hepatocytes ($P < 0.05$). Kupffer cells isolated from ethanol-fed mice produced high amounts of tumor necrosis factor alpha, whereas Kupffer cells from paeonol treatment ethanol-fed mice produced less tumor necrosis factor alpha ($P < 0.05$).
Conclusions
These findings suggest that paeonol may represent a novel, protective strategy against alcoholic liver injury by attenuating hepatic steatosis, inflammatory response and apoptosis.

4.777 Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment
Beck, K.D., Nguyen, H.X., Galvan, M.D., Salazar, D.L., Woodruff, T.M. and Anderson, A.J.
*Brain, **133(2)**, 433-447 (2010)*

Traumatic injury to the central nervous system results in the disruption of the blood brain/spinal barrier, followed by the invasion of cells and other components of the immune system that can aggravate injury and affect subsequent repair and regeneration. Although studies of chronic neuroinflammation in the injured spinal cord of animals are clinically relevant to most patients living with traumatic injury to the brain or spinal cord, very little is known about chronic neuroinflammation, though several studies have tested the role of neuroinflammation in the acute period after injury. The present study characterizes a novel cell preparation method that assesses, quickly and effectively, the changes in the principal immune cell types by flow cytometry in the injured spinal cord, daily for the first 10 days and periodically up to 180 days after spinal cord injury. These data quantitatively demonstrate a novel time-dependent multiphasic response of cellular inflammation in the spinal cord after spinal cord injury and are verified by quantitative stereology of immunolabelled spinal cord sections at selected time points. The early phase of cellular inflammation is comprised principally of neutrophils (peaking 1 day post-injury), macrophages/microglia (peaking 7 days post-injury) and T cells (peaking 9 days post-injury). The late phase of cellular inflammation was detected after 14 days post-injury, peaked after 60 days post-injury and remained detectable throughout 180 days post-injury for all three cell types. Furthermore, the late phase of cellular inflammation (14–180 days post-injury) did not coincide with either further improvements, or new decrements, in open-field locomotor function after spinal cord injury. However, blockade of chemoattractant C5a-mediated inflammation after 14 days post-injury reduced locomotor recovery and myelination in the injured spinal cord, suggesting that the late inflammatory response serves a reparative function. Together, these data provide new insight into cellular inflammation of spinal cord injury and identify a surprising and extended multiphasic response of cellular inflammation. Understanding the role of this multiphasic response in the pathophysiology of spinal cord injury could be critical for the design and implementation of rational therapeutic treatment strategies, including both cell-based and pharmacological interventions.

4.778 Adjuvant Activity on Murine and Human Macrophages
Quesniaux, V., Erard, F. and Ryffel, B.
*Methods in Mol. Biol., **626**, 117-130 (2010)*

Activation of cells of the innate immunity such as macrophages and dendritic cells is critical to mount an adaptive immune response. Recent advances on the understanding of innate immune receptors such as the Toll-like receptors (TLR) and NOD-like receptors (NLR) and the demonstration that microbial products activate specific receptors. This discovery represented a major advance and provided tools to test novel adjuvants in vitro to investigate activation on innate immune cells. Here the isolation and culture of murine macrophages is described, and the use of macrophages derived from gene-deficient mice is proposed to define receptor usage. Novel adjuvants may be tested for their capacity to induce cytokines, chemokines and the expression of costimulatory molecules. The basic methods to assess macrophage activation are given, which may predict an in vivo activity of a novel adjuvant.

4.779 Pathogenic cysteine mutations affect progranulin function and production of mature granulins
Wang, J., Van Damme, P., Cruchaga, C., Gitcho, M.A., Vidal, J.M., Seijo-Martinez, M., Wang, L., Wu, J.Y., Robberecht, W. and Goate, A.
*J. Neurochem., **112**, 1305-1315 (2010)*

Frontotemporal dementia with ubiquitin-positive inclusions (FTLD-U) can be caused by mutations in the progranulin gene (GRN). Progranulin (PGRN) is a cysteine-rich growth factor, which is proteolytically cleaved by elastase to produce several granulins (GRNs). All FTLD-U mutations in GRN characterized to date result in reduced secreted PGRN protein. We recently reported a Spanish family with progressive non-fluent aphasia and dementia in which a novel C521Y mutation segregates with disease. A second cysteine mutation (C139R) has also been reported to be disease specific. Allele-specific mRNA expression assays in brain reveal that the C521Y mutant allele is expressed at similar levels to the wild-type allele. Furthermore, plasma PGRN levels in C521Y carriers are comparable with non-carrier family relatives, suggesting that the mutation does not affect PGRN protein expression and secretion in vivo. Despite normal PGRN levels C521Y and C139R mutant GRNs show reduced neurite growth-stimulating activity in vitro. Further study revealed that these mutations also cause impaired cleavage of PGRN by elastase. Our data suggest that these mutations affect the function of full-length PGRN as well as elastase cleavage

of PGRN into GRNs, leading to neurodegeneration.

4.780 Microvesicle entry into marrow cells mediates tissue-specific changes in mRNA by direct delivery of mRNA and induction of transcription
Aliotta, J.M., Pereira, M., Johnson, K.W., de Paz, N., Dooner, M.S., Puente, N., Ayala, C., Brilliant, K., Berz, D., Lee, D., Ramratman, B., McMillan, P.N., Hixson, D.C., Josic, D. and Quesenberry, P.J.
Exp. Hematol., ***38***, *233-245 (2010)*

Objective
Microvesicles have been shown to mediate intercellular communication. Previously, we have correlated entry of murine lung-derived microvesicles into murine bone marrow cells with expression of pulmonary epithelial cell-specific messenger RNA (mRNA) in these marrow cells. The present studies establish that entry of lung-derived microvesicles into marrow cells is a prerequisite for marrow expression of pulmonary epithelial cell-derived mRNA.
Materials and Methods
Murine bone marrow cells cocultured with rat lung, but separated from them using a cell-impermeable membrane (0.4-μm pore size), were analyzed using species-specific primers (for rat or mouse).
Results
These studies revealed that surfactant B and C mRNA produced by murine marrow cells were of both rat and mouse origin. Similar results were obtained using murine lung cocultured with rat bone marrow cells or when bone marrow cells were analyzed for the presence of species-specific albumin mRNA after coculture with rat or murine liver. These studies show that microvesicles both deliver mRNA to marrow cells and mediate marrow cell transcription of tissue-specific mRNA. The latter likely underlies the longer-term stable change in genetic phenotype that has been observed. We have also observed microRNA in lung-derived microvesicles, and studies with RNase-treated microvesicles indicate that microRNA negatively modulates pulmonary epithelial cell-specific mRNA levels in cocultured marrow cells. In addition, we have also observed tissue-specific expression of brain, heart, and liver mRNA in cocultured marrow cells, suggesting that microvesicle-mediated cellular phenotype change is a universal phenomena.
Conclusion
These studies suggest that cellular systems are more phenotypically labile than previously considered.

4.781 Lethal Antibody Enhancement of Dengue Disease in Mice Is Prevented by Fc Modification
Balsitis, S.J., Williams, K.L., Lachica, R., Flores, D., Kyle, J.L., Mehlhop, E., Johnson, S., Diamond, M.S., Beatty, P.R. and Harris, E.
PloSPathogens, ***6(2)***, *e1000790 (2010)*

Immunity to one of the four dengue virus (DV) serotypes can increase disease severity in humans upon subsequent infection with another DV serotype. Serotype cross-reactive antibodies facilitate DV infection of myeloid cells *in vitro* by promoting virus entry via Fcγ receptors (FcγR), a process known as antibody-dependent enhancement (ADE). However, despite decades of investigation, no *in vivo* model for antibody enhancement of dengue disease severity has been described. Analogous to human infants who receive anti-DV antibodies by transplacental transfer and develop severe dengue disease during primary infection, we show here that passive administration of anti-DV antibodies is sufficient to enhance DV infection and disease in mice using both mouse-adapted and clinical DV isolates. Antibody-enhanced lethal disease featured many of the hallmarks of severe dengue disease in humans, including thrombocytopenia, vascular leakage, elevated serum cytokine levels, and increased systemic viral burden in serum and tissue phagocytes. Passive transfer of a high dose of serotype-specific antibodies eliminated viremia, but lower doses of these antibodies or cross-reactive polyclonal or monoclonal antibodies all enhanced disease *in vivo* even when antibody levels were neutralizing *in vitro*. In contrast, a genetically engineered antibody variant (E60-N297Q) that cannot bind FcγR exhibited prophylactic and therapeutic efficacy against ADE-induced lethal challenge. These observations provide insight into the pathogenesis of antibody-enhanced dengue disease and identify a novel strategy for the design of therapeutic antibodies against dengue.

4.782 TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration
Wils, H., Kleinberger, G., Janssens, J., Pereson, S., Joris, G., Cuijt, I., Smits, V., Ceuterick-de Groote, C., Van Broechoven, C and Kumar-Singh, S.
PNAS, ***107(8)***, *3858-3863 (2010)*

Neuronal cytoplasmic and intranuclear aggregates of RNA-binding protein TDP-43 are a hallmark feature

of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). ALS and FTLD show a considerable clinical and pathological overlap and occur as both familial and sporadic forms. Though missense mutations in TDP-43 cause rare forms of familial ALS, it is not yet known whether this is due to loss of TDP-43 function or gain of aberrant function. Moreover, the role of wild-type (WT) TDP-43, associated with the majority of familial and sporadic ALS/FTLD patients, is also currently unknown. Generating homozygous and hemizygous WT human TDP-43 transgenic mouse lines, we show here a dose-dependent degeneration of cortical and spinal motor neurons and development of spastic quadriplegia reminiscent of ALS. A dose-dependent degeneration of nonmotor cortical and subcortical neurons characteristic of FTLD was also observed. Neurons in the affected spinal cord and brain regions showed accumulation of TDP-43 nuclear and cytoplasmic aggregates that were both ubiquitinated and phosphorylated as observed in ALS/FTLD patients. Moreover, the characteristic ≈25-kDa C-terminal fragments (CTFs) were also recovered from nuclear fractions and correlated with disease development and progression in WT TDP-43 mice. These findings suggest that ≈25-kDa TDP-43 CTFs are noxious to neurons by a gain of aberrant nuclear function.

4.783 Deletion of the Mucin-Like Molecule Muc1 Enhances Dendritic Cell Activation in Response to Toll-Like Receptor Ligands

Williams, M.A., Bauer, S., Lu, W., Guo, J., Walter, S., Brushnell, T.P., Lillehoj, E.P. and Georas, S.N.
*J. Innate Immun., **2(2)**, 123-143 (2010)*

Dendritic cells (DC) are potent professional antigen-presenting cells that drive primary immune responses to infections or other agonists perceived as 'dangerous'. Muc1 is the only cell surface mucin or MUC gene product that is expressed in DC. Unlike other members of this glycoprotein family, Muc1 possesses a unique cytosolic region capable of signal transduction and attenuating toll-like receptor (TLR) activation. The expression and function of Muc1 has been intensively investigated on epithelial and tumor cells, but relatively little is known about its function on DC. We hypothesized that Muc1 would influence in vitro generation and primary DC activation in response to the TLR4 and TLR5 ligands lipopolysaccharide and flagellin. Compared with $Muc1^{+/+}$ DC, we found that $Muc1^{-/-}$ DC were constitutively activated, as determined by higher expression of co-stimulatory molecules (CD40, CD80 and CD86), greater secretion of immunoregulatory cytokines (TNF-α and VEGF), and better stimulation of allogeneic naïve CD4+ T cell proliferation. After activation by either LPS or flagellin and co-culture with allogeneic CD4+ T cells, $Muc1^{-/-}$ DC also induced greater secretion of TNF-α and IFN-γ compared to similarly activated $Muc1^{+/+}$ DC. Taken together, our results indicate that deletion of Muc1 promotes a heightened functional response of DC in response to TLR4 and TLR5 signaling pathways, and suggests a previously under-appreciated role for Muc1 in regulating innate immune responses of DC.

4.784 Enhanced Infection of Liver Sinusoidal Endothelial Cells in a Mouse Model of Antibody-Induced Severe Dengue Disease

Zellweger, R.M., Prestwood, T.R. and Shresta, S.
*Cell Host & Microbe, **7(2)**, 128-139 (2010)*

Dengue virus (DENV) causes disease ranging from dengue fever (DF), a self-limited febrile illness, to the potentially lethal dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). DHF/DSS usually occurs in patients who have acquired DENV-reactive antibodies prior to infection, either from a previous infection with a heterologous DENV serotype or from an immune mother. Hence, it has been hypothesized that subneutralizing levels of antibodies exacerbate disease, a phenomenon termed antibody-dependent enhancement (ADE). However, given the lack of suitable animal models for DENV infection, the mechanism of ADE and its contribution to pathology remain elusive. Here we demonstrate in mice that DENV-specific antibodies can sufficiently increase severity of disease so that a mostly nonlethal illness becomes a fatal disease resembling human DHF/DSS. Antibodies promote massive infection of liver sinusoidal endothelial cells (LSECs), resulting in increased systemic levels of virus. Thus, a subprotective humoral response may, under some circumstances, have pathological consequences.

4.785 Use of Iodixanol Self-Generated Density Gradients to Enrich for Viable Urothelial Cells from Nonneurogenic and Neurogenic Bladder Tissue

Bruce, A.T., Sangha, N., Richmond, A., Johnson, K., Jones, S., Spencer, T. and Ludlow, J.W.
*Tissue Engineering: Part C, **16(1)**, 33-40 (2010)*

Suspensions of viable urothelial cells (UC) isolated from patient bladder biopsies often contain considerable amounts of extraneous materials comprised of cellular debris, dead and dying UC, and red

blood cells. We have consistently observed an inversely proportional relationship between UC attachment efficiency and the amount of extraneous materials in the suspension; viable UC cell attachment efficiency decreases as the amount of extraneous materials in the cell suspension increases. Processing the initial cell isolate to reduce the amount of extraneous materials can enrich for viable UC capable of attaching and proliferating in *ex vivo* cultures. In this report, we describe the isolation of an enriched population of viable UC from nonneurogenic and neurogenic bladder tissue biopsies using iodixanol self-generated density gradients (OptiPrep™), and characterization by trypan blue exclusion, fluorescence-activated cell sorting, immunofluorescence, and growth kinetics.

4.786 A Physiological Function of Inflammation-Associated SerpinB2 Is Regulation of Adaptive Immunity
Schroder, W.A., Le, T.T.T., Major, L., Street, S., Gardner, J., Lambley, E., Markey, K., MacDonald, K.P., Fish, R.J., Thomas, R. and Suhrbier, A.
J. Immunol., ***184***, *2663-2670 (2010)*

SerpinB2 (plasminogen activator inhibitor-2) is widely described as an inhibitor of urokinase plasminogen activator; however, SerpinB2$^{-/-}$ mice show no detectable increase in urokinase plasminogen activator activity. In this study, we describe an unexpected immune phenotype in SerpinB2$^{-/-}$ mice. After immunization with OVA in CFA, SerpinB2$^{-/-}$ mice made ≈6-fold more IgG2c and generated ≈2.5-fold more OVA-specific IFN-γ–secreting T cells than SerpinB2$^{+/+}$ littermate controls. In SerpinB2$^{+/+}$ mice, high inducible SerpinB2 expression was seen at the injection site and in macrophages low levels in draining lymph nodes and conventional dendritic cells, and no expression was seen in plasmacytoid dendritic, B, T, or NK cells. SerpinB2$^{-/-}$ macrophages promoted greater IFN-γ secretion from wild-type T cells in vivo and in vitro and, when stimulated with anti-CD40/IFN-γ or cultured with wild-type T cells in vitro, secreted more Th1-promoting cytokines than macrophages from littermate controls. Draining lymph node SerpinB2$^{-/-}$ myeloid APCs similarly secreted more Th1-promoting cytokines when cocultured with wild-type T cells. Regulation of Th1 responses thus appears to be a physiological function of inflammation-associated SerpinB2; an observation that may shed light on human inflammatory diseases like pre-eclampsia, lupus, asthma, scleroderma, and periodontitis, which are associated with SerpinB2 polymorphisms or dysregulated SerpinB2 expression.

4.787 MeCP2 Controls an Epigenetic Pathway That Promotes Myofibroblast Transdifferentiation and Fibrosis
Mann, J., Chu, D.C.K., Maxwell, A., Oakley, F., Zhu, N-L., Tsukamoto, H. and Mann, D.A.
Gastroenterology, ***138***, *705-714 (2010)*

Background & Aims
Myofibroblast transdifferentiation generates hepatic myofibroblasts, which promote liver fibrogenesis. The peroxisome proliferator-activated receptor γ (PPARγ) is a negative regulator of this process. We investigated epigenetic regulation of PPARγ and myofibroblast transdifferentiation.
Methods
Chromatin immunoprecipitation (ChIP) assays assessed the binding of methyl-CpG binding protein 2 (MeCP2) to PPARγ and chromatin modifications that silence this gene. MeCP2$^{-/y}$ mice and an inhibitor (DZNep) of the epigenetic regulatory protein EZH2 were used in the carbon tetrachloride model of liver fibrosis. Liver tissues from mice were assessed by histologic analysis; markers of fibrosis were measured by quantitative polymerase chain reaction (qPCR). Reverse transcription PCR detected changes in expression of the microRNA miR132 and its target, elongated transcripts of MeCP2. Myofibroblasts were transfected with miR132; PPARγ and MeCP2 expressions were analyzed by qPCR or immunoblotting.
Results
Myofibroblast transdifferentiation of hepatic stellate cells is controlled by a combination of MeCP2, EZH2, and miR132 in a relay pathway. The pathway is activated by down-regulation of miR132, releasing the translational block on MeCP2. MeCP2 is recruited to the 5′ end of PPARγ, where it promotes methylation by H3K9 and recruits the transcription repressor HP1α. MeCP2 also stimulates expression of EZH2 and methylation of H3K27 to form a repressive chromatin structure in the 3′ exons of PPARγ. Genetic and pharmacologic disruptions of MeCP2 or EZH2 reduced the fibrogenic characteristics of myofibroblasts and attenuated fibrogenesis.
Conclusions
Liver fibrosis is regulated by an epigenetic relay pathway that includes MeCP2, EZH2, and miR132. Reagents that interfere with this pathway might be developed to reduce fibrogenesis in chronic liver disease.

4.788 CD36 Is a Novel Serum Amyloid A (SAA) Receptor Mediating SAA Binding and SAA-induced Signaling in Human and Rodent Cells

Baranova, I.N., Bochatrov, A.V., Vishnyakova, T.G., Kurlander, R., Chen, Z., Fu, D., Arias, I.M., Csako, G., Patterson, A.P. and Eggerman, T.L.
J. Biol. Chem., ***285(11)***, *8942-8506 (2010)*

Serum amyloid A (SAA) is a major acute phase protein involved in multiple physiological and pathological processes. This study provides experimental evidence that CD36, a phagocyte class B scavenger receptor, functions as a novel SAA receptor mediating SAA proinflammatory activity. The uptake of Alexa Fluor® 488 SAA as well as of other well established CD36 ligands was increased 5–10-fold in HeLa cells stably transfected with CD36 when compared with mock-transfected cells. Unlike other apolipoproteins that bind to CD36, only SAA induced a 10–50-fold increase of interleukin-8 secretion in CD36-overexpressing HEK293 cells when compared with control cells. SAA-mediated effects were thermolabile, inhibitable by anti-SAA antibody, and also neutralized by association with high density lipoprotein but not by association with bovine serum albumin. SAA-induced cell activation was inhibited by a CD36 peptide based on the CD36 hexarelin-binding site but not by a peptide based on the thrombospondin-1-binding site. A pronounced reduction (up to 60–75%) of SAA-induced pro-inflammatory cytokine secretion was observed in *cd36*$^{-/-}$ rat macrophages and Kupffer cells when compared with wild type rat cells. The results of the MAPK phosphorylation assay as well as of the studies with NF-κB and MAPK inhibitors revealed that two MAPKs, JNK and to a lesser extent ERK1/2, primarily contribute to elevated cytokine production in CD36-overexpressing HEK293 cells. In macrophages, four signaling pathways involving NF-κB and three MAPKs all appeared to contribute to SAA-induced cytokine release. These observations indicate that CD36 is a receptor mediating SAA binding and SAA-induced pro-inflammatory cytokine secretion predominantly through JNK- and ERK1/2-mediated signaling.

4.789 Distinct Roles for CCR4 and CXCR3 in the Recruitment and Positioning of Regulatory T Cells in the Inflamed Human Liver

Oo, Y., Weston, C.J., Lalor, P.F., Curbishley, S.M., Withers, D.R., Reynolds, G.M., Shetty, S., Harki, J., Shaw, J.C., Eksteen, B., Hubscher, S.G., Walker, L.S. and Adams, D.H.
J. Immunol., ***184***, *2886-2898 (2010)*

Regulatory T cells (T_{regs}) are found at sites of chronic inflammation where they mediate bystander and Ag-specific suppression of local immune responses. However, little is known about the molecular control of T_{reg} recruitment into inflamed human tissues. We report that up to 18% of T cells in areas of inflammation in human liver disease are forkhead family transcriptional regulator box P3 (FoxP3)$^+$ T_{regs}. We isolated $CD4^+CD25^+CD127^{low}FoxP3^+$ T_{regs} from chronically inflamed human liver removed at transplantation; compared with blood-derived T_{regs}, liver-derived T_{regs} express high levels of the chemokine receptors CXCR3 and CCR4. In flow-based adhesion assays using human hepatic sinusoidal endothelium, T_{regs} used CXCR3 and α4β1 to bind and transmigrate, whereas CCR4 played no role. The CCR4 ligands CCL17 and CCL22 were absent from healthy liver, but they were detected in chronically inflamed liver where their expression was restricted to dendritic cells (DCs) within inflammatory infiltrates. These DCs were closely associated with CD8 T cells and $CCR4^+$ T_{regs} in the parenchyma and septal areas. Ex vivo, liver-derived T_{regs} migrated to CCR4 ligands secreted by intrahepatic DCs. We propose that CXCR3 mediates the recruitment of T_{regs} via hepatic sinusoidal endothelium and that CCR4 ligands secreted by DCs recruit T_{regs} to sites of inflammation in patients with chronic hepatitis. Thus, different chemokine receptors play distinct roles in the recruitment and positioning of T_{regs} at sites of hepatitis in chronic liver disease.

4.790 Blockade of Programmed Death Ligand 1 Enhances the Therapeutic Efficacy of Combination Immunotherapy against Melanoma

Pilon-Thomas, S., Mackay, A., Vohra, N. and Mule, J.J.
J. Immunol., ***184***, *3442-3449 (2010)*

Inhibition of antitumor T cell responses can be mediated by the productive interaction between the programmed death-1 (PD-1) receptor on T cells and its ligand PD-L1. PD-L1 is highly expressed on both murine bone marrow-derived dendritic cells (DCs) and B16 melanoma. In this study, in vitro blockade of PD-L1 interaction on DCs led to enhanced IFN-γ production and cytotoxicity by Ag-specific T cells. In vivo, the systemic administration of anti–PD-L1 Ab plus melanoma peptide-pulsed DCs resulted in a higher number of melanoma peptide-specific $CD8^+$ T cells, but this combination was insufficient to delay the growth of established B16 melanoma. Although the addition of 600 rad of total body irradiation

delayed tumor growth, further adoptive transfer of Ag-specific $CD8^+$ T cells was needed to achieve tumor regression and long-term survival of the treated mice. Lymphopenic mice treated with anti–PD-L1 Ab demonstrated increased activation and persistence of adoptively transferred T cells, including a higher number of $CD8^+$ T cells infiltrating the tumor mass. Together, these studies support the blocking of PD-L1 signaling as a means to enhance combined immunotherapy approaches against melanoma.

4.791 Human Peripheral Lymphoid Tissues Contain Autoimmune Regulator-Expressing Dendritic Cells
Poliani, P.L., Kisand, K., Marrella, V., Ravanini, M., Notarangelo, L.D., Villa, A., Peterson, P. and Facchetti, F.
Am. J. Pathol., ***176(3)***, *1104-1112 (2010)*

Autoimmune regulator (AIRE) modulates the expression of tissue-restricted antigens (TSAs) and promotes central tolerance in the thymus. However, few autoreactive T cells escape negative selection and reach the periphery, where peripheral tolerance is required to avoid autoimmunity. Murine lymph nodes (LNs) have been shown to contain "stromal" cells expressing AIRE and TSAs. Here we report the occurrence of AIRE-expressing cells in human peripheral lymphoid tissues, including LNs, tonsils, and gut-associated lymphoid tissue, with the exception of the spleen. Notably, $AIRE^+$ cells are absent in fetal LNs and, in postnatal life, they are more numerous in abdominal than in superficial LNs, thus suggesting that their development in periphery may depend on instructive signals from microenvironment and antigen challenge. Extrathymic $AIRE^+$ cells show a dendritic morphology, consistently express human leukocyte antigen-DR (HLADR) and fascin, and are largely positive for CD11c and S100 and for the dendritic cell-activation markers CD40, CD83, DC-LAMP/CD208, and CCR7. Lymphoid, myelomonocytic, mesenchymal, and epithelial cell lineage markers are negative. The $HLADR^{high}/AIRE^+$ cell fraction isolated from mesenteric LNs expressed TSAs (insulin, CYP17A1, and CYP21A2), as well as molecules associated with tolerogenic functions, such as interleukin-10 and indoleamine 2,3-dioxygenase. Data indicate that $AIRE^+$ cells in human peripheral lymphoid tissues correspond to a subset of activated interdigitating dendritic cells expressing TSAs and the tolerogenic molecules indoleamine 2,3-dioxygenase and interleukin-10, suggestive of a potential tolerogenic function.

4.792 Islet Isolation for Clinical Transplantation
Kin, T.
Adv. Exp. Med. and Biol., ***654***, *683-710 (2010)*

Islet transplantation is emerging as a viable treatment option for selected patients with type 1 diabetes. Following the initial report in 2000 from Edmonton of insulin independence in seven out of seven consecutive recipients, there has been a huge expansion in clinical islet transplantation. The challenge we now face is the apparent decline in graft function over time. Isolating high-quality human islets which survive and function for a longer period will no doubt contribute to further improvement in long-term clinical outcome. This chapter reviews the selection of appropriate donors for islet isolation and transplantation, describes each step during islet isolation, and discusses the scope for further improvements.

4.793 Tumour necrosis factor alpha induces rapid reduction in AMPA receptor-mediated calcium entry in motor neurones by increasing cell surface expression of the GluR2 subunit: relevance to neurodegeneration
Rainey-Smith, S.R., Andersson, D.A., Williams, R.J. and Rattray, M.
J. Neurochem., ***113***, *692-703 (2010)*

The [alpha]-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPAR) subunit GluR2, which regulates excitotoxicity and the inflammatory cytokine tumour necrosis factor alpha (TNF[alpha]) have both been implicated in motor neurone vulnerability in amyotrophic lateral sclerosis/motor neurone disease. TNF[alpha] has been reported to increase cell surface expression of AMPAR subunits to increase synaptic strength and enhance excitotoxicity, but whether this mechanism occurs in motor neurones is unknown. We used primary cultures of mouse motor neurones and cortical neurones to examine the interaction between TNF[alpha] receptor activation, GluR2 availability, AMPAR-mediated calcium entry and susceptibility to excitotoxicity. Short exposure to a physiologically relevant concentration of TNF[alpha] (10 ng/mL, 15 min) caused a marked redistribution of both GluR1 and GluR2 to the cell surface as determined by cell surface biotinylation and immunofluorescence. Using fura-2-acetoxymethyl ester microfluorimetry, we showed that exposure to TNF[alpha] caused a rapid reduction in the peak amplitude of AMPA-mediated calcium entry in a PI3-kinase and p38 kinase-dependent manner, consistent with

increased insertion of GluR2-containing AMPAR into the plasma membrane. This resulted in a protection of motor neurones against kainate-induced cell death. Our data therefore, suggest that TNF[alpha] acts primarily as a physiological regulator of synaptic activity in motor neurones rather than a pathological drive in amyotrophic lateral sclerosis.

4.794 Survival effect of PDGF-CC rescues neurons from apoptosis in both brain and retina by regulating GSK3β phosphorylation
Tang, Z., Arjunan, P., Lee, C., Li, Y., Kumar, A., Hou, X., Wang, B., Wardega, P., Zhang, F., Ddong, L., Zhang, Y., Zhang, S-Z., Ding, H., Fariss, R.N., Becker, K.G., Lennartsson, J., Nagai, N., Cao, Y. and Li, X.
J: Exp. Med., ***207(4)***, *867-880 (2010)*

Platelet-derived growth factor CC (PDGF-CC) is the third member of the PDGF family discovered after more than two decades of studies on the original members of the family, PDGF-AA and PDGF-BB. The biological function of PDGF-CC remains largely to be explored. We report a novel finding that PDGF-CC is a potent neuroprotective factor that acts by modulating glycogen synthase kinase 3β (GSK3β) activity. In several different animal models of neuronal injury, such as axotomy-induced neuronal death, neurotoxin-induced neuronal injury, 6-hydroxydopamine–induced Parkinson's dopaminergic neuronal death, and ischemia-induced stroke, PDGF-CC protein or gene delivery protected different types of neurons from apoptosis in both the retina and brain. On the other hand, loss-of-function assays using *PDGF-C* null mice, neutralizing antibody, or short hairpin RNA showed that PDGF-CC deficiency/inhibition exacerbated neuronal death in different neuronal tissues in vivo. Mechanistically, we revealed that the neuroprotective effect of PDGF-CC was achieved by regulating GSK3β phosphorylation and expression. Our data demonstrate that PDGF-CC is critically required for neuronal survival and may potentially be used to treat neurodegenerative diseases. Inhibition of the PDGF-CC–PDGF receptor pathway for different clinical purposes should be conducted with caution to preserve normal neuronal functions.

4.795 A defined long-term *in vitro* tissue engineered model of neuromuscular junctions
Das, M., Rumsey, J.W., Bhaargava, N., Stancescu, M. and Hickman, J.J.
Biomaterials, ***31***, *4880-4888 (2010)*

Neuromuscular junction (NMJ) formation, occurring between motoneurons and skeletal muscle, is a complex multistep process involving a variety of signaling molecules and pathways. *In vitro* motoneuron-muscle co-cultures are powerful tools to study the role of different growth factors, hormones and cellular structures involved in NMJ formation. In this study, a serum-free culture system utilizing defined temporal growth factor application and a non-biological substrate resulted in the formation of robust NMJs. The system resulted in long-term survival of the co-culture and selective expression of neonatal myosin heavy chain, a marker of myotube maturation. NMJ formation was verified by colocalization of dense clusters of acetylcholine receptors visualized using alpha-bungarotoxin and synaptophysin containing vesicles present in motoneuron axonal terminals. This model will find applications in basic NMJ research and tissue engineering applications such as bio-hybrid device development for limb prosthesis and regenerative medicine as well as for high-throughput drug and toxin screening applications.

4.796 Apoptosis induced by ozone and oxysterols in human alveolar epithelial cells
Kosmider, B., Loader, J.E., Murphy, R.C. and Mason, R.J.
Free Radical Biol. & Med., ***48***, *1513-1524 (2010)*

The mechanism of ozone-induced lung cell injury is poorly understood. One hypothesis is that ozone induces lipid peroxidation and that these peroxidated lipids produce oxidative stress and DNA damage. Oxysterols are lipid peroxides formed by the direct effects of ozone on pulmonary surfactant and cell membranes. We studied the effects of ozone and the oxysterol 5β,6β-epoxycholesterol (β-epoxide) and its metabolite cholestan-6-oxo-3,5-diol (6-oxo-3,5-diol) on human alveolar epithelial type I-like cells (ATI-like cells) and type II cells (ATII cells). Ozone and oxysterols induced apoptosis and cytotoxicity in ATI-like cells. They also generated reactive oxygen species and DNA damage. Ozone and β-epoxide were strong inducers of nuclear factor erythroid 2-related factor 2, heat shock protein 70, and Fos-related antigen 1 protein expression. Furthermore, we found higher sensitivity of ATI-like cells compared to ATII cells exposed to ozone or treated with β-epoxide or 6-oxo-3,5-diol. In general the response to the cholesterol epoxides was similar to the effect of ozone. Understanding the response of human ATI-like cells and ATII cells to oxysterols may be useful for further studies, because these compounds may represent useful biomarkers in other diseases.

4.797 Neurons and Oligodendrocytes Recycle Sphingosine 1-Phosphate to Ceramide: SIGNIFICANCE FOR APOPTOSIS AND MULTIPLE SCLEROSIS
Qin, J., Berdyshev, E., Goya, J., Natarajan, V. and Dawson, G.
J. Biol. Chem., ***285(19)***, *14134-14143 (2010)*

Both cultured neonatal rat hippocampal neurons and differentiated oligodendrocytes rapidly metabolized exogenous C_2- and C_6-ceramides to sphingosine (Sph) and sphingosine 1-phosphate (S1P) but only minimally to $C_{16–24}$-ceramides. Dihydrosphinolipids were unaffected but were increased by exogenous C_6-dihydroceramide. Conversely, quantitative liquid chromatography-tandem mass spectrometry technology showed that exogenous S1P (0.25–10 μM) was rapidly metabolized to both Sph (a >200-fold increase) and predominantly C_{18}-ceramide (a >2-fold increase). Longer treatments with either C_2-ceramide (>2.5 μM) or S1P (10 μM) led to apoptotic cell death. Thus, there is an active sphingolipid salvage pathway in both neurons and oligodendrocytes. Staurosporine-induced cell death was shown to be associated with decreased S1P and increased Sph and $C_{16/18}$-ceramide levels. The physiological significance of this observation was confirmed by the analysis of affected white matter and plaques from brains of multiple sclerosis patients in which reduced S1P and increased Sph and $C_{16/18}$-ceramides were observed.

4.798 Adult human brain cell culture for neuroscience research
Gibbons, H.M. and Dragunow, M.
Int. J. Biochem. & Cell Biol., ***42***, *844-856 (2010)*

Studies of the brain have progressed enormously through the use of in vivo and in vitro non-human models. However, it is unlikely such studies alone will unravel the complexities of the human brain and so far no neuroprotective treatment developed in animals has worked in humans. In this review we discuss the use of adult human brain cell culture methods in brain research to unravel the biology of the normal and diseased human brain. The advantages of using adult human brain cells as tools to study human brain function from both historical and future perspectives are discussed. In particular, studies using dissociated cultures of adult human microglia, astrocytes, oligodendrocytes and neurons are described and the applications of these types of study are evaluated. Alternative sources of human brain cells such as adult neural stem cells, induced pluripotent stem cells and slice cultures of adult human brain tissue are also reviewed. These adult human brain cell culture methods could benefit basic research and more importantly, facilitate the translation of basic neuroscience research to the clinic for the treatment of brain disorders.

4.799 Distinct populations of metastases-enabling myeloid cells expand in the liver of mice harboring invasive and preinvasive intra-abdominal tumor
Conolly, M.K., Mallen-St. Clair, J., Bedrosian, A.S., Malhotra, A., Vera, V., Ibrahim, J., Henning, J., Pachter, H.L., Bar-Sagi, D., Frey, A.B. and Miller, G.
J. Leukoc. Biol., ***87***, *713-725 (2010)*

The liver is the most common site of adenocarcinoma metastases, even in patients who initially present with early disease. We postulated that immune-suppressive cells in the liver of tumor-bearing hosts inhibit anti-tumor T cells, thereby accelerating the growth of liver metastases. Using models of early preinvasive pancreatic neoplasia and advanced colorectal cancer, aims of this study were to determine immune phenotype, stimulus for recruitment, inhibitory effects, and tumor-enabling function of immune-suppressive cells in the liver of tumor-bearing hosts. We found that in mice with intra-abdominal malignancies, two distinct $CD11b^+Gr1^+$ populations with divergent phenotypic and functional properties accumulate in the liver, becoming the dominant hepatic leukocytes. Their expansion is contingent on tumor expression of KC. These cells are distinct from $CD11b^+Gr1^+$ populations in other tissues of tumor-bearing hosts in terms of cellular phenotype and cytokine and chemokine profile. Liver $CD11b^+Gr1^+$ cells are highly suppressive of T cell activation, proliferation, and cytotoxicity and induce the development of Tregs. Moreover, liver myeloid-derived suppressor cells accelerate the development of hepatic metastases by inactivation of cytotoxic T cells. These findings may explain the propensity of patients with intra-abdominal cancers to develop liver metastases and suggest a promising target for experimental therapeutics.

4.800 Glucose Intolerance and Reduced Proliferation of Pancreatic β-Cells in Transgenic Pigs With Impaired Glucose-Dependent Insulinotropic Polypeptide Function
Renner, S., Fehlings, C., Herbach, N., Hofmann, A., von Waldthausen, D.C., Kessler, B., Ulrichs, K., Chodnevskaja, I., Moskalenko, V., Amselgruber, W., Göke, B., Pfeifer, A., wanke, R. and Wolf, E.
Diabetes, ***59***, *1228-1238 (2010)*

OBJECTIVE The insulinotropic action of the incretin glucose-dependent insulinotropic polypeptide (GIP) is impaired in type 2 diabetes, while the effect of glucagon-like peptide-1 (GLP-1) is preserved. To evaluate the role of impaired GIP function in glucose homeostasis and development of the endocrine pancreas in a large animal model, we generated transgenic pigs expressing a dominant-negative GIP receptor ($GIPR^{dn}$) in pancreatic islets.
RESEARCH DESIGN AND METHODS $GIPR^{dn}$ transgenic pigs were generated using lentiviral transgenesis. Metabolic tests and quantitative stereological analyses of the different endocrine islet cell populations were performed, and β-cell proliferation and apoptosis were quantified to characterize this novel animal model.
RESULTS Eleven-week-old $GIPR^{dn}$ transgenic pigs exhibited significantly reduced oral glucose tolerance due to delayed insulin secretion, whereas intravenous glucose tolerance and pancreatic β-cell mass were not different from controls. The insulinotropic effect of GIP was significantly reduced, whereas insulin secretion in response to the GLP-1 receptor agonist exendin-4 was enhanced in $GIPR^{dn}$ transgenic versus control pigs. With increasing age, glucose control deteriorated in $GIPR^{dn}$ transgenic pigs, as shown by reduced oral and intravenous glucose tolerance due to impaired insulin secretion. Importantly, β-cell proliferation was reduced by 60% in 11-week-old $GIPR^{dn}$ transgenic pigs, leading to a reduction of β-cell mass by 35% and 58% in 5-month-old and 1- to 1.4-year-old transgenic pigs compared with age-matched controls, respectively.
CONCLUSIONS The first large animal model with impaired incretin function demonstrates an essential role of GIP for insulin secretion, proliferation of β-cells, and physiological expansion of β-cell mass.

4.801 Langerhans Cells Prime IL-17–Producing T Cells and Dampen Genital Cytotoxic Responses following Mucosal Immunization
Hervouet, C., Luci, C., Rol., N., Rlousseau, D., Kissenpfennig, A., Malissen, B., Czerkinsky, C. and Anjuere, F.
J. Immunol., ***184***, *4842-4851 (2010)*

Langerhans cells (LCs) are dendritic cells (DCs) localized in stratified epithelia, such as those overlaying skin, buccal mucosa, and vagina. The contribution of LCs to the promotion or control of immunity initiated at epithelial sites remains debated. We report in this paper that an immunogen comprising OVA linked to the B subunit of cholera toxin, used as delivery vector, was efficient to generate CTLs after vaginal immunization. Using *Lang-EGFP* mice, we evaluated the contribution of distinct DC subsets to the generation of CD4 and CD8 T cell responses. We demonstrate that the vaginal epithelium, unlike the skin epidermis, includes a minor population of LCs and a major subset of $langerin^-$ DCs. Intravaginally administered Ag is taken up by LCs and $langerin^-$ DCs and carried up to draining lymph nodes, where both subsets prime CD8 T cells, unlike blood-derived DCs, although with distinct capabilities. LCs prime CD8 T cells with a cytokine profile dominated by IL-17, whereas $Lang^-$ DCs induce IFN-γ–producing T cells. Using *Lang-DTR-EGFP* mice to ensure a transient ablation of LCs, we found that these cells not only are dispensable for the generation of genital CTL responses but also downregulate these responses, by a mechanism that may involve IL-10 and IL-17 cytokines. This finding has implications for the development of mucosal vaccines and immunotherapeutic strategies designed for the targeting of DCs.

4.802 High-efficiency transfection of cultured primary motor neurons to study protein localization, trafficking, and function
Fallini, C., Bassell, G.J. and Rossoll, W.
Mol. Neurodegeneration, ***5***, *17-26 (2010)*

Background
Cultured spinal motor neurons are a valuable tool to study basic mechanisms of development, axon growth and pathfinding, and, importantly, to analyze the pathomechanisms underlying motor neuron diseases. However, the application of this cell culture model is limited by the lack of efficient gene transfer techniques which are available for other neurons. To address this problem, we have established magnetofection as a novel method for the simple and efficient transfection of mouse embryonic motor neurons. This technique allows for the study of the effects of gene expression and silencing on the development and survival of motor neurons.
Results
We found that magnetofection, a novel transfection technology based on the delivery of DNA-coated magnetic nanobeads, can be used to transfect primary motor neurons. Therefore, in order to use this method as a new tool for studying the localization and transport of axonal proteins, we optimized

conditions and determined parameters for efficient transfection rates of >45% while minimizing toxic effects on survival and morphology. To demonstrate the potential of this method, we have used transfection with plasmids encoding fluorescent fusion-proteins to show for the first time that the spinal muscular atrophy-disease protein Smn is actively transported along axons of live primary motor neurons, supporting an axon-specific role for Smn that is different from its canonical function in mRNA splicing. We were also able to show the suitability of magnetofection for gene knockdown with shRNA-based constructs by significantly reducing Smn levels in both cell bodies and axons, opening new opportunities for the study of the function of axonal proteins in motor neurons.
Conclusions
In this study we have established an optimized magnetofection protocol as a novel transfection method for primary motor neurons that is simple, efficient and non-toxic. We anticipate that this novel approach will have a broad applicability in the study of motor neuron development, axonal trafficking, and molecular mechanisms of motor neuron diseases.

4.803 Successful Human Islet Isolation and Transplantation Indicating the Importance of Class 1 Collagenase and Collagen Degradation Activity Assay
Balamurugan, A.N., Breite, A.G., Anazawa, T., Loganathan, G., Wilhelm, J.J., Papas, K.K., Dwulet, F.E., McCarthy, R.C. and Hering, B.J.
*Transplantation**, 89,** 954-961 (2010)*

Background. Purified tissue dissociation enzymes (TDEs) are critical to successful human islet isolation required for clinical transplantation, but little is known about the characteristics of the key enzymes-class I (C1) and class II (C2) collagenase from Clostridium histolyticum-used in these procedures. Here, we show the differences between the C1 collagenase found in purified collagenase products manufactured by three suppliers and the impact of differences in C1 between two suppliers on human islet yield.
Methods. Collagenase from Roche, Serva/Nordmark (Uetersen, Germany), and VitaCyte (Indianapolis, IN) were analyzed by analytical high-performance liquid chromatography and collagen degradation activity (CDA), an assay that preferentially detects intact C1 collagenase. Human islet isolations were performed using current standard practices.
Results. These studies showed that the highest amount of intact C1 that correlated with a high specific CDA (CDA unit per milligram of protein). The highest specific CDA was found in VitaCyte product followed by the Roche and Serva/Nordmark products. The products of VitaCyte were used successfully for human islet isolation (n=14) with an average final islet yield obtained was 419,100+/-150,900 islet equivalent number (IEQ) (4147+/-1759 IEQ/g pancreas). Four of these preparations were used successfully in clinical transplantation procedures. These TDEs gave significantly better results when compared with earlier data where 27 isolations were performed using Serva NB1 collagenase and NB neutral protease where the final islet yield was 217,500+/-152,400 IEQ (2134+/-1524 IEQ/g pancreas).
Conclusions. These data indicate the importance of intact C1 and the use of the appropriate analytical assays to correlate biochemical characteristics of TDEs to islet quality and yield.

4.804 Expression of Cell Cycle–Related Genes With Cytokine-Induced Cell Cycle Progression of Primitive Hematopoietic Stem Cells
Quesenberry, P.J., Dooner, G.J., Del Tatto, M., Colvin, G.A., Johnson, K. and Dooner, M.S.
*Stem Cells and Development, **19(4)**, 453-460 (2010)*

Primitive marrow lineage-negative rhodamine low and Hoechst low (LRH) stem cells isolated on the basis of quiescence respond to the cytokines thrombopoietin, FLT3L, and steel factor by synchronously progressing through cell cycle. We have now profiled the mRNA expression, as determined by real-time RT-PCR, of 47 hematopoietic or cell cycle-related genes, focusing on the variations in the cell cycle regulators with cycle transit. LRH stem cells, at isolation, showed expression of all interrogated genes, but at relatively low levels. In our studies, there was a good deal of consistency with regard to cell cycle regulatory genes involved in the G1/S progression point of LRH murine stem cells. The observed pattern of expression of cyclin A2 is consistent with actions at these phases of cell cycle. Minimal elevations were seen at 16 h with higher elevations at 24, 32, 40, and 48 h times encompassing S, G2, and M phases. CDK2 expression pattern was also consistent with a role in G1/S transition with a modest elevation at 24 h and more substantial elevation at 32 h. The observed pattern of expression of cyclin F mRNA with marked elevations at 16–40 h was also consistent with actions in S and G2 phases. Cyclin D1 expression pattern was less consistent with its known role in G1 progression. The alterations in multiple other cell cycle regulators were consistent with previous information obtained in other cell systems. The cycle regulatory

mechanics appears to be preserved across broad ranges of cell types.

4.805 Cultured hypothalamic neurons are resistant to inflammation and insulin resistance induced by saturated fatty acids
Choi, S.J., Kim, F., Schwartz, M.W. and Wisse, B.E.
*Am. J. Physiol. Endocrinol. Metab., **298**, E1122-E1130 (2010)*

Hypothalamic inflammation induced by high-fat feeding causes insulin and leptin resistance and contributes to the pathogenesis of obesity. Since in vitro exposure to saturated fatty acids causes inflammation and insulin resistance in many cultured cell types, we determined how cultured hypothalamic neurons respond to this stimulus. Two murine hypothalamic neuronal cell cultures, N43/5 and GT1–7, were exposed to escalating concentrations of saturated fatty acids for up to 24 h. Harvested cells were evaluated for activation of inflammation by gene expression and protein content. Insulin-treated cells were evaluated for induction of markers of insulin receptor signaling (p-IRS, p-Akt). In both hypothalamic cell lines, inflammation was induced by prototypical inflammatory mediators LPS and TNFα, as judged by induction of IκBα (3- to 5-fold) and IL-6 (3- to 7-fold) mRNA and p-IκBα protein, and TNFα pretreatment reduced insulin-mediated p-Akt activation by 30% ($P < 0.05$). By comparison, neither mixed saturated fatty acid (100, 250, or 500 μM for ≤6 h) nor palmitate exposure alone (200 μM for ≤24 h) caused inflammatory activation or insulin resistance in cultured hypothalamic neurons, whereas they did in control muscle and endothelial cell lines. Despite the lack of evidence of inflammatory signaling, saturated fatty acid exposure in cultured hypothalamic neurons causes endoplasmic reticulum stress, induces mitogen-activated protein kinase, and causes apoptotic cell death with prolonged exposure. We conclude that saturated fatty acid exposure does not induce inflammatory signaling or insulin resistance in cultured hypothalamic neurons. Therefore, hypothalamic neuronal inflammation in the setting of DIO may involve an indirect mechanism mediated by saturated fatty acids on nonneuronal cells.

4.806 Transport of *Bacillus anthracis* from the lungs to the draining lymph nodes is a rapid process facilitated by CD11c+ cells
Shetron-Rama, L.M., Herring-Palmer, A.C., Huffnagle, G.B. and Hanna, P.
*Microbial Pathogenesis, **49**, 38-46 (2010)*

Inhalational anthrax is established after inhaled *Bacillus anthracis* spores are transported to the lung associated lymph nodes. Dendritic cells (CD11c+ cells) located in the lungs are phagocytes that maintain many capabilities consistent with transport. This study investigates the role of dendritic cells as conduits of spores from the lung to the draining lymph nodes. The intratracheally spore-challenged mouse model of inhalational anthrax was utilized to investigate in vivo activities of CD11c+ cells. FITC labeled spores were delivered to the lungs of mice. Subsequently lung associated lymph nodes were isolated after infection and CD11c+ cells were found in association with the labeled spores. Further investigation of CD11c+ cells in early anthrax events was facilitated by use of the CD11c-diphtheria toxin (DT) receptor-green fluorescent protein transgenic mice in which CD11c+ cells can be transiently depleted by treatment with DT. We found that the presence of CD11c+ cells was necessary for efficient traffic of the spore to lung associated lymph nodes at early times after infection. Cultured dendritic cells were used to determine that these cells are capable of *B. anthracis* spore phagocytosis, and support germination and outgrowth. This data demonstrates that CD11c+ cells are likely carriers of *B. anthracis* spores from the point of inhalation in the lung to the lung associated lymph nodes. The cultured dendritic cell allows for spore germination and outgrowth supporting the concept that the CD11c+ cell responsible for this function can be a dendritic cell.

4.807 The CagA protein of *Helicobacter pylori* suppresses the functions of dendritic cell in mice
Tanaka, H., Yoshida, M., Nishiumi, S., Ohnishi, N., Kobayashi, K., Yamamoto, K., Fujita, T., Hatakeyama, M. and Azuma, T.
*Arch. Biochem. Biophys., **498**, 35-42 (2010)*

CagA protein is the most assessed effecter molecule of *Helicobacter pylori*. In this report, we demonstrate how CagA protein regulates the functions of dendritic cells (DC) against *H. pylori* infection. In addition, we found that CagA protein was tyrosine-phosphorylated in DC. The responses to *cagA*-positive *H. pylori* in DC were reduced in comparison to those induced by *cagA*-negative *H. pylori*. CagA-overexpressing DC also exhibited a decline in the responses against LPS stimulation and the differentiation of $CD4^+$ T cells toward Th1 type cells compared to wild type DC. In addition, the level of phosphorylated IRF3 decreased in CagA-overexpressing DC stimulated with LPS, indicating that activated SHP-2 suppressed the

enzymatic activity of TBK1 and consequently IRF3 phosphorylation. These data suggest that CagA protein negatively regulates the functions of DC via CagA phosphorylation and that *cagA*-positive *H. pylori* strains suppress host immune responses resulting in their chronic colonization of the stomach.

4.808 The PXR is a drug target for chronic inflammatory liver disease
Wallace, K., Cowie, D.E., Konstantinou, D.K., Hill, S.J., Tjelle, T.E., Axon, A., Koruth, M., White, S.A., Carlsen, H., Mann, D.A. and Wright, M.C.
J. Steroid Biochem. Mol. Biol., ***120***, *137-148 (2010)*

PXR activators are used to treat pruritus in chronic inflammatory liver diseases such as primary biliary cirrhosis (PBC). The aims of this study were to determine whether PXR activators could have an additional benefit of inhibiting inflammation in the liver, and determine whether cyclosporin A – which more effectively prevents PBC recurrence in transplanted patients than FK506 – is a PXR activator. In SJL/J mice (which have constitutively high levels of hepatic portal tract inflammatory cell recruitment), feeding a PXR activator inhibited inflammation, TNFα and Il-1α mRNA expression in SJL/J-$PXR^{+/+}$, but not SJL/J-$PXR^{-/-}$. Monocytic cells – a major source of inflammatory mediators such as TNFα – expressed the PXR and PXR activators inhibited endotoxin-induced NF-κB activation and TNFα expression. PXR activation also inhibited endotoxin-stimulated TNFα secretion from liver monocytes/macrophages isolated from $PXR^{+/+}$ mice, but not from cells isolated from $PXR^{-/-}$ mice. To confirm that PXR activation inhibits NF-κB *in vivo*, 3x-κB-luc fibrotic mice (which express a luciferase gene regulated by NF-κB) were imaged after treatment with the hepatotoxin CCl_4. PXR activator inhibited the induction of hepatic NF-κB activity without affecting CCl_4 toxicity/hepatic damage. Using a PXR reporter gene assay, cyclosporin A – but not FK506 – was shown to be a direct PXR activator, and also to induce expression of the classic PXR-regulated CYP3A4 gene in human hepatocytes and in a cell line null for the FXR, a nuclear receptor with similar properties to the PXR. *Conclusion*: PXR activation is anti-inflammatory in the liver and the effects of cyclosporin A in PBC disease recurrence may be mediated in part *via* the PXR. Since PXR activation promotes hepatocyte growth and is also anti-fibrogenic, the PXR may be an excellent drug target for the treatment of chronic inflammatory liver disease.

4.809 trans-10,cis-12-Conjugated Linoleic Acid Instigates Inflammation in Human Adipocytes Compared with Preadipocytes
Martinez, K., Kennedy, A., West, T., Milatovic, D., Aschner, M. and McIntosh, M.
J. Biol. Chem., ***285(23)***, *17701-17721 (2010)*

We showed previously in cultures of primary human adipocytes and preadipocytes that lipopolysaccharide and *trans*-10,*cis*-12-conjugated linoleic acid (10,12-CLA) activate the inflammatory signaling that promotes insulin resistance. Because our published data demonstrated that preadipocytes are the primary instigators of inflammatory signaling in lipopolysaccharide-treated cultures, we hypothesized that they played the same role in 10,12-CLA-mediated inflammation. To test this hypothesis, we employed four distinct models. In model 1, a differentiation model, CLA activation of MAPK and induction of interleukin-8 (IL-8), IL-6, IL-1β, and cyclo-oxygenase-2 (COX-2) were greatest in differentiated compared with undifferentiated cultures. In model 2, a cell separation model, the mRNA levels of these inflammatory proteins were increased by 10,12-CLA compared with bovine serum albumin vehicle in the adipocyte fraction and the preadipocyte fraction. In model 3, a co-culture insert model, inserts containing ~ 50% adipocytes (AD50) or ~ 100% preadipocytes (AD0) were suspended over wells containing AD50 or AD0 cultures. 10,12-CLA-induced IL-8, IL-6, IL-1β, and COX-2 mRNA levels were highest in AD50 cultures when co-cultured with AD0 inserts. In model 4, a conditioned medium (CM) model, CM collected from CLA-treated AD50 but not AD0 cultures induced IL-8 and IL-6 mRNA levels and activated phosphorylation of MAPK in naive AD0 and AD50 cultures. Consistent with these data, 10,12-CLA-mediated secretions of IL-8 and IL-6 from AD50 cultures were higher than from AD0 cultures. Notably, blocking adipocytokine secretion prevented the inflammatory capacity of CM from 10,12-CLA-treated cultures. These data suggest that CLA instigates the release of inflammatory signals from adipocytes that subsequently activate adjacent preadipocytes.

4.810 CCR5 Signaling Suppresses Inflammation and Reduces Adverse Remodeling of the Infarcted Heart, Mediating Recruitment of Regulatory T Cells
Dobaczewski, M., Xia, Y., Bujak, M., Gonzales-Quesada, C. and Frangogiannis, N.G.
Am. J. Pathol., ***176(5)***, *2177-2187 (2010)*

Myocardial infarction triggers an inflammatory reaction that is involved in cardiac remodeling. Cardiac

repair is dependent on regulatory mechanisms that suppress inflammation and prevent excessive matrix degradation. Chemokine induction in the infarcted heart mediates recruitment of leukocyte subsets with distinct properties. We demonstrate that signaling through the CC chemokine receptor 5 (CCR5) prevents uncontrolled postinfarction inflammation and protects from adverse remodeling by recruiting suppressive mononuclear cells. CCR5 and its ligands macrophage inflammatory protein (MIP)–1α and MIP-1β were markedly induced in the infarcted mouse myocardium. In addition, almost 40% of the mononuclear cells infiltrating the infarct expressed CCR5. $CCR5^{-/-}$ mice exhibited marked upregulation of proinflammatory cytokine and chemokine expression in the infarct. In wild-type infarcts $CCR5^{+}$ mononuclear cells had anti-inflammatory properties, expressing higher levels of IL-10 than $CCR5^{-}$ cells. In contrast, mononuclear cells isolated from $CCR5^{-/-}$ infarcts had reduced IL-10 expression. Moreover, enhanced inflammation in the absence of CCR5 was associated with impaired recruitment of $CD4^{+}$/$foxp3^{+}$ regulatory T cells (Tregs). The $CCR5^{+}$ Treg subset exhibited increased IL-10 expression, reflecting potent anti-inflammatory activity. Accentuated inflammation in $CCR5^{-/-}$ infarcts was associated with increased matrix metalloproteinase (MMP) expression, reduced TIMP levels, and enhanced MMP-2 and MMP-9 activity, resulting in worse cardiac dilation. These results suggest that CCR5-mediated Treg recruitment may restrain postinfarction inflammation, preventing excessive matrix degradation and attenuating adverse remodeling.

4.811 Comparison of density gradient and simple centrifugation of equine spermatozoa: effect on fertility of an oligospermic-subfertile stallion

Mari, G., Castagnetti, C., Morganti, M., Rizzato, G., Mislei, B., Iacono, E. and Merlo, B.
Animal Reprod., ***121S***, *S153-S154 (2010)*

Low dose insemination methods have recently evolved to satisfy the desire to exploit newly available technology such as sex pre-selection of spermatozoa. Additional benefits, such as improvements in the efficiency of the use of frozen semen and the potential to enhance the fertility of subfertile stallions, have become apparent during the development and evaluation of these techniques (Morris and Allen, 2002). Vazquez et al. (1998) investigated the use of hysteroscopic insemination in an attempt to bypass the uterine transport phase of sperm migration and to increase the fertility of subfertile stallions. Inseminating only the highest quality sperm by selecting for quality prior to insemination may further improve the outcome of techniques delivering low numbers of sperm. Macpherson et al. (2002) demonstrated that EquiPure™, a proprietary silan-coated silica particle density gradient, can improve semen quality in stallions. In two studies, fresh spermatozoa from fertile stallions were processed through density gradients to enhance selection of those with intact plasma membranes (Morris et al., 2000; Lindsey et al., 2002) and successful low dose hysteroscopic inseminations were achieved. Similarly, frozen-thawed ejaculated (Alvarenga and Leão, 2002) and epididymal (Morris and Allen, 2002) spermatozoa have been centrifuged through density gradients to remove those damaged by the cryopreservation process. However, no beneficial effect of this treatment was observed on the fertility of low numbers

4.812 Cortical Overexpression of Neuronal Calcium Sensor-1 Induces Functional Plasticity in Spinal Cord Following Unilateral Pyramidal Tract Injury in Rat

Yip, P.K., Wong, L-F., Sears, T.A., Yanez-Munoz, R.J. and McMahon, S.B.
PloSBiology, ***8(6)***, *e1000399 (2010)*

Following trauma of the adult brain or spinal cord the injured axons of central neurons fail to regenerate or if intact display only limited anatomical plasticity through sprouting. Adult cortical neurons forming the corticospinal tract (CST) normally have low levels of the neuronal calcium sensor-1 (NCS1) protein. In primary cultured adult cortical neurons, the lentivector-induced overexpression of NCS1 induces neurite sprouting associated with increased phospho-Akt levels. When the PI3K/Akt signalling pathway was pharmacologically inhibited the NCS1-induced neurite sprouting was abolished. The overexpression of NCS1 in uninjured corticospinal neurons exhibited axonal sprouting across the midline into the CST-denervated side of the spinal cord following unilateral pyramidotomy. Improved forelimb function was demonstrated behaviourally and electrophysiologically. In injured corticospinal neurons, overexpression of NCS1 induced axonal sprouting and regeneration and also neuroprotection. These findings demonstrate that increasing the levels of intracellular NCS1 in injured and uninjured central neurons enhances their intrinsic anatomical plasticity within the injured adult central nervous system.

4.813 Dicer-Dependent MicroRNAs Control Maturation, Function, and Maintenance of Langerhans Cells In Vivo

Kuipers, H., Schnorfeil, F.M., Fehling, H-J., Bartels, H. and Brocker, T.

J. Immunol., ***185***, *400-409 (2010)*

Dendritic cells (DCs) are central for the induction of T cell immunity and tolerance. Fundamental for DCs to control the immune system is their differentiation from precursors into various DC subsets with distinct functions and locations in lymphoid organs and tissues. In contrast to the differentiation of epidermal Langerhans cells (LCs) and their seeding into the epidermis, LC maturation, turnover, and MHC class II Ag presentation capacities are strictly dependent on the presence of Dicer, which generates mature microRNAs (miRNAs). Absence of miRNAs caused a strongly disturbed steady-state homeostasis of LCs by increasing their turnover and apoptosis rate, leading to progressive ablation of LCs with age. The failure to maintain LCs populating the epidermis was accompanied by a proapoptotic gene expression signature. Dicer-deficient LCs showed largely increased cell sizes and reduced expression levels of the C-type lectin receptor Langerin, resulting in the lack of Birbeck granules. In addition, LCs failed to properly upregulate MHC class II, CD40, and CD86 surface molecules upon stimulation, which are critical hallmarks of functional DC maturation. This resulted in inefficient induction of CD4 T cell proliferation, whereas Dicer-deficient LCs could properly stimulate CD8 T cells. Taken together, Dicer-dependent generation of miRNAs affects homeostasis and function of epidermal LCs.

4.814 **Innate immunity defines the capacity of antiviral T cells to limit persistent infection**
Andrews, D.M., Estcourt, M.J., Andoniou, C.E., Wikstrom, M.E., Khong, A., Voigt, V., Fleming, P., Tabarias, H., Hill, G.R., vander Most, R.G., Scalzo, A.A., Smyth, M.J. and Degli-Esposti, M.A.
J. Exp. Med., ***207(6)***, *1333-1343 (2010)*

Effective immunity requires the coordinated activation of innate and adaptive immune responses. Natural killer (NK) cells are central innate immune effectors, but can also affect the generation of acquired immune responses to viruses and malignancies. How NK cells influence the efficacy of adaptive immunity, however, is poorly understood. Here, we show that NK cells negatively regulate the duration and effectiveness of virus-specific CD4+ and CD8+ T cell responses by limiting exposure of T cells to infected antigen-presenting cells. This impacts the quality of T cell responses and the ability to limit viral persistence. Our studies provide unexpected insights into novel interplays between innate and adaptive immune effectors, and define the critical requirements for efficient control of viral persistence.

4.815 **Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8α+ dendritic cells**
Poulin, L.F., Salio, M., Griessinger, E., Anjos-Afonso, F., Craciun, L., Chen, J-L., Keller, A.M., Joffre, O., Zelenay, S., Nye, E., Moine, A.L., Faure, F., Donckier, V., Sancho, D., Cerundolo, V., Bonnet, D. and Reis e Sousa, C.
J. Exp. Med., ***207(6)***, *1261-1271 (2010)*

In mouse, a subset of dendritic cells (DCs) known as $CD8\alpha^+$ DCs has emerged as an important player in the regulation of T cell responses and a promising target in vaccination strategies. However, translation into clinical protocols has been hampered by the failure to identify $CD8\alpha^+$ DCs in humans. Here, we characterize a population of human DCs that expresses DNGR-1 (CLEC9A) and high levels of BDCA3 and resembles mouse $CD8\alpha^+$ DCs in phenotype and function. We describe the presence of such cells in the spleens of humans and humanized mice and report on a protocol to generate them in vitro. Like mouse $CD8\alpha^+$ DCs, human DNGR-1^+ BDCA3^{hi} DCs express Necl2, CD207, BATF3, IRF8, and TLR3, but not CD11b, IRF4, TLR7, or (unlike $CD8\alpha^+$ DCs) TLR9. DNGR-1^+ BDCA3^{hi} DCs respond to poly I:C and agonists of TLR8, but not of TLR7, and produce interleukin (IL)-12 when given innate and T cell–derived signals. Notably, DNGR-1^+ BDCA3^+ DCs from in vitro cultures efficiently internalize material from dead cells and can cross-present exogenous antigens to $CD8^+$ T cells upon treatment with poly I:C. The characterization of human DNGR-1^+ BDCA3^{hi} DCs and the ability to grow them in vitro opens the door for exploiting this subset in immunotherapy.

4.816 **Predicting islet yield in pediatric patients undergoing pancreatectomy and autoislet transplantation for chronic pancreatitis**
Bellin, M.D., Blondet, J.J., Beilman, G.J., Dunn, Ty.B., Balamurugan, A.N., Thomas, W., Sutherland, D.E.R. and Moran, A.
Pedriatric Diabetes, ***11***, *227-234 (2010)*

Background/Objective: Chronic pancreatitis (CP) in children is associated with significant morbidity and can lead to narcotic dependence. Total pancreatectomy (TP) may be indicated in refractory CP to relieve

pain; simultaneous islet autotransplant (IAT) may prevent postsurgical diabetes. About half of pediatric patients are insulin independent 1 yr after IAT. Insulin independence correlates best with the number of islets available for transplantation (islet yield). Currently there is no known method to predict islet yield in a given patient. We assessed the ability of preoperative metabolic tests to predict islet yields in 10 children undergoing TP/IAT.
Design/Methods: Hemoglobin A1c (HbA1c) and mixed meal tolerance tests (MMTT) were obtained prior to surgery in 10 patients age <= 18 yr. Fasting glucose, C-peptide, and creatinine were used to calculate the C-peptide to glucose* creatinine ratio (CPGCR). C-peptide peak and area under the curve (AUC) were determined from 2 h MMTT. Linear regressions were performed to predict islet yield from baseline test results.
Results: Islet yield ranged from 7000 to 434 000 islet equivalents (IE) (mean 222 452 +/- 148 697 IE). Islet yield was well predicted from body weight and fasting plasma glucose (R 2 = 57%, adjusted for overfitting by bootstrap). Islet yield was positively associated with CPGCR, peak C-peptide, and AUC C-peptide and negatively associated with HbA1c.
Conclusions: Pilot data from 10 pediatric patients suggest that simple preoperative measurement of fasting plasma glucose may give a useful prediction of islet yield. Islet yield correlates with HbA1c and C-peptide levels. This information allows individual candidates to weigh the specific risk of becoming diabetic against the benefit of pain relief should they undergo TP-IAT.

4.817 Transplantation of Long-term Cultured Porcine Islets in the Rat: Prolonged Graft Survival and Recipient Growth on Reduced Immunosuppression
Rijkelijkhuizen, J.K.R.A., Töns, A., Terpstra, O.T. and Bouwman, E.
*Cell Transplantation, **19**, 387-398 (2010)*

To evaluate whether further improvement in porcine islet xenotransplantation is feasible, a number of questions were addressed. Earlier we showed significant improvement in the nude mouse of the porcine islets by selection through long-term culture. Now these islets were tested in the stringent pig-to-rat model. Islets were isolated from adult pigs, cultured for 1.5-3 weeks and transplanted to rats. Possible rejection mechanisms were assessed by interference of the cellular response with cyclosporine A (CsA), blocking macrophages with gadolinium chloride (GdCl), and suppressing the humoral response with cyclophosphamide. Modifications in graft size and condition were analyzed. Untreated control recipients showed primary nonfunction (PNF). CsA treatment could fully overcome PNF and resulted in graft survival from 10 to over 134 days. Rejection was the main cause of function loss. Although rejection could not be prevented by intensifying the induction therapy, increased maintenance immunosuppression effectively blocked rejection, albeit at the expense of toxicity. Blocking the humoral response was ineffective; all grafts showed PNF. In contrast, depletion of macrophages fully prevented PNF. Combination of GdCl and CsA gave no additional effect, and grafts were rejected between 57 and 162 days. Generally, graft survivals were similar to those reported in the literature; however, long-term cultured islets required much less maintenance immunosuppression. Cessation of graft function was not always due to rejection; in some cases “islet exhaustion” was found, possibly caused by discrepancy between the graft size and the rapidly growing recipient. Neither the presence of damaged islet tissue in the graft nor the size of the graft exerted any influence on graft survival. On rejection, no real infiltration of the graft was seen; destruction gradually processed from the outside. The good functional capability of the cultured islets was illustrated by disappearance of the clinical symptoms and increase in body weight, which almost doubled in the long-term survivors.

4.818 Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes
Radom-Aizik, S., Zaldivar Jr., F., oliver, S., Galassetti, P. and Cooper, D.M.
*J. Appl. Physiol., **109(1)**, 252-261 (2010)*

Exercise leads to a rapid change in the profile of gene expression in circulating neutrophils. MicroRNAs (miRNAs) have been discovered to play important roles in immune function and often act to attenuate or silence gene translation. We hypothesized that miRNA expression in circulating neutrophils would be affected by brief exercise. Eleven healthy men (19–30 yr old) performed 10, 2-min bouts of cycle ergometer exercise interspersed with 1-min rest at a constant work equivalent to ~76% of maximal oxygen uptake ($\dot{V}_{O_{2max}}$). We used the Agilent Human miRNA V2 Microarray. A conservative statistical approach was used to determine that exercise significantly altered 38 miRNAs (20 had lower expression). Using RT-PCR, we verified the expression level changes from before to after exercise of seven miRNAs. In silico analysis showed that collectively 36 miRNAs potentially targeted 4,724 genes (2 of the miRNAs had no apparent gene targets). Moreover, when we compared the gene expression changes (n = 458) in neutrophils

that have been altered by exercise, as previously reported, with the miRNAs altered by exercise, we identified three pathways, Ubiquitin-mediated proteolysis, Jak-STAT signaling pathway, and Hedgehog signaling pathway, in which an interaction of miRNA and gene expression was plausible. Each of these pathways is known to play a role in key mechanisms of inflammation. Brief exercise alters miRNA profile in circulating neutrophils in humans. These data support the hypothesis that exercise-associated changes in neutrophil miRNA expression play a role in neutrophil gene expression in response to physical activity.

4.819 Addition of glutamate to serum-free culture promotes recovery of electrical activity in adult hippocampal neurons *in vitro*
Edwards, D., Das, M., Molnar, P. and Hickman, J.J.
*J. Neurosci. Methods, **190**, 155-163 (2010)*

A long-term cell culture system utilizing normal adult hippocampal neurons would represent an important tool that could be useful in research on the mature brain, neurological disorders and age-related neurological diseases. Historically, *in vitro* neuronal systems are derived from embryonic rather than mature brain tissue, a practice predicated upon difficulties in supporting regeneration, functional recovery and long-term survival of adult neurons *in vitro*. A few studies have shown that neurons derived from the hippocampal tissue of adult rats can survive and regenerate *in vitro* under serum-free conditions. However, while the adult neurons regenerated morphologically under these conditions, both the electrical activity characteristic of *in vivo* neurons as well as long-term neuronal survival was not consistently recovered *in vitro*. In this study, we report on the development of a defined culture system with the ability to support functional recovery and long-term survival of adult rat hippocampal neurons. In this system, the cell-adhesive substrate, *N*-1 [3-(trimethoxysilyl) propyl]-diethylenetriamine, supported neuronal attachment, regeneration, and long-term survival of adult neurons for more than 80 days *in vitro*. Additionally, the excitatory neurotransmitter glutamate, applied at 25 μM for 1–7 days after morphological neuronal regeneration *in vitro*, enabled full recovery of neuronal electrical activity. This low concentration of glutamate promoted the recovery of neuronal electrical activity but with minimal excitotoxicity. These improvements allowed electrically active adult neurons to survive *in vitro* for several months, providing a stable test-bed for the long-term study of regeneration in adult-derived neuronal systems, especially for traumatic brain injury (TBI).

4.820 Enhanced Prediction of Porcine Islet Yield and Posttransplant Outcome Using a Combination of Quantitative Histomorphometric Parameters and Flow Cytometry
Jin, S-M., Kim, K.S., Lee, S-Y., Gong, C-H., Park, S.K., Yu, J.E., Yeom, S-C., Yoon, T.W., Ha, J., Park, C-G. and Kim, S-J.
*Cell Transplant., **19**, 299-311 (2010)*

Prediction of islet yield and posttransplant outcome is essential for clinical porcine islet xenotransplantation. Although several histomorphometric parameters of biopsied porcine pancreases are predictive of islet yield, their role in the prediction of in vivo islet potency is unknown. We investigated which histomorphometrical parameter best predicts islet yield and function, and determined whether it enhanced the predictive value of in vitro islet function tests for the prediction of posttransplant outcome. We analyzed the histomorphometry of pancreases from which 60 adult pig islet isolations were obtained. Islet function was assessed using the β-cell viability index based on flow cytometry analysis, oxygen consumption rate, ADP/ATP ratio, and/or concurrent transplantation into NOD/SCID mice. Receiver operating characteristic (ROC) analysis revealed that only islet equivalent (IEQ)/cm^2 and the number of islets >200 μm in diameter significantly predicted an islet yield of >2000 IEQ/g ($p < 0.001$ for both) and in vivo islet potency ($p = 0.024$ and $p = 0.019$, respectively). Although not predictive of islet yield, a high proportion of large islets (>100 μm in diameter) best predicted diabetes reversal ($p = 0.001$). Multiple regression analysis revealed that the β-cell viability index ($p = 0.003$) and the proportion of islets >100 μm in diameter ($p = 0.048$) independently predicted mean posttransplant blood glucose level (BGL). When BGL was estimated using both these parameters [area under the ROC curve (AUC), 0.868; 95% confidence interval (CI), 0.730-1.006], it predicted posttransplant outcome more accurately than the β-cell viability index alone (AUC, 0.742; 95% CI, 0.544-0.939). In conclusion, we identified the best histomorphometric predictors of islet yield and posttransplant outcome. This further enhanced the predictive value of the flow cytometry analysis. These parameters should be useful for predicting islet yield and in vivo potency before clinical adult porcine islet xenotransplantation.

4.821 Seven Consecutive Successful Clinical Islet Isolations With Pancreatic Ductal Injection
Matsumoto, S., Noguichi, H., Shimoda, M., Ikemoto, T., Nazirruddin, B., Jackson, A., Tamura, Y., Olson,

G., Fujita, Y., Chujo, D., Takita, M., Kobayashi, N., Onaca, N. and Levy, M.
Cell Transplant., ***19***, *291-297 (2010)*

Inconsistent islet isolation is one of the issues of clinical islet transplantation. In the current study, we applied ductal injection to improve the consistency of islet isolation. Seven islet isolations were performed with the ductal injection of ET-Kyoto solution (DI group) and eight islet isolations were performed without the ductal injection (standard group) using brain-dead donor pancreata. Isolated islets were evaluated based on the Edmonton protocol for transplantation. The DI group had significantly higher islet yields (588,566 ± 64,319 vs. 354,836 ± 89,649 IE, $p < 0.01$) and viability (97.3 ± 1.2% vs. 92.6 ± 1.2%, $p < 0.02$) compared with the standard group. All seven isolated islet preparations in the DI group (100%), versus only three out of eight isolated islet preparations (38%) in the standard group met transplantation criteria. The islets from the DI group were transplanted into three type 1 diabetic patients and all three patients became insulin independent. Ductal injection significantly improved quantity and quality of isolated islets and resulted in high success rate of clinical islet transplantation. This simple modification will reduce the risk of failure of clinical islet isolation.

4.822 Mutant HSPB8 causes motor neuron-specific neurite degeneration
Irobi, J., Almeida-Souza, L., Asselbergh, B., De Winter, V., Goethais, S., Dierick, I., Krishnan, J., Timmermans, J-P., Robberecht, W., De Johnghe, P., van den Bosch, L., Janssens, S. and Timmerman, V.
Hum. Mol. Genet., ***19(16)***, *3254-3265 (2010)*

Missense mutations (K141N and K141E) in the α-crystallin domain of the small heat shock protein HSPB8 (HSP22) cause distal hereditary motor neuropathy (distal HMN) or Charcot-Marie-Tooth neuropathy type 2L (CMT2L). The mechanism through which mutant HSPB8 leads to a specific motor neuron disease phenotype is currently unknown. To address this question, we compared the effect of mutant HSPB8 in primary neuronal and glial cell cultures. In motor neurons, expression of both HSPB8 K141N and K141E mutations clearly resulted in neurite degeneration, as manifested by a reduction in number of neurites per cell, as well as in a reduction in average length of the neurites. Furthermore, expression of the K141E (and to a lesser extent, K141N) mutation also induced spheroids in the neurites. We did not detect any signs of apoptosis in motor neurons, showing that mutant HSPB8 resulted in neurite degeneration without inducing neuronal death. While overt in motor neurons, these phenotypes were only very mildly present in sensory neurons and completely absent in cortical neurons. Also glial cells did not show an altered phenotype upon expression of mutant HSPB8. These findings show that despite the ubiquitous presence of HSPB8, only motor neurons appear to be affected by the K141N and K141E mutations which explain the predominant motor neuron phenotype in distal HMN and CMT2L.

4.823 Islet Transplantation: Lessons Learned Since the Edmonton Breakthrough
Langer, R.M.
Transplant. Proceedings, ***42***, *1421-1424 (2010)*
This work sought to summarize the main issues of the last decade in the field of clinical islet transplantation. Ten years ago in Edmonton, a new protocol initiated for islet transplantation brought a breakthrough to the field. The earlier, rather poor results were in a sharp contrast to the first published results of 100% insulin freedom at 1 year. However, later it became clear that the promising initial results decline with time; at around 5 years, only about 10% of the patients maintain freedom from external insulin. Despite that fact, a milestone was set and intensive research started worldwide. New hopes were raised for patients. Modifications of the original protocol have been implemented to improve clinical results; however, islet transplantation remains an experimental procedure to date.

4.824 Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress
Lee, Y.Y., Hong, S.H., Lee, Y.J., Chung, S.S., Jung, H.S., Park, S.G. and Park, K.S.
Biochem. Biophys. Res. Comm., ***397***, *735-739 (2010)*

The exposure to acute or chronic endoplasmic reticulum (ER) stress has been known to induce dysfunction of islets, leading to apoptosis. The reduction of ER stress in islet isolation for transplantation is critical for islet protection. In this study, we investigated whether tauroursodeoxycholate (TUDCA) could inhibit ER stress induced by thapsigargin, and restore the decreased glucose stimulation index of islets. In pig islets, thapsigargin decreased the insulin secretion by high glucose stimulation in a time-dependent manner (1 h, 1.35 ± 0.16; 2 h, 1.21 ± 0.13; 4 h, 1.17 ± 0.16 vs. 0 h, 1.81 ± 0.15, $n = 4$, $p < 0.05$, respectively). However, the treatment of TUDCA restored the decreased insulin secretion index induced by thapsigargin

(thapsigargin, 1.25 ± 0.12 vs. thapsigargin + TUDCA, 2.13 ± 0.19, $n = 5$, $p < 0.05$). Furthermore, the culture of isolated islets for 24 h with TUDCA significantly reduced the rate of islet regression (37.4 ± 5.8% vs. 14.5 ± 6.4%, $n = 12$, $p < 0.05$). The treatment of TUDCA enhanced ATP contents in islets (27.2 ± 3.2 pmol/20IEQs vs. 21.7 ± 2.8 pmol/20IEQs, $n = 9$, $p < 0.05$). The insulin secretion index by high glucose stimulation is also increased by treatment of TUDCA (2.42 ± 0.15 vs. 1.92 ± 0.12, $n = 12$, $p < 0.05$). Taken together, we suggest that TUDCA could be a useful agent for islet protection in islet isolation for transplantation.

4.825 Live attenuated *Salmonella enterica* serovar Typhimurium expressing swine interferon-α has antiviral activity and alleviates clinical signs induced by infection with transmissible gastroenteritis virus in piglets

Kim, S.J., Han, Y.W., Rahman, M.M., Kim, S.B., Uyangaa, E., Lee, B.M., Kim, J.H., Roh, Y.S., Kang, S.H., Kim, K., Lee, J.H., Kim, B., Park, K.I. and Eo, S.K.
*Vaccine, **28**, 5031-5037 (2010)*

Enhancing innate and acquired immunity by cytokines such as IFN-α appears to be useful as a first line of defense against viral infection. However, the practical use of cytokines in livestock is not evident due to cost and production issues associated with mass administration. In this study, we tested the efficacy of live attenuated *Salmonella enterica* serovar Typhimurium designed to secrete swine IFN-α (swIFN-α) protein for preventing the clinical signs caused by infection with transmissible gastroenteritis virus (TGEV), one of the diarrhea-causing viruses in the swine industry. Attenuated *Salmonella* vaccine (χ8501) containing swIFN-α-encoding pYA3560 vector (χ8501/swIFN-α) successfully induced the secretion of swIFN-α protein into the culture supernatants, as confirmed by SDS-PAGE and Western blot. The culture supernatants of χ8501/swIFN-α had antiviral activity against TGEV with 50% effective dose (ED_{50}) of 320 per mg of supernatant protein. In addition, oral administration of χ8501/swIFN-α reduced the severity of clinical signs caused by TGEV infection with the effect more apparent at 6–8 days post-infection, and reduced excretion of TGEV in feces. Similarly, the amount of TGEV in intestinal tissues and mesenteric lymph node of χ8501/swIFN-α-administered piglets was lower than in piglets that were treated with control bacteria. These results indicate the value of attenuated *Salmonella* vaccines as delivery systems of cytokines that can be used for mass administration, thereby overcoming cost and production issues.

4.826 Angiotensin-converting Enzyme Inhibition Down-regulates the Pro-atherogenic Chemokine Receptor 9 (CCR9)-Chemokine Ligand 25 (CCL25) Axis

Alla, J.A., Langer, A., Elzahwy, S.S., Arman-Kalcek, G., Streichert, T. and Quitterer, U.
*J. Biol. Chem., **285(30)**, 23496-23505 (2010)*

Many experimental and clinical studies suggest a relationship between enhanced angiotensin II release by the angiotensin-converting enzyme (ACE) and the pathophysiology of atherosclerosis. The atherosclerosis-enhancing effects of angiotensin II are complex and incompletely understood. To identify anti-atherogenic target genes, we performed microarray gene expression profiling of the aorta during atherosclerosis prevention with the ACE inhibitor, captopril. Atherosclerosis-prone apolipoprotein E (apoE)-deficient mice were used as a model to decipher susceptible genes regulated during atherosclerosis prevention with captopril. Microarray gene expression profiling and immunohistology revealed that captopril treatment for 7 months strongly decreased the recruitment of pro-atherogenic immune cells into the aorta. Captopril-mediated inhibition of plaque-infiltrating immune cells involved down-regulation of the C-C chemokine receptor 9 (CCR9). Reduced cell migration correlated with decreased numbers of aorta-resident cells expressing the CCR9-specific chemoattractant factor, chemokine ligand 25 (CCL25). The CCL25-CCR9 axis was pro-atherogenic, because inhibition of CCR9 by RNA interference in hematopoietic progenitors of apoE-deficient mice significantly retarded the development of atherosclerosis. Analysis of coronary artery biopsy specimens of patients with coronary artery atherosclerosis undergoing bypass surgery also showed strong infiltrates of CCR9-positive cells in atherosclerotic lesions. Thus, the C-C chemokine receptor, CCR9, exerts a significant role in atherosclerosis.

4.827 Contributions of selective knockout studies to understanding cholinesterase disposition and function

Camp, S., Zhang, L., Krejci, E., Dobbertin, A., Bernanrd, V., Girard, E., Duysen, E., Lockridge, O., De Jaco, A. and Taylor, P.
*Chemico-Biological Interantions, **187**, 72-77 (2010)*

The complete knockout of the acetylcholinesterase gene (*AChE*) in the mouse yielded a surprising phenotype that could not have been predicted from deletion of the cholinesterase genes in *Drosophila*, that

of a living, but functionally compromised animal. The phenotype of this animal showed a sufficient compromise in motor function that precluded precise characterization of central and peripheral nervous functional deficits. Since AChE in mammals is encoded by a single gene with alternative splicing, additional understanding of gene expression might be garnered from selected deletions of the alternatively spliced exons. To this end, transgenic strains were generated that deleted exon 5, exon 6, and the combination of exons 5 and 6. Deletion of exon 6 reduces brain AChE by 93% and muscle AChE by 72%. Deletion of exon 5 eliminates AChE from red cells and the platelet surface. These strains, as well as knockout strains that selectively eliminate the AChE anchoring protein subunits PRiMA or ColQ (which bind to sequences specified by exon 6) enabled us to examine the role of the alternatively spliced exons responsible for the tissue disposition and function of the enzyme. In addition, a knockout mouse was made with a deletion in an upstream intron that had been identified in differentiating cultures of muscle cells to control AChE expression. We found that deletion of the intronic regulatory region in the mouse essentially eliminated AChE in muscle and surprisingly from the surface of platelets. The studies generated by these knockout mouse strains have yielded valuable insights into the function and localization of AChE in mammalian systems that cannot be approached in cell culture or *in vitro*.

4.828 Superiority of Visipaque (Iodixanol)-Controlled Density Gradient Over Ficoll-400 in Adult Porcine Islet Purification

Min, T., Yi, L., Chao, Z., Haitoa, Z., Wei, W., Liang, Y and Bo, W.
*Transplant. Proccedings, **42(5)**, 1825-1829 (2010)*

Objective
Sufficient and favorable biological functions of islets are major problems hindering xenotransplantation. The aim of the present study was to evaluate the effects on harvesting, purity, viability, and function of using improved Visipaque (iodixanol) and Ficoll-400 for adult porcine islet purification.
Methods
Twelve adult porcine pancreata were randomly divided into an Iodixanol-University of Wisconsin (UW) group and a Ficoll-400-UW group according to the purification method. Porcine pancreata were isolated by collagenase digestion. After isolation and purification, the islet yield and purity were evaluated by dithizone staining, and islet function assessed by in vitro insulin release assays and in vivo islet xenotransplantation.
Results
There were no marked differences in the islet yield before purification (5254.67 ± 189.44 IEQ/g vs 5092.67 ± 178.94 IEQ/g, $P > .05$). After purification, there were significantly more islets harvested in Iodixanol-UW group than in the Ficoll-400-UW group: 4222.00 ± 228.84 IEQ/g vs 3036.83 ± 79.60 IEQ/g ($P < .05$). Islets from the two groups showed satisfactory insulin secretory ability. There were no significant differences in islet survival times between the two groups in diabetic rats: 8.2 ± 1.619 days vs 6.9 ± 1.197 days ($P > .05$).
Conclusion
The improved iodixanol-UW density gradient method was superior to Ficoll-400 method to improve the number, viability, and insulin secret of purified adult porcine islets although the benefits did not improve in vivo survival.

4.829 Improved Method of Porcine Pancreas Procurement With Arterial Flush and Ductal Injection Enhances Islet Isolation Outcome

Anazawa, T., Balamuragan, A.N., Papas, K.K., Avgoustiniatos, E.S., Ferrer, J., Matsumoto, S., Sutherland, D.E.R. and Hering, B.J.
*Transplant. Proceedings, **42(6)**, 2032-2035 (2010)*

Background
Several pancreas procurement procedures have been used for porcine islet isolation; however, their impact on outcomes has not been extensively studied. We evaluated an advanced procurement technique for porcine islet isolation designed to reduce warm ischemia, to remove blood content, and enhance cooling of the pancreas by implementing a vascular flush and ductal preservation.
Method
Pancreata procured from adult Landrace pigs were divided into 3 different surgical protocols: Pancreatectomy utilizing only surface cooling (group 1; $n = 24$); surface cooling and ductal injection with cold preservation solution before pancreatectomy (group 2; $n = 12$); or surface cooling, ductal injection, and an approach by selectively flushing through the celiac trunk and the superior mesenteric artery (group

3; *n* = 14). We assessed the islet isolation results and quality using in vitro and in vivo assays.
Results
Significantly higher overall yield and islet yield per gram pancreas were obtained from group 3 pigs compared with the other groups. Measurements of islet viability after 7 days of culture, as assessed by oxygen consumption rate per DNA, showed that group 3 islets displayed the highest values. Sustained normoglycemia was observed in diabetic nude mice transplanted with 2000 islet equivalents from all 3 groups.
Discussion
This study demonstrated that an advanced pancreas procurement technique including ductal preservation and selective arterial flush with cold preservation solution provided significant improvements in porcine islet isolation outcomes.

4.830 Rapid Quantitative Assessment of the Pig Pancreas Biopsy Predicts Islet Yield
Anazawa, T., Balamurugan, A.N., Matsumoto, S., LaFreniere, S.A., O'Brien, T.D., Sutherland, D.E.R. and Hering, B.J.
Transplant. Proceedings, ***42(6)****, 2036-2039 (2010)*

Background
The cost of islet procurement from donor pigs is increased by the use of organs that produce low yields. We developed an assessment system using dithizone-stained pig pancreas biopsies to enable the preselection of donor organs.
Methods
Pig pancreas biopsy slices were soaked in dithizone solution. The islets were evaluated before islet isolation by converting the islet counts (IC) to islet equivalents (IE), and then determining the IE/cm^2, IE/IC, % islets >150 μm, and % islets >200 μm. These parameters were evaluated in 3 different areas of the pancreas (duodenal, splenic, and connecting lobe; n = 42 each). Stepwise multivariate linear regression analysis was performed to assess for correlations with islet yield and decide which area of the pancreas had the most predictive value. To identify other predictors, including donor and islet isolation variables, we performed binary logistic regression analysis with significant variables from the univariate analysis (n = 67). For this analysis, the pigs were categorized into high (n = 23) and low (n = 44) yield groups.
Results
Stepwise multivariate linear regression analysis revealed that IE/cm^2 of the splenic lobe significantly predicted islet yield. Binary logistic regression analysis indicated that the IE/mm^2 of the splenic lobe was the only parameter that significantly correlated with successful pig islet isolations (*P* = .01; odds ratio 3.605). Variables associated with donor and islet isolation, such as age, gender, ischemic time, or enzyme lot, were not significantly correlated with islet yield.
Conclusion
Our study suggests that the islet distribution of splenic lobe biopsies can be a reliable predictor of islet yield from pig pancreata.

4.831 Assessment of Human Islet Isolation With Four Different Collagenases
Shimoda, M., Noguchi, H., Naziruddin, BG., Fujita, Y., Chujo, D., Takita, M., Peng, H., Tamura, Y., Olsen, G.S., Sugimoto, K., Itoh, T., Onaca, N., Levy, M.F., Grayburn, P.A. and Matsumoto, S.
Transplant. Proceedings, ***42(6)****, 2049-2051 (2010)*

Background
The isolation of islets from the human pancreas critically depends on the efficiency of the digestive enzymes. Liberase HI had been used as a standard preparation until the issues concerning bovine spongiform encephalopathy. Thus, we must now use other collagenases for clinical islet transplantation, four of which we have evaluated herein.
Methods
The digestion of each of 17 pancreata from brain-dead donors was performed using the following collagenases: Liberase HI (HI; Roche, *n* = 9); Liberase MTF C/T (MTF; Roche, *n* = 4); Collagenase NB1 Premium Grade (NB1; Serva, *n* = 7); or Clzyme Collagenase HA (CI, VitaCyte, *n* = 4). Islet isolations were based on the Edmonton protocol for HI, whereas our modified islet isolation method was used for the three new enzymes (MTF, NB1, and CI).
Results
There were no significant differences in donor age, body mass index, pancreas size, and cold ischemic time among the four groups. The phase I time in the NB1 group was significantly shorter than in the CI group (*P* = .0014). The prepurification IEQ/g in the HI group was significantly lower than the others (*P* = .0003

vs MTF, .0007 vs NB1, and .0009 vs CI, respectively). The postpurification IEQ/g in the MTF group was significantly higher than in the HI group (P = .006). The viability in the NB1 group was significantly greater than the HI group (P = .003).
Conclusion
Three new enzymes (MTF, NB1, and CI) may enable us to obtain higher islet yields than with HI.

4.832 ET-Kyoto Ductal Injection and Density-Adjusted Purification Combined With Potent Anti-Inflammatory Strategy Facilitated Single-Donor Islet Transplantation: Case Reports
Matsumoto, S., Noguchi, H., Takita, M., Shimoda, M., Tamura, Y., Olsen, G., Naziruddin, B., Onaca, N. and Levy, M.F.
Transplant. Proceedings, ***42(6)****, 2159-2161 (2010)*

Background
The necessity to use multiple donors for achieving insulin independence in clinical islet transplantation is still a major issue. We have developed a modified islet isolation method for non–heart-beating donors (Kyoto method) to significantly increase islet yield. In this study, we further modified the method for brain-dead donors and in addition, introduced a potent anti-inflammatory strategy aiming for single-donor islet transplantation.
Materials and methods
Two islet isolations used pancreatic ductal preservation with the modified Kyoto solution and a density-adjusted purification method. Anti-interleukin-1-beta antibody (Anakinra) and anti-tumor necrosis factor-alpha (Eternacept) were administered during and after transplantation. The efficacy of the islet transplantation was assessed by the insulin requirement and SUITO (Secretory Unit of Islet Transplant Objects) index, wherein a value of more than 26.0 seems to be associated with insulin independence.
Results
Both isolated islet preparations met the criteria for transplantation. They were transplanted into two type 1 diabetic patients, both of whom became insulin independent with stable glycemic control. The average SUITO index within 1 month was 29.2 and 45.3.
Conclusion
The islet isolation method combined with a potent anti-inflammation strategy made it possible to achieve single-donor islet transplantation achieving a high SUITO index.

4.833 Neural precursor-derived astrocytes of wobbler mice induce apoptotic death of motor neurons through reduced glutamate uptake
Diana, V., Ottalina, A., Botti, F., Fumagalli, E., Calcagno, E., De Paola, M., Cagnotto, A., Invernici, G., Parati, E., Curti, D. and Mennini, T.
Exp. Neurol., ***225****, 163-172 (2010)*

In the present study, we investigated whether cultured astrocytes derived from adult neural precursor cells (NPCs) obtained from the subventricular zone (SVZ) of wobbler mice display metabolic traits of the wobbler astrocytes *in situ* and in primary culture. We also utilized NPC-derived astrocytes as a tool to investigate the involvement of astrocytes in the molecular mechanism of MND focusing on the possible alteration of glutamate reuptake since excitotoxicity glutamate-mediated may be a contributory pathway. NPC-derived wobbler astrocytes are characterized by high immunoreactivity for GFAP, significant decrease of glutamate uptake and reduced immunoreactivity for glutamate transporters GLT1 and GLAST. Spinal cord motor neurons obtained from healthy mouse embryos, when co-cultured with wobbler NPC-derived astrocytes, show reduced viability and morphologic alterations. These suffering motor neurons are caspase-7 positive, and treatment with anti-apoptotic drug V5 increases cell survival. Physical contact with wobbler astrocytes is not essential because purified motor neurons display reduced survival also when treated with the medium conditioned by wobbler NPC-derived astrocytes. Toxic levels of glutamate were revealed by HPLC assay in the extracellular medium of wobbler NPC-derived astrocytes, whereas the level of intracellular glutamate is reduced if compared with controls. Moreover, glutamate receptor antagonists are able to enhance motor neuron survival. Therefore, our results demonstrate that astrocytes derived from wobbler neural precursor cells display impaired glutamate homeostasis that may play a crucial role in motor neuron degeneration. Finally, the cultured astrocytes derived from NPCs of adult mice may offer a useful alternative *in vitro* model to study the molecular mechanisms involved in neurodegeneration.

4.834 The NF-κB p50:p50:HDAC-1 repressor complex orchestrates transcriptional inhibition of multiple pro-inflammatory genes
Elsharkawy, A.M., Oakley, F., Lin, F., Packham, G., Mann, D.A. and Mann, J.

J. Hepatol., ***53***, *519-527 (2010)*

Background & Aims
The pro-inflammatory functions of NF-κB must be tightly regulated to prevent inappropriate tissue damage and remodelling caused by activated inflammatory and wound-healing cells. The p50 subunit of NF-κB is emerging as an important repressor of immune and inflammatory responses, but by mechanisms that are poorly defined. This study aims to delineate p50 target genes in activated hepatic stellate cells and to outline mechanisms utilised in their repression.
Methods
Hepatic stellate cells were isolated from *nfkb1*(p50)-deficient or Wt mice and gene expression compared using microarray. Target genes were verified by qRT-PCR and p50-mediated HDAC-1 recruitment to the target genes demonstrated using chromatin immunoprecipitation.
Results
We identify p50 as transcriptional repressor of multiple pro-inflammatory genes including *Ccl2*, *Cxcl10*, *Gm-csf*, and *Mmp-13*. These genes are over-expressed in *nfkb1*(p50)-deficient mice suffering from chronic hepatitis and in fibrogenic/inflammatory hepatic stellate cells isolated from $nfkb1^{-/-}$ liver. We identify *Mmp-13* as a *bona-fide* target gene for p50 and demonstrate that p50 is required for recruitment of the transcriptional repressor histone deacetylase (HDAC)-1 to κB sites in the *Mmp-13* promoter. Chromatin immunoprecipitations identified binding of HDAC-1 to specific regulatory regions of the *Ccl2*, *Cxcl10*, *Gm-csf* genes that contain predicted κB binding motifs. Recruitment of HDAC-1 to these genes was not observed in $nfkb1^{-/-}$ cells suggesting a requirement for p50 in a manner similar to that described for *Mmp-13*.
Conclusions
Recruitment of HDAC-1 to inflammatory genes provides a widespread mechanism to explain the immunosuppressive properties of p50.

4.835 **Diesel exhaust particles override natural injury-limiting pathways in the lung**
Chaudhuri, N., Paiva, C., Donaldsen, K., Duffin, r., Parker, L.C. and Sabroe, I.
Am. J. Physiol. Lung Cell. Mol. Phsyiol., ***299***, *L263-L271 (2010)*

Induction of effective inflammation in the lung in response to environmental and microbial stimuli is dependent on cooperative signaling between leukocytes and lung tissue cells. We explored how these inflammatory networks are modulated by diesel exhaust particles (DEP) using cocultures of human monocytes with epithelial cells. Cocultures, or monoculture controls, were treated with DEP in the presence or absence of LPS or flagellin. Production of cytokines was explored by Western blotting and ELISA; cell signaling was analyzed by Western blotting. Here, we show that responses of epithelial cells to DEP are amplified by the presence of monocytes. DEP amplified the responses of cellular cocultures to very low doses of TLR agonists. In addition, in the presence of DEP, the responses induced by LPS or flagellin were less amenable to antagonism by the physiological IL-1 antagonist, IL-1ra. This was paralleled by the uncoupling of IL-1 production and release from monocytes, potentially attributable to an ability of DEP to sequester or degrade extracellular ATP. These data describe a model of inflammation where DEP amplifies responses to low concentrations of microbial agonists and alters the nature of the inflammatory milieu induced by TLR agonists.

4.836 **In Hepatic Fibrosis, Liver Sinusoidal Endothelial Cells Acquire Enhanced Immunogenicity**
Conolly, M.K., Bedrosian, A.S., Malhotra, A., Henning, J.R., Ibrahim, J., Vera, V., Cieza-Rubio, N.E., Hassan, B.U., Pachter, H.L., Cohen, S., Frey, A.B. and Miller, G.
J. Immunol., ***185***, *2200-2208 (2010)*

The normal liver is characterized by immunologic tolerance. Primary mediators of hepatic immune tolerance are liver sinusoidal endothelial cells (LSECs). LSECs block adaptive immunogenic responses to Ag and induce the generation of T regulatory cells. Hepatic fibrosis is characterized by both intense intrahepatic inflammation and altered hepatic immunity. We postulated that, in liver fibrosis, a reversal of LSEC function from tolerogenic to proinflammatory and immunogenic may contribute to both the heightened inflammatory milieu and altered intrahepatic immunity. We found that, after fibrotic liver injury from hepatotoxins, LSECs become highly proinflammatory and secrete an array of cytokines and chemokines. In addition, LSECs gain enhanced capacity to capture Ag and induce T cell proliferation. Similarly, unlike LSECs in normal livers, in fibrosis, LSECs do not veto dendritic cell priming of T cells. Furthermore, whereas in normal livers, LSECs are active in the generation of T regulatory cells, in hepatic fibrosis LSECs induce an immunogenic T cell phenotype capable of enhancing endogenous CTLs and

generating potent de novo CTL responses. Moreover, depletion of LSECs from fibrotic liver cultures mitigates the proinflammatory milieu characteristic of hepatic fibrosis. Our findings offer a critical understanding of the role of LSECs in modulating intrahepatic immunity and inflammation in fibro-inflammatory liver disease.

4.837 AAV-mediated expression of wild-type and ALS-linked mutant VAPB selectively triggers death of motoneurons through a Ca2+-dependent ER-associated pathway

Langou, K., Moumen, A., Pellegrino, C., Aebischer, J., Medina, I., Aebischer, P. and Raoul, C.
J. Neurochem., ***114***, *795-809 (2010)*

A dominant mutation in the gene coding for the vesicle-associated membrane protein-associated protein B (VAPB) was associated with amyotrophic lateral sclerosis, a fatal paralytic disorder characterized by the selective loss of motoneurons in the brain and spinal cord. Adeno-associated viral vectors that we show to transduce up to 90% of motoneurons in vitro were used to model VAPB-associated neurodegenerative process. We observed that Adeno-associated viral-mediated over-expression of both wild-type and mutated form of human VAPB selectively induces death of primary motoneurons, albeit with different kinetics. We provide evidence that ER stress and impaired homeostatic regulation of calcium (Ca2+) are implicated in the death process. Finally, we found that completion of the motoneuron death program triggered by the over-expression of wild-type and mutant VAPB implicates calpains, caspase 12 and 3. Our viral-based in vitro model, which recapitulates the selective vulnerability of motoneurons to the presence of mutant VAPB and also to VAPB gene dosage effect, identifies aberrant Ca2+ signals and ER-derived death pathways as important events in the motoneuron degenerative process.

4.838 Induction of Indoleamine 2,3-Dioxygenase by Gene Delivery in Allogeneic Islets Prolongs Allograft Survival

Delle, H. and Noronha, L.
Am. J: Transplant., ***10***, *1918-1924 (2010)*

Indoleamine 2,3-dioxygenase (IDO), an enzyme that plays a critical role in fetomaternal tolerance, exerts immunoregulatory functions suppressing T-cell responses. The aims of this study were to promote IDO expression in rat islets using a nonviral gene transfer approach, and to analyze the effect of the in vivo induction of IDO in a model of allogeneic islet transplantation. The IDO cDNA was isolated from rat placenta, subcloned into a plasmid and transfected into rat islets using Lipofectamine. The efficiency of transfection was confirmed by qRT-PCR and functional analysis. The in vivo effect of IDO expression was analyzed in streptozotocin-induced diabetic Lewis rats transplanted with allogeneic islets under the renal capsule. Transplantation of IDO-allogeneic islets reversed diabetes and maintained metabolic control, in contrast to transplantation of allogeneic nontransfected islets, which failed shortly after transplantation in all animals. Graft survival of allograft islets transfected with IDO transplanted without any immunosuppression was superior to that observed in diabetic rats receiving nontransfected islets. These data demonstrated that IDO expression induced in islets by lipofection improved metabolic control of streptozotocin-diabetic rats and prolonged allograft survival.

4.839 Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress

Lv, X., Chen, Z., Li, J., Zhang, L., Liu, H., Huang, C. and Zhu, P.
Inflamm. Res., ***59***, *635-645 (2010)*

Objective and design
The present investigation was designed to determine the effects of caffeine on alcohol-induced hepatic injury in mice.
Material
Five groups of mice (8 each) were used.
Treatment
The mice treated with different doses of caffeine (5, 10, and 20 mg/kg, respectively).
Methods
The degree of alcoholic liver injury was evaluated biochemically by measuring serum markers and pathological examination. Real time PCR and ELISA methods were used to check the expression of cytokines and CYP 450.
Results
Treatment with caffeine significantly attenuated the elevated serum aminotransferase enzymes and reduced

the severe extent of hepatic cell damage, steatosis and the immigration of inflammatory cells. Interestingly, caffeine decreased hepatic mRNA expression of lipogenic genes, while it had no effect on protein expression of hepatic CYP2E1. Furthermore, caffeine decreased serum and tissue inflammatory cytokines levels, tissue lipid peroxidation and inhibited the necrosis of hepatocytes. Kupffer cells isolated from ethanol-fed mice produced high amounts of reactive oxygen species (ROS) and tumor necrosis factor alpha (TNF-α), whereas Kupffer cells from caffeine treatment mice produced less ROS and TNF-α.
Conclusions
These findings suggest that caffeine may represent a novel, protective strategy against alcoholic liver injury by attenuating oxidative stress and inflammatory response.

4.840 PD-1 on Immature and PD-1 Ligands on Migratory Human Langerhans Cells Regulate Antigen-Presenting Cell Activity

Pena-Cruz, V., McDonough, S.M., Diaz-Grieffero, F., Crum, C.P., Carrasco, R.D. and Freeman, G.J.
J. Invest. Dermatol., ***130***, *2222-2230 (2010)*

Langerhans cells (LCs) are known as "sentinels" of the immune system that function as professional antigen-presenting cells (APCs) after migration to draining lymph node. LCs are proposed to have a role in tolerance and the resolution of cutaneous immune responses. The Programmed Death-1 (PD-1) receptor and its ligands, PD-L1 and PD-L2, are a co-inhibitory pathway that contributes to the negative regulation of T-lymphocyte activation and peripheral tolerance. Surprisingly, we found PD-1 to be expressed on immature LCs (iLCs) in situ. PD-1 engagement on iLCs reduced IL-6 and macrophage inflammatory protein (MIP)-1α cytokine production in response to TLR2 signals but had no effect on LC maturation. PD-L1 and PD-L2 were expressed at very low levels on iLCs. Maturation of LCs upon migration from epidermis led to loss of PD-l expression and gain of high expression of PD-L1 and PD-L2 as well as co-stimulatory molecules. Blockade of PD-L1 and/or PD-L2 on migratory LCs (mLCs) and DDCs enhanced T-cell activation, as has been reported for other APCs. Thus the PD-1 pathway is active in iLCs and inhibits iLC activities, but expression of receptor and ligands reverses upon maturation and PD-L1 and PD-L2 on mLC function to inhibit T-cell responses.

4.841 Existence of CD8α-Like Dendritic Cells with a Conserved Functional Specialization and a Common Molecular Signature in Distant Mammalian Species

Contreras, V., Urien, C., Guiton, R., Alexandre, Y., Vu Manh, T-P., Andrieu, T., Crozat, K., Jouneau, L., Bertho, N., Epardaud, M., Hope, J., Savina, A., Amigorena, S., Bonneau, M., Dalod, M. and Schwartz-Cornil, I.
J. Immunol., ***185***, *3313-3325 (2010)*

The mouse lymphoid organ-resident CD8α^+ dendritic cell (DC) subset is specialized in Ag presentation to CD8$^+$ T cells. Recent evidence shows that mouse nonlymphoid tissue CD103$^+$ DCs and human blood DC Ag 3$^+$ DCs share similarities with CD8α^+ DCs. We address here whether the organization of DC subsets is conserved across mammals in terms of gene expression signatures, phenotypic characteristics, and functional specialization, independently of the tissue of origin. We study the DC subsets that migrate from the skin in the ovine species that, like all domestic animals, belongs to the Laurasiatheria, a distinct phylogenetic clade from the supraprimates (human/mouse). We demonstrate that the minor sheep CD26$^+$ skin lymph DC subset shares significant transcriptomic similarities with mouse CD8α^+ and human blood DC Ag 3$^+$ DCs. This allowed the identification of a common set of phenotypic characteristics for CD8α-like DCs in the three mammalian species (i.e., SIRP^{lo}, CADM1^{hi}, CLEC9A^{hi}, CD205^{hi}, XCR1^{hi}). Compared to CD26$^-$ DCs, the sheep CD26$^+$ DCs show 1) potent stimulation of allogeneic naive CD8$^+$ T cells with high selective induction of the *Ifn*γ and *Il22* genes; 2) dominant efficacy in activating specific CD8$^+$ T cells against exogenous soluble Ag; and 3) selective expression of functional pathways associated with high capacity for Ag cross-presentation. Our results unravel a unifying definition of the CD8α^+-like DCs across mammalian species and identify molecular candidates that could be used for the design of vaccines applying to mammals in general.

4.842 *In Vitro* Cultured Cardiomyocytes for Evaluating Cardiotoxicity

Liu, S.J. and Melchert, R.B.
Comprehensive Toxicology, ***6***, *113-131 (2010)*

Cardiotoxicity has often been observed with the advent of pharmaceuticals for conditions such as cancer and characterized by abnormality of cardiac electrical activity and contractile dysfunction, ultimately leading to heart failure. Finding and understanding the primary cause of cardiotoxicity would improve

pharmaceutical development for effective treatment without cardiac side effects. However, complexities of muscle structure and the heterogeneity of cell populations in the heart make *in vivo* studies and *in vitro* studies of the whole-heart preparations problematic to identify the primary cause and mechanisms underlying cardiotoxicity at the cellular level. Thus, isolated cardiomyocytes have been powerful tools to discover functional changes of individual cardiac myocytes in response to stimuli. With careful design and control of cell growth, isolated cardiomyocytes can be maintained for a long time in culture and can provide stable model systems for both short-term and long-term studies of genetic physiology, evaluation of cardiotoxicity, and reparative medicine. Understanding limitations of these cell cultures and utilizing multidisciplinary technologies in refined experimental conditions would enable one to take best advantage of unique varieties of these *in vitro* model systems. Then, cultured cardiomyocytes become the best systems and the choice for physiological, pharmacological, and toxicological studies to evaluate direct effects and underlying mechanisms of xenobiotics on the heart at cellular, subcellular, and molecular levels. Knowledge obtained from using *in vitro* cultured cardiomyocytes is essential and critical for further *in vivo* studies in whole animals and drug development to reduce cardiotoxicity.

4.843 The Morphology of Poly(3,4-Ethylenedioxythiophene)

Martin, D.C., Wu, J., Shaw, C.M., King, Z., Spanninga, S.A., Richardson-Burns, S., Hendricks, J. and Yang, J.

*Polymer Reviews, **50**, 340-384 (2010)*

Poly(3,4-ethylene dioxythiophene) (PEDOT) is a chemically stable, conjugated polymer that is of considerable interest for a variety of applications including coatings for interfacing electronic biomedical devices with living tissue. Here, we describe recent work from our laboratory and elsewhere to investigate the morphology of PEDOT in the solid state. We discuss the importance of oxidative chemical and electrochemical polymerization, as well as the critical role of the counterion used during synthesis and film deposition. We have obtained information about the morphology of PEDOT from a number of different complimentary techniques including X-ray diffraction, optical microscopy, scanning electron microscopy, transmission high-resolution electron microscopy, and low-voltage electron microscopy. We also discuss results from ultraviolet-visible light spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), and X-ray photoelectron spectroscopy (XPS).
PEDOT is a relatively rigid polymer that packs in the solid state at a characteristic face-to-face distance (010) of ~ 0.34 nm, similar to graphite. These sheets of oriented PEDOT molecules are separated from one another by ~ 1.4 nm laterally, with the (100) distance between layers quite sensitive to the choice of counterion used during sample preparation. The order in the films is typically modest, although this also depends on the counterion used and the method of film deposition. The films can be organized into useful structures with a variety of nanoscale dissolvable templates (including fibers, particles, and lyotropic mesophases). When PEDOT is electrochemically deposited in the presence of bromine counterions, highly ordered crystalline phases are observed. It is also possible to deposit PEDOT around living cells, both in vitro and in vivo.

4.844 Islet cell transplantation for Type 1 diabetes

Matsumoto, S.

*J. Diabetes, **2**, 16-22 (2010)*

Islet transplantation is an attractive concept for the treatment of Type 1 diabetes because of its potential high efficacy and minimal invasion to patients. The treatment may effectively control blood glucose for brittle Type 1 diabetes, resulting in a marked reduction in hypoglycemic episodes and improvements in HbA**1**c. In addition, approximately 70% of transplanted Type 1 diabetic patients have achieved insulin independence. However, there are still important issues to be addressed before this treatment is widely applicable, including difficulty in maintaining insulin independence, low islet isolation success rate, multiple donor requirements, and side effects associated with the use of immunosuppressants. Donor shortage is another dilemma. To address the issue of donor shortage, living donor islet transplantation and bioartificial islet transplantation using pig islets are being evaluated. Bioartificial islet transplantation could be the ultimate solution of the donor shortage. Currently, overcoming immunological hurdles, establishing reliable islet isolation methods, and controlling porcine endogenous retrovirus are the primary obstacles to the implementation of this treatment. If bioartificial islet transplant becomes a clinical reality, it may even be applicable in the treatment of select Type 2 diabetic patients. β-Cell regeneration from naïve pancreas and β-cell generation from embryonic stem cells or induced pluripotent stem cells are the next-generation treatments for Type 1 diabetes.

4.845 Identifying Homing Interactions in T-Cell Traffic in Human Disease
Lalor, P.F., Curbishley, S.M. and Adams, D.H.
*Methods in Mol. Biol., **616(4)**, 231-252 82010)*

Description of the molecular mechanisms which regulate the traffic of lymphocyte populations over recent years [for useful reviews see (1, 2)] has significantly enhanced our understanding of the processes underlying acquired immunity and also permitted the development of therapies targeted at specific leukocyte subpopulations. Such therapies are dependent upon a detailed knowledge of the molecular regulation of lymphocyte adhesion to and migration through endothelium in specific tissues. Whereas animal models have been central to understanding the underlying mechanisms, it is crucial to confirm and extend observations in man by using analysis of tissues and in vitro cell-based models. In this chapter, we discuss expertise developed in our laboratory for the isolation of specific lymphocyte and endothelial populations from explanted human liver tissue specimens. We then move on to provide specific examples of assays such as the Stamper–Woodruff assay, the transmigration assay and the tissue-specific endothelial static and flow-based adhesion assays, which can be used to interrogate the tissue-specific adhesion and migration of lymphocyte subsets. Although our own experience is with human liver tissue, the general principles apply to analysing any organ of interest.

4.846 Isolation and Culture of Adult Human Liver Progenitor Cells: In Vitro Differentiation to Hepatocyte-Like Cells
Gerbal-Chaloin, S., Duret, C., Raulet, E., Navarro, F., Blanc, P., Ramos, J., Maurel, P. and Daujat-Chavanieu, M.
*Methods in Mol. Biol., **640**, 247-260 (2010)*

Highly differentiated normal human hepatocytes represent the gold standard cellular model for basic and applied research in liver physiopathology, pharmacology, toxicology, virology, and liver biotherapy. Nowadays, although livers from organ donors or medically required resections represent the current sources of hepatocytes, the possibility to generate hepatocytes from the differentiation of adult and embryonic stem cells represents a promising opportunity. The aim of this chapter is to describe our experience with the isolation from adult human liver and culture of non-parenchymal epithelial cells. Under appropriate conditions, these cells differentiate in vitro in hepatocyte-like cells and therefore appear to behave as liver progenitor cells.

4.847 Pediatric Islet Autotransplantation: Indication, Technique, and Outcome
Bellin, M.D. and Sutherland, D.E.R.
*Cur. Diab. Rep., **10**, 326-331 (2010)*

Chronic pancreatitis is a rare disease in childhood. However, when severe, a total pancreatectomy may be the only option to relieve pain and restore quality of life. An islet autotransplant performed at the time of pancreatectomy can prevent or minimize the postsurgical diabetes that would otherwise result from pancreatectomy alone. In this procedure, the resected pancreas is mechanically disrupted and enzymatically digested to separate the islets from the surrounding exocrine tissue, and the isolated islets are infused into the portal vein and engraft in the liver. Because patients are receiving their own tissue, no immunosuppression is required. Islet autotransplant is successful in two thirds of children—these patients are insulin independent or require little insulin to maintain euglycemia. Factors associated with a more successful outcome include a younger age at transplant (<13 years), more islets transplanted, and lack of prior surgical procedures on the pancreas (partial pancreatectomy or surgical drainage procedures).

4.848 ADP mediates inhibition of insulin secretion by activation of P2Y13 receptors in mice
Amisten, S., Meidute-Abaraviciene, S., Tan, C., Olde, B., Lundquist, I., Salehi, A. and Erlinge, D.
*Diabetologia, **53**, 1927-1934 (2010)*

Aims/hypotheses
To investigate the effects of extracellular purines on insulin secretion from mouse pancreatic islets.
Methods
Mouse islets and beta cells were isolated and examined with mRNA real-time quantification, cAMP quantification and insulin and glucagon secretion. ATP release was measured in MIN6c4 cells. Insulin and glucagon secretion were measured in vivo after glucose injection.
Results

Enzymatic removal of extracellular ATP at low glucose levels increased the secretion of both insulin and glucagon, while at high glucose levels insulin secretion was reduced and glucagon secretion was stimulated, indicating an autocrine effect of purines. In MIN6c4 cells it was shown that glucose does induce release of ATP into the extracellular space. Quantitative real-time PCR demonstrated the expression of the ADP receptors P2Y1 and P2Y13 in both intact mouse pancreatic islets and isolated beta cells. The stable ADP analogue 2-MeSADP had no effect on insulin secretion. However, co-incubation with the P2Y1 antagonist MRS2179 inhibited insulin secretion, while co-incubation with the P2Y13 antagonist MRS2211 stimulated insulin secretion, indicating that ADP acting via P2Y1 stimulates insulin secretion, while signalling via P2Y13 inhibits the secretion of insulin. P2Y13 antagonism through MRS2211 per se increased the secretion of both insulin and glucagon at intermediate (8.3 mmol/l) and high (20 mmol/l) glucose levels, confirming an autocrine role for ADP. Administration of MRS2211 during glucose injection in vivo resulted in both increased secretion of insulin and reduced glucose levels.
Conclusions/interpretation
In conclusion, ADP acting on the P2Y13 receptors inhibits insulin release. An antagonist to P2Y13 increases insulin release and could be evaluated for the treatment of diabetes.

4.849 Human primary cultured hepatic stellate cells can be cryopreserved
Nakamura, A., Ueno, T., Yagi, Y., Okuda, K., Ogata, T., Nakamura, T., Torimura, T., Iwamoto, H., Ramadoss, S., Sata, M., Tsutsumi, V., Yasuda, K., Tomiyashi, K., Tashiro, K. and Kuhara, S.
*Med. Mol. Morphol., **43**, 107-115 (2010)*

We compared the morphological and functional characteristics of cultured unfrozen hepatic stellate cells (HSCs) and cryopreserved HSCs obtained from human livers. We used liver tissues obtained by surgical resection from patients with metastatic liver cancer or with hepatocellular carcinoma. HSCs were isolated and allowed to spread in culture. Comparison of morphological and functional features between the unfrozen HSCs and cryopreserved HSCs was performed at each passage using the following techniques: light microscopy, immunohistochemistry, cell growth curve, metallothionein (MTT) assay, and PI staining, Western blot, real-time polymerase chain reaction (PCR), and gene expression analysis using microarrays. The purity of HSCs was more than 90% in all passages. α-Smooth muscle actin (SMA-)positive HSCs gradually increased in successive passages, and the positive cell rate and rate of increase in cell number were similar in both groups. Expression of platelet-derived growth factor (PDGF) receptor, transforming growth factor (TGF)-β receptor, and α-SMA mRNAs and protein was similar during each passage in the two groups. Gene expression was nearly identical at each passage in unfrozen and frozen/thawed samples obtained from the same patient. In conclusion, an adequate protocol for the cryopreservation of human primary cultured HSCs could be established.

4.850 Modulation of Synaptic Transmission and Analysis of Neuroprotective Effects of Valproic Acid and Derivates in Rat Embryonic Motoneurons
Ragancokova, D., Song, Y., Nau, H., Dengler, R., Krammpfl, K. and Petri, S.
*Cell. Mol. Neurobiol., **30**, 891-900 (2010)*

Amyotrophic lateral sclerosis is a devastating motoneuron disorder for which no effective treatment exists. There is some evidence for neuroprotective effects of valproic acid (VPA). The beneficial effects, however, are limited due to the adverse effects of VPA. To overcome this problem, a number of VPA derivates with fewer side effects have been synthesized. In the present study, we investigated the viability of highly purified embryonic motoneurons cultured on glial feeder layers, composed of either astrocytes or Schwann cells, or in monoculture, in presence of VPA and its three derivates 3-propyl-heptanoic acid (3-PHA), PE-4-yn enantiomers (R- and S-PE-4-yn). An excitotoxic stimulus, kainate (KA), was added at day in vitro 9 (DIV9) and the neuroprotective effect of either simultaneous incubation (DIV9) or pre-incubation (DIV1) of VPA and its derivates was tested. The survival of motoneurons under simultaneous application of KA and VPA derivates was not remarkably increased. Pre-incubation with VPA and even more with the derivates before the addition of KA, however, significantly reduced their vulnerability against the KA-induced neurotoxic effect. Our data suggest that the neuroprotective capacities of VPA and its three derivates tested here drastically increase when they are added several days before KA. Most prominent neuroprotective effects were seen for the PE-4-yn enantiomers. Patch-clamp experiments revealed an antiexcitotoxic effect of the S-PE-4-yn enantiomer that reduces the frequency of postsynaptic currents and enhances the inhibitory postsynaptic transmission dependent on the co-culture condition.

4.851 CX3CR1 and vascular adhesion protein-1-dependent recruitment of CD16+ monocytes across human liver sinusoidal endothelium

Aspinall, A., Curbishley, S.M., Lalor, P:F., Weston, C.J., Blahova, M., Liaskou, E., Adams, R.M., Holt, A.P. ansd Adams, D.H.

*Hepatology, **51**, 2030-2039 (2010)*

The liver contains macrophages and myeloid dendritic cells (mDCs) that are critical for the regulation of hepatic inflammation. Most hepatic macrophages and mDCs are derived from monocytes recruited from the blood through poorly understood interactions with hepatic sinusoidal endothelial cells (HSECs). Human CD16+ monocytes are thought to contain the precursor populations for tissue macrophages and mDCs. We report that CD16+ cells localize to areas of active inflammation and fibrosis in chronic inflammatory liver disease and that a unique combination of cell surface receptors promotes the transendothelial migration of CD16+ monocytes through human HSECs under physiological flow. CX3CR1 activation was the dominant pertussis-sensitive mechanism controlling transendothelial migration under flow, and expression of the CX3CR1 ligand CX3CL1 is increased on hepatic sinusoids in chronic inflammatory liver disease. Exposure of CD16+ monocytes to immobilized purified CX3CL1 triggered β1-integrin-mediated adhesion to vascular cell adhesion molecule-1 and induced the development of a migratory phenotype. Following transmigration or exposure to soluble CX3CL1, CD16+ monocytes rapidly but transiently lost expression of CX3CR1. Adhesion and transmigration across HSECs under flow was also dependent on vascular adhesion protein-1 (VAP-1) on the HSECs. Conclusion: Our data suggest that CD16+ monocytes are recruited by a combination of adhesive signals involving VAP-1 and CX3CR1 mediated integrin-activation. Thus a novel combination of surface molecules, including VAP-1 and CX3CL1 promotes the recruitment of CD16+ monocytes to the liver, allowing them to localize at sites of chronic inflammation and fibrosis.

4.852 The hepatic vagus nerve stimulates hepatic stellate cell proliferation in rat acute hepatitis via muscarinic receptor type 2

Bockx, I., Vander elst, I., Roskams, T. and Cassiman, D.

*Liver Int., **30(5)**, 693-702 (2010)*

Background & aims: We have previously shown that the hepatic vagus nerve stimulates the activation of hepatic progenitor cells (HPC), via muscarinic acetylcholine receptor type 3. Given the coproliferation of HPC and hepatic stellate cells (HSC) in acute hepatitis, we determined whether HSC proliferation is also modulated by vagal activity.
Methods: We induced acute hepatitis in Wistar rats by injection of galactosamine and lipopolysaccharides. Hepatitis was preceded by hepatic branch vagotomy or sham vagotomy, by electrical stimulation or sham stimulation and by muscarinic receptor antagonist atropine, nicotinic receptor antagonist mecamylamine or saline injection. Rats were sacrificed after 12 and 48 h and HSC numbers were quantified on immunohistochemical stainings. Furthermore, we performed reverse transcriptase-polymerase chain reaction with receptor-specific primers on total RNA from isolated HSC and determined the *in vitro* proliferation of HSC in response to acetylcholine, atropine and mecamylamine.
Results: HSC numbers were significantly lower after vagotomy than after sham vagotomy. Conversely, more HSC were seen after electrical stimulation than after sham stimulation. Atropine resulted in less HSC than saline at both time points, while mecamylamine treatment only diminished HSC after 12 h, suggesting a predominant involvement of muscarinic receptors. Moreover, HSC express muscarinic receptor type 2 mRNA and protein, as well as nicotinic receptor α1, α5, β1 and vasoactive intestinal peptide receptor 1 mRNA. Furthermore, acetylcholine enhanced the *in vitro* proliferation of HSC, which was inhibited by atropine, but not by mecamylamine.
Conclusions: We show here that the hepatic vagus nerve stimulates HSC proliferation, most likely through binding of acetylcholine on muscarinic receptor type 2.

4.853 Toll-like receptor expression in the peripheral nerve

Goethals, S., Ydens, E., Timmerman, V. and Janssens, S.

*Glia, **58**, 1701-1709 (2010)*

Toll-like receptors comprise a family of evolutionary conserved pattern recognition receptors that act as a first defense line in the innate immune system. Upon stimulation with microbial ligands, they orchestrate the induction of a host defense response by activating different signaling cascades. Interestingly, they appear to detect the presence of endogenous signals of danger as well and as such, neurodegeneration is thought to trigger an immune response through ligation of TLRs. Though recent data report the expression

of various TLRs in the central nervous system, TLR expression patterns in the peripheral nervous system have not been determined yet. We observed that Schwann cells express relatively high levels of TLRs, with especially TLR3 and TLR4 being prominent. Sensory and motor neurons hardly express TLRs at all. Through the use of NF-κB signaling as read-out, we could show that all TLRs are functional in Schwann cells and that bacterial lipoprotein, a ligand for TLR1/TLR2 receptors yields the strongest response. In sciatic nerve, basal levels of TLRs closely reflect the expression patterns as determined in Schwann cells. TLR3, TLR4, and TLR7 are majorly expressed, pointing to their possible role in immune surveillance. Upon axotomy, TLR1 becomes strongly induced, while most other TLR expression levels remain unaffected. Altogether, our data suggest that similar to microglia in the brain, Schwann cells might act as sentinel cells in the PNS. Furthermore, acute neurodegeneration induces a shift in TLR expression pattern, most likely illustrating specialized functions of TLRs in basal versus activated conditions of the peripheral nerve.

4.854 Multicenter Analysis of Novel and Established Variables Associated with Successful Human Islet Isolation Outcomes

Kaddis, J.S., Danobeitia, J.S., Niland, J.C., Stiller, T. and Fernandez, L.A.
Am. J. Transplant., ***10***, *646-656 (2010)*

Islet transplantation is a promising therapy used to achieve glycometabolic control in a select subgroup of individuals with type I diabetes. However, features that characterize human islet isolation success prior to transplantation are not standardized and lack validation. We conducted a retrospective analysis of 806 isolation records from 14 pancreas-processing laboratories, considering variables from relevant studies in the last 15 years. The outcome was defined as postpurification islet equivalent count, dichotomized into yields ≥315 000 or ≤220 000. Univariate analysis showed that donor cause of death and use of hormonal medications negatively influenced outcome. Conversely, pancreata from heavier donors and those containing elevated levels of surface fat positively influence outcome, as did heavier pancreata and donors with normal amylase levels. Multivariable logistic regression analysis identified the positive impact on outcome of surgically intact pancreata and donors with normal liver function, and confirmed that younger donors, increased body mass index, shorter cold ischemia times, no administration of fluid/electrolyte medications, absence of organ edema, use of University of Wisconsin preservation solution and a fatty pancreas improves outcome. In conclusion, this multicenter analysis highlights the importance of carefully reviewing all donor, pancreas and processing parameters prior to isolation and transplantation.

4.855 Primary Sensory and Motor Neuron Cultures

Vincent, A.M. and Feldman, E.L.
Protocols for Neural Cell Culture, 161-173 (2010) Springer Protocols Handbook

The ability to culture primary neurons is critical to the study of neuronal biochemistry and molecular biology. Because committed neurons do not proliferate, neuronal cell lines are fundamentally altered in all areas of activity. Transformation of neuronal lines necessarily alters their survival, signaling, and metabolism, so for these and many other studies primary cells are essential. The issue of cell identity is also an important advantage of primary neuron cultures. An investigator tends to have high confidence in the identity of neurons reproducibly harvested from a specific site from an animal. Issues of senescence and enrichment of more robust population subtypes are eliminated in primary cell culture. We have particularly focused on enriched neuronal cultures so that we can study neuronal biology in isolation. These cultures are amenable to extraction of protein, lipid, and nucleic acid for assessment of expression and post-translational modifications with high confidence that the measurements are neuronal and not occurring in accessory cells.
We have established protocols for many embryonic neuronal cultures and also a limited number of adult neurons. In some studies, co-culture with glial cells is important, so we also culture various glial cells. These cultures are used for immunohistochemistry (*1*), biochemical enzyme assays (*2*), gene expression at mRNA and protein level (*3*, *4*), live cell fluorescence-based metabolic assays (*5*, *6*), survival (*5*, *6*), and signaling measurements (*1*, *7*). We have found that the most effective method for gene transfer to primary neurons is viral infection. Using adeno- and adeno-associated viruses, we achieve high expression levels and greater than 90% infection rates within 24–48 h (*3*, *8*). This fits well within our 3-day culture period. These gene transfer experiments prove powerful for the rescue of a knockout phenotype or the study of gene overexpression (*2*, *8*). We also can use lower multiplicities of infection to achieve 10% or 50% infection. With a bicistronic vector that expresses GFP as well as the gene of interest, we can microscopically compare neurons in the same dish with and without the transgene and separate them by green fluorescence.

We also have devised serum-free defined media for our neuronal cultures. This development confers many advantages over other protocols. The media permits the culture of neurons at very low density in the absence of a glial feeder layer. This is important for our studies of neurite growth on different extracellular matrix proteins and aligned nanofibers (9) and for immunohistochemistry (6, 7). The absence of serum also is important for our studies into the effects of growth factors on signaling and survival (1, 3).
We and others have found that embryonic and neonatal neurons require basal 25 mM glucose in order to remain viable in culture, and this is contained in the formulation of neurobasal media (Gibco, #21103). This is consistent with many neuronal cells, but contrasts with most other primary cell types (5, 10). Adult DRG neurons may be grown in either low- or high-glucose media, although very large neurons are decreased in high glucose and fluctuations in glucose are detrimental to the neurons (11).
Representative examples of the cells isolated in the following protocols are shown in **Fig.** 9.1.

4.856 Preparation of Normal and Reactive Astrocyte Cultures

De Villes, J., Ghiani, C.A., Wanner, I.B. and Cole, R.

Protocols for Neural Cell Culture, 193-215 (2010) SpringerProtocols Handbooks

The study of glial cell development and function has been considerably enhanced by the development of methods to culture oligodendrocytes, astrocytes, and microglia from central nervous system tissue. A primary mixed glial culture, composed of astrocytes, oligodendrocytes, and microglia, is obtained when newborn disaggregated cerebral brain cells from rat are plated at high cell density ($2 \times 10^5/cm^2$) in serum-supplemented medium (1). Neurons fail to develop or survive in this culture model.
At low cell density (e.g., 5×10^4 cells/cm^2), few oligodendrocytes develop, and the culture consists mostly of astrocytes. At high cell density, phase-dark, process-bearing spindle or spider-shaped cells appear by 4 days and stratify into clusters and individual cells above the bed-layer of cells. The bed layer consists of astrocytes rich in glial filaments. This observation led to the development of the shaking procedure, which results in selective removal of the process-bearing cells from the underlying astrocytes (1).Thus, highly purified cultures of astrocytes and oligodendrocytes, as wells as microglia, can be obtained from the same piece of brain tissue. Microglia cells (2) can be harvested from the stationary cultures by harvesting the medium on days 6 and 7, when they can be microscopically observed to be suspended in the medium. The remaining microglia and loosely adhering astrocytes are then removed from the mixed culture by a 6-h pre-shake, before the oligodendrocyte lineage cells are removed. The microglia cultures are about 95% pure, as characterized with immunocytochemistry using the microglia marker, ED 1 (3).
At the time of harvesting the process-bearing cells from the 7- to 9-day-old cultures, the cell population is mostly composed of oligodendrocyte progenitor cells and immature oligodendrocytes (4). If the process-bearing cells are placed in a chemically defined serum-free medium, <4% of the cells express astrocyte markers, such as glial fibrillary acidic protein (GFAP) (5). In fetal bovine serum (FBS)-supplemented medium, 25–35% of the cells express GFAP, originally called astrocyte type II. It has now been determined that astrocytes type II are an in vitro phenomenon with no in vivo counterpart (6). The bed-layer cells can be maintained as pure astrocyte cultures by keeping the flasks shaking slowly, to keep removing dividing oligodendrocyte progenitors and microglia. When cultured in the presence of horse serum, astrocytes grow flat processes and acquire a highly differentiated quiescent phenotype while continuing to form a lawn (7).
This primary culture of astrocytes can be used to set up pure secondary astrocyte cultures in serum or serum-free medium (8, 9). Astroglial and oligodendroglial cell lines have been developed from the cultures described above (10, 11). The usage of astrocyte cultures derived from neonatal brains has the advantage of using a population of astroglial cells with a purity of 99% (1).
Primary cultures of astrocytes offer an invaluable tool to characterize the changes that may occur in these cells following, for instance, a brain injury. Astrocytes respond to injury by becoming hypertrophic, hyperplastic, and increasing GFAP. This process, named reactive gliosis or astrogliosis, occurs in the brain and spinal cord in response to different types of injury including ischemia and inflammatory diseases. Its hallmark is an increase in GFAP (12, 13). Astrogliosis leads to the formation of a glial scar, a boundary necessary to contain the injury, but also an inhibitory obstacle to successful regeneration of damaged neuronal networks and axon tracts. The differences in the response to a harmful event, either chemical or physical, have been examined in astrocytes cultured from either the neonatal or the adult brain (12). It is important to point out that neonatal astrocytes in culture as well as in vivo display higher levels of GFAP compared to adult astrocytes. Yet, cultured astrocytes derived from adult brain or spinal cord were shown to arise from glial progenitor cells, similarly to those prepared from newborn brains (14–16). Hence, a protocol to obtain purified and highly differentiated astrocytes in culture is desirable to study mechanisms of astrogliosis and scar formation. Here, we provide a set of detailed protocols preparing and using purified astrocytes that were kept in culture for up to 8 weeks, as well as of reactive astrocytes (1, 7 , 12, 17).

4.857 Production of IL-10 and IL-12 by antigen-presenting cells in periapical lesions
Colic, M., Gazivoda, D., Vasilijic, S., Vucevic, D. and Lukic, A.
J. Oral Pathol. Med., ***39(9)***, *690-696 (2010)*

Background: Interferon-γ (IFN-γ) plays an important role in the pathogenesis of periapical lesions. Its expression is up-regulated by interleukin (IL)-12) and down-regulated by IL-10. The aim of this work was to study the cellular source of these cytokines and their mutual interactions in human periapical lesions.
Methods: Mononuclear cells, macrophages and dendritic cells were isolated from periapical lesions using plastic adherence and osmotic gradients. Cytokines were measured in culture supernatants by a microbeads fluorescence assay. Phenotypic characteristics of cells were studied by immunocytochemistry, whereas allostimulatory activity of antigen-presenting cells was tested using a mixed leukocyte reaction.
Results: We observed the positive correlations between the levels of IL-12 and IFN-γ as well as IL-12 and IL-10 in cultures of mononuclear cells. As IL-10 and IL-12 are produced by dendritic cells and activated macrophages, we examined their contribution to the production of these cytokines. Macrophages, $CD14^+$ adherent cells, produced high levels of IL-10 and very low levels of IL-12. In contrast, non-adherent, strongly $HLA\text{-}DR^+$ dendritic cells, potent stimulators of the alloreactive T-cell response, produced low levels of IL-10 and moderate levels of IL-12. Dendritic cells stimulated the production of IFN-γ by allogeneic $CD4^+$ T cells. In contrast, the level of IFN-γ was significantly decreased and the production of IL-10 was enhanced by addition of macrophages to the culture system.
Conclusion: Our results suggest that a fine balance between the production of IL-10 and IL-12 by different antigen-presenting cells, through IFN-γ, may control the course of chronic inflammation in periapical lesions.

4.858 Surfactant Protein-A Inhibits Mycoplasma-Induced Dendritic Cell Maturation through Regulation of HMGB-1 Cytokine Activity
Ledford, J.G., Lo, B., Kislan, M.M., Thomas, J.M., Evans, K., Cain, D.W., Kraaft, M., Williams, K.L. and Wright, J.R.
J. Immunol., ***185***, *3884-3894 (2010)*

During pulmonary infections, a careful balance between activation of protective host defense mechanisms and potentially injurious inflammatory processes must be maintained. Surfactant protein A (SP-A) is an immune modulator that increases pathogen uptake and clearance by phagocytes while minimizing lung inflammation by limiting dendritic cell (DC) and T cell activation. Recent publications have shown that SP-A binds to and is bacteriostatic for Mycoplasma pneumoniae in vitro. In vivo, SP-A aids in maintenance of airway homeostasis during M. pneumoniae pulmonary infection by preventing an overzealous proinflammatory response mediated by TNF-α. Although SP-A was shown to inhibit maturation of DCs in vitro, the consequence of DC/SP-A interactions in vivo has not been elucidated. In this article, we show that the absence of SP-A during M. pneumoniae infection leads to increased numbers of mature DCs in the lung and draining lymph nodes during the acute phase of infection and, consequently, increased numbers of activated T and B cells during the course of infection. The findings that glycyrrhizin, a specific inhibitor of extracellular high-mobility group box-1 (HMGB-1) abrogated this effect and that SP-A inhibits HMGB-1 release from immune cells suggest that SP-A inhibits M. pneumoniae-induced DC maturation by regulating HMGB-1 cytokine activity.

4.859 Cu,Zn-Superoxide Dismutase Increases Toxicity of Mutant and Zinc-deficient Superoxide Dismutase by Enhancing Protein Stability
Sahawneh, M.A., Ricart, K.C., Roberts, B.R., Bomben, V.C., Basso, M., Ye, Y., Sahawneh, J., Franco, M.C., Beckman, J.S. and Estevez, A.G.
J. Biol. Chem., ***285(44)***, *33885-33897 (2010)*

When replete with zinc and copper, amyotrophic lateral sclerosis (ALS)-associated mutant SOD proteins can protect motor neurons in culture from trophic factor deprivation as efficiently as wild-type SOD. However, the removal of zinc from either mutant or wild-type SOD results in apoptosis of motor neurons through a copper- and peroxynitrite-dependent mechanism. It has also been shown that motor neurons isolated from transgenic mice expressing mutant SODs survive well in culture but undergo apoptosis when exposed to nitric oxide via a Fas-dependent mechanism. We combined these two parallel approaches for understanding SOD toxicity in ALS and found that zinc-deficient SOD-induced motor neuron death required Fas activation, whereas the nitric oxide-dependent death of G93A SOD-expressing motor neurons required copper and involved peroxynitrite formation. Surprisingly, motor neuron death doubled when

Cu,Zn-SOD protein was either delivered intracellularly to G93A SOD-expressing motor neurons or co-delivered with zinc-deficient SOD to nontransgenic motor neurons. These results could be rationalized by biophysical data showing that heterodimer formation of Cu,Zn-SOD with zinc-deficient SOD prevented the monomerization and subsequent aggregation of zinc-deficient SOD under thiol-reducing conditions. ALS mutant SOD was also stabilized by mutating cysteine 111 to serine, which greatly increased the toxicity of zinc-deficient SOD. Thus, stabilization of ALS mutant SOD by two different approaches augmented its toxicity to motor neurons. Taken together, these results are consistent with copper-containing zinc-deficient SOD being the elusive "partially unfolded intermediate" responsible for the toxic gain of function conferred by ALS mutant SOD.

4.860 Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications

Bao, W., Min, D., Twigg, S.M., Shackel, N.A., Warner, F.J., Yue, D.K. and McLennan, S.V.
Am. J. Physiol. Cell Physiol., ***299***, *C1212-C1219 (2010)*

CD147 is a highly glycosylated transmembrane protein that is known to play a role in regulation of many protein families. It has the unique ability to maintain functional activity in both the membrane bound state and in the soluble form. CD147 is known to play a role in regulation of matrix metalloproteinase (MMP) expression, but whether its expression is affected by the diabetic milieu is not known, and its role in regulation of monocyte MMPs in this environment has not been investigated. Therefore, in this study we investigated the effect of advanced glycation end products (AGEs) and high glucose (HG; 25 mM), on monocyte CD147 expression. Culture of THP-1 monocytes in the presence of AGEs or HG significantly increased CD147 at the gene and protein level. THP-1 cell results were confirmed using freshly isolated monocytes from human volunteers. The effect of AGEs and HG on CD147 expression was also mimicked by addition of proinflammatory cytokines. Addition of AGEs or HG also increased expression of monocyte MMP-1 and MMP-9 but not MMP-2. This increase in MMPs was significantly attenuated by inhibition of CD147 using either a small interfering RNA or an anti-CD147 antibody. Inhibition of NF-κB or addition of antibodies to either TNF-α or the receptor for AGE (RAGE) each significantly prevented in a dose-dependent manner the induction of CD147 gene and protein by AGE and also decreased MMP-1 and MMP-9. This novel result shows that AGEs can induce monocyte CD147 expression, an effect mediated by inflammatory pathways and RAGE. Because MMPs play a role in monocyte migration, inhibition of their regulator CD147 may assist in the prevention of diabetic complications, particularly those where monocyte infiltration is an early initiating event.

4.861 Isolation of periportal, midlobular, and centrilobular rat liver sinusoidal endothelial cells enables study of zonated drug toxicity

Xie, G., Wang, L., Wang, X., Wang, L. and DeLeve, L.D.
Am. J. Physiol. Gastrointest. Liver Physiol., ***299***, *G1204-G1210 (2010)*

Many liver sinusoidal endothelial cell (LSEC)-dependent processes, including drug-induced liver injury, ischemia-reperfusion injury, acute and chronic rejection, fibrosis, and the HELLP (hemolytic anemia, elevated liver enzymes, low platelet count) syndrome, may have a lobular distribution. Studies of the mechanism of this distribution would benefit from a reliable method to isolate LSEC populations from different regions. We established and verified a simple method to isolate periportal, midlobular, and centrilobular LSEC. Three subpopulations of LSEC were isolated by immunomagnetic separation on the basis of CD45 expression. Flow cytometry showed that 78.2 ± 2.3% of LSEC were CD45 positive and that LSEC could be divided into CD45 bright (28.6 ± 2.7% of total population), dim (49.6 ± 1.0%), and negative populations (21.8 ± 2.3%). Immunohistochemistry confirmed that in vivo expression of CD45 in LSEC had a lobular distribution with enhanced CD45 staining in periportal LSEC. Cell diameter, fenestral diameter, number of fenestrae per sieve plate and per cell, porosity, and lectin uptake were significantly different in the subpopulations, consistent with the literature. Endocytosis of low concentrations of the LSEC-specific substrate, formaldehyde-treated serum albumin, was restricted to CD45 bright and dim LSEC. Acetaminophen was more toxic to the CD45 dim and negative populations than to the CD45 bright population. In conclusion, CD45 is highly expressed in periportal LSEC, low in midlobular LSEC, and negative in centrilobular LSEC, and this provides an easy separation method to isolate LSEC from the three different hepatic regions. The LSEC subpopulations obtained by this method are adequate for functional studies and drug toxicity testing.

4.862 Tubular cell-enriched subpopulation of primary renal cells improves survival and augments kidney function in rodent model of chronic kidney disease

Kelley, R., Werdin, E.S., Bruce, A.T., Choudhury, S., Wallace, S.M., Ilagan, R.M., Cox, B.R., Tatsumi-Ficht, P., Rivera, E.A., Spencer, T., Rapoport, H., Wagner, B.J., Guthrie, K., Jayo, M.J., Bertram, T.A. and Presnell, S.C.

Am. J. Physiol. Renal Physiol., ***299***, *F1026-F1039 (2010)*

Established chronic kidney disease (CKD) may be identified by severely impaired renal filtration that ultimately leads to the need for dialysis or kidney transplant. Dialysis addresses only some of the sequelae of CKD, and a significant gap persists between patients needing transplant and available organs, providing impetus for development of new CKD treatment modalities. Some postulate that CKD develops from a progressive imbalance between tissue damage and the kidney's intrinsic repair and regeneration processes. In this study we evaluated the effect of kidney cells, delivered orthotopically by intraparenchymal injection to rodents 4–7 wk after CKD was established by two-step 5/6 renal mass reduction (NX), on the regeneration of kidney function and architecture as assessed by physiological, tissue, and molecular markers. A proof of concept for the model, cell delivery, and systemic effect was demonstrated with a heterogeneous population of renal cells (UNFX) that contained cells from all major compartments of the kidney. Tubular cells are known contributors to kidney regeneration in situ following acute injury. Initially tested as a control, a tubular cell-enriched subpopulation of UNFX (B2) surprisingly outperformed UNFX. Two independent studies (3 and 6 mo in duration) with B2 confirmed that B2 significantly extended survival and improved renal filtration (serum creatinine and blood urea nitrogen). The specificity of B2 effects was verified by direct comparison to cell-free vehicle controls and an equivalent dose of non-B2 cells. Quantitative histological evaluation of kidneys at 6 mo after treatment confirmed that B2 treatment reduced severity of kidney tissue pathology. Treatment-associated reduction of transforming growth factor (TGF)-β1, plasminogen activator inhibitor (PAI)-1, and fibronectin (FN) provided evidence that B2 cells attenuated canonical pathways of profibrotic extracellular matrix production.

4.863 Cdc42-mediated MTOC polarization in dendritic cells controls targeted delivery of cytokines at the immune synapse

Pulecio, J., Petrovic, J., Prete, F., Chiaruttini, G., Lennon-Dumenil, A-M., Desdouets, C., Gasman, S., Burrone, O.R. and Benvenuti, F.

J. Exp. Med., ***207(12)***, *2719-2732 (2010)*

The immune synapse (IS) forms as dendritic cells (DCs) and T cells interact in lymph nodes during initiation of adaptive immunity. Factors that contribute to the formation and maintenance of IS stability and function have been mostly studied in T cells, whereas little is known about events occurring during synapse formation in DCs. Here, we show that DCs activated by Toll-like receptor (TLR) agonists reorient the microtubule-organizing center (MTOC) toward the interacting T cell during antigen-specific synapse formation through a mechanism that depends on the Rho GTPase Cdc42. IL-12, a pivotal cytokine produced by DCs, is found enriched around the MTOC at early time points after TLR ligation and is dragged to the DC–T cell interface in antigen-specific synapses. Synaptic delivery of IL-12 induces activation of pSTAT4 and IFN-γ neosynthesis in $CD8^+$ naive T cells engaged in antigen-specific conjugates and promotes the survival of antigen-primed T cells. We propose that DC polarization increases the local concentration of proinflammatory mediators at the IS and that this represents a new mechanism by which T cell priming is controlled.

4.864 Regulation by SIRPα of dendritic cell homeostasis in lymphoid tissues

Saito, Y., Iwamura, H., Kaneko, T., Ohnishi, H., Murata, Y., Okazawa, H., Kanazawa, Y., Sato-Hashimoto, M., Kobayashi, H., Oldenborg, P-A., Naito, M., Kaneko, Y., Nojima, Y. and Matozaki, T.

Blood, ***116(18)***, *3517-3525 (2010)*

The molecular basis for regulation of dendritic cell (DC) development and homeostasis remains unclear. Signal regulatory protein α(SIRPα), an immunoglobulin superfamily protein that is predominantly expressed in DCs, mediates cell-cell signaling by interacting with CD47, another immunoglobulin superfamily protein. We now show that the number of $CD11c^{high}$ DCs (conventional DCs, or cDCs), in particular, that of $CD8^-CD4^+$ ($CD4^+$) cDCs, is selectively reduced in secondary lymphoid tissues of mice expressing a mutant form of SIRPα that lacks the cytoplasmic region. We also found that SIRPα is required intrinsically within cDCs or DC precursors for the homeostasis of splenic $CD4^+$ cDCs. Differentiation of bone marrow cells from SIRPα mutant mice into DCs induced by either macrophage-granulocyte colony-stimulating factor or Flt3 ligand in vitro was not impaired. Although the accumulation

of the immediate precursors of cDCs in the spleen was also not impaired, the half-life of newly generated splenic $CD4^+$ cDCs was markedly reduced in SIRPα mutant mice. Both hematopoietic and nonhematopoietic CD47 was found to be required for the homeostasis of $CD4^+$ cDCs and $CD8^-CD4^-$ (double negative) cDCs in the spleen. SIRPα as well as its ligand, CD47, are thus important for the homeostasis of $CD4^+$ cDCs or double negative cDCs in lymphoid tissues. The molecular basis for regulation of dendritic cell (DC) development and homeostasis remains unclear. Signal regulatory protein α(SIRPα), an immunoglobulin superfamily protein that is predominantly expressed in DCs, mediates cell-cell signaling by interacting with CD47, another immunoglobulin superfamily protein. We now show that the number of $CD11c^{high}$ DCs (conventional DCs, or cDCs), in particular, that of $CD8^-CD4^+$ ($CD4^+$) cDCs, is selectively reduced in secondary lymphoid tissues of mice expressing a mutant form of SIRPα that lacks the cytoplasmic region. We also found that SIRPα is required intrinsically within cDCs or DC precursors for the homeostasis of splenic $CD4^+$ cDCs. Differentiation of bone marrow cells from SIRPα mutant mice into DCs induced by either macrophage-granulocyte colony-stimulating factor or Flt3 ligand in vitro was not impaired. Although the accumulation of the immediate precursors of cDCs in the spleen was also not impaired, the half-life of newly generated splenic $CD4^+$ cDCs was markedly reduced in SIRPα mutant mice. Both hematopoietic and nonhematopoietic CD47 was found to be required for the homeostasis of $CD4^+$ cDCs and $CD8^-CD4^-$ (double negative) cDCs in the spleen. SIRPα as well as its ligand, CD47, are thus important for the homeostasis of $CD4^+$ cDCs or double negative cDCs in lymphoid tissues.

4.865 Trim17, a novel E3 ubiquitin-ligase, initiates neuronal apoptosis

Lassot, I., Robbins, I., Kristiansen, M., Rahmeh, R., Jaudon, F., Magiera, M.M., Mora, S., Vanhille, L., Lidkin, A., Pettmann, B., Ham, J. and Desagher, S.
*Cell Death and Differentiation, **17(12)**, 1928-1941 (2010)*

Accumulating data indicate that the ubiquitin–proteasome system controls apoptosis by regulating the level and the function of key regulatory proteins. In this study, we identified Trim17, a member of the TRIM/RBCC protein family, as one of the critical E3 ubiquitin ligases involved in the control of neuronal apoptosis upstream of mitochondria. We show that expression of Trim17 is increased both at the mRNA and protein level in several *in vitro* models of transcription-dependent neuronal apoptosis. Expression of *Trim17* is controlled by the PI3K/Akt/GSK3 pathway in cerebellar granule neurons (CGN). Moreover, the Trim17 protein is expressed *in vivo*, in apoptotic neurons that naturally die during post-natal cerebellar development. Overexpression of active Trim17 in primary CGN was sufficient to induce the intrinsic pathway of apoptosis in survival conditions. This pro-apoptotic effect was abolished in $Bax^{-/-}$ neurons and depended on the E3 activity of Trim17 conferred by its RING domain. Furthermore, knock-down of endogenous Trim17 and overexpression of dominant-negative mutants of Trim17 blocked trophic factor withdrawal-induced apoptosis both in CGN and in sympathetic neurons. Collectively, our data are the first to assign a cellular function to Trim17 by showing that its E3 activity is both necessary and sufficient for the initiation of neuronal apoptosis.

4.866 Phenotypic Heterogeneity among Tumorigenic Melanoma Cells from Patients that Is Reversible and Not Hierarchically Organized

Quintana, E., Shackleton, M., Foster, H.R., Fullen, D.R., Sabel, M.S., Johnson, T,M. and Morrison, S.J.
*Cancer Cell, **18(5)**, 510-523 (2010)*

We investigated whether melanoma is hierarchically organized into phenotypically distinct subpopulations of tumorigenic and nontumorigenic cells or whether most melanoma cells retain tumorigenic capacity, irrespective of their phenotype. We found 28% of single melanoma cells obtained directly from patients formed tumors in NOD/SCID IL2Rγ^{null} mice. All stage II, III, and IV melanomas obtained directly from patients had common tumorigenic cells. All tumorigenic cells appeared to have unlimited tumorigenic capacity on serial transplantation. We were unable to find any large subpopulation of melanoma cells that lacked tumorigenic potential. None of 22 heterogeneously expressed markers, including CD271 and ABCB5, enriched tumorigenic cells. Some melanomas metastasized in mice, irrespective of whether they arose from $CD271^-$ or $CD271^+$ cells. Many markers appeared to be reversibly expressed by tumorigenic melanoma cells.

4.867 Accelerated neuritogenesis and maturation of primary spinal motor neurons in response to nanofibers

Gertz, C., Leach, M.K., Birrell, L.K., Martin, D.C., Feldman, E.L. and Corey, J.M.
Develop. Neurobiol., ***70(8)****, 589-603 (2010)*

Neuritogenesis, neuronal polarity formation, and maturation of axons and dendrites are strongly influenced by both biochemical and topographical extracellular components. The aim of this study was to elucidate the effects of polylactic acid electrospun fiber topography on primary motor neuron development, because regeneration of motor axons is extremely limited in the central nervous system and could potentially benefit from the implementation of a synthetic scaffold to encourage regrowth. In this analysis, we found that both aligned and randomly oriented submicron fibers significantly accelerated the processes of neuritogenesis and polarity formation of individual cultured motor neurons compared to flat polymer films and glass controls, likely due to restricted lamellipodia formation observed on fibers. In contrast, dendritic maturation and soma spreading were inhibited on fiber substrates after 2 days in vitro. This study is the first to examine the effects of electrospun fiber topography on motor neuron neuritogenesis and polarity formation. Aligned nanofibers were shown to affect the directionality and timing of motor neuron development, providing further evidence for the effective use of electrospun scaffolds in neural regeneration applications.

4.868 The effect of interleukin-13 (IL-13) and interferon-γ (IFN-γ) on expression of surfactant proteins in adult human alveolar type II cells *in vitro*

Ito, Y. and Mason, R.J.
Respiratory Res., ***11****, 157-169 (2010)*

Background
Surfactant proteins are produced predominantly by alveolar type II (ATII) cells, and the expression of these proteins can be altered by cytokines and growth factors. Th1/Th2 cytokine imbalance is suggested to be important in the pathogenesis of several adult lung diseases. Recently, we developed a culture system for maintaining differentiated adult human ATII cells. Therefore, we sought to determine the effects of IL-13 and IFN-γ on the expression of surfactant proteins in adult human ATII cells *in vitro*. Additional studies were done with rat ATII cells.
Methods
Adult human ATII cells were isolated from deidentified organ donors whose lungs were not suitable for transplantation and donated for medical research. The cells were cultured on a mixture of Matrigel and rat-tail collagen for 8 d with differentiation factors and human recombinant IL-13 or IFN-γ.
Results
IL-13 reduced the mRNA and protein levels of surfactant protein (SP)-C, whereas IFN-γ increased the mRNA level of SP-C and proSP-C protein but not mature SP-C. Neither cytokine changed the mRNA level of SP-B but IFN-γ slightly decreased mature SP-B. IFN-γ reduced the level of the active form of cathepsin H. IL-13 also reduced the mRNA and protein levels of SP-D, whereas IFN-γ increased both mRNA and protein levels of SP-D. IL-13 did not alter SP-A, but IFN-γ slightly increased the mRNA levels of SP-A.
Conclusions
We demonstrated that IL-13 and IFN-γ altered the expression of surfactant proteins in human adult ATII cells *in vitro*. IL-13 decreased SP-C and SP-D in human ATII cells, whereas IFN-γ had the opposite effect. The protein levels of mature SP-B were decreased by IFN-γ treatment, likely due to the reduction in active form cathpesin H. Similarly, the active form of cathepsin H was relatively insufficient to fully process proSP-C as IFN-γ increased the mRNA levels for SP-C and proSP-C protein, but there was no increase in mature SP-C. These observations suggest that in disease states with an overexpression of IL-13, there would be some deficiency in mature SP-C and SP-D. In disease states with an excess of IFN-γ or therapy with IFN-γ, these data suggest that there might be incomplete processing of SP-B and SP-C.

4.869 Islet Transplantation Using Donors After Cardiac Death: Report of the Japan Islet Transplantation Registry

Saito, T., Gotoh, M., Satomi, S., Uemoto, S., Kenmochi, T., Itoh, T., Kuroda, Y., Yasunami, Y., Matsumoto, S. and Teraoka, S.
Transplantation, ***90(7)****, 740-747 (2010)*

Background. This report summarizes outcomes of islet transplantation employing donors after cardiac

death (DCD) between 2004 and 2007 as reported to the Japan Islet Transplantation Registry.
Method. Sixty-five islet isolations were performed for 34 transplantations in 18 patients with insulin-dependent diabetes mellitus, including two patients who had prior kidney transplantation. All but one donor (64/65) was DCD at the time of harvesting.
Results. Factors influencing criteria for islet release included duration of low blood pressure of the donor, cold ischemic time, and usage of Kyoto solution for preservation. Multivariate analysis selected usage of Kyoto solution as most important. Of the 18 recipients, 8, 4, and 6 recipients received 1, 2, and 3 islet infusions, respectively. Overall graft survival defined as C-peptide level more than or equal to 0.3 ng/mL was 76.5%, 47.1%, and 33.6% at 1, 2, and 3 years, respectively, whereas corresponding graft survival after multiple transplantations was 100%, 80.0%, and 57.1%, respectively. All recipients remained free of severe hypoglycemia while three achieved insulin independence for 14, 79, and 215 days. HbA1c levels and requirement of exogenous insulin were significantly improved in all patients.
Conclusion. Islet transplantation employing DCD can ameliorate severe hypoglycemic episodes, significantly improve HbA1c levels, sustain significant levels of C-peptide, and achieve insulin independence after multiple transplantations. Thus, DCD can be an important resource for islet transplantation if used under strict releasing criteria and in multiple transplantations, particularly in countries where heart-beating donors are not readily available.

4.870 miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice
Meng, Z., Fu, X., Chen, X., Zeng, S., Tian, Y., Jove, R., Xu, R. and Huang, W.
Hepatology, ***52(6)****, 2148-2157 (2010)*

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by interacting with the 3′ untranslated region (3′-UTR) of multiple mRNAs. Recent studies have linked miRNAs to the development of cancer metastasis. In this study, we show that miR-194 is specifically expressed in the human gastrointestinal tract and kidney. Moreover, miR-194 is highly expressed in hepatic epithelial cells, but not in Kupffer cells or hepatic stellate cells, two types of mesenchymal cells in the liver. miR-194 expression was decreased in hepatocytes cultured in vitro, which had undergone a dedifferentiation process. Furthermore, expression of miR-194 was low in liver mesenchymal-like cancer cell lines. The overexpression of miR-194 in liver mesenchymal-like cancer cells reduced the expression of the mesenchymal cell marker N-cadherin and suppressed invasion and migration of the mesenchymal-like cancer cells both in vitro and in vivo. We further demonstrated that miR-194 targeted the 3′-UTRs of several genes that were involved in epithelial-mesenchymal transition and cancer metastasis. Conclusion: These results support a role of miR-194, which is specifically expressed in liver parenchymal cells, in preventing liver cancer cell metastasis.

4.871 B7-H4 mediates inhibition of T cell responses by activated murine hepatic stellate cells
Chinnadurai, R. and Grakoui, A.
Hepatology, ***52(6)****, 2177-2185 (2010)*

Liver fibrosis is mediated by the transformation of hepatic stellate cells (HSC) from a quiescent to an activated state. To understand the role of HSC in liver immunity, we investigated the effect of this transition on T cell stimulation in vitro. Unlike quiescent HSC, activated HSC did not induce proliferation of antigen-specific T cells. Phenotypic analysis of quiescent and activated HSC revealed that activated HSC expressed the coinhibitory molecule B7-H4. Silencing B7-H4 by small interfering RNA (siRNA) in activated HSC restored the ability of T cells to proliferate, differentiate, and regain effector recall responses. Furthermore, expression of B7-H4 on HSC inhibits early T cell activation and addition of exogenous interleukin (IL)-2 reversed the T cell anergy induced by activated HSC. *Conclusion:* These studies reveal a novel role for activated HSC in the attenuation of intrahepatic T cell responses by way of expression of the coinhibitory molecule B7-H4, and may provide fundamental insight into intrahepatic immunity during liver fibrogenesis.

4.872 Calcium and Calmodulin-dependent Kinase Kinase Beta Signalling Controls Human Platelet Aggregation
Onselaer, M-B., eeckoudt, S., de Meester, C., Hue, L., Vanoverschelde, J-L., Bertrand, L., Oury, C., Beauloye, C., and Norman, S.
Circulation, ***122,*** *Abstract A17868 (2010)*

Background: Several agonists, thrombin (T), thromboxane (TXA2) and collagen (C) induce platelet activation and aggregation. Their effects are mediated by increase in intracellular calcium (Ca2+) and

remodelling of actin cytoskeleton. Ca2+/calmodulin-dependent protein kinase kinase beta (CaMKKbeta) controls actin cytoskeleton organization through AMP-activated protein kinase (AMPK) in epithelial cells, extending the role of AMPK beyond metabolism. Therefore, our hypothesis was that activation of CaMKKbeta-AMPK pathway by these agonists mediated platelet aggregation by regulating cytoskeletal targets. Methods: Blood freshly drawn was collected from healthy donors. Platelet aggregation was analyzed on the platelet rich fraction (PRP) using a lumi-aggregometer (Chrono-log). For in vitro treatments and western blot analysis, PRP was purified by the OptiPrep method. Results: 10μM STO609, a selective inhibitor of CaMKKbeta, inhibited thrombin-induced platelet aggregation (0.025U/ml T: 69±3; T + STO609: 11±4 % of aggregation, P<0.001). STO609 also reduced platelet aggregation induced by TXA2 and collagen (1 μM TXA2: 79±5; TXA2+STO609, 29±11, 2μg/ml C: 50±4; C+STO609, 19±2 % o aggregation, P<0.01). Thrombin induced an increase in acetyl CoA carboxylase (ACC) phosphorylation, a substrate of AMPK (control: 15±10; 0.1 U/ml T:81±17 A.U., P<0.05). TXA2 and collagen also increased ACC phosphorylation to a lower extent. STO609 prevented thrombin effect on ACC phosphorylatio (Control: 15±10 ; Control+STO609: 16±11; 0.1U/ml T: 81±17; T+STO609: 16±6 A.U., P<0.05). Finally, inhibition of CaMKKbeta was associated with a decrease in the phosphorylation of two AMPK cytoskeletal targets, namely myosin light chains (MLC) and vasodilatator-phosphoprotein (VASP), known to be modulated during platelet aggregation. Conclusion: Inhibition of CaMKKbeta by STO609 blocked platelet aggregation induced by thrombin, TXA2 and collagen. The molecular mechanism by which CaMKKbeta control aggregation could be a direct activation of AMPK and subsequent phosphorylation of MLC and VASP. CaMKKbeta is a potential target for new anti-platelet therapies.

4.873 Combined Transplantation of Pancreatic Islets and Adipose Tissue-Derived Stem Cells Enhances the Survival and Insulin Function of Islet Grafts in Diabetic Mice

Ohmura, Y., tanemura, M., Kawaguchi, N., Machida, T., Tanida, T., Deguchi, T., Wada, H., Kobayashi, S., Marubashi, S., Eguchi, H., Takeda, Y., Matsuura, N., Ito, T., Nagano, H., Doki, Y. and Mori, M.
*Transplantation, **90(12)**, 1366-1373 (2010)*

Background. Overcoming significant loss of transplanted islet mass is important for successful islet transplantation. Adipose tissue-derived stem cells (ADSCs) seem to have angiogenic potential and antiinflammatory properties. We hypothesized that the inclusion of ADSCs with islet transplantation should enhance the survival and insulin function of the islet graft.
Methods. Syngeneic ADSCs and allogeneic islets were transplanted simultaneously under the kidney capsules of diabetic C57BL/6J mice. Rejection of the graft was examined by measurement of blood glucose level. Revascularization and inflammatory cell infiltration were examined by immunohistochemistry.
Results. Transplantation of 400 islets only achieved normoglycemia with graft survival of 13.6±1.67 days (mean±standard deviation), whereas that of 100 or 200 allogeneic islets never reversed diabetes. Transplantation of 200 islets with 2×10^5 ADSCs reversed diabetes and significantly prolonged graft survival (13.0±5.48 days). Results of glucose tolerance tests performed on day 7 were significantly better in islets-ADSCs than islets-alone recipients. Immunohistochemical analysis confirmed the presence of insulin-stained islet grafts with well-preserved structure in islets-ADSCs transplant group. Significant revascularization (larger number of von Willebrand factor-positive cells) and marked inhibition of inflammatory cell infiltration, including $CD4^+$ and $CD8^+$ T cells and macrophages, were noted in the islets-ADSCs transplant group than islets-alone transplant group.
Conclusions. Our results indicated that cotransplantation of ADSCs with islet graft promoted survival and insulin function of the graft and reduced the islet mass required for reversal of diabetes. This innovative protocol may allow “one donor to one recipient” islet transplantation.

4.874 *In vivo* trafficking and immunostimulatory potential of an intranasally-administered primary dendritic cell-based vaccine

Vilekar, P., Awasthi, V., Lagisetty, P., King, C., Shankar, N. and Awasthi, S.
*BMC Immunol., **11**, 60-76 (2010)*

Background
Coccidioidomycosis or Valley fever is caused by a highly virulent fungal pathogen: *Coccidioides posadasii* or *immitis*. Vaccine development against *Coccidioides* is of contemporary interest because a large number of relapses and clinical failures are reported with antifungal agents. An efficient Th1 response engenders protection. Thus, we have focused on developing a dendritic cell (DC)-based vaccine for coccidioidomycosis. In this study, we investigated the immunostimulatory characteristics of an intranasal primary DC-vaccine in BALB/c mouse strain that is most susceptible to coccidioidomycosis.

The DCs were transfected nonvirally with *Coccidioides*-Ag2/PRA-cDNA. Expression of DC-markers, Ag2/PRA and cytokines were studied by flow cytometry, dot-immunoblotting and cytometric bead array methods, respectively. The T cell activation was studied by assessing the upregulation of activation markers in a DC-T cell co-culture assay. For trafficking, the DCs were co-transfected with a plasmid DNA encoding HSV1 thymidine kinase (TK) and administered intranasally into syngeneic mice. The trafficking and homing of TK-expressing DCs were monitored with positron emission tomography (PET) using ^{18}F-FIAU probe. Based on the PET-probe accumulation in vaccinated mice, selected tissues were studied for antigen-specific response and T cell phenotypes using ELISPOT and flow cytometry, respectively.
Results
We found that the primary DCs transfected with *Coccidioides*-Ag2/PRA-cDNA were of immature immunophenotype, expressed Ag2/PRA and activated naïve T cells. In PET images and subsequent biodistribution, intranasally-administered DCs were found to migrate in blood, lung and thymus; lymphocytes showed generation of T effector memory cell population (T_{EM}) and IFN-γ release.
Conclusions
In conclusion, our results demonstrate that the intranasally-administered primary DC vaccine is capable of inducing Ag2/PRA-specific T cell response. Unique approaches utilized in our study represent an attractive and novel means of producing and evaluating an autologous DC-based vaccine.

4.875 Antiproinflammatory Effects of Iodixanol (OptiPrep)-Based Density Gradient Purification on Human Islet Preparations

Mita, A., Ricordi, C., Messinger, S., Miki, A., Misawa, R., Barker, S., Molano, R.D., Haertter, R., Khan, A., Miyagawa, S., PIleggi, A., Inverardi, L., Ellejandro, R., Hering, B.J. and Ichii, H.
Cell Transplant., ***19(12)***, *1537-1546 (2010)*

Islet isolation and purification using a continuous density gradient may reduce the volume of tissue necessary for implantation into patients, therefore minimizing the risks associated with intraportal infusion in islet transplantation. On the other hand, the purification procedure might result in a decreased number of islets recovered due to various stresses such as exposure to cytokine/chemokine. While a Ficoll-based density gradient has been widely used in purification for clinical trials, purification with iodixanol (OptiPrep) has been recently reported in islet transplant series with successful clinical outcomes. The aim of the current study was to compare the effects of the purification method using OptiPrep-based and Ficoll-based density gradients. Human islet isolations were performed using a modified automated method. After the digestion phase, prepurification digests were divided into two groups and purified using a semiautomated cell processor with either a continuous Ficoll- or OptiPrep-based density gradient. The quantity, purity, viability, and cellular composition of islet preparations from each group were assessed. Cytokine/chemokine and tissue factor production from islet preparations after 48-h culture were also measured. Although islet purity, postpurification IEQ, islet recovery rate, FDA/PI, and fractional β-cell viability were comparable, β-cell mass after 48-h culture significantly improved in the OptiPrep group when compared to the Ficoll group. The production of cytokine/chemokine including IL-1β, TNF-α, IFN-γ, IL-6, IL-8, MIP-1β, MCP-1, and RANTES but not tissue factor from the OptiPrep group was significantly lower during 48-h culture after isolation. Each preparation contained the similar number of ductal cells and macrophages. Endotoxin level in both gradient medium was also comparable. The purification method using OptiPrep gradient media significantly reduced cytokine/chemokine production but not tissue factor from human islet preparations and improved β-cell survival during pretransplant culture. Our results suggest that the purification method using OptiPrep gradient media may be of assistance in increasing successful islet transplantation.

4.876 Growth temperature-dependent expression of structural variants of *Listeria monocytogenes* lipoteichoic acid

Dehus, O., Pfitzenmaier, M., Stuebs, G., Fischer, N., Schwaeble, W., Morath, S., Hartung, T., Geyer, A. and Hermann, C.
Immunobiol., ***216***, *23-31 (2011)*

Investigating the expression of lipoteichoic acid (LTA) from *Listeria monocytogenes*, we found two distinct structural variants of LTA (LTA1 and LTA2) using NMR and MS technology. While both LTA consisted of a poly-glycerophosphate backbone (differing in length) bound via a disaccharide to a diacyl-glycerol moiety, one LTA type (LTA2) possessed a second diacyl-glycerol moiety linked to the disaccharide via a phosphodiester. As examined *in vitro*, LTA2 in contrast to LTA1 failed to activate the L-ficolin dependent pathway of complement. Most interestingly, growth temperature had a strong influence

on the expression levels of LTA1 and LTA2 in the cell wall: while the amount of LTA1 was comparable, the expression of LTA2 was low when *Listeria* had grown at room temperature (ratio of LTA1 to LTA2 was 1:0.06), but increased when *Listeria* had been cultivated at 37 °C (ratio of LTA1 to LTA2 was 1:0.68). The observed shift in LTA expression, probably accompanying the switch from the saprophytic to the virulent entity, indicates an important adaptation to the different structural requirements inside the host cells.

4.877 Indole-3-carbinol inhibits hepatic stellate cells proliferation by blocking NADPH oxidase/reactive oxygen species/p38 MAPK pathway
Ping, J., Li, J-t., Liao, Z-x., Shang, L. and Wang, H.
Eur. J. Pharmocol., ***650****, 656-662 (2011)*

During the course of liver fibrogenesis, hepatic stellate cell (HSC) proliferation as well as subsequent synthesis of excessive extracellular matrix components is known to be the central events. Thus, factors that could limit HSC proliferation are potential anti-fibrotic agents. The aim of this study was to investigate the effects of indole-3-carbinol (I3C), a nutritional component derived from *Brassica* family vegetables, on the proliferation of cultured HSC and to clarify the underline molecular mechanism. HSC-T6, an activated rat HSC line, was treated with I3C (50, 100 and 200 μM) for 24 h. The results indicated that I3C can significantly inhibit HSC proliferation in a concentration-dependent manner with or without platelet-derived growth factor-BB (PDGF-BB) stimulation ($P < 0.01$). I3C could also block HSC in the G_0/G_1 phase from entering the S phase. The expressions of α-smooth muscle actin in HSC treated with I3C, were significantly decreased at levels of protein and mRNA ($P < 0.01$). In addition, the type I collagen level, cyclin D_1 and cyclin-dependent kinase 4 mRNA expressions, intracellular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and reactive oxygen species generation were significantly decreased by I3C ($P < 0.05$). We also observed that the phosphorylated p38 MAPK in HSC-T6 was inhibited by I3C in a concentration-dependent manner; however, the phosphorylated ERK1/2 was unaltered. In conclusion, I3C could inhibit the proliferation of HSC by blocking the NADPH oxidase/reactive oxygen species/p38 MAPK signal pathway. These findings suggest that dietary I3C might play a novel role in prevention and treatment of chronic liver diseases.

4.878 Hyperbranched polyglycerols on the nanometer and micrometer scale
Steinhilber, D., Seiffert, S., Heyman, J.A., Paulus, F., Weitz, D.A. and Haag, r.
Biomaterials, ***32****, 1311-1316 (2011)*

We report the preparation of polyglycerol particles on different length scales by extending the size of hyperbranched polyglycerols (3 nm) to nanogels (32 nm) and microgels (140 and 220 μm). We use miniemulsion templating for the preparation of nanogels and microfluidic templating for the preparation of microgels, which we obtain through a free-radical polymerization of hyperbranched polyglycerol decaacrylate and polyethylene glycol-diacrylate. The use of mild polymerization conditions allows yeast cells to be encapsulated into the resultant microgels with cell viabilities of approximately 30%.

4.879 Circulating cytokines and growth factors in professional soccer players: correlation with *in vitro*-induced motor neuron death
De Paola, M., Visconti, L., Vianello, E., Mattana, F., Banfi, G., Corsi, M.M., Beghi, E. and Mennini, T.
Eur. J. Neurol., ***18****, 85-92 (2011)*

Background: Professional soccer players are susceptible to amyotrophic lateral sclerosis. Strenuous physical activity has been associated with persistent inflammatory conditions and elevation of systemic cytokine levels, which could contribute to the vulnerability of these athletes. To investigate changes induced by playing soccer in the systemic profiles of growth factors and of the principal cytokines involved in the inflammatory response, we compared the serum concentrations of these factors in Italian professional soccer players and sedentary subjects. We also investigated the effects of the sera on primary cultured motor neurons in relation to their cytokine and growth factor content.
Methods: Serum concentrations of cytokines and growth factors were measured by a biochip array analyzer. Neurotoxicity of sera was assessed by immunocytochemical assays in primary motor neuron cultures from mouse embryos.
Results: Circulating levels of interleukin-8, tumor necrosis factor-alpha and interleukin-4 were lower in soccer players than controls. However, the viability of primary cultured mouse motor neurons treated with sera from the two groups did not differ significantly. Vascular endothelial growth factor (VEGF)

independently emerged as a systemic protective factor for motor neurons.
Conclusions: We found significant alterations in circulating pro-inflammatory cytokines in Italian professional soccer players, showing an unbalanced inflammatory condition in these subjects. VEGF was a protective serum factor affecting motor neuron survival.

4.880 Mechanisms by Which Chronic Ethanol Feeding Limits the Ability of Dendritic Cells to Stimulate T-Cell Proliferation
Fan, J., Edsen-Moore, R., Turner, L.E., Cook, R.T., Legge, K.L., Waldschmidt, T.J. and Schkuter, A.J.
Alcoholism: Clin. Exp. Res., ***35(1)***, *47-59 (2011)*

Background: As initiators of immune responses, dendritic cells (DCs) are required for antigen (Ag)-specific activation of naïve T cells in the defense against infectious agents. The increased susceptibility to and severity of infection seen in chronic alcoholics could be because of impaired DCs initiation of naïve T-cell responses. Specifically, these DCs may not provide adequate Signals 1 (Ag presentation), 2 (costimulation), or 3 (cytokine production) to these T cells.
Methods: Using the Meadows-Cook murine model of chronic alcohol abuse, the ability of ethanol (EtOH)-exposed DCs to stimulate T-cell proliferation, acquire and process Ag, express costimulatory molecules, and produce inflammatory cytokines was assessed.
Results: Normal naïve T cells primed by EtOH-exposed DCs showed decreased proliferation in vitro and in vivo, compared to water-fed control mice. These EtOH-exposed DCs, after activation by CpG or tumor necrosis factor alpha (TNFα), were less able to upregulate costimulatory molecules CD40, CD80, or CD86, and produced less IL-12 p40, TNFα, and IFNα than DCs from water-fed mice. TLR9 and TNF receptor expression were also reduced in/on EtOH-exposed DCs. No evidence of defective Ag acquisition or processing as a result of EtOH feeding was identified.
Conclusions: Inadequate proliferation of normal T cells following stimulation by EtOH-exposed DCs is likely a result of diminished Signal 2 and Signal 3. Lack of adequate inflammatory stimulation of EtOH-exposed DCs because of diminished receptors for inflammatory mediators appears to be at least partially responsible for their dysfunction. These findings provide a mechanism to explain increased morbidity and mortality from infectious diseases in alcoholics and suggest targets for therapeutic intervention.

4.881 SOD1 targeted to the mitochondrial intermembrane space prevents motor neuropathy in the Sod1 knockout mouse
Fischer, L.R., Igoudjil, A., Magrane, J., Li, Y., Hansen, J.M., Manfredi, g. and Glass, J.D.
Brain, ***134***, *196-209 (2011)*

Motor axon degeneration is a critical but poorly understood event leading to weakness and muscle atrophy in motor neuron diseases. Here, we investigated oxidative stress-mediated axonal degeneration in mice lacking the antioxidant enzyme, Cu,Zn superoxide dismutase (SOD1). We demonstrate a progressive motor axonopathy in these mice and show that Sod1−/− primary motor neurons extend short axons in vitro with reduced mitochondrial density. Sod1−/− neurons also show oxidation of mitochondrial—but not cytosolic—thioredoxin, suggesting that loss of SOD1 causes preferential oxidative stress in mitochondria, a primary source of superoxide in cells. SOD1 is widely regarded as the cytosolic isoform of superoxide dismutase, but is also found in the mitochondrial intermembrane space. The functional significance of SOD1 in the intermembrane space is unknown. We used a transgenic approach to express SOD1 exclusively in the intermembrane space and found that mitochondrial SOD1 is sufficient to prevent biochemical and morphological defects in the Sod1−/− model, and to rescue the motor phenotype of these mice when followed to 12 months of age. These results suggest that SOD1 in the mitochondrial intermembrane space is fundamental for motor axon maintenance, and implicate oxidative damage initiated at mitochondrial sites in the pathogenesis of motor axon degeneration.

4.882 Marek's Disease Virus Type 1 MicroRNA miR-M3 Suppresses Cisplatin-Induced Apoptosis by Targeting SMAD2 of the Transforming Growth Factor Beta Signal Pathway
Xu, S., Xue, C., Li, J., Bi, Y. and Cao, Y.
J. Virol., ***85(1)***, *276-285 (2011)*

Viruses cause about 15% of the cancers that are still the leading causes of human mortality. The discovery of viral oncogenes has enhanced our understanding of viral oncogenesis. However, the underlying molecular mechanisms of virus-induced cancers are complex and require further investigation. The present study has attempted to investigate the effects of the microRNAs (miRNAs) encoded by Marek's disease virus 1 (MDV1), a chicken herpesvirus causing acute T-cell lymphomas and solid visceral tumors in

chickens, on anti-cancer drug-induced apoptosis and identify the targets of the miRNAs. The results showed that of the total 14 miRNAs encoded by MDV1, MDV1-miR-M3 significantly promoted cell survival under treatment with cisplatin, a widely used chemotherapy drug. MDV1-miR-M3 suppressed cisplatin-induced apoptosis by directly downregulating expression at the protein but not the mRNA level of Smad2, a critical component in the transforming growth factor β signal pathway. Our data suggest that latent/oncogenic viruses may encode miRNAs to directly target cellular factors involved in antiviral processes including apoptosis, thus proactively creating a cellular environment beneficial to viral latency and oncogenesis. Furthermore, the knowledge of the apoptosis resistance conferred by viral miRNAs has great practical implications for improving the efficacy of chemotherapies for treating cancers, especially those induced by oncogenic viruses.

4.883 Effective Caspase Inhibition Blocks Neutrophil Apoptosis and Reveals Mcl-1 as Both a Regulator and a Target of Neutrophil Caspase Activation
Wardle, D.J., Burgon, J., Sabroe, I., Bingle, C.D., Whyte, M.K.B. and Renshaw, S.A.
*PloSOne, **6(1)**, e15768 (2011)*

Human tissue inflammation is terminated, at least in part, by the death of inflammatory neutrophils by apoptosis. The regulation of this process is therefore key to understanding and manipulating inflammation resolution. Previous data have suggested that the short-lived pro-survival Bcl-2 family protein, Mcl-1, is instrumental in determining neutrophil lifespan. However, Mcl-1 can be cleaved following caspase activity, and the possibility therefore remains that the observed fall in Mcl-1 levels is due to caspase activity downstream of caspase activation, rather than being a key event initiating apoptosis in human neutrophils. We demonstrate that apoptosis in highly purified neutrophils can be almost completely abrogated by caspase inhibition with the highly effective di-peptide caspase inhibitor, Q-VD.OPh, confirming the caspase dependence of neutrophil apoptosis. Effective caspase inhibition does not prevent the observed fall in Mcl-1 levels early in ultrapure neutrophil culture, suggesting that this fall in Mcl-1 levels is not a consequence of neutrophil apoptosis. However, at later timepoints, declines in Mcl-1 can be reversed with effective caspase inhibition, suggesting that Mcl-1 is both an upstream regulator and a downstream target of caspase activity in human neutrophils.

4.884 Regulation of Neutrophil Senescence by MicroRNAs
Ward, J.R., Heath, P.R., Catto, J.W., Whyte, M.K.B., Milo, M. and renshaw, S.A.
*PlosOne, **6(1)**, e15810 (2011)*

Neutrophils are rapidly recruited to sites of tissue injury or infection, where they protect against invading pathogens. Neutrophil functions are limited by a process of neutrophil senescence, which renders the cells unable to respond to chemoattractants, carry out respiratory burst, or degranulate. In parallel, aged neutrophils also undergo spontaneous apoptosis, which can be delayed by factors such as GMCSF. This is then followed by their subsequent removal by phagocytic cells such as macrophages, thereby preventing unwanted inflammation and tissue damage. Neutrophils translate mRNA to make new proteins that are important in maintaining functional longevity. We therefore hypothesised that neutrophil functions and lifespan might be regulated by microRNAs expressed within human neutrophils. Total RNA from highly purified neutrophils was prepared and subjected to microarray analysis using the Agilent human miRNA microarray V3. We found human neutrophils expressed a selected repertoire of 148 microRNAs and that 6 of these were significantly upregulated after a period of 4 hours in culture, at a time when the contribution of apoptosis is negligible. A list of predicted targets for these 6 microRNAs was generated from http://mirecords.biolead.org and compared to mRNA species downregulated over time, revealing 83 genes targeted by at least 2 out of the 6 regulated microRNAs. Pathway analysis of genes containing binding sites for these microRNAs identified the following pathways: chemokine and cytokine signalling, Ras pathway, and regulation of the actin cytoskeleton. Our data suggest that microRNAs may play a role in the regulation of neutrophil senescence and further suggest that manipulation of microRNAs might represent an area of future therapeutic interest for the treatment of inflammatory disease.

4.885 CpG Promotes Cross-Presentation of Dead Cell-Associated Antigens by Pre-CD8α$^+$ Dendritic Dells
De Brito, C., Tomkowiak, M., Ghittoni, R., Caux, C., Leverrier, Y. and Marvel, J.
*J. Immunol., **186**, 1503-1511 (2011)*

Cross-presentation of cell-associated Ags by dendritic cells (DC) plays an important role in immunity. DC in lymphoid tissues are short lived, being continuously replaced by precursors that proliferate and differentiate locally. Paradoxically, although TLR ligands promote immune responses and stimulate DC

replenishment, they impair the cross-priming capacity of terminally differentiated splenic CD8α$^+$ DC, the major subset involved in cross-priming. In this study, we have investigated the cross-presentation capacity of newly generated murine DC and especially immediate precursors of CD8α$^+$ DC. We show that these DC do not cross-present Ag from dead cells unless stimulated by TLR ligands before Ag capture. TLR ligand CpG induced the expression of costimulatory molecules required for CD8 T cell activation but also regulated the intracellular mechanisms of cross-presentation such as Ag degradation rates without regulating Ag uptake. GM-CSF, an inflammatory cytokine associated with infections, also promoted cross-presentation acquisition by pre-CD8α$^+$ DC and synergized with TLR9 ligand. The concept that TLR ligands as well as inflammatory cytokines promote the acquisition of cross-presenting properties by pre-CD8α$^+$ DC has important implications during immune responses and when considering the use of these cells for vaccination.

4.886 Rhesus macaque θ-defensin isoforms: expression, antimicrobial activities, and demonstration of a prominent role in neutrophil granule microbicidal activities
Tongaonkar, P., Tran, P., Roberts, K., Schaal, J., Ösapay, G., Tran, D., Ouellette, A.J. and Selsted, M.E.
J. Leukoc. Biol., ***89(2)***, *283-290 (2011)*

Mammalian defensins are cationic, antimicrobial peptides that play a central role in innate immunity. The peptides are composed of three structural subfamilies: α-, β-, and θ-defensins. θ-Defensins are macrocyclic octadecapeptides expressed only in Old World monkeys and Orangutans and are produced by the pair-wise, head-to-tail splicing of nonapeptides derived from their respective precursors. The existence of three active θ-defensin genes predicts that six different RTDs (1–6) are produced in this species. In this study, we isolated and quantified RTDs 1–6 from the neutrophils of 10 rhesus monkeys. RTD-1 was the most abundant θ-defensin, constituting ~ 50% of the RTD content; total RTD content varied by as much as threefold between animals. All peptides tested were microbicidal at ~ 1 μM concentrations. The contribution of θ-defensins to macaque neutrophil antimicrobial activity was assessed by analyzing the microbicidal properties of neutrophil granule extracts after neutralizing θ-defensin content with a specific antibody. θ-Defensin neutralization markedly reduced microbicidal activities of the corresponding extracts. Macaque neutrophil granule extracts had significantly greater microbicidal activity than those of human neutrophils, which lack θ-defensins. Supplementation of human granule extracts with RTD-1 markedly increased the microbicidal activity of these preparations, further demonstrating a prominent microbicidal role for θ-defensins.

4.887 Neuroprotective signaling pathways are modulated by adenosine in the anoxia tolerant turtle
Nayak, G.H., Prentice, H.M. and Milton, S.L.
J. Cerebral Blood Flow & Metabolism, ***31(2)***, *467-475 (2011)*

Cumulative evidence shows a protective role for adenosine A1 receptors (A1R) in hypoxia/ischemia; A1R stimulation reduces neuronal damage, whereas blockade exacerbates damage. The signal transduction pathways may involve the mitogen-activated protein kinase (MAPK) pathways and serine/threonine kinase (AKT), with cell survival depending on the timing and degree of upregulation of these cascades as well as the balance between pro-survival and pro-death pathways. Here, we show *in vitro* that extracellular signal-regulated kinase (ERK1/2) and phosphatidylinositol 3-kinase (PI3-K/AKT) activation is dependent on A1R stimulation, with further downstream effects that promote neuronal survival. Phosphorylated ERK1/2 (p-ERK) and AKT (p-AKT) as well as Bcl-2 are upregulated in anoxic neuronally enriched primary cultures from turtle brain. This native upregulation is further increased by the selective A1R agonist 2-chloro-*N*-cyclopentyladenosine (CCPA), whereas the selective antagonist 8-cyclopentyl-1,3-dihydropylxanthine (DPCPX) decreases p-ERK and p-AKT expression. Conversely, A1R antagonism resulted in increases in phosphorylated JNK (p-JNK), p38 (p-p38), and Bax. As pathological and adaptive changes occur simultaneously during anoxia/ischemia in mammalian neurons, the turtle provides an alternative model to analyze protective mechanisms in the absence of evident pathologies.

4.888 Rapid brain-derived neurotrophic factor-dependent sequestration of amygdala and hippocampal $GABA_A$ receptors via different tyrosine receptor kinase B-mediated phosphorylation pathways
Mou, L., heldt, S.A. and Ressler, K.J.
Neuroscience, ***176***, *72-85 (2011)*

During the consolidation of fear memory, it has been shown that $GABA_A$ receptors ($GABA_AR$) are rapidly

downregulated in amygdala. This rapid decrease in $GABA_AR$ functioning may permit transient hyperexcitablity, contributing to cellular mechanisms of memory consolidation. Memory consolidation also requires brain-derived neurotrophic factor (BDNF) activation of tyrosine receptor kinase B (TrkB) receptors in the amygdala and hippocampus. We hypothesized that rapid internalization of $GABA_AR\alpha1$ is mediated via TrkB activation of PKA and PKC-dependent processes. Primary neuronal cell cultures, from postnatal day 14–21 mouse amygdala and hippocampus, were analyzed with immunofluorescence using cell-surface, whole-cell permeabilization, and antibody internalization techniques, as well as with ^3H-muscimol binding assays. In both hippocampal and amygdala cultures, we found a >60% reduction in surface $GABA_AR\alpha1$ within 5 min of BDNF treatment. Notably, the rapid decrease in surface $GABA_AR\alpha1$ was confirmed biochemically using surface biotinylation assays followed by western blotting. This rapid effect was accompanied by TrkB phosphorylation and increased internal $GABA_AR\alpha1$ immunofluorescence, and was blocked by k252a, a broad-spectrum tyrosine kinase antagonist. To further demonstrate TrkB specificity, we used previously characterized $TrkB^{F616A}$ mice, in which the highly selective TrkB-mutant specific antagonist, 1NMPP1, prevented the BDNF-dependent $GABA_AR\alpha1$ internalization. In hippocampus, we found both PKA and PKC inhibition, using Rp-8-Br-cAMP and Calphostin C, respectively, blocked $GABA_AR\alpha1$ internalization, whereas inhibition of MAPK (U0126) and PI3K (LY294002) did not prevent rapid internalization. By contrast in amygdala cultures, Rp-8-Br-cAMP had no effect. Together, these data suggest that rapid $GABA_AR$ internalization during memory consolidation is BDNF-TrkB dependent. Further, it appears that hippocampal $GABA_AR$ internalization is PKA and PKC dependent, while it may be primarily PKC dependent in amygdala, implying differential roles for TrkB-dependent kinase activation in BDNF-dependent memory formation.

4.889 Characterization of Dendritic Cells Subpopulations in Skin and Afferent Lymph in the Swine Model
Marquet, F., Bonneau, M., Pascale, F., Urien, C., Kang, C., Schwartz-Cornil, I. and Bertho, N.
PloSOne, ***6(1)***, *e16320 (2011)*

Transcutaneous delivery of vaccines to specific skin dendritic cells (DC) subsets is foreseen as a promising strategy to induce strong and specific types of immune responses such as tolerance, cytotoxicity or humoral immunity. Because of striking histological similarities between human and pig skin, pig is recognized as the most suitable model to study the cutaneous delivery of medicine. Therefore improving the knowledge on swine skin DC subsets would be highly valuable to the skin vaccine field. In this study, we showed that pig skin DC comprise the classical epidermal langerhans cells (LC) and dermal DC (DDC) that could be divided in 3 subsets according to their phenotypes: (1) the $CD163^{neg}/CD172a^{neg}$, (2) the $CD163^{high}CD172a^{pos}$ and (3) the $CD163^{low}CD172a^{pos}$ DDC. These subtypes have the capacity to migrate from skin to lymph node since we detected them in pseudo-afferent lymph. Extensive phenotyping with a set of markers suggested that the $CD163^{high}$ DDC resemble the antibody response-inducing human skin DC/macrophages whereas the $CD163^{neg}CD172^{low}$ DDC share properties with the $CD8^+$ T cell response-inducing murine skin $CD103^{pos}$ DC. This work, by showing similarities between human, mouse and swine skin DC, establishes pig as a model of choice for the development of transcutaneous immunisation strategies targeting DC.

4.890 p75NTR Regulates Aβ Deposition by Increasing Aβ Production But Inhibiting Aβ Aggregation with Its Extracellular Domain
Wang, Y-J., Wang, X., Lu, J-J., Li, Q-X., Gao, C-Y., Liu, X-H., Sun, Y., Yang, M., Lim, Y., Evin, G., Zhong, J-H., Masters, C. and Zhou, X-F.
J. Neurosci., ***31(6)***, *2292-2304 (2011)*

Accumulation of toxic amyloid-β (Aβ) in the cerebral cortex and hippocampus is a major pathological feature of Alzheimer's disease (AD). The neurotrophin receptor p75NTR has been proposed to mediate Aβ-induced neurotoxicity; however, its role in the development of AD remains to be clarified. The p75NTR/ExonIII–/– mice and APPSwe/PS1dE9 mice were crossed to generate transgenic AD mice with deletion of p75NTR gene. In APPSwe/PS1dE9 transgenic mice, p75NTR expression was localized in the basal forebrain neurons and degenerative neurites in neocortex, increased with aging, and further activated by Aβ accumulation. Deletion of the p75NTR gene in APPSwe/PS1dE9 mice reduced soluble Aβ levels in the brain and serum, but increased the accumulation of insoluble Aβ and Aβ plaque formation. There was no change in the levels of amyloid precursor protein (APP) and its proteolytic derivatives, or α-, β-, and γ-secretase activities, or in levels of BACE1, neprilysin (NEP), and insulin-degrading enzyme (IDE) proteins. Aβ production by cortical neurons of APPSwe/PS1dE9 mice was reduced by deletion of p75NTR gene *in vitro*. Recombinant extracellular domain of p75NTR attenuated the oligomerization and fibrillation of synthetic $A\beta_{42}$ peptide *in vitro*, and reduced local Aβ plaques after hippocampus injection *in vivo*. In

addition, deletion of p75NTR attenuated microgliosis but increased the microhemorrhage profiles in the brain. The deletion of p75NTR did not significantly change the cognitive function of the mice up to the age of 9 months. Our data suggest that p75NTR plays a critical role in regulating Aβ levels by both increasing Aβ production and attenuating its aggregation, and they caution that a therapeutic intervention simply reducing p75NTR may exacerbate AD pathology.

4.891 Lung Effector Memory and Activated CD4+ T Cells Display Enhanced Proliferation in Surfactant Protein A-Deficient Mice during Allergen-Mediated Inflammation

Pastva, A.M., Mukherjee, S., Giamberardino, C., Hsia, B., Lo, B., Sempowski, G.D. and Wright, J.R.
J. Immunol., ***186***, *2842-2849 (2011)*

Although many studies have shown that pulmonary surfactant protein (SP)-A functions in innate immunity, fewer studies have addressed its role in adaptive immunity and allergic hypersensitivity. We hypothesized that SP-A modulates the phenotype and prevalence of dendritic cells (DCs) and $CD4^+$ T cells to inhibit Th2-associated inflammatory indices associated with allergen-induced inflammation. In an OVA model of allergic hypersensitivity, SP-$A^{-/-}$ mice had greater eosinophilia, Th2-associated cytokine levels, and IgE levels compared with wild-type counterparts. Although both OVA-exposed groups had similar proportions of $CD86^+$ DCs and $Foxp3^+$ T regulatory cells, the SP-$A^{-/-}$ mice had elevated proportions of $CD4^+$ activated and effector memory T cells in their lungs compared with wild-type mice. Ex vivo recall stimulation of $CD4^+$ T cell pools demonstrated that cells from the SP-$A^{-/-}$ OVA mice had the greatest proliferative and IL-4–producing capacity, and this capability was attenuated with exogenous SP-A treatment. Additionally, tracking proliferation in vivo demonstrated that $CD4^+$ activated and effector memory T cells expanded to the greatest extent in the lungs of SP-$A^{-/-}$ OVA mice. Taken together, our data suggested that SP-A influences the prevalence, types, and functions of $CD4^+$ T cells in the lungs during allergic inflammation and that SP deficiency modifies the severity of inflammation in allergic hypersensitivity conditions like asthma.

4.892 Similar Islet Function in Islet Allotransplant and Autotransplant Recipients, Despite Lower Islet Mass in Autotransplants

Bellin, M.D., Sutherland, D.E.R., Beilman, G:J., Hong-Mcatee, I., Balamurugan, A.N., Hering, B.J. and Moran, A.
T*ransplantation*, ***91(3)***, *367-372 (2011)*

Background. Despite high initial rates of insulin independence after islet allotransplant for type 1 diabetes, long-term islet function is suboptimal. Possible contributing factors include autoimmune recurrence, alloimmune rejection, or immunosuppressant medication toxicity. In contrast, islet autografts, infused at the time of pancreatectomy for chronic pancreatitis, are not subject to these variables. Islet function was compared in autograft and allograft recipients.
Methods. Eight autograft and eight allograft recipients, insulin independent or requiring minimal insulin, were matched for similar duration posttransplant (mean 2.1±1.2 years). Eleven healthy control subjects were also enrolled. Subjects underwent oral and intravenous glucose tolerance testing and arginine stimulation testing.
Results. Age, gender, body mass index, duration posttransplant, and hemoglobin A_{1c} levels were similar between groups. Glucose tolerance was worse in transplant recipients compared with controls. Alloislet recipients received significantly more islet equivalents per kg body weight (IE/kg) than autograft recipients (9958±6229 IE/kg vs. 4589±1232 IE/kg, P=0.03). However, the glycemic response to oral glucose tolerance testing, the acute insulin response to glucose, and the acute insulin response to arginine did not differ significantly between islet allograft and autograft recipients.
Conclusions. Insulin secretion and glucose excursion were similar in allograft and autograft recipients, despite the latter group receiving less than half as many islets. Better preservation of islet mass in the autograft setting is likely related to the lack of autoimmunity, alloimmunity, and immunosuppressive drug toxicity, highlighting the potential for better outcomes in islet allotransplant for type 1 diabetes mellitus with refinements in immunosuppression.

4.893 Migration and immunological reaction after the administration of αGalCer-pulsed antigen-presenting cells into the submucosa of patients with head and neck cancer

Kurosaki, M., Horiguchi, S., Yamasaki, K., Uchida, Y., Motohashi, S., Nakayama, T., Sugimoto, A. and Okamoto, Y.
Cancer Immunol. Immunother., ***60(2)***, *207-215 (2011)*

Background
Antigen-presenting cells (APCs) play a crucial role in the induction of immune responses. However, the optimal administration route of tumor-specific APCs for inducing effective immunological responses via cancer immunotherapy remains to be elucidated. Human NKT cells are known to have strong anti-tumor activities and are activated by the specific ligand, namely, α-galactosylceramide (αGalCer).
Methods
Seventeen patients with head and neck squamous cell carcinoma (HNSCC) were enrolled in this study. Patients received an injection of αGalCer-pulsed APCs into the nasal, or the oral floor submucosa. Then total body image and single photon emission computed tomography (SPECT) images were examined. The immunological responses including the number of peripheral blood NKT cells, anti-tumor activities and the $CD4^+$ $CD25^{high}$ $Foxp3^+$ T cells (Tregs) induced following APCs were also compared.
Results
APCs injected into the nasal submucosa quickly migrated to the lateral lymph nodes and those injected into the oral floor submucosa dominantly migrated to the submandibular nodes rather than the lateral lymph nodes. An increase in the absolute number of NKT cells and the IFN-γ producing cells was observed in peripheral blood after injection of the APCs into the nasal submucosa, however, these anti-tumor activities were not detected and the increased frequency of Treg cells were observed after administration into oral floor.
Conclusions
These results indicate that a different administration route of APCs has the potential to bring a different immunological reaction. The submucosal administration of αGalCer into the oral submucosa tends to induce immunological suppression.

4.894 COX-2 Inhibition and Inhibition of Cytosolic Phospholipase A2 Increase CD36 Expression and Foam Cell Formation in THP-1 Cells

Anwar, K., Voloshyna, I., Littlefield, M.J., Carsons, S.E., Wirkowski, P.A., jaber, N.L., Sohn, A., Eapen, S. and Reiss, A.B.

*Lipids, 4**6(2)**, 131-142 (2011)*

Cardiovascular safety of cyclooxygenase (COX)-2-selective inhibitors and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) is of worldwide concern. COX-2 inhibitors and NSAIDs act by inhibiting arachidonic acid metabolism to prostaglandins. They confer a cardiovascular hazard manifested as an elevated risk of myocardial infarction. Mechanisms underlying these cardiovascular effects are uncertain. Here we determine whether interference with cytosolic phospholipase A2 (cPLA-2) or COX-2 through pharmacologic blockade or silencing RNA impacts expression of scavenger receptor CD36 and scavenger receptor A, both involved in cholesterol uptake in monocytes and macrophages. THP-1 human monocytes and human peripheral blood mononuclear cells were exposed to celecoxib, a COX-2 selective inhibitor currently in clinical use, and to arachidonyl trifluoromethyl ketone (AACOCF3), an arachidonic acid analog that selectively inhibits cPLA-2. Celecoxib and AACOCF3 each upregulated expression of CD36, but not scavenger receptor A, as determined by quantitative PCR and immunoblotting. Silencing of cPLA-2 or COX-2 had comparable effects to pharmacologic treatments. Oil red O staining revealed a profound increase in foam cell transformation of THP-1 macrophages exposed to either celecoxib or AACOCF3 (both 25 μM), supporting a role for the COX pathway in maintaining macrophage cholesterol homeostasis. Demonstration of disrupted cholesterol balance by AACOCF3 and celecoxib provides further evidence of the possible mechanism by which COX inhibition may promote lipid overload leading to atheromatous lesion formation and increased cardiovascular events.

4.895 Loss of Caspase-2-dependent Apoptosis Induces Autophagy after Mitochondrial Oxidative Stress in Primary Cultures of Young Adult Cortical Neurons

Tiwari, M., Lopez-Cruzaqn, M., Morgan, W.W. and Herman, B.

*J. Biol. Chem., **286(10)**, 8493-8506 (2011)*

Mitochondrial dysfunctions have been associated with neuronal apoptosis and are characteristic of neurodegenerative conditions. Caspases play a central role in apoptosis; however, their involvement in mitochondrial dysfunction-induced neuronal apoptosis remains elusive. In the present report using rotenone, a complex I inhibitor that causes mitochondrial dysfunction, we determined the initiator caspase and its role in cell death in primary cultures of cortical neurons from young adult mice (1–2 months old). By pretreating the cells with a cell-permeable, biotinylated pan-caspase inhibitor that irreversibly binds to and traps the active caspase, we identified caspase-2 as an initiator caspase activated in rotenone-treated primary neurons. Loss of caspase-2 inhibited rotenone-induced apoptosis; however, these neurons

underwent a delayed cell death by necrosis. We further found that caspase-2 acts upstream of mitochondria to mediate rotenone-induced apoptosis in neurons. The loss of caspase-2 significantly inhibited rotenone-induced activation of Bid and Bax and the release of cytochrome *c* and apoptosis inducing factor from mitochondria. Rotenone-induced downstream activation of caspase-3 and caspase-9 were also inhibited in the neurons lacking caspase-2. Autophagy was enhanced in caspase-2 knock-out neurons after rotenone treatment, and this response was important in prolonging neuronal survival. In summary, the present study identifies a novel function of caspase-2 in mitochondrial oxidative stress-induced apoptosis in neurons cultured from young adult mice.

4.896 The N-methyl-D-aspartate-evoked cytoplasmic calcium increase in adult rat dorsal root ganglion neuronal somata was potentiated by substance P pretreatment in a protein kinase C-dependent manner

Castillo, C., Norcini, M., Baquero-Buitrago, J., levavic, D., Medina, R., Montoya-Gacharna. J.V., Blanck, T.J.J., Dubois, M. and Recio-Pinto, E.
Neuroscience, ***177***, *308-320 (2011)*

The involvement of substance P (SP) in neuronal sensitization through the activation of the neurokinin-1-receptor (NK1r) in postsynaptic dorsal horn neurons has been well established. In contrast, the role of SP and NK1r in primary sensory dorsal root ganglion (DRG) neurons, in particular in the soma, is not well understood. In this study, we evaluated whether SP modulated the NMDA-evoked transient increase in cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) in the soma of dissociated adult DRG neurons. Cultures were treated with nerve growth factor (NGF), prostaglandin E_2 (PGE_2) or both NGF+PGE_2. Treatment with NGF+PGE_2 increased the percentage of N-methyl-D-aspartate (NMDA) responsive neurons. There was no correlation between the percentage of NMDA responsive neurons and the level of expression of the NR1 and NR2B subunits of the NMDA receptor or of the NK1r. Pretreatment with SP did not alter the percentage of NMDA responsive neurons; while it potentiated the NMDA-evoked $[Ca^{2+}]_{cyt}$ transient by increasing its magnitude and by prolonging the period during which small- and some medium-sized neurons remained NMDA responsive. The SP-mediated potentiation was blocked by the SP-antagonist ([D-Pro^4, D-$Trp^{7,9}$]-SP (4–11)) and by the protein kinase C (PKC) blocker bisindolylmaleimide I (BIM); and correlated with the phosphorylation of PKCε. The Nk1r agonist [Sar^9, $Met(O_2)^{11}$]-SP (SarMet-SP) also potentiated the NMDA-evoked $[Ca^{2+}]_{cyt}$ transient. Exposure to SP or SarMet-SP produced a rapid increase in the labeling of phosphorylated-PKCε. In none of the conditions we detected phosphorylation of the NR2B subunit at Ser-1303. Phosphorylation of the NR2B subunit at Tyr1472 was enhanced to a similar extent in cells exposed to NMDA, SP or NMDA+SP, and that enhancement was blocked by BIM. Our findings suggest that NGF and PGE_2 may contribute to the injury-evoked sensitization of DRG neurons in part by enhancing their NMDA-evoked $[Ca^{2+}]_{cyt}$ transient in all sized DRG neurons; and that SP may further contribute to the DRG sensitization by enhancing and prolonging the NMDA-evoked increase in $[Ca^{2+}]_{cyt}$ in small- and medium-sized DRG neurons.

4.897 Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages

Harada, N., Kanayama, M., Maruyama, A., Yoshida, A., Tazumi, K., Hosoya, T., Mimura, J., Toki, T., Maher, J.M., Yamamoto, M. and Itoh, K.
Arch. Biochem. Biophys., ***508***, *101-109 (2011)*

Iron is an essential element of hemoglobin, and efficient iron recycling from senescent erythrocytes by splenic macrophages is required for erythrocyte hemoglobin synthesis during erythropoiesis. Ferroportin 1 (Fpn1) is the sole iron exporter in mammals, and it also regulates iron reutilization. In this study, we demonstrated genetically that a redox-sensitive transcription factor, Nrf2, regulates *Fpn*1 mRNA expression in macrophages. Nrf2 activation by several electrophilic compounds commonly resulted in the upregulation of *Fpn*1 mRNA in bone marrow-derived and peritoneal macrophages obtained from wild-type mice but not from *Nrf*2 knockout mice. Further, Nrf2 activation enhanced iron release from the J774.1 murine macrophage cell line. Previous studies showed that inflammatory stimuli, such as LPS, downregulates macrophage Fpn1 by transcriptional and hepcidin-mediated post-translational mechanisms leading to iron sequestration by macrophages. We showed that two Nrf2 activators, diethyl maleate and sulforaphane (SFN; a natural Nrf2 activator found in broccoli), restored the LPS-induced suppression of *Fpn*1 mRNA in human and mouse macrophages, respectively. Furthermore, SFN counteracted the LPS-induced increase of *Hepcidin* mRNA by an Nrf2-independent mechanism in mouse peritoneal macrophages. These results demonstrate that Nrf2 regulates iron efflux from macrophages through *Fpn*1 gene transcription and suggest that Nrf2 may control iron metabolism during inflammation.

4.898 Scavenger receptor CD36 mediates uptake of high density lipoproteins in mice and by cultured cells
Brundert, M., Heeren, J., Merkel, M., Carambia, A., herkel, J., Groitl, P., Dobner, T., Ramakrishnan, R., Moore, K.J. and Rinninger, F.
*J. Lipid Res., **52**, 745-758 (2011)*

The mechanisms of HDL-mediated cholesterol transport from peripheral tissues to the liver are incompletely defined. Here the function of scavenger receptor cluster of differentiation 36 (CD36) for HDL uptake by the liver was investigated. CD36 knockout (KO) mice, which were the model, have a 37% increase (P = 0.008) of plasma HDL cholesterol compared with wild-type (WT) littermates. To explore the mechanism of this increase, HDL metabolism was investigated with HDL radiolabeled in the apolipoprotein (^{125}I) and cholesteryl ester (CE, [^{3}H]) moiety. Liver uptake of [^{3}H] and ^{125}I from HDL decreased in CD36 KO mice and the difference, i. e. hepatic selective CE uptake ([^{3}H]^{125}I), declined (–33%, P = 0.0003) in CD36 KO compared with WT mice. Hepatic HDL holo-particle uptake (^{125}I) decreased (–29%, P = 0.0038) in CD36 KO mice. In vitro, uptake of ^{125}I-/[^{3}H]HDL by primary liver cells from WT or CD36 KO mice revealed a diminished HDL uptake in CD36-deficient hepatocytes. Adenovirus-mediated expression of CD36 in cells induced an increase in selective CE uptake from HDL and a stimulation of holo-particle internalization. In conclusion, CD36 plays a role in HDL uptake in mice and by cultured cells. A physiologic function of CD36 in HDL metabolism in vivo is suggested.

4.899 Prolonged survival of E coli but not *Staphylococcus aureus* in monocytes from patients with Crohn's disease
Elliott, T.R., Hudspith, b., Karaiskos, C., Rayment, N. and Sanderson, J.D.
*Gut, **60**, A61-A62 (2011)*

Introduction A primary macrophage defect has been proposed to play a role in Crohn's disease (CD) pathogenesis.1 In CD, Escherichia coli persist within lamina propria macrophages and peripheral blood monocytes are unable to effectively kill E coli in vitro.2 3 It is uncertain whether this abnormal bacterial handling by monocytes and macrophages in CD is limited to certain strains of E coli or whether a broader bacterial killing defect exists. The aim of this study was to compare intracellular survival of E coli with Staphylococcus aureus (not a usual resident of gut flora) within CD-derived and control monocytes.
Methods Peripheral blood was taken from eight healthy controls and nine CD patients. CD distribution was ileal (n=1) and ileocolonic (n=8). Three CD patients were on thiopurines. Monocyte isolation was performed by iodixanol barrier flotation. Monocytes were challenged with a CD-derived strain of E coli and methicillin sensitive Staphylococcus aureus (MSSA). After 1 h incubation, the gentamicin protection assay was performed. Monocytes were lysed to release internalized bacteria after further incubation for 1 and 4 h. Cell lysates were plated on agar and incubated for 24 h at 37°C. Colony forming units (CFU) were counted after both 1 and 4h incubation. The CFU counts at these time points allowed relative replication to be determined.
Results Viable CD-derived E coli were cultured from cell lysates at 1 and 4 h from 9/9 CD patients, and in 3/8 HC's (mean increase CFUs from 1 to 4 h: CD +394%, HC −7%). Viable S aureus was cultured in cell lysates at 1 and 4 h respectively from 1/9 and 0/9 CD patients and 0/8 healthy controls at both time points. The difference in the number of CFU's present for each subject at 1 and 4 h was calculated and there was a statistically significant difference in mean rank of (CFU (4 h) – CFU (1 h)) between CD and HC's for E coli but not for staph aureus (Mann–Whitney U test).
Conclusion Within CD-derived peripheral monocytes, intracellular survival is prolonged for E coli but not for S aureus, illustrating that impairment of killing by monocytes in CD is restricted to particular bacteria. This is consistent with the clinical observation that CD primarily affects the gut which has an extensive but specific microbiome, rather than causing systemic immunodeficiency characterised by infections in other systems.

4.900 Prediction of Pancreatic Tissue Densities by an Analytical Test Gradient System Before Purification Maximizes Human Islet Recovery for Islet Autotransplantation/Allotransplantation
Anazawa, T., Matsumoto, S., Yonekawa, Y., Loganathan, G., Wilhelm, J.J., Soltani, S.M., Papas, K.K., Sutherland, D.E.R., hering, B.J. and Balamurugan, A.N.
*Transplantation, **91(5)**, 508-514 (2011)*

Background. Using standard density gradient (SDG) ranges for human islet purification frequently results in islet loss and transplantation of lower islet mass. Measuring the densities of islet and acinar tissue beforehand to customize the gradient range for the actual COBE 2991 cell processor (COBE) purification

is likely to maximize the recovery of islets. We developed an analytical test gradient system (ATGS) for predicting pancreatic tissue densities before COBE purification to minimize islet loss during purification. Methods. Human islets were isolated from deceased donor (n=30) and chronic pancreatitis pancreata (n=30). Pancreatic tissue densities were measured before purification by the ATGS, and the density gradient range for islet purification in a COBE was customized based on density profiles determined by the ATGS. The efficiency of custom density gradients (CDGs) to recover high islet yield was compared with predefined SDGs.
Results. Pancreatic tissue densities from autografts were significantly higher than in allograft preparations. In allograft purifications, a higher proportion of islets were recovered using ATGS-guided CDGs (85.9%±18.0%) compared with the SDG method (69.2%±27.0%; *P*=0.048). Acinar contamination at 60%, 70%, and 80% cumulative islet yield for allografts was significantly lower in the CDG group. In autograft purifications, more islets were recovered with CDGs (81.9%±28.0%) than SDGs (55.8%±22.8%; *P*=0.03). CDGs effectively reduced islet loss by minimizing islet sedimentation in the COBE bag.
Conclusions. Using ATGS-guided CDGs maximizes the islet recovery for successful transplantations by reducing acinar contamination in allograft preparations and by reducing sedimentation of islets in the COBE bag in autograft preparations.

4.901 Enhanced proliferation of primary rat type II pneumocytes by Jaagsiekte sheep retrovirus envelope protein
Johnson, C., Jahid, S., Voelker, D.R. and Fan, H.
Virology, ***412***, *349-356 (2011)*

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of a contagious lung cancer in sheep. The envelope protein (Env) is the oncogene, as it can transform cell lines in culture and induce tumors in animals, although the mechanisms for transformation are not yet clear because a system to perform transformation assays in differentiated type II pneumocytes does not exist. In this study we report culture of primary rat type II pneumocytes in conditions that favor prolonged expression of markers for type II pneumocytes. Env-expressing cultures formed more colonies that were larger in size and were viable for longer periods of time compared to vector control samples. The cells that remained in culture longer were confirmed to be derived from type II pneumocytes because they expressed surfactant protein C, cytokeratin, displayed alkaline phosphatase activity and were positive for Nile red. This system will be useful to study JSRV Env in the targets of transformation.

4.902 Characterization of mouse sperm TMEM190, a small transmembrane protein with the trefoil domain: evidence for co-localization with IZUMO1 and complex formation with other sperm proteins
Nishimura, H., Gupta, S., Myles, D.G. and Primakoff, P.
Reproduction, ***141***, *437-451 (2011)*

TMEM190, a small transmembrane protein containing the trefoil domain, was previously identified by our proteomic analysis of mouse sperm. Two structural features of TMEM190, 'trefoil domain' and 'small transmembrane protein', led us to hypothesize that this protein forms a protein–protein complex required during fertilization, and we characterized TMEM190 by biochemical, cytological, and genetic approaches. We showed in this study that the mouse Tmem190 gene exhibits testis-specific mRNA expression and that the encoded RNA is translated into a 19-kDa protein found in both testicular germ cells and cauda epididymal sperm. Treatment of the cell surface with proteinase K, subcellular fractionation, and immunofluorescence assay all revealed that mouse TMEM190 is an inner-acrosomal membrane protein of cauda epididymal sperm. During the acrosome reaction, TMEM190 partly relocated onto the surface of the equatorial segment, on which sperm–oocyte fusion occurs. Moreover, TMEM190 and IZUMO1, which is an immunoglobulin-like protein required for gamete fusion, co-localized in mouse sperm both before and after the acrosome reaction. However, immunoprecipitates of TMEM190 contained several sperm proteins, but did not include IZUMO1. These findings suggest that a mouse sperm protein complex(es) including TMEM190 plays an indirect role(s) in sperm–oocyte fusion. The role(s), if any, is probably dispensable since Tmem190-null male mice were normally fertile.

4.903 Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens
DePaolo, R.W., Abadie, V., Tang, F., Fehlner-Peach, H., Hall, J.A., Wang, W., Marietta, E.V., Kaserda, D.D., Waldmann, T.A., Murray, J.A., Semrad, C., Kupfer, S.S., Belkaid, Y., Guandalini, S. and Jabri, B.
Nature, ***471***, *220-225 (2011)*

Under physiological conditions the gut-associated lymphoid tissues not only prevent the induction of a local inflammatory immune response, but also induce systemic tolerance to fed antigens[1, 2]. A notable exception is coeliac disease, where genetically susceptible individuals expressing human leukocyte antigen (HLA) HLA-DQ2 or HLA-DQ8 molecules develop inflammatory T-cell and antibody responses against dietary gluten, a protein present in wheat[3]. The mechanisms underlying this dysregulated mucosal immune response to a soluble antigen have not been identified. Retinoic acid, a metabolite of vitamin A, has been shown to have a critical role in the induction of intestinal regulatory responses[4, 5, 6]. Here we find in mice that in conjunction with IL-15, a cytokine greatly upregulated in the gut of coeliac disease patients[3, 7], retinoic acid rapidly activates dendritic cells to induce JNK (also known as MAPK8) phosphorylation and release the proinflammatory cytokines IL-12p70 and IL-23. As a result, in a stressed intestinal environment, retinoic acid acted as an adjuvant that promoted rather than prevented inflammatory cellular and humoral responses to fed antigen. Altogether, these findings reveal an unexpected role for retinoic acid and IL-15 in the abrogation of tolerance to dietary antigens.

4.904 **MyD88-dependent pathway is essential for the innate immunity to *Enterocytozoon bieneusi***
Zhinag, Q., Feng, X., Nie, W., Golenbock, D.T., Mayanja-Kizza, H., Tzipori, S. and Feng, H.
Parasite Immunol., ***33***, *217-225 (2011)*

Enterocytozoon bieneusi is clinically the most significant microsporidian parasite associated with persistent diarrhoea, wasting and cholangitis in 30–50% of individuals with HIV/AIDS, as well as in malnutritional children and in the recipients of immunosuppressive therapy. However, the host immune responses to *E. bieneusi* have not been investigated until recently because of lack of sources of spores, cell culture system and animal models. In this study, we purified spores from heavily infected human or monkey faeces by serial salt-Percoll-sucrose-iodixanol centrifugation, and the purity of spores was confirmed by FACS and scanning electron microscopy. Exposure of dendritic cells to *E. bieneusi* spores induced the upregulation of the surface markers and production of pro-inflammatory cytokines. The cytokine production was independent of toll-like receptor 4, but MyD88 dependent, because dendritic cells from MyD88 knockout mice failed to secrete these pro-inflammatory cytokines, whereas dendritic cells from C3H/HeJ (a toll-like receptor 4 mutant) were activated by *E. bieneusi* and secreted these cytokines. Furthermore, MyD88-deficient mice were susceptible to *E. bieneusi* infection, in contrast to wild-type mice that resisted the infection. Collectively, the data demonstrate innate recognition of *E. bieneusi* by dendritic cells and the importance of MyD88-dependent signalling in resisting infection in a murine challenge model.

4.905 **Activation of the mitochondrial permeability transition pore modulates Ca^{2+} responses to physiological stimuli in adult neurons**
Barsukova, A., Komarov, A., Hajnoczky, G., Bernardi, P,m Bourdette, D. and Forte, M.
Eur. J. Neurosci., ***33***, *831-842 (2011)*

The participation of mitochondria in cellular and neuronal Ca^{2+} homeostatic networks is now well accepted. Yet, critical tests of specific mitochondrial pathways in neuronal Ca^{2+} responses have been hampered because the identity of mitochondrial proteins that must be integrated within this dynamic system remain uncertain. One putative pathway for Ca^{2+} efflux from mitochondria exists through the formation of the permeability transition pore (PTP) that is often associated with cellular and neuronal death. Here, we have evaluated neuronal Ca^{2+} dynamics and the PTP in single adult neurons in wild-type mice and those missing cyclophilin D (CyPD), a key regulator of the PTP. Using high-resolution time-lapse imaging, we demonstrate that PTP opening only follows simultaneous activation with two physiological stimuli that generate critical threshold levels of cytosolic and mitochondrial Ca^{2+}. Our results are the first to demonstrate CyPD-dependent PTP opening in normal neuronal Ca^{2+} homeostatic mechanisms not leading to activation of cell death pathways. As neurons in mice lacking CyPD are protected in a number of neurodegenerative disease models, the results suggest that improved viability of CyPD-knockout animals in these pathological states may be due to the transient, rather than persistent, activation of the PTP in mutant mitochondria, thereby shielding neurons from cytoplasmic Ca^{2+} overload.

4.906 **Isolation, Characterization, and Expansion Methods for Defined Primary Renal Cell Populations from Rodent, Canine, and Human Normal and Diseased Kidneys**
Presnell, S.C., Bruce, A.T., Wallace, S.M., Choudury, S., Genheimer, C.W., Cox, B., Guthrie, K., Werdin, E.S., Tatsumi-Ficht, P., Ilagan, R.M., Kelley, R.W., Rivera, E.A., ludlow, J.W., Wagner, B.J., Jayo, M.J. and Bertram, T.A.
Tissue Engineering: Part C, ***17(3)***, *261-273 (2011)*

Chronic kidney disease (CKD) is a global health problem; the growing gap between the number of patients awaiting transplant and organs actually transplanted highlights the need for new treatments to restore renal function. Regenerative medicine is a promising approach from which treatments for organ-level disorders (e.g., neurogenic bladder) have emerged and translated to clinics. Regenerative templates, composed of biodegradable material and autologous cells, isolated and expanded *ex vivo*, stimulate native-like organ tissue regeneration after implantation. A critical step for extending this strategy from bladder to kidney is the ability to isolate, characterize, and expand functional renal cells with therapeutic potential from diseased tissue. In this study, we developed methods that yield distinct subpopulations of primary kidney cells that are compatible with process development and scale-up. These methods were translated to rodent, large mammal, and human kidneys, and then to rodent and human tissues with advanced CKD. Comparative *in vitro* studies demonstrated that phenotype and key functional attributes were retained consistently in *ex vivo* cultures regardless of species or disease state, suggesting that autologous sourcing of cells that contribute to *in situ* kidney regeneration after injury is feasible, even with biopsies from patients with advanced CKD.

4.907 Porcine CTLA4-Ig prolong islet xenografts in rats by downregulating the direct pathway of T-cell activation

Zhai, C., Yu, L., Zhu, H., Tian, M., Xiaogang, Z. and Bo, W.
*Xenotransplantation, **18**, 40-45 (2011)*

Abstract: Aim: Porcine pancreatic islets fused with pCTLA4-Ig were transplanted into diabetic rats. Xenografts survival was observed, and the underlying immunological rejection mechanisms were investigated.
Methods: Control porcine islets, empty vector (Adv-GFP)–transfected, and gene-modified porcine islets were transplanted into the renal capsule of diabetic rats. The survival rates of the xenografts were observed. Changes in serum levels of IL-4 and ϒ-IFN in the recipients were assessed.
Results: The survival time of xenografts in the gene-modified porcine islets group was 34.50 ± 4.14 days, which was longer than those in the control group (34.50 ± 4.14 days vs. 7.43 ± 1.72 days and 7.22 ± 1.72 days; $P < 0.01$). Changes in the serum levels of IL-4 and ϒ-IFN between the groups of rats post-transplantation indicated the differentiation bias of T helper cells.
Conclusions: The donor-originated pCTLA-IgG4 fusion protein inhibits the direct pathway of recipient T-cell priming, which might prolong xenograft survival.

4.908 Tristetraprolin-dependent Post-transcriptional Regulation of Inflammatory Cytokine mRNA Expression by Apolipoprotein A-I: ROLE OF ATP-BINDING MEMBRANE CASSETTE TRANSPORTER A1 AND SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3

Yin, K., Deng, X., Mo, Z-C., Zhao, G-J., Jiang, J., Cui, L-B., Tan, C-Z., Wen, G-B., Fu, Y. and Tang, C-K.
*J. Biol. Chem., **286(16)**, 13834-13845 (2011)*

Atherosclerosis is an inflammatory disease characterized by the accumulation of macrophages in the arterial intima. The activated macrophages secreted more pro-inflammatory cytokines, such as tumor necrosis factor (*TNF*)-α, which promote the development of the disease. Apolipoprotein A-I (*apoA-I*), the major component of high density lipoprotein, is involved in reverse cholesterol transport of lipid metabolism. Recently, it has been found that *apoA-I* suppresses inflammation via repression of inflammatory cytokine expression; the mechanisms of the *apoA-I*-suppressive action, however, are not yet well characterized. In this study, we have for the first time found that *apoA-I* suppresses the expression of some inflammatory cytokines induced by lipopolysaccharide via a specific post-transcriptional regulation process, namely mRNA destabilization, in macrophages. Our further studies have also shown that AU-rich elements in the 3′-untranslated region of *TNF*-α mRNA are responsive to the *apoA-I*-mediated mRNA destabilization. The *apoA-I*-induced inflammatory cytokine mRNA destabilization was associated with increased expression of mRNA-destabilizing protein tristetraprolin through a *JAK2/STAT3* signaling pathway-dependent manner. When blocking interaction of *apoA-I* with ATP-binding membrane cassette transporter A1 (*ABCA1*), a major receptor for *apoA-I* in macrophages, it would almost totally abolish the effect of *apoA-I* on tristetraprolin expression. These results present not only a novel mechanism for the *apoA-I*-mediated inflammation suppression in macrophages but also provide new insights for developing strategies for modulating vascular inflammation and atherosclerosis.

4.909 Bovine Plasmacytoid Dendritic Cells Are the Major Source of Type I Interferon in Response to Foot-and-Mouth Disease Virus In Vitro and In Vivo

Reid, E., Juleff, N., Gubbins, S., Prentice, H., Seago, J and Charleston, B.

J. Virol., ***85(9)***, *4297-4308 (2011)*

Type I interferons (alpha/beta interferons [IFN-α/β]) are the main innate cytokines that are able to induce a cellular antiviral state, thereby limiting viral replication and disease pathology. Plasmacytoid dendritic cells (pDCs) play a crucial role in the control of viral infections, especially in response to viruses that have evolved mechanisms to block the type I IFN signal transduction pathway. Using density gradient separation and cell sorting, we have highly enriched a population of bovine cells capable of producing high levels of biologically active type I IFN. These cells represented less than 0.1% of the total lymphocyte population in blood, pseudoafferent lymph, and lymph nodes. Phenotypic analysis identified these cells as bovine pDCs (CD3– CD14– CD21– CD11c– NK– TCRδ– CD4+ MHC II+ CD45RB+ CD172a+ CD32+). High levels of type I IFN were generated by these cells in vitro in response to Toll-like receptor 9 (TLR-9) agonist CpG and foot-and-mouth disease virus (FMDV) immune complexes. In contrast, immune complexes formed with UV-inactivated FMDV or FMDV empty capsids failed to elicit a type I IFN response. Depletion of CD4 cells in vivo resulted in levels of type I IFN in serum early during FMDV infection that were significantly lower than those for control animals. In conclusion, pDCs interacting with immune-complexed virus are the major source of type I interferon production during acute FMDV infection in cattle.

4.910 Surfactant protein-A and toll-like receptor-4 modulate immune functions of preterm baboon lung dendritic cell precursor cells

Awasthi, S., Madhusoodhanan, R. and Wolf, R.
Cell. Immunol., ***268***, *87-96 (2011)*

Lung infections are important risk factors for an increased morbidity and mortality in prematurely-delivered babies. Immaturity of the innate immune components makes them extremely susceptible to infection. Recently, we isolated lung dendritic cell (DC)-precursor cells from preterm fetal baboons. The isolated cells were found to be defective in phagocytosing *Escherichia coli* under basal conditions. In this study, we investigated the effects of exogenously-added purified native lung surfactant protein (SP)-A and recombinant toll-like receptor (TLR)-4-MD2 proteins on phagocytic uptake and cytokine secreting ability of fetal baboon lung DC-precursor cells. The cells were pulsed with SP-A and/or TLR4-MD2 proteins and the phagocytic function was investigated by incubating the cells with fluorescent-labeled *E. coli* bioparticles and analyzed by spectrofluorometry. The amounts of TNF-α secreted in cell-free supernatants were measured by ELISA. Our results demonstrate that SP-A and TLR4-MD2 proteins, whether added alone or together, induce phagocytosis of *E. coli* ($p < 0.05$). The SP-A does not affect TNF-α secretion, while the TLR4-MD2 protein induces TNF-α. However, simultaneous addition of SP-A with TLR4-MD2 protein reduces the TLR4-MD2-protein induced TNF-α to basal level. In conclusion, our results indicate that an exogenous administration of SP-A can potentially induce phagocytic activity and anti-inflammatory effect in preterm babies, and help control infection and inflammation.
Keywords: Dendritic cells; Surfactant protein-A; Toll-like receptor-4; Preterm babies; Innate immunity
Abbreviations: DC(s), dendritic cell(s); dGA, days of gestation age; D-PBS, Dulbecco's phosphate buffered saline; FACS, fluorescence activated cell sorting; FBS, fetal bovine serum; FITC, fluorescien isothiocyanate; HBSS, Hanks balanced salt solution; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MFI, mean fluorescent intensity; PE, phycoerythrinin; SEM, standard error of measurement

4.911 A new model to study spinal muscular atrophy: Neurite degeneration and cell death is counteracted by BCL-X_L Overexpression in motoneurons

Garcera, A., Mincheva, S., Gou-Fabregas, M., Caraballo-Miralles, V., Llado, J., Comella, J.X. and Soler, R.M.
Neurobiology of Disease, ***42***, *415-426 (2011)*

Spinal muscular atrophy (SMA) is a motoneuron disorder characterized by deletions or specific mutations in the *Survival Motor Neuron* gene (*SMN*). SMN is ubiquitously expressed and has a general role in the assembly of small nuclear ribonucleoprotein (snRNP) and pre-mRNA splicing requirements. However, in motoneuron axons SMN deficiency results in inappropriate levels of certain transcripts in the distal axon, suggesting that the specific susceptibility of motoneurons to SMN deficiency is related to a specialized function in these cells. Although mouse models of SMA have been generated and are useful for *in vivo* and *in vitro* studies, the limited number of isolated MNs that could be obtained from them makes it difficult to perform biochemical, genetic and pharmacological approaches. We describe here an *in vitro* model of isolated embryonic mouse motoneurons in which the cellular levels of endogenous SMN are reduced.

These cells show neurite degeneration and cell death after several days of SMN knockdown. We found that the over-expression of the anti-apoptotic protein Bcl-x_L into motoneurons rescues these cells from the phenotypic changes observed. This result demonstrates that Bcl-x_L signaling could be a possible pharmacological target of SMA therapeutics.

4.912 Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signaling and response, oxidative/nitrative stress, and cell death
Mukhopadhyay, P., Rajesh, M., Horvath, B., Batkai, S., Park, O., Tanchian, G., Gao, R.Y., Patel, V., Wink, D.A., Liaudet, L., Hasko, G., Mechoulam, R. and Pachet, P.
*Free Radical Biology & Medicine, **50**, 1368-1381 (2011)*

Ischemia/reperfusion (I/R) is a pivotal mechanism of liver damage after liver transplantation or hepatic surgery. We have investigated the effects of cannabidiol (CBD), the nonpsychotropic constituent of marijuana, in a mouse model of hepatic I/R injury. I/R triggered time-dependent increases/changes in markers of liver injury (serum transaminases), hepatic oxidative/nitrative stress (4-hydroxy-2-nonenal, nitrotyrosine content/staining, and gp91phox and inducible nitric oxide synthase mRNA), mitochondrial dysfunction (decreased complex I activity), inflammation (tumor necrosis factor α (TNF-α), cyclooxygenase 2, macrophage inflammatory protein-1α/2, intercellular adhesion molecule 1 mRNA levels; tissue neutrophil infiltration; nuclear factor κB (NF-κB) activation), stress signaling (p38MAPK and JNK), and cell death (DNA fragmentation, PARP activity, and TUNEL). CBD significantly reduced the extent of liver inflammation, oxidative/nitrative stress, and cell death and also attenuated the bacterial endotoxin-triggered NF-κB activation and TNF-α production in isolated Kupffer cells, likewise the adhesion molecule expression in primary human liver sinusoidal endothelial cells stimulated with TNF-α and attachment of human neutrophils to the activated endothelium. These protective effects were preserved in CB_2 knockout mice and were not prevented by $CB_{1/2}$ antagonists in vitro. Thus, CBD may represent a novel, protective strategy against I/R injury by attenuating key inflammatory pathways and oxidative/nitrative tissue injury, independent of classical $CB_{1/2}$ receptors.

4.913 Silencing tissue inhibitors of metalloproteinases (TIMPs) with short interfering RNA reveals a role for TIMP-1 in hepatic stellate cell proliferation
Fowell, A.J., Collins, J.E., Duncombe, D.R., Pickering, J.A., Rosenberg, W.M.C. and Benyon, R.C.
*Biochem. Biophys. Res. Comm., **407**, 277-282 (2011)*

Myofibroblastic, activated hepatic stellate cells (HSC) play a pivotal role in the development of liver fibrosis through the secretion of fibrillar collagens and the tissue inhibitors of metalloproteinase (TIMP)-1 and -2. TIMPs are believed to promote hepatic fibrosis by inhibiting both matrix degradation and apoptosis of HSC. In other cell types, there is evidence that TIMP-1 has effects on proliferation, however the role of TIMPs in the regulation of HSC proliferation remains unexplored. Therefore, we have used short interfering RNA (siRNA) to investigate the effects of autocrine TIMP-1 and -2 on HSC proliferation. TIMP-1 and -2 siRNA were highly effective, producing peak target protein knockdown compared to negative control siRNA of 92% and 63%, respectively. Specific silencing of TIMP-1, using siRNA, significantly reduced HSC proliferation. TIMP-1 was localised in part to the HSC nucleus and TIMP-1 siRNA resulted in loss of both cytoplasmic and nuclear TIMP-1. Attenuated proliferation was associated with reduced Akt phosphorylation and was partially rescued by addition of recombinant TIMP-1. We have revealed a novel autocrine mitogenic effect of TIMP-1 on HSC, which may involve Akt-dependent and specific nuclear mechanisms of action. We suggest that TIMP-1 might promote liver fibrosis by means other than its previously described anti-apoptotic effect on HSC. Moreover, these findings, together with our previous reports and the emerging data from in vivo studies of TIMP inhibition, provide strong evidence that TIMP-1 is mechanistically central to liver fibrosis and an important potential therapeutic target.

4.914 GalR2/3 mediates proliferative and trophic effects of galanin on postnatal hippocampal precursors
Abbosh, C., Lawkowski, A., Zaben, M. and Gray, W.
*J. Neurochem., **117(3)**, 425-436 (2011)*

Understanding how neural activity is functionally linked to the stem cell niche, is assuming ever increasing importance as hippocampal neurogenesis is shown to be important for modulating the behavioural responses to stress and for certain forms of learning and memory. Neuropeptides such as neuropeptide Y and vasoactive intestinal peptide have emerged as important mediators for signalling local interneuron activity to subgranular zone precursors, however, little is known regarding the effects of neuropeptides that

are extrinsic modulators of hippocampal information processing. Here, we show that the galanin GalR2/3 agonist Gal2–11 is both trophic and proliferative for postnatal subgranular precursors and proliferating neuroblasts at 10 nM and is purely trophic at doses as low as 100 pM. We found no effect mediated via GalR1. As galanin is co-released from noradrenergic and serotonergic projection neurons to the dentate gyrus, these findings support a direct effect of galanin on hippocampal neurogenesis, which may partly mediate its antidepressant effect via GalR2/3 receptors.

4.915 IFNγ triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1

Aebischer, J., Cassina, P., Otsmane, B., Moumen, A., Seilhean, D., Meininger, V., Barbeito, L., Pettmann, B. and Raoul, C.

*Cell Death and Differentiation, **18**, 754-768 (2011)*

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease that primarily affects motoneurons in the brain and spinal cord. Dominant mutations in superoxide dismutase-1 (SOD1) cause a familial form of ALS. Mutant SOD1-damaged glial cells contribute to ALS pathogenesis by releasing neurotoxic factors, but the mechanistic basis of the motoneuron-specific elimination is poorly understood. Here, we describe a motoneuron-selective death pathway triggered by activation of lymphotoxin-β receptor (LT-βR) by LIGHT, and operating by a novel signaling scheme. We show that astrocytes expressing mutant SOD1 mediate the selective death of motoneurons through the proinflammatory cytokine interferon-γ (IFNγ), which activates the LIGHT-LT-βR death pathway. The expression of LIGHT and LT-βR by motoneurons in vivo correlates with the preferential expression of IFNγ by motoneurons and astrocytes at disease onset and symptomatic stage in ALS mice. Importantly, the genetic ablation of Light in an ALS mouse model retards progression, but not onset, of the disease and increases lifespan. We propose that IFNγ contributes to a cross-talk between motoneurons and astrocytes causing the selective loss of some motoneurons following activation of the LIGHT-induced death pathway.

4.916 The Canonical Nuclear Factor-κB Pathway Regulates Cell Survival in a Developmental Model of Spinal Cord Motoneurons

Mincheva, S., Garcera, A., Gou-Fabregas, M., Encinas, M., Dolcet, X. and Soler, R.M.

*J. Neurosci., **31(17)**, 6493-6503 (2011)*

In vivo and *in vitro* motoneuron survival depends on the support of neurotrophic factors. These factors activate signaling pathways related to cell survival or inactivate proteins involved in neuronal death. In the present work, we analyzed the involvement of the nuclear factor-κB (NF-κB) pathway in mediating mouse spinal cord motoneuron survival promoted by neurotrophic factors. This pathway comprises ubiquitously expressed transcription factors that could be activated by two different routes: the canonical pathway, associated with IKKα/IKKβ kinase phosphorylation and nuclear translocation RelA (p65)/p50 transcription factors; and the noncanonical pathway, related to IKKα kinase homodimer phosphorylation and RelB/p52 transcription factor activation. In our system, we show that neurotrophic factors treatment induced IKKα and IKKβ phosphorylation and RelA nuclear translocation, suggesting NF-κB pathway activation. Protein levels of different members of the canonical or noncanonical pathways were reduced in a primary culture of isolated embryonic motoneurons using an interference RNA approach. Even in the presence of neurotrophic factors, selective reduction of IKKα, IKKβ, or RelA proteins induced cell death. In contrast, RelB protein reduction did not have a negative effect on motoneuron survival. Together these results demonstrated that the canonical NF-κB pathway mediates motoneuron survival induced by neurotrophic factors, and the noncanonical pathway is not related to this survival effect. Canonical NF-κB blockade induced an increase of Bim protein level and apoptotic cell death. Bcl-x_L overexpression or Bax reduction counteracted this apoptotic effect. Finally, RelA knockdown causes changes of CREB and Smn protein levels.

4.917 The Inhibition of Neutrophil Elastase Ameliorates Islet Yield and Islet Graft Survival

Tanemura, M., Machida, T., Nagano, H., Wada, S., Kobayashi, S., Murabashi, S., Eguchi, H., Ito, T., Mori, M. and Doki, Y.

*Am. J. Transplant., **11**, 249 (2011)*

Introduction: One important key to achieve successful insulin-independence after islet transplantation (ITx) is acquiring a suffi cient donor islet mass. Activated neutrophils play an important role for acute tissue injury in transplant organs. Neutrophil elastase (NE), released from activated neutrophils can

directly cause tissue injury. We focused on the crucial role of NE in the cytotoxic effects against islets during islet isolation and after ITx. Objectives: The objectives are to determine whether the addition of the specifi c NE inhibitor, sivelestat (Si) into the islet isolation solution could improve islet yield and, to investigate the cytoprotective effects of the administration of Si in islet recipients.
Materials and Methods: ***Islet isolation:***
Anesthetized male C57BL/6 mice underwent bile duct cannulation with pancreatic inflation using either UW, UW containing 20 μM of Si (ie: S-UW), ET-Kyoto or ET-Kyoto containing 20 μM of Si (ie: S-Kyoto). The infl ated pancreas was digested with collagenase, followed by purifi cation using a discontinuous Iodixanol gradient.
Assessment of islet viability: Fluorescence labeling with TMRE was performed.
Glucose-Stimulated Insulin Release: To assess *in vitro* potency of isolated islets, static glucose change was performed. ***Measurement of NE activity:*** NE activity during isolation was measured by the incubation with *N*-methoxysuccinyl-Ala-Ala- Pro-Val-*p*-nitroanilide as the substrate. ***IT experiments:*** The diabetic recipient, male Balb c/A mice received 500 of allogeneic islets isolated from C57BL/6J mice under kidney capsule. To assess the benefi cial effects of Si, intraperitoneal administration of 100 mg/kg of Si per one day was performed at 1 day before ITx and every day until 14 day after ITx.
Results: Data are summarized in the Table.
Conclusion: NE inhibitor (ie: sivelestat) treatment should be considered a potential strategy for recovering transplantable islets and long-term islet allograft survival, and patients may be cured with islets from one donor.

4.918 Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment
Wu, Y., Lousberg, E.L., Moldenhauer, L.M., Hayball, J.D., Robertson, S.A., Coller, J.K., Watkins, L.R., Somogyi, A.A. and Hutchinson, M.R.
*Brain, Behavior, and immunity, **25**, 5155-5164 (2011)*

Alcohol-induced proinflammatory central immune signaling has been implicated in the chronic neurotoxic actions of alcohol, although little work has examined if these non-neuronal actions contribute to the acute behavioral responses elicited by alcohol administration. The present study examined if acute alcohol-induced sedation (loss of righting reflex, sleep time test) and motor impairment (rotarod test) were influenced by acute alcohol-induced microglial-dependent central immune signaling. Inhibition of acute alcohol-induced central immune signaling, through the reduction of proinflammatory microglial activation with minocycline, or by blocking interleukin-1 (IL-1) receptor signaling using IL-1 receptor antagonist (IL-1ra), reduced acute alcohol-induced sedation in mice. Mice treated with IL-1ra recovered faster from acute alcohol-induced motor impairment than control animals. However, minocycline led to greater motor impairment induced by alcohol, implicating different mechanisms in alcohol-induced sedation and motor impairment. At a cellular level, IκBα protein levels in mixed hippocampal cells responded rapidly to alcohol in a time-dependent manner, and both minocycline and IL-1ra attenuated the elevated levels of IκBα protein by alcohol. Collectively these data suggest that alcohol is capable of rapid modification of proinflammatory immune signaling in the brain and this contributes significantly to the pharmacology of alcohol.

4.919 Immunological tolerance in a mouse model of immune-mediated liver injury induced by 16,16 dimethyl PGE2 and PGE2-containing nanoscale hydrogels
Okamoto, T., Saito, T., Tabata, Y. and Uemoto, S.
*Biomaterials, **32**, 4925-4935 (2011)*

Although immunosuppressive agents play a pivotal role in the success of organ transplantation, chronic toxicity has been a major issue for long-term treatment. The development of therapies that induce donor-specific immunological tolerance remains an important clinical challenge. In the present study, we investigated the underlying mechanisms and applications of prostaglandin (PG) E2 for the induction of immunological tolerance in mice with concanavalin A(Con A)-induced immune-mediated liver injury. The immunological tolerogenic effect of 16,16 dimethyl PGE2 (dmPGE2) pretreatment in C57B/6 male mice with Con A-induced liver injury was observed, and it was revealed that its response was partially associated with the expression of interleukin (IL)-10, an anti-inflammatory cytokine, in Kupffer cells. To apply native eicosanoids of PGE2 for tolerance induction *in vivo*, PGE2 was incorporated into L-lactic acid oligomer-grafted pullulan of an amphiphilic polymer to form a nano-sized hydrogel (PGE2-nanogel). Pharmacokinetics studies revealed that nanogel incorporation enabled PGE2 to have a prolonged life-time in circulating blood, and a tolerogenic effect was also observed in Con A-induced liver injury, the same as

with dmPGE2 pretreatment. Nanogel-based prostaglandin administration might be developed as a therapeutic agent to induce immunological tolerance, which is necessary in allogenic organ and cell transplantation.

4.920 New Insight into the Antifibrotic Effects of Praziquantel on Mice in Infection with *Schistosoma japonicum*

Liang, Y-J., Luo, J., Yuan, Q., Zheng, D., Liu, Y-P., Shi, L., Zhou, Y., Chen, A-L., Ren, Y-Y., Sun, K-Y., Sun, Y., Wang, T. and Zhang, Z-S.
*PloS One, **6(5),** e20247 (2011)*

Background
Schistosomiasis is a parasitic disease infecting more than 200 million people in the world. Although chemotherapy targeting on killing schistosomes is one of the main strategies in the disease control, there are few effective ways of dealing with liver fibrosis caused by the parasite infection in the chronic and advanced stages of schistosomiasis. For this reason, new strategies and prospective drugs, which exert antifibrotic effects, are urgently required.
Methods and Findings
The antifibrotic effects of praziquantel were assessed in the murine models of schistosomiasis japonica. Murine fibrosis models were established by cutaneous infection with 14±2 Schistosoma japonicum cercariae. Then, the mice of both chronic (8 weeks post-infection) and advanced (15 weeks post-infection) schistosomiasis were treated by gavage of praziquantel (250 mg/kg, once daily for 3 days) to eliminate worms, and followed by praziquantel anti-fibrosis treatment (300 mg/kg, twice daily for 30 days). The fibrosis-related parameters assessed were areas of collagen deposition, content of hydroxyproline and mRNA expressions of Col1α1, Col3α1, α-SMA, TGF-β, MMP9, TIMP1, IL-4, IL-10, IL-13 and IFN-γ of liver. Spleen weight index, alanine aminotransferase activity and liver portal venous pressure were also measured. The results showed that anti-fibrosis treatment improved liver fibrosis, splenomegaly, hepatic function, as well as liver portal hypertension. In order to confirm the anti-fibrotic properties of praziquantel, we established a CCL4-induced model and revealed that CCL4-induced liver fibrosis was inhibited by PZQ treatment for 30 days. Furthermore, we analyzed the effects of praziquantel on mouse primary hepatic stellate cells (HSCs). It is indicated that mRNA expressions of Col1α1, Col3α1, α-SMA, TGF-β, MMP9 and TIMP1 of HSCs were all inhibited after praziquantel anti-parasite treatments.
Conclusions
The significant amelioration of hepatic fibrosis by praziquantel treatment validates it as a promising drug of anti-fibrosis and offers potential of a new chemotherapy for hepatic fibrosis resulting from schistosomiasis.

4.921 Cellular and intracellular distribution of growth hormone in the adult chicken testis

Martinez-Moreno, C.G., Palma, L., Carranza, M., Harvey, S., Aramburo, C. and Luna, M.
*Gen. Comp. Endocrinol., **172**, 344-357 (2011)*

Endocrine actions of growth hormone (GH) have been implicated during the development of adult testicular function in several mammalian species, and recently intracrine, autocrine, and paracrine effects have been proposed for locally expressed GH. Previous reports have shown the distribution of GH mRNA and the molecular heterogeneity of GH protein in both adult chicken testes and vas deferens. This study provides evidence of the presence and distribution of GH and its receptor (GHR) during all stages of spermatogenesis in adult chicken testes. This hormone and its receptor are not restricted to the cytoplasm; they are also found in the nuclei of spermatogonia, spermatocytes, and spermatids. The pattern of GH isoforms was characterized in the different, isolated germ cell subpopulations, and the major molecular variant in all subpopulations was 17 kDa GH, as reported in other chicken extra-pituitary tissues. Another molecular variant, the 29 kDa moiety, was found mainly in the enriched spermatocyte population, suggesting that it acts at specific developmental stages. The co-localization of GH with the proliferative cell nuclear antigen PCNA (a DNA replication marker present in spermatogonial cells) was demonstrated by immunohistochemistry. These results show for the first time that GH and GHR are present in the nuclei of adult chicken germinal cells, and suggest that GH could participate in proliferation and differentiation during the complex process of spermatogenesis.

4.922 Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis

Neymotin, A., Calingasan, N:Y., Wille, E., Naseri, N., Petri, S., Damiano, M., Liby, K.T., Risingsong, R., Sporn, M., Beal, M.F. and Kiaei, M.

*Free Radical Biology & Medicine, **51**, 88-96 (2011)*

Oxidative damage, neuroinflammation, and mitochondrial dysfunction contribute to the pathogenesis of amyotrophic lateral sclerosis (ALS), and these pathologic processes are tightly regulated by the Nrf2/ARE (NF-E2-related factor 2/antioxidant response element) signaling program. Therefore, modulation of the Nrf2/ARE pathway is an attractive therapeutic target for neurodegenerative diseases such as ALS. We examined two triterpenoids, CDDO (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid) ethylamide and CDDO trifluoroethylamide (CDDO-TFEA), that potently activate Nrf2/ARE in a cell culture model of ALS and in the G93A SOD1 mouse model of ALS. Treatment of NSC-34 cells stably expressing mutant G93A SOD1 with CDDO-TFEA upregulated Nrf2 expression and resulted in translocation of Nrf2 into the nucleus. Western blot analysis showed an increase in the expression of Nrf2/ARE-regulated proteins. When treatment started at a "presymptomatic age" of 30 days, both of these compounds significantly attenuated weight loss, enhanced motor performance, and extended the survival of G93A SOD1 mice. Treatment started at a "symptomatic age," as assessed by impaired motor performance, was neuroprotective and slowed disease progression. These findings provide further evidence that compounds that activate the Nrf2/ARE signaling pathway may be useful in the treatment of ALS.

4.923 A5-Positive Primary Sensory Neurons Are Nonpermissive for Productive Infection with Herpes Simplex Virus 1 In Vitro▽

Bertke, A.S., Swanson, S.M., Chen, J., Imai, Y., Kinchington, P.R. and Margolis, T.P.
*J. Virol., **85(13)**, 6699-6677 (2011)*

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) establish latency and express the latency-associated transcript (LAT) preferentially in different murine sensory neuron populations, with most HSV-1 LAT expression in A5+ neurons and most HSV-2 LAT expression in KH10+ neurons. To study the mechanisms regulating the establishment of HSV latency in specific subtypes of neurons, cultured dissociated adult murine trigeminal ganglion (TG) neurons were assessed for relative permissiveness for productive infection. In contrast to that for neonatal TG, the relative distribution of A5+ and KH10+ neurons in cultured adult TG was similar to that seen in vivo. Productive infection with HSV was restricted, and only 45% of cultured neurons could be productively infected with either HSV-1 or HSV-2. A5+ neurons supported productive infection with HSV-2 but were selectively nonpermissive for productive infection with HSV-1, a phenomenon that was not due to restricted viral entry or DNA uncoating, since HSV-1 expressing β-galactosidase under the control of the neurofilament promoter was detected in ~90% of cultured neurons, with no preference for any neuronal subtype. Infection with HSV-1 reporter viruses expressing enhanced green fluorescent protein (EGFP) from immediate early (IE), early, and late gene promoters indicated that the block to productive infection occurred before IE gene expression. Trichostatin A treatment of quiescently infected neurons induced productive infection preferentially from non-A5+ neurons, demonstrating that the nonpermissive neuronal subtype is also nonpermissive for reactivation. Thus, HSV-1 is capable of entering the majority of sensory neurons in vitro; productive infection occurs within a subset of these neurons; and this differential distribution of productive infection is determined at or before the expression of the viral IE genes.

4.924 Isolation of Inflammatory Cells from Human Tumours

Polak, M.E.
*Methods in Mol. Biol., **731**, 201-208 (2011)*

Inflammatory cells are present in many tumours, and understanding their function is of increasing importance, particularly to studies of tumour immunology. The tumour-infiltrating leukocytes encompass a variety of cell types, e.g. T lymphocytes, macrophages, dendritic cells, NK cells, and mast cells. Choice of the isolation method greatly depends on the tumour type and the leukocyte subset of interest, but the protocol usually includes tissue disaggregation and cell enrichment. We recommend density centrifugation for initial enrichment, followed by specific magnetic bead negative or positive panning with leukocyte and tumour cell selective antibodies.

4.925 Increased Expression of CD69 on T Cells as an Early Immune Marker for Human Cytomegalovirus Reactivation in Chronic Lymphocytic Leukemia Patients

Petersen, C.C., Nederby, L., Roug, A.S., Skovbo, A., Peterslund, N.A., Hokland, P., Nielsen, B. and Hokland, M.
*Viral Immunol., **24(2)**, 165-169 (2011)*

Reactivation of human cytomegalovirus (HCMV) remains a serious problem in immunosuppressed individuals. To investigate whether a change in the immune status can be used as an earlier marker for HCMV reactivation than the traditional PCR analysis, eight chronic lymphocytic leukemia (CLL) patients at risk for reactivation due to commencement of alemtuzumab (anti-CD52) treatment were longitudinally followed. Five series of consecutive weekly blood samples were immunophenotyped by flow cytometry to cover both the innate and adaptive immune responses. Concurrently, patients were monitored by PCR for HCMV reactivation. We found a minor upregulation of the early activation marker CD69 on NK cells immediately before HCMV was detected in circulation by PCR. Interestingly, for the specific immune response, CD69 was highly upregulated on $CD3^+$ T cells, especially for the $CD8^+$ subset, in the two patients experiencing an HCMV reactivation between 6 and 20 d before HCMV viremia was measured by PCR. Moreover, a $CD4^+$:$CD8^+$ ratio lower than 0.6 may indicate a trend toward an increased risk for viral reactivation. In conclusion, an increase in CD69 expression is a promising candidate as an early predictor of HCMV reactivation.

4.926 Viral Replication and Innate Host Responses in Primary Human Alveolar Epithelial Cells and Alveolar Macrophages Infected with Influenza H5N1 and H1N1 Viruses

Yu, W.C.L., Chan, R.W.Y., Wang, J., Travanty, E.A., Nicholls, J.M., Peiris, J.S.M., Mason, R.J. and Chan, M.C.W.

J. Virol., ***85(14)****, 6844-6855 (2011)*

Highly pathogenic influenza H5N1 virus continues to pose a threat to public health. Although the mechanisms underlying the pathogenesis of the H5N1 virus have not been fully defined, it has been suggested that cytokine dysregulation plays an important role. As the human respiratory epithelium is the primary target cell for influenza viruses, elucidating the viral tropism and innate immune responses of influenza H5N1 virus in the alveolar epithelium may help us to understand the pathogenesis of the severe pneumonia associated with H5N1 disease. Here we used primary cultures of differentiated human alveolar type II cells, alveolar type I-like cells, and alveolar macrophages isolated from the same individual to investigate viral replication competence and host innate immune responses to influenza H5N1 (A/HK/483/97) and H1N1 (A/HK/54/98) virus infection. The viral replication kinetics and cytokine and chemokine responses were compared by quantitative PCR (qPCR) and enzyme-linked immunosorbent assay (ELISA). We demonstrated that influenza H1N1 and H5N1 viruses replicated productively in type II cells and type I-like cells although with different kinetics. The H5N1 virus replicated productively in alveolar macrophages, whereas the H1N1 virus led to an abortive infection. The H5N1 virus was a more potent inducer of proinflammatory cytokines and chemokines than the H1N1 virus in all cell types. However, higher levels of cytokine expression were observed for peripheral blood monocyte-derived macrophages than for alveolar macrophages in response to H5N1 virus infection. Our findings provide important insights into the viral tropisms and host responses of different cell types found in the lung and are relevant to an understanding of the pathogenesis of severe human influenza disease.

4.927 Expression and Function of Fibroblast Growth Factor (FGF) 7 during Liver Regeneration

Tsai, S-M. and Wang, W-P.

Cell. Physiol. Biochem., ***27(6)****, 641-652 (2011)*

Background/Aim: Previous studies have shown that fibroblast growth factors (FGFs) are involved in the process of liver injury repair. Liver regeneration after partial hepatectomy (PH) is impaired in transgenic mice expressing dominant-negative FGFR2b in hepatocytes. Although FGF7, a ligand specifically bound to FGFR2b, is expressed by activated hepatic stellate cells (HSCs) in fibrotic livers, the expressions and functions of FGF7 and FGFR2b after PH remain unexplored. Therefore, this study sought to examine the potential role of FGF7 signaling during liver regeneration. Methods: We examined the expression of FGF7 and FGFR2b in normal and regenerating livers. Effects of FGF7 on hepatocytes were examined *in vitro* using primary hepatocyte culture with FGF7 recombinant protein and *in vivo* by hydrodynamic-based gene transfer method. Results: We found that FGF7 expression was increased according to the activation status of HSCs after PH. The receptor, FGFR2b, was also increased in hepatocytes during liver regeneration. *In vitro* treatment with FGF7 protein activated ERK1/2 and promoted proliferation of hepatocytes isolated from regenerating livers. *In vivo* overexpression of exogenous FGF7 could notably promote hepatic proliferation and activate MAPKs after PH. Conclusion: This study suggests a role for activated HSC-expressed FGF7 in stimulating FGF signaling pathways in hepatocytes and regulating liver regeneration.

4.928 Thymosin-β4 (Tβ4) Blunts PDGF-Dependent Phosphorylation and Binding of AKT to Actin in Hepatic Stellate Cells

Reyes-Gordillo, K., Shah, R., Popratiloff, A., Fu, S., Hindle, A., Brody, F. and Rojkind, M.
Am. J. Pathol., ***178(5)***, *2100-2108 (2011)*

Hepatic stellate cell transdifferentiation is a key event in the fibrogenic cascade. Therefore, attempts to prevent and/or revert the myofibroblastic phenotype could result in novel therapeutic approaches to treat liver cirrhosis. The expression of platelet-derived growth factor (PDGF)-β receptor and the proliferative response to platelet-derived growth factor-ββ (PDGF-ββ) are hallmarks of the transdifferentiation of hepatic stellate cells (HSC). In this communication, we investigated whether thymosin-β4 (Tβ4), a chemokine expressed by HSC could prevent PDGF-BB-mediated proliferation and migration of cultured HSC. Using early passages of human HSC, we showed that Tβ4 inhibited cell proliferation and migration and prevented the expression of PDGF-β receptor (PDGF-βr), α-smooth muscle actin and α1(I) collagen mRNAs. Tβ4 also inhibited the reappearance of PDGF-βr after its PDGF-BB-dependent degradation. These PDGF-dependent events were associated with the inhibition of AKT phosphorylation at both T308 and S473 amino acid residues. The lack of AKT phosphorylation was not due to the inhibition of PDGF-βr phosphorylation, the activation of phosphoinositide 3-kinase (PI3K), pyruvate dehydrogenase kinase isozyme 1 (PDK1), and mammalian target of rapamycin (mTOR). We found that PDGF-BB induced AKT binding to actin, and that Tβ4 prevented this effect. Tβ4 also prevented the activation of freshly isolated HSC cultured in the presence of Dulbecco's modified Eagle's medium or Dulbecco's minimal essential medium containing 10% fetal bovine serum. In conclusion, overall, our findings suggest that Tβ4 by sequestering actin prevents binding of AKT, thus inhibiting its phosphorylation. Therefore, Tβ4 has the potential to be an antifibrogenic agent.

4.929 Enhancement of dentate gyrus neurogenesis, dendritic and synaptic plasticity and memory by a neurotrophic peptide

Chohan, M.O., Li, B., Blanchard, J., Tung, Y-C., Heaney, A.T., Rabe, A., Izbal, K. and Grundke-Iabal, I.
Neurobiology of Aging, ***32***, *1420-1434 (2011)*

Pharmacological enhancement of hippocampal neurogenesis is a therapeutic approach for improvement of cognition in learning and memory disorders such as Alzheimer's disease. Here we report the development of an 11-mer peptide that we designed based on a biologically active region of the ciliary neurotrophic factor. This peptide, Peptide 6, induced proliferation and increased survival and maturation of neural progenitor cells into neurons in the dentate gyrus of normal adult C57BL6 mice. Furthermore, Peptide 6 increased the MAP2 and synaptophysin immunoreactivity in the dentate gyrus. Thirty-day treatment of the mice with a slow release bolus of the peptide implanted subcutaneously improved reference memory of the mice in Morris water maze. Peptide 6 has a plasma half life of over 6 h, is blood–brain barrier permeable, and acts by competitively inhibiting the leukemia inhibitory factor signaling. The fact that Peptide 6 is both neurogenic and neurotrophic and that this peptide is effective when given peripherally, demonstrates its potential for prevention and treatment of learning and memory disorders.

4.930 Hepatic Stellate Cells Function as Regulatory Bystanders
Ichikawa, S., Mucida, D., Tyznik, A.J., Kronenberg, M. and Cheroutre, H.
*J. Immunol., **186**, 5549-5555 (2011)*

Regulatory T cells (Tregs) contribute significantly to the tolerogenic nature of the liver. The mechanisms, however, underlying liver-associated Treg induction are still elusive. We recently identified the vitamin A metabolite, retinoic acid (RA), as a key controller that promotes TGF-β–dependent Foxp3+ Treg induction but inhibits TGF-β–driven Th17 differentiation. To investigate whether the RA producing hepatic stellate cells (HSC) are part of the liver tolerance mechanism, we investigated the ability of HSC to function as regulatory APC. Different from previous reports, we found that highly purified HSC did not express costimulatory molecules and only upregulated MHC class II after in vitro culture in the presence of exogenous IFN-γ. Consistent with an insufficient APC function, HSC failed to stimulate naive OT-II TCR transgenic CD4+T cells and only moderately stimulated α-galactosylceramide–primed invariant NKT cells. In contrast, HSC functioned as regulatory bystanders and promoted enhanced Foxp3 induction by OT-II TCR transgenic T cells primed by spleen dendritic cells, whereas they greatly inhibited the Th17 differentiation. Furthermore, the regulatory bystander capacity of the HSC was completely dependent on their ability to produce RA. Our data thus suggest that HSC can function as regulatory bystanders, and therefore, by promoting Tregs and suppressing Th17 differentiation, they might represent key players in the mechanism that drives liver-induced tolerance.

4.931 Development of an In Vitro Model to Evaluate the Regenerative Capacity of Adult Brain-Derived Tyrosine Hydroxylase-Expressing Dopaminergic Neurons
Majd, S., Smardencas, A., Parish, C.L. and Drago, J.
*Neurochem. Res., **36(6)**, 967-977 (2011)*

The loss of nigral dopaminergic (DA) neurons is the disease-defining pathological change responsible for progressive motor dysfunction in Parkinson's disease. In this study, we sought to establish a culture method for adult rat tyrosine hydroxylase (TH)-immunoreactive DA neurons. In this context, we investigated the role of fibroblast growth factor 2 (FGF2), brain-derived neurotrophic factor (BDNF), transforming growth factor-β3 (TGF-β3), glial-derived neurotrophic factor (GDNF) and dibutyryl-cyclic AMP (dbcAMP) in these cultures. Culturing in the presence of FGF2, BDNF and GDNF enhanced the survival of DA neurons by 15-fold and promoted neurite growth. In contrast, dbcAMP promoted neurite growth in all neurons but did not enhance DA cell survival. This study demonstrates that long-term cultures of DA neurons can be established from the mature rat brain and that survival and regeneration of DA neurons can be manipulated by epigenetic factors such as growth factors and intracellular cAMP pathways.

4.932 Assessment of the protective effects of oral tocotrienols in arginine chronic-like pancreatitis
Gonzales, A.M., Garcia, T., Samper, E., Rickmann, M., Vaquero, E.C. and Molero, X.
Am. J. Physiol. Gastrointest. Liver Physiol., 301, G846-G855 (2011)

Tocotrienols exhibit anti-inflammatory properties over macrophages and promote cytotoxicity in activated pancreatic stellate cells, suggesting that they may limit chronic pancreatitis progression. We aimed to quantitate the effect of oral tocotrienols on a rat model of chronic pancreatic injury. Chronic-like pancreatitis was induced by repeated arginine pancreatitis. Palm oil tocotrienol-rich fraction (TRF) was given by gavage before and after pancreatitis inductions. Amylase and hydroxyproline were determined in pancreatic homogenates; collagen, fibronectin, α-smooth muscle actin (SMA), glial fibrillary acidic protein (GFAP), and phosphorylated Smad3 were assessed by Western blotting. Transforming growth factor (TGF)-β1 was measured in plasma. Morphological assessment included light microscopy, fibrosis area fraction, and collagen network fractal analysis. Arginine pancreatitis induced pancreatic atrophy and increased hydroxyproline that ameliorated after TRF. Arginine increased TGF-β1 (185 ± 40 vs. 15 ± 2 ng/ml; $P < 0.01$) that was blunted by TRF (53 ± 19; $P < 0.01$). TRF reduced protease and Smad3 activation, collagen, and fibronectin. α-SMA increased and GFAP diminished in arginine pancreatitis, consistent with long-term stellate cell activation, and TRF reverted these changes to basal. Arginine pancreatitis increased fibrosis area fraction (4.5 ± 0.3% vs. 0.2 ± 0.2%), collagen network complexity (fractal dimension 1.52 ± 0.03 vs. 1.42 ± 0.01; $P < 0.001$), and inhomogeneity (lacunarity 0.63 ± 0.03 vs. 0.40 ± 0.02; $P < 0.001$), which were all reduced by TRF (1.3 ± 0.4%, 1.43 ± 0.02%, and 0.51 ± 0.03%, respectively; $P < 0.01$). Best correlation coefficients were obtained when comparing fibrosis area fraction with lacunarity ($r = 0.88$) and both parameters with pancreatic weight ($r = -0.91$ and -0.79, respectively). TRF administered only before pancreatitis best, but not fully, recapitulated the beneficial effects of TRF. Tocotrienols improve

quantitative measures of chronic pancreatic damage. They may be of benefit in human chronic pancreatitis.

4.933 Rodent blood-stage Plasmodium survive in dendritic cells that infect naive mice
Wykes, M.N. et al
*PNAS, **108(27)**, 11205-11210 (2011)*

Plasmodium spp. parasites cause malaria in 300 to 500 million individuals each year. Disease occurs during the blood-stage of the parasite's life cycle, where the parasite is thought to replicate exclusively within erythrocytes. Infected individuals can also suffer relapses after several years, from *Plasmodium vivax* and *Plasmodium ovale* surviving in hepatocytes. *Plasmodium falciparum* and *Plasmodium malariae* can also persist after the original bout of infection has apparently cleared in the blood, suggesting that host cells other than erythrocytes (but not hepatocytes) may harbor these blood-stage parasites, thereby assisting their escape from host immunity. Using blood stage transgenic *Plasmodium berghei*-expressing GFP (PbGFP) to track parasites in host cells, we found that the parasite had a tropism for $CD317^+$ dendritic cells. Other studies using confocal microscopy, in vitro cultures, and cell transfer studies showed that blood-stage parasites could infect, survive, and replicate within $CD317^+$ dendritic cells, and that small numbers of these cells released parasites infectious for erythrocytes in vivo. These data have identified a unique survival strategy for blood-stage *Plasmodium*, which has significant implications for understanding the escape of *Plasmodium* spp. from immune-surveillance and for vaccine development.

4.934 Role of Interleukin-1 and MyD88-Dependent Signaling in Rhinovirus Infection
Stokes, C.A., Ismail, S., Dick, E.P., Bennett, J.A., Johnston, S.L., Edwards, M.R., Sabroe, I. and Parker, L.C.
*J. Virol., **85(15),** 7912-7921 (2011)*

Rhinoviral infection is an important trigger of acute inflammatory exacerbations in patients with underlying airway disease. We have previously established that interleukin-1β (IL-1β) is central in the communication between epithelial cells and monocytes during the initiation of inflammation. In this study we explored the roles of IL-1β and its signaling pathways in the responses of airway cells to rhinovirus-1B (RV-1B) and further determined how responses to RV-1B were modified in a model of bacterial coinfection. Our results revealed that IL-1β dramatically potentiated RV-1B-induced proinflammatory responses, and while monocytes did not directly amplify responses to RV-1B alone, they played an important role in the responses observed with our coinfection model. MyD88 is the essential signaling adapter for IL-1β and most Toll-like receptors. To examine the role of MyD88 in more detail, we created stable MyD88 knockdown epithelial cells using short hairpin RNA (shRNA) targeted to MyD88. We determined that IL-1β/MyD88 plays a role in regulating RV-1B replication and the inflammatory response to viral infection of airway cells. These results identify central roles for IL-1β and its signaling pathways in the production of CXCL8, a potent neutrophil chemoattractant, in viral infection. Thus, IL-1β is a viable target for controlling the neutrophilia that is often found in inflammatory airway disease and is exacerbated by viral infection of the airways.

4.935 Classical Swine Fever Virus Npro Limits Type I Interferon Induction in Plasmacytoid Dendritic Cells by Interacting with Interferon Regulatory Factor 7
Fiebach, A.R., Guzylack-Piriou. L., Python, S., Summerfield, A. and Ruggli, N.
*J. Virol., **85(16)**, 8002-8011 (2011)*

Viruses are detected by different classes of pattern recognition receptors that lead to the activation of interferon regulatory factors (IRF) and consequently to the induction of alpha/beta interferon (IFN-α/β). In turn, efficient viral strategies to escape the type I IFN-induced antiviral mechanisms have evolved. Previous studies established that pestivirus N^{pro} antagonizes the early innate immune response by targeting the transcription factor IRF3 for proteasomal degradation. Here, we report that N^{pro} of classical swine fever virus (CSFV) interacts also with IRF7, another mediator of type I IFN induction. We demonstrate that the Zn-binding domain of N^{pro} is essential for the interaction of N^{pro} with IRF7. For IRF3 and IRF7, the DNA-binding domain, the central region, and most of the regulatory domain are required for the interaction with N^{pro}. Importantly, the induction of IRF7-dependent type I IFN responses in plasmacytoid dendritic cells (pDC) is reduced after wild-type CSFV infection compared with infection with virus mutants unable to interact with IRF7. This is associated with lower levels of IRF7 in pDC. Consequently, wild-type but not N^{pro} mutant CSFV-infected pDC show reduced responses to other stimuli. Taken together, the results of this study show that CSFV N^{pro} is capable of manipulating the function of IRF7 in pDC and provides the virus with an additional strategy to circumvent the innate defense.

4.936 Enhanced protection against infection with transmissible gastroenteritis virus in piglets by oral co-administration of live attenuated *Salmonella enterica* serovar Typhimurium expressing swine interferon-α and interleukin-18
Lee, B.M. et al
*Comp. Immunol. Microbiol. Infect. Dis., **34**, 369-380 (2011)*

The enhanced effect of cytokine combinations has been assessed empirically, based on their immunobiological mechanisms. However, far less is known of the enhanced protection of practical cytokine combinations against viral infection in the livestock industry, due to cost and production issues associated with mass administration. This study demonstrates the enhanced protection of oral co-administration of swine interferon-α (swIFN-α) and interleukin-18 (swIL-18) against infection with transmissible gastroenteritis virus (TGEV) in piglets using attenuated *Salmonella enterica* serovar Typhimurium as carrier of cytokine proteins. A single oral co-administration of *S. enterica* serovar Typhimurium expressing swIFN-α and swIL-18 induced enhanced alleviation of the severity of diarrhea caused by TGEV infection, compared to piglets administered *S. enterica* serovar Typhimurium expressing swIFN-α or swIL-18 alone. This enhancement was further observed by the reduction of TGEV shedding and replication, and the expression of IFN-stimulated gene products in the intestinal tract. The results suggest that the combined administration of the swIFN-α and swIL-18 cytokines using attenuated *S. enterica* serovar Typhimurium as an oral carrier provides enhanced protection against intestinal tract infection with TGEV.

4.937 iNOS potentiates mouse Ig isotype switching through AID expression
Lee, M-R., Seo, G-Y., Kim, Y-M. and Kim, P-H.
*Biochem. Biophys. Res. Comm., **410**, 602-607 82011)*

The IgA antibody plays an important role in protecting mucosal surfaces against pathogens. It has recently been shown that nitric oxide (NO) plays a critical role in mouse IgA synthesis. In the present study, we further characterized inducible-nitric oxide synthase-deficient ($iNOS^{-/-}$) mice in the context of Ig expression. The amount of IgA in fecal pellets was substantially diminished in $iNOS^{-/-}$ mice and was paralleled by a decrease in IgA production by Peyer's patch cells. Interestingly, the amount of all IgG subisotypes, as well as IgA, was substantially diminished in sera and in cultured spleen B cells from $iNOS^{-/-}$ mice. Moreover, the synthesis of TGF-β1-inducible IgA and IgG2b in $iNOS^{-/-}$ mice was also lower than that in WT mice. However, levels of Ig germ-line transcripts, and expression of TGF-β receptor type II (TβRII) and BAFF/APRIL, were comparable between $iNOS^{-/-}$ and WT mice. Expression of activation-induced cytidine deaminase (AID) was diminished in $iNOS^{-/-}$ B cells, but restored by a NO donor, SNAP. These results indicate that iNOS regulates Ig isotype switching events at the level of AID gene expression.

4.938 Decreased glutathione accelerates neurological deficit and mitochondrial pathology in familial ALS-linked $hSOD1^{G93A}$ mice model
Vargas, M.R., Johnson, D.A. and Johnson, J.A.
*Neurobiol. of Disease, **43**, 543-551 (2011)*

Dominant mutations in Cu/Zn-superoxide dismutase (SOD1) cause familial forms of amyotrophic lateral sclerosis (ALS), a fatal disorder characterized by the progressive loss of motor neurons. To investigate the role of antioxidant defenses in ALS we used knockout mice for the glutamate-cysteine ligase modifier subunit (GCLM−/−), which have a 70–80% reduction in total glutathione. Although GCLM(−/−) mice are viable and fertile, the life span of GCLM(−/−)/$hSOD1^{G93A}$ mice decreased in 55% when compared to GCLM(+/+)/$hSOD1^{G93A}$ mice. Decreased life span in GCLM(−/−)/$hSOD1^{G93A}$ mice was associated to increased oxidative stress, aggravated mitochondrial pathology and increased association of hSOD1 with the mitochondria. Interestingly, when the GCLM(−/−) animals were mated with a different ALS-model which overexpress the experimental mutation $hSOD1^{H46R/H48Q}$, no effect was observed in survival of GCLM(−/−)/$hSOD1^{H46R/H48Q}$ mice; and little or no mitochondrial pathology was observed. Since a specific disease modifier, such as glutathione deficiency, may affect only certain hSOD1 mutants, these findings contribute to our understanding of the potential difference in the molecular pathways by which different hSOD1 mutants generate disease.

4.939 Effect of cryopreservation protocol on postthaw characteristics of stallion sperm
Salazar Jr., J.L., Teague, S.R., Love, C.C., Brinsko, S.P., Blanchard, T.L. and Varner, D.D.

Theriogenology, ***76***, *409-418 (2011)*

Three ejaculates from each of eight stallions were subjected to cryopreservation in a milk/egg yolk-based freezing extender or an egg yolk-based freezing extender. Semen was exposed to a fast prefreeze cooling rate (FAST; semen immediately subjected to cryopreservation) or a slow prefreeze cooling rate (SLOW; semen pre-cooled at a controlled rate for 80 min prior to cryopreservation). Postthaw semen was diluted in initial freezing medium (FM) or INRA 96 (IMV Technologies, L'Aigle, France) prior to analysis of 10 experimental end points: total motility (MOT; %), progressive motility (PMOT; %), curvilinear velocity (VCL; μm/s), linearity (LIN; %), intact acrosomal and plasma membranes (AIMI; %), intact acrosomal membranes (AI; %), intact plasma membranes (MI; %), and DNA quality. Eight of 10 experimental endpoints (MOT, PMOT, average-path velocity [VAP], mean straight-line velocity [VSL], LIN AIMI, AI, and MI) were affected by extender type, with egg yolk-based extender yielding higher values than milk/egg yolk-based extender ($P < 0.05$). Exposure of extended semen to a slow prefreeze cooling period resulted in increased values for six of eight endpoints (MOT, PMOT, VCL, AIMI, AI, and MI), as compared with a fast prefreeze cooling period ($P < 0.05$). As a postthaw diluent, INRA 96 yielded higher mean values than FM for MOT, PMOT, VCL, average-path velocity, and mean straight-line velocity ($P < 0.05$). Treatment group FM yielded slightly higher values than INRA 96 for LIN and MI ($P < 0.05$). In conclusion, a slow prefreeze cooling rate was superior to a fast prefreeze cooling rate, regardless of freezing extender used, and INRA 96 served as a satisfactory postthaw diluent prior to semen analysis.

4.940 Obstructive Jaundice Expands Intrahepatic Regulatory T Cells, Which Impair Liver T Lymphocyte Function but Modulate Liver Cholestasis and Fibrosis

Katz, S., Ryan, K., Ahmed, N., Plitas, G., Chaudhry, U.I., Kingham, T.P., Naheed, S., Nguyen, C., Somasundar, P., espat, N.J., Junghans, R.P. and DeMatteo, r.P

J. Immunol., ***187***, *1150-1156 (2011)*

Although obstructive jaundice has been associated with a predisposition toward infections, the effects of bile duct ligation (BDL) on bulk intrahepatic T cells have not been clearly defined. The aim of this study was to determine the consequences of BDL on liver T cell phenotype and function. After BDL in mice, we found that bulk liver T cells were less responsive to allogeneic or syngeneic Ag-loaded dendritic cells. Spleen T cell function was not affected, and the viability of liver T cells was preserved. BDL expanded the number of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg), which were anergic to direct CD3 stimulation and mediated T cell suppression in vitro. Adoptively transferred $CD4^+CD25^-$ T cells were converted into Treg within the liver after BDL. In vivo depletion of Treg after BDL restored bulk liver T cell function but exacerbated the degrees of inflammatory cytokine production, cholestasis, and hepatic fibrosis. Thus, BDL expands liver Treg, which reduce the function of bulk intrahepatic T cells yet limit liver injury.

4.941 Evidence for proteolysis of a recombinant prion protein in a lamb brain-amended loamy soil

Rapp, D., Richaume, A., Jame, P., Rigou, P., Rezaei, H., Alcouffe, P., Chapel, J-P., Quiquampoix, H. and POtier, P.

Eur. J. Soil Sci., ***62(4)***, *607-616 (2011)*

Soils contaminated by prions, the infectious agents responsible for transmissible spongiform encephalopathy diseases, remain infectious to grazing animals for many years. In this study, the ability of enzymes produced by soil microbes to degrade a recombinant prion protein (recPrP) was investigated in a loamy soil. A ^{15}N-labelled recPrP was added to soil in which microbial biomass and soil proteolytic activity had been increased by either simultaneous or prior amendment with lamb brain, and distribution of ^{15}N among soil solid particles, soluble molecules and bacterial biomass was determined. After 1 day the proportions of recovered recPrP-N associated with microbial biomass and soluble molecules were 6–9 and 15–19%, respectively, which is consistent with the hypothesis of degradation. A greater incorporation of ^{15}N-derived β-sheeted recPrP into the microbial biomass pool occurred when the soil proteolytic activity was pre-stimulated by a lamb brain amendment, suggesting that the recPrP degradation in soil is mediated by the activity level of proteolytic enzymes produced by the microbial biomass. The majority (35–87%) of the recovered recPrP-N was associated with the soil particles. An observed partial degradation of recPrP deposited on a mica surface by soil soluble enzymes indicated a sorption-related resistance to proteolysis. In conclusion, integration of the stimulation and turnover of the soil microbial component, after an input of a large amount of animal organic matter with the sorption properties of prion protein, is required to model and predict prion survivability, transformation and transmissibility in soil.

4.942 The earliest intrathymic precursors of CD8α+ thymic dendritic cells correspond to myeloid-type

double-negative 1c cells
Luche, H., Ardouin, L., Teo, P., See, P., henri, S., Merad, M., Ginhoux, F and Malissen, B.
Eur. J. Immunol., ***41(8)***, *21645-2175 (2011)*

The dendritic cells (DCs) present in lymphoid and non-lymphoid organs are generated from progenitors with myeloid-restricted potential. However, in the thymus a major subset of DCs expressing CD8α and langerin (CD207) appears to stand apart from all other DCs in that it is thought to derive from progenitors with lymphoid potential. Using mice expressing a fluorescent reporter and a diphtheria toxin receptor under the control of the *cd207* gene, we demonstrated that $CD207^{+}CD8\alpha^{+}$ thymic DCs do not share a common origin with T cells but originate from intrathymic precursors that express markers that are normally present on all ($CD11c^{+}$ and MHCII molecules) or on some (CD207, CD135, CD8α, CX3CR1) DC subsets. Those intrathymic myeloid-type precursors correspond to $CD44^{+}CD25^{-}$ double-negative 1c (DN1c) cells and are continuously renewed from bone marrow-derived canonical DC precursors. In conclusion, our results demonstrate that the earliest intrathymic precursors of $CD8\alpha^{+}$ thymic DCs correspond to myeloid-type DN1c cells and support the view that under physiological conditions myeloid-restricted progenitors generate the whole constellation of DCs present in the body including the thymus.

4.943 Effects of Mycobacterium bovis on monocyte-derived macrophages from bovine tuberculosis infection and healthy cattle
Wang, Y., Zhou, X., Lin, J., Yin, F., Xu, L., Huang, Y., Ding, T. and Zhao, D.
FEMS Microbiol. Lett., ***321(1)***, *30-36 (2011)*

Bovine tuberculosis (BTB) is a chronic infectious disease caused by the pathogen *Mycobacterium bovis* and poses a long-standing threat to livestock worldwide. To further elucidate the poorly defined BTB immune response in cattle, we utilized monocyte-derived macrophages (MDMs) to assess the gene expression related to *M. bovis Beijing* strain stimulation. Here, we demonstrate the existence of distinctive gene expression patterns between macrophages of healthy cattle and those exposed to BTB. In comparing MDMs cells from healthy cattle (n=5) and cattle with tuberculosis (n=5) 3 h after *M. bovis* stimulation, the differential expressions of seven genes (IL1β, IL1R1, IL1A, TNF-α, IL10, TLR2 and TLR4) implicated in *M. bovis* response were examined. The expressions of these seven genes were increased in both the tuberculosis-infected and the healthy cattle to *M. bovis* stimulation, and two of them (TLR2 and IL10) were significantly different in the tuberculosis and the healthy control groups ($P \leq 0.05$). The increase in the expression of the TLR2 gene is more significant in healthy cattle response to stimulation, and the change of IL10 gene expression is more significant in tuberculosis cattle. Additionally, we investigated the cytopathic effect caused by *M. bovis* stimulation and the relationship between *M. bovis* and MDMs cells to obtain a general profile of pathogen–host interaction.

4.944 A Francisella tularensis Locus Required for Spermine Responsiveness Is Necessary for Virulence
Russo, B.C., Horzempa, J., O'Dee, D.M., Schmitt, D.M., Brown, M.J., Carlson Jr., P.E., Xavier, R.J. and Nau, G.J.
Infect. Immun., ***79(9)***, *3665-3676 (2011)*

Tularemia is a debilitating febrile illness caused by the category A biodefense agent *Francisella tularensis*. This pathogen infects over 250 different hosts, has a low infectious dose, and causes high morbidity and mortality. Our understanding of the mechanisms by which *F. tularensis* senses and adapts to host environments is incomplete. Polyamines, including spermine, regulate the interactions of *F. tularensis* with host cells. However, it is not known whether responsiveness to polyamines is necessary for the virulence of the organism. Through transposon mutagenesis of *F. tularensis* subsp. *holarctica* live vaccine strain (LVS), we identified FTL_0883 as a gene important for spermine responsiveness. In-frame deletion mutants of FTL_0883 and FTT_0615c, the homologue of FTL_0883 in *F. tularensis* subsp. *tularensis* Schu S4 (Schu S4), elicited higher levels of cytokines from human and murine macrophages compared to wild-type strains. Although deletion of FTL_0883 attenuated LVS replication within macrophages *in vitro*, the Schu S4 mutant with a deletion in FTT_0615c replicated similarly to wild-type Schu S4. Nevertheless, both the LVS and the Schu S4 mutants were significantly attenuated *in vivo*. Growth and dissemination of the Schu S4 mutant was severely reduced in the murine model of pneumonic tularemia. This attenuation depended on host responses to elevated levels of proinflammatory cytokines. These data associate responsiveness to polyamines with tularemia pathogenesis and define FTL_0883/FTT_0615c as an *F. tularensis* gene important for virulence and evasion of the host immune response.

4.945 The repair function of the multifunctional DNA repair/redox protein APE1 is neuroprotective after

ionizing radiation
Vasko, M.R., Guo, C., Thompson, E.L. and Kelley, M.R.
*DNA Repair, **10**, 942-952 (2011)*

Although exposure to ionizing radiation (IR) can produce significant neurotoxicity, the mechanisms mediating this toxicity remain to be determined. Previous studies using neurons isolated from the central nervous system show that IR produces reactive oxygen species and oxidative DNA damage in those cells. Because the base excision DNA repair pathway repairs single-base modifications caused by ROS, we asked whether manipulating this pathway by altering APE1 expression would affect radiation-induced neurotoxicity. In cultures of adult hippocampal and sensory neurons, IR produces DNA damage as measured by phosphorylation of histone H2A.X and results in dose-dependent cell death. In isolated sensory neurons, we demonstrate for the first time that radiation decreases the capsaicin-evoked release of the neuropeptide CGRP. Reducing APE1 expression in cultured cells augments IR-induced neurotoxicity, whereas overexpressing APE1 is neuroprotective. Using lentiviral constructs with a neuronal specific promoter that selectively expresses APE1s different functions in neurons, we show that selective expression of the DNA repair competent (redox inactive) APE1 constructs in sensory neurons resurrects cell survival and neuronal function, whereas use of DNA-repair deficient (redox active) constructs is not protective. Use of an APE1 redox-specific inhibitor, APX3330, also facilitates neuronal protection against IR-induced toxicity. These results demonstrate for the first time that the repair function of APE1 is required to protect both hippocampal and DRG neuronal cultures—specifically neuronal cells—from IR-induced damage, while the redox activity of APE1 does not appear to be involved.

4.946 Sample preparation method for isolation of single-cell types from mouse liver for proteomic studies
Liu, W., Hou, Y., Chen, H., Wei, H., Lin, W., Li, J., Zhang, M., He, F. and Jiang, Y.
*Proteomics, **11(17)**, 3556-3564 (2011)*

It becomes increasingly clear that separation of pure cell populations provides a uniquely sensitive and accurate approach to protein profiling in biological systems and opens up a new area for proteomic analysis. The method we described could simultaneously isolate population of hepatocytes (HCs), hepatic stellate cells (HSCs), Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) by a combination of collagenase-based density gradient centrifugation and magnetic activated cell sorting with high purity and yield for the first time. More than 98% of the isolated HCs were positive for cytokeratin 18, with a viability of 91%. Approximately 97% of the isolated HSCs expressed glial fibrillary acidic protein with a viability of 95%. Nearly 98% of isolated KCs expressed F4/80 with a viability of 94%. And the purity of LSECs reached up to 91% with a viability of 94%. And yield for HCs, HSCs, LSECs and KCs were 6.3, 1.3, 2.6 and 5.0 million per mouse. This systematic isolation method enables us to study the proteome profiling of different types of liver cells with high purity and yield, which is especially useful for sample preparation of Human Liver Proteome Project.

4.947 NOX1/nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase promotes proliferation of stellate cells and aggravates liver fibrosis induced by bile duct ligation
Cui, W., Matsuno, K., Iwata, K., Ibi, M., Matsumoto, M., Zhang, J., Zhu, K., Katsuyama, M., Torok, N.J. and Yabe-Nishimura, C.
*Hepatology, **54(3)**, 949-958 (2011)*

Among multiple isoforms of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase expressed in the liver, the phagocytic NOX2 isoform in hepatic stellate cells (HSCs) has been demonstrated to play a key role in liver fibrogenesis. The aim of this study was to clarify the role of NOX1, a nonphagocytic form of NADPH oxidase, in the development of fibrosis using *Nox1*-deficient mice (Nox1KO). Liver injury and fibrosis were induced by bile duct ligation (BDL) and carbon tetrachloride in Nox1KO and wildtype littermate mice (WT). Primary HSCs were isolated to characterize the NOX1-induced signaling cascade involved in liver fibrogenesis. Following BDL, a time-dependent increase in NOX1 messenger RNA (mRNA) was demonstrated in WT liver. Compared with those in WT, levels of collagen-1α mRNA and hydroxyproline were significantly suppressed in Nox1KO with a reduced number of activated HSCs and less severe fibrotic lesions. The expression levels of α-smooth muscle actin, a marker of HSCs activation, were similar in cultured HSCs isolated from both genotypes. However, cell proliferation was significantly attenuated in HSCs isolated from Nox1KO. In these cells, the expression of

$p27^{kip1}$, a cell cycle suppressor, was significantly up-regulated. Concomitantly, a significant reduction in phosphorylated forms of Akt and forkhead box O (FOXO) 4, a downstream effector of Akt that regulates the transcription of $p27^{kip1}$ gene, was demonstrated in Nox1KO. Finally, the level of the oxidized inactivated form of phosphatase and tensin homolog (PTEN), a negative regulator of PI3K/Akt pathway, was significantly attenuated in HSCs of Nox1KO.

4.948 Dendritic cell depletion exacerbates acetaminophen hepatotoxicity
Conolly, M.K. et al
Hepatology, ***54(3)***, *959-968 (2011)*

Acetaminophen (APAP) overdose is one of the most frequent causes of acute liver failure in the United States and is primarily mediated by toxic metabolites that accumulate in the liver upon depletion of glutathione stores. However, cells of the innate immune system, including natural killer (NK) cells, neutrophils, and Kupffer cells, have also been implicated in the centrilobular liver necrosis associated with APAP. We have recently shown that dendritic cells (DCs) regulate intrahepatic inflammation in chronic liver disease and, therefore, postulated that DC may also modulate the hepatotoxic effects of APAP. We found that DC immune-phenotype was markedly altered after APAP challenge. In particular, liver DC expressed higher MHC II, costimulatory molecules, and Toll-like receptors, and produced higher interleukin (IL)-6, macrophage chemoattractant protein-1 (MCP-1), and tumor necrosis factor alpha (TNF-α). Conversely, spleen DC were unaltered. However, APAP-induced centrilobular necrosis, and its associated mortality, was markedly exacerbated upon DC depletion. Conversely, endogenous DC expansion using FMS-like tyrosine kinase 3 ligand (Flt3L) protected mice from APAP injury. Our mechanistic studies showed that APAP liver DC had the particular capacity to prevent NK cell activation and induced neutrophil apoptosis. Nevertheless, the exacerbated hepatic injury in DC-depleted mice challenged with APAP was independent of NK cells and neutrophils or numerous immune modulatory cytokines and chemokines. *Conclusion:* Taken together, these data indicate that liver DC protect against APAP toxicity, whereas their depletion is associated with exacerbated hepatotoxicity

4.949 Neuroprotective Signaling Mechanisms of Telomerase Are Regulated by Brain-Derived Neurotrophic Factor in Rat Spinal Cord Motor Neurons
Niu, C. and Yip, H.K.
J. Neuropathol. Exp. Neurol., ***70(7)***, *634-652 (2011)*

Telomerase can promote neuron survival and can be regulated by growth factors such as brain-derived neurotrophic factor (BDNF). Increases of BDNF expression and telomerase activity after brain injury suggest that telomerase may be involved in BDNF-mediated neuroprotection. We investigated BDNF regulation of telomerase in rat spinal cord motor neurons (SMNs). Our results indicate that BDNF increases telomerase expression and activity levels in SMNs and activates mitogen-activated protein kinase/extracellular signal-regulated kinases 1 and 2 and phosphatidylinositol-3-OH kinase/protein kinase B signals, and their downstream transcription factors nuclear factor-κB, c-Myc, and Sp1. Administration of the tyrosine kinase receptor B inhibitor K-252a, the mitogen-activated protein kinase 1 inhibitor PD98059, and the phosphatidylinositol-3-OH kinase inhibitor LY294002 abolished BDNF-induced upregulation of these transcription factors and telomerase expression. The nuclear factor-κB inhibitor Bay11-7082 also attenuated c-Myc and Sp1 expression and increased telomerase promoter activity. Spinal cord motor neurons with higher telomerase levels induced by BDNF became more resistant to apoptosis; survival of SMNs that overexpressed the catalytic protein component of telomerase with reverse transcriptase activity was also enhanced against apoptosis. The neuronal survival-promoting effect of telomerase was mediated through the regulation of Bcl-2, Bax, p53, and maintenance of mitochondrial membrane potential. Taken together, these data suggest that the neuroprotective effect of BDNF via telomerase is mediated by inhibition of apoptotic pathways.

4.950 5′ Triphosphorylated Small Interfering RNAs Control Replication of Hepatitis B Virus and Induce an Interferon Response in Human Liver Cells and Mice
Ebert, G., Poeck, H., Lucifora, J., Baschuk, N., Esser, K., Essposito, I., Hartmann, G. and Protzer, U.
Gastroenterology, ***141(2)***, *696-706 (2011)*

Background & Aims
Approved therapies for chronic hepatitis B include systemic administration of interferon (IFN)-alfa and inhibitors of hepatitis B virus (HBV) reverse-transcription. Systemic application of IFN-alfa is limited by side effects. Reverse-transcriptase inhibitors effectively control HBV replication, but rarely eliminate the

virus and can select drug-resistant variants. We aimed to develop an alternative therapeutic approach that combines gene silencing with induction of IFN in the liver.
Methods
To stimulate an immune response while inhibiting HBV activity, we designed 3 small interfering (si)RNAs that target highly conserved sequences and multiple HBV transcripts of all genotypes. A 5′-triphosphate (3p) was added to the siRNAs, turning them into a ligand for the cytosolic helicase retinoic acid–inducible protein I, which becomes activated and induces expression of type-I IFNs. Antiviral activity was investigated in cell lines that replicate HBV, in HBV-infected primary human hepatocytes, and in HBV transgenic mice.
Results
3p-double-stranded RNA (3p-RNA) activated retinoic acid–inducible protein I, induced a strong type I IFN response (expression of IFN-β) in liver cells and showed transient but strong antiviral activity. Bifunctional, HBV-specific, 3p-siRNAs controlled replication of HBV more efficiently and for longer periods of time than 3p-RNAs without silencing capacity or siRNAs that targeted identical sequences but did not contain 3p.
Conclusions
HBV-specific 3p-siRNAs are bifunctional antiviral molecules that induce production of type I IFNs in the liver and target HBV RNAs to inhibit viral replication.

4.951 Thymic but not splenic CD8+ DCs can efficiently cross-prime T cells in the absence of licensing factors

Dresch, C., Ackermann, M., Vogt, B., de Andrada Pereira, B., Shortman, K. and Fraefel, C.
Eur. J. Immunol., ***41(9)****, 2544-2555 (2011)*

Cross-presentation is an important mechanism to elicit both immune defenses and tolerance. Although only a few DC subsets possess the machinery required for cross-presentation, little is known about differences in cross-presenting capabilities of DCs belonging to the same subpopulation but localized in different lymphoid organs. In this study, we demonstrate that steady-state thymic $CD8^+$ DCs can efficiently cross-prime naïve $CD8^+$ T cells in the absence of costimulation. Surprisingly, cross-priming by splenic $CD8^+$ DCs was dependent on licensing factors such as GM-CSF. In the absence of GM-CSF, antigen–MHC-class-I complexes were detected on thymic but not on splenic $CD8^+$ DCs, indicating that the cross-presentation capacity of the thymic subpopulation was higher. The observed cross-priming differences between thymic and splenic $CD8^+$ DCs did not correlate with differential antigen capture or costimulatory molecules found on the surface of DCs. Moreover, we did not detect overall impairment of antigen presentation, as peptide-loaded splenic $CD8^+$ DCs were able to induce $CD8^+$ T-cell proliferation. The observation that thymic $CD8^+$ DCs are more efficient than splenic $CD8^+$ DCs in T-cell cross-priming in the absence of licensing factors indicates that the requirements for efficient antigen presentation differ between these cells.

4.952 Immunization with dendritic cells transfected in vivo with HIV-1 plasmid DNA induces HIV-1-specific immune responses

Malm, M., Krohn, K. and Blazevic, V.
Arch. Virol., ***156(9)****, 1607-1610 (2011)*

We evaluated the importance of dendritic cells (DCs) in the induction of the immune response after immunization of mice with DNA plasmid Auxo-GTU®-MultiHIV. First, GTU®-encoded protein was shown to be expressed by DCs of the draining lymph nodes (LNs) following intradermal (i.d.) immunization. Next, donor mice were immunized with the MultiHIV DNA plasmid, and DCs were enriched and further used to immunize naïve recipient mice. For the first time, the results show that i.d. immunization with Auxo-GTU®-MultiHIV transfects DCs *in vivo*, enabling them to present antigens and induce HIV-specific immune responses in recipient mice.

4.953 The role of dystroglycan in PDGF-BB-dependent migration of activated hepatic stellate cells/myofibroblasts

Kastanis, G.J., Hernandez-Nazara, Z., Nieto, N., Rincon-Sanchez, A.R., Popratiloff, A., Dominguez-Rosales, J.A., Lechuga, C.G. and Rojkind, M.
Am. J. Physiol. Gastrintest. Liver Physiol., 301,G464-G474 (2011)

Hepatic stellate cells are embedded in the loose connective tissue matrix within the space of Disse. This extracellular matrix contains several basement membrane components including laminin, but its

composition changes during liver injury because of the production of extracellular matrix components found in scar tissue. These changes in extracellular matrix composition and in cell-extracellular matrix interactions may play a key role in hepatic stellate cell transdifferentiation. In this communication we used early passages of mouse hepatic stellate cells (activated HSC/myofibroblasts) to study the platelet-derived growth factor BB (PDGF-BB)-dependent expression and regulation of β-dystroglycan and its role in activated HSC/myofibroblast migration. We used Northern and Western analysis to study dystroglycan expression and confocal microscopy to investigate changes in subcellular distribution of the protein. Activated HSC migration was investigated using an in vitro wound-healing assay. PDGF-BB induced significant changes in dystroglycan regulation and subcellular distribution of the protein. Whereas steady-state levels of dystroglycan mRNA remained constant, PDGF-BB increased dystroglycan transcription but shortened the $t_{1/2}$ by 50%. Moreover, PDGF-BB changed dystroglycan and α5-integrin cellular distribution. Cell migration experiments revealed that PDGF-BB-dependent migration of activated HSC/myofibroblasts was completely blocked by neutralizing antibodies to fibronectin, α5-integrin, laminin, and β-dystroglycan. Overall, these findings suggest that both laminin and fibronectin and their receptors play a key role in PDGF-BB-induced activated HSC migration.

4.954 Quality of Life Improves for Pediatric Patients After Total Pancreatectomy and Islet Autotransplant for Chronic Pancreatitis

Bellin, M.D., Freeman, M.L., Schwarzenberg, S.J., Dunn, T.B., Beilman, G.J., Vickers, S.M., Chinnakotla, S., Balamurugan, A.N., Hering, B.J., Radosevich, D.M., Moran, A. and Sutherland, D.E.R.
Clin. Gastroenterol. Hepatol., ***9***, *793-799 (2011)*

Background & Aims
Total pancreatectomy (TP) and islet autotransplant (IAT) have been used to treat patients with painful chronic pancreatitis. Initial studies indicated that most patients experienced significant pain relief, but there were few validated measures of quality of life. We investigated whether health-related quality of life improved among pediatric patients undergoing TP/IAT.
Methods
Nineteen consecutive children (aged 5–18 years) undergoing TP/IAT from December 2006 to December 2009 at the University of Minnesota completed the Medical Outcomes Study 36-item Short Form (SF-36) health questionnaire before and after surgery. Insulin requirements were recorded.
Results
Before TP/IAT, patients had below average health-related quality of life, based on data from the Medical Outcomes Study SF-36; they had a mean physical component summary (PCS) score of 30 and mental component summary (MCS) score of 34 (2 and 1.5 standard deviations, respectively, below the mean for the US population). By 1 year after surgery, PCS and MCS scores improved to 50 and 46, respectively (global effect, PCS $P < .001$, MCS $P = .06$). Mean scores improved for all 8 component subscales. More than 60% of IAT recipients were insulin independent or required minimal insulin. Patients with prior surgical drainage procedures (Puestow) had lower yields of islets ($P = .01$) and greater incidence of insulin dependence ($P = .04$).
Conclusions
Quality of life (physical and emotional components) significantly improve after TP/IAT in subsets of pediatric patients with severe chronic pancreatitis. Minimal or no insulin was required for most patients, although islet yield was reduced in patients with previous surgical drainage operations.
Keywords: Pancreas; Inflammation; Therapy; Clinical Trial
Abbreviations: CP, chronic pancreatitis; HbA, hemoglobin A; HRQOL, health-related quality of life; IAT, islet autotransplant; IE, islet equivalent; MCS, mental component summary; PCS, physical component summary; SF-36, 36-Item Short Form Medical Outcomes Survey; TP, total pancreatectomy

4.955 A knockout of the caspase 2 gene produces increased resistance of the nigrostriatal dopaminergic pathway to MPTP-induced toxicity

Tiwari, M., Herman, B. and Morgan, W.W.
Exp. Neurol., ***229***, *421-428 (2011)*

This study investigated the effect of a knockout of the caspase 2 gene on the sensitivity of murine nigral dopaminergic neurons to 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP)-induced toxicity. Female wild type (WT), heterozygous caspase 2 NL (HET) and homozygous caspase 2 null (NL) mice were treated with cumulative dosages of 0, 10, 15 or 20 mg/kg MPTP free base. Without MPTP treatment, one week later dopamine (DA) levels were not significantly different in HET or NL versus WT mice. Twenty mg/kg MPTP reduced striatal DA in WT and HET ($p < 0.01$) but not NL mice. This same MPTP dosage regimen

also induced a significantly greater decrease in tyrosine hydroxylase immunopositive (TH+) protein in striata of WT compared to NL mice ($p < 0.001$).
Subsequently, WT and NL mice were treated daily with 20 mg/kg MPTP for 3 days and 25 mg/kg MPTP for 2 additional days, and TH+ neurons in the substantia nigra (SN) were estimated using unbiased stereology. When compared to untreated WT, the numbers of TH+ neurons were significantly lower in the SN of untreated NL mice ($p < 0.05$). Treatment with the MPTP regimen significantly reduced TH+ neurons in WT mice but not NL mice.
In primary mesencephalic cultures both the cell bodies and the neuronal processes of TH immunopositive (TH+) neurons from NL embryos were significantly ($p < 0.001$) more resistant to 10 μM MPP+ compared to WT. Following MPP+ treatment, features of apoptotic cell death were also significantly ($p < 0.001$) more prevalent in nuclei of TH+ neurons in cultures prepared from WT versus NL mouse pups.
These results suggest that caspase 2 may play a role in modulating the MPTP-induced damage to the nigrostriatal dopaminergic system.

4.956 Integrin expression and function in the response of primary culture hepatic stellate cells to connective tissue growth factor (CCN2)
Huang, G. and Brigstock, D.R.
*J. Cell. Mol. Med., **15(5)**, 1087-1095 (2011)*

Production of connective tissue growth factor (CCN2, also known as CTGF) is a hallmark of hepatic fibrosis. This study examined early primary cultures of hepatic stellate cells (HSC) for (*i*) CCN2 regulation of its cognate receptor integrin subunits; and (*ii*) interactions between CCN2 and integrin $\alpha_5\beta_1$, heparan sulphate proteoglycans (HSPG) or fibronectin (FN) in supporting cell adhesion. HSC were isolated from healthy male Balb/c mice. mRNA levels of CCN2 or α_5, β_1, αv or β_3 integrin subunits were measured in days 1–7 primary culture HSC, and day 3 or day 7 cells treated with recombinant CCN2 or CCN2 small interfering RNA. Interactions between CCN2 and integrin $\alpha_5\beta_1$, HSPG or FN were investigated using an *in vitro* cell adhesion assay. Co-incident with autonomous activation over the first 7 days, primary culture HSC increasingly expressed mRNA for CCN2 or integrin subunits. Addition of exogenous CCN2 or knockdown of endogenous CCN2 differentially regulated integrin gene expression in day 3 *versus* day 7 cells. Either full length CCN2 ('$CCN2_{1–4}$') or residues 247–349 containing module 4 alone ('$CCN2_4$') supported day 3 cell adhesion in an integrin $\alpha_5\beta_1$- and HSPG-dependent fashion. Adhesion of day 3 cells to FN was promoted in an integrin $\alpha_5\beta_1$-dependent manner by $CCN2_{1–4}$ or $CCN2_4$, whereas FN promoted HSPG-dependent HSC adhesion to $CCN2_{1–4}$ or $CCN2_4$. These findings suggest CCN2 regulates integrin expression in primary culture HSC and supports HSC adhesion *via* its binding of cell surface integrin $\alpha_5\beta_1$, a novel CCN2 receptor in primary culture HSC which interacts co-operatively with HSPG or FN.

4.957 Necdin Protects Embryonic Motoneurons from Programmed Cell Death
Aebisher, J., Sturny, R., Andrieu, D., Rieusset, A., Schaller, F., Geib, S., Raoul, C. and Muscatelli, F.
*PloS One, **6(9)**, e23764 (2011)*

NECDIN belongs to the type II Melanoma Associated Antigen Gene Expression gene family and is located in the Prader-Willi Syndrome (PWS) critical region. *Necdin*-deficient mice develop symptoms of PWS, including a sensory and motor deficit. However, the mechanisms underlying the motor deficit remain elusive. Here, we show that the genetic ablation of *Necdin*, whose expression is restricted to post-mitotic neurons in the spinal cord during development, leads to a loss of 31% of specified motoneurons. The increased neuronal loss occurs during the period of naturally-occurring cell death and is not confined to specific pools of motoneurons. To better understand the role of *Necdin* during the period of programmed cell death of motoneurons we used embryonic spinal cord explants and primary motoneuron cultures from *Necdin*-deficient mice. Interestingly, while *Necdin*-deficient motoneurons present the same survival response to neurotrophic factors, we demonstrate that deletion of *Necdin* leads to an increased susceptibility of motoneurons to neurotrophic factor deprivation. We show that by neutralizing TNFα this increased susceptibility of *Necdin*-deficient motoneurons to trophic factor deprivation can be reduced to the normal level. We propose that Necdin is implicated through the TNF-receptor 1 pathway in the developmental death of motoneurons.

4.958 Nonoverlapping functions for Notch1 and Notch3 during murine steady-state thymic lymphopoiesis
Shi, J., Fallahi, M., Luo, J-L. and Petrie, H.T.
*Blood, **118(9)**, 2511-2519 (2011)*

Notch1 signaling is absolutely essential for steady-state thymic lymphopoiesis, but the role of other Notch

receptors, and their potential overlap with the function of Notch1, remains unclear. Here we show that like Notch1, Notch3 is differentially expressed by progenitor thymocytes, peaking at the DN3 progenitor stage. Using mice carrying a gene-trapped allele, we show that thymic cellularity is slightly reduced in the absence of Notch3, although progression through the defined sequence of TCR-αβ development is normal, as are NKT and TCRγδ cell production. The absence of a profound effect from Notch3 deletion is not explained by residual function of the gene-trapped allele because insertion mapping suggests that the targeted allele would not encode functional signaling domains. We also show that although Notch1 and Notch3 are coexpressed on some early intrathymic progenitors, the relatively mild phenotype seen after Notch3 deletion does not result from the compensatory function of Notch1, nor does Notch3 function explain the likewise mild phenotype seen after conditional (intrathymic) deletion of Notch1. Our studies indicate that Notch1 and Notch3 carry out nonoverlapping functions during thymocyte differentiation, and that while Notch1 is absolutely required early in the lymphopoietic process, neither receptor is essential at later stages.

4.959 Quantitative Assessment of Immune Cells in the Injured Spinal Cord Tissue by Flow Cytometry: a Novel Use for a Cell Purification Method

Nguyen, H.X., Beck, K.D. and Anderson, A.J.

J. Vis. Exp., ***50***, *(2011)* http://www.jove.com/details.php?id=2698

Detection of immune cells in the injured central nervous system (CNS) using morphological or histological techniques has not always provided true quantitative analysis of cellular inflammation. Flow cytometry is a quick alternative method to quantify immune cells in the injured brain or spinal cord tissue. Historically, flow cytometry has been used to quantify immune cells collected from blood or dissociated spleen or thymus, and only a few studies have attempted to quantify immune cells in the injured spinal cord by flow cytometry using fresh dissociated cord tissue. However, the dissociated spinal cord tissue is concentrated with myelin debris that can be mistaken for cells and reduce cell count reliability obtained by the flow cytometer. We have advanced a cell preparation method using the OptiPrep gradient system to effectively separate lipid/myelin debris from cells, providing sensitive and reliable quantifications of cellular inflammation in the injured spinal cord by flow cytometry. As described in our recent study (*Beck & Nguyen et al., Brain. 2010 Feb; 133 (Pt 2): 433-47*), the OptiPrep cell preparation had increased sensitivity to detect cellular inflammation in the injured spinal cord, with counts of specific cell types correlating with injury severity. Critically, novel usage of this method provided the first characterization of acute and chronic cellular inflammation after SCI to include a complete time course for polymorphonuclear leukocytes (PMNs, neutrophils), macrophages/microglia, and T-cells over a period ranging from 2 hours to 180 days post-injury (dpi), identifying a surprising novel second phase of cellular inflammation. Thorough characterization of cellular inflammation using this method may provide a better understanding of neuroinflammation in the injured CNS, and reveal an important multiphasic component of neuroinflammation that may be critical for the design and implementation of rational therapeutic treatment strategies, including both cell-based and pharmacological interventions for SCI.

4.960 The Culture of Primary Motor and Sensory Neurons in Defined Media on Electrospun Poly-L-lactide Nanofiber Scaffolds

Leach, M.K., Feng, Z-Q., Gertz, C.C., Tuck, S.J., Regan, T.M., Naim, Y., Vincent, A.M. and Corey, J.M.

J. Vis. Exp., ***48***, *(2011)*, http://www.jove.com/details.php?id=2389

Electrospinning is a technique for producing micro- to nano-scale fibers. Fibers can be electrospun with varying degrees of alignment, from highly aligned to completely random. In addition, fibers can be spun from a variety of materials, including biodegradable polymers such as poly-L-lactic acid (PLLA). These characteristics make electrospun fibers suitable for a variety of scaffolding applications in tissue engineering. Our focus is on the use of aligned electrospun fibers for nerve regeneration. We have previously shown that aligned electrospun PLLA fibers direct the outgrowth of both primary sensory and motor neurons *in vitro*. We maintain that the use of a primary cell culture system is essential when evaluating biomaterials to model real neurons found *in vivo* as closely as possible. Here, we describe techniques used in our laboratory to electrospin fibrous scaffolds and culture dorsal root ganglia explants, as well as dissociated sensory and motor neurons, on electrospun scaffolds. However, the electrospinning and/or culture techniques presented here are easily adapted for use in other applications.

4.961 Transcriptional profiling of peripheral lymphoid tissue reveals genes and networks linked to SSBP/1 scrapie pathology in sheep

Gossner, A., Roupaka, S., Foster, J., Hunter, N. and Hopkins, J.

Vet. Microbiol., ***153***, *218-228 (2011)*

Transmissible spongiform encephalopathies (TSEs) are slow and progressive neurodegenerative diseases of humans and animals. The major target organ for all TSEs is the brain but some TSE agents are associated with prior accumulation within the peripheral lymphoid system. Many studies have examined the effects of scrapie infection on the expression of central nervous system (CNS) genes, but this study examines the progression of scrapie pathology in the peripheral lymphoid system and how scrapie infection affects the transcriptome of the lymph nodes and spleen.
Infection of sheep with SSBP/1 scrapie resulted in PrP^{Sc} deposition in the draining prescapular lymph node (PSLN) by 25 days post infection (dpi) in VRQ/VRQ genotype sheep and 75 dpi in tonsils and spleen. Progression of PrP^{Sc} deposition in VRQ/ARR animals was 25 dpi later in the PSLN and 250 dpi later in spleen. Microarray analysis of 75 dpi tissues from VRQ/VRQ sheep identified 52 genes in PSLN and 37 genes in spleen cells that showed significant difference ($P \leq 0.05$) between scrapie-infected and mock-infected animals. Transcriptional pathway analysis highlighted immunological disease, cell death and neurological disease as the biological pathways associated with scrapie pathogenesis in the peripheral lymphoid system. PrP^{Sc} accumulation of lymphoid tissue resulted in the repression of genes linked to inflammation and oxidative stress, and the up-regulation of genes related to apoptosis.

4.962 Dysregulation of astrocyte–motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis
Ferraiuolo, L., Higginbottom, A., Hath, P.R., Barber, S., Greenald, D., Kirby, J. and Shaw, P.J.
Brain, ***134****, 2627-2641 (2011)*

Amyotrophic lateral sclerosis is a neurodegenerative disease in which death of motoneurons leads to progressive failure of the neuromuscular system resulting in death frequently within 2–3 years of symptom onset. Focal onset and propagation of the disease symptoms to contiguous motoneuron groups is a striking feature of the human disease progression. Recent work, using mutant superoxide dismutase 1 murine models and *in vitro* culture systems has indicated that astrocytes are likely to contribute to the propagation of motoneuron injury and disease progression. However, the basis of this astrocyte toxicity and/or failure of motoneuron support has remained uncertain. Using a combination of *in vivo* and *in vitro* model systems of superoxide dismutase 1-related amyotrophic lateral sclerosis, linked back to human biosamples, we set out to elucidate how astrocyte properties change in the presence of mutant superoxide dismutase 1 to contribute to motoneuron injury. Gene expression profiling of spinal cord astrocytes from presymptomatic transgenic mice expressing mutant superoxide dismutase 1 revealed two striking changes. First, there was evidence of metabolic dysregulation and, in particular, impairment of the astrocyte lactate efflux transporter, with resultant decrease of spinal cord lactate levels. Second, there was evidence of increased nerve growth factor production and dysregulation of the ratio of pro-nerve growth factor to mature nerve growth factor, favouring p75 receptor expression and activation by neighbouring motoneurons. Functional *in vitro* studies showed that astrocytes expressing mutant superoxide dismutase 1 are toxic to normal motoneurons. We provide evidence that reduced metabolic support from lactate release and activation of pro-nerve growth factor-p75 receptor signalling are key components of this toxicity. Preservation of motoneuron viability could be achieved by increasing lactate provision to motoneurons, depletion of increased pro-nerve growth factor levels or p75 receptor blockade. These findings are likely to be relevant to human amyotrophic lateral sclerosis, where we have demonstrated increased levels of pro-nerve growth factor in cerebrospinal fluid and increased expression of the p75 receptor by spinal motoneurons. Taken together, these data confirm that altered properties of astrocytes are likely to play a crucial role in the propagation of motoneuron injury in superoxide dismutase 1-related amyotrophic lateral sclerosis and indicate that manipulation of the energy supply to motoneurons as well as inhibition of p75 receptor signalling may represent valuable neuroprotective strategies.

4.963 Motor neuron impairment mediated by a sumoylated fragment of the glial glutamate transporter EAAT2
Foran, E., Bogush, A., Goffredo, M., Roncaglia, P., Gustincich, S., Pasinell, P. and Trotti, D.
Glia, ***59(11)****, 1719-1731 (2011)*

Dysregulation of glutamate handling ensuing downregulation of expression and activity levels of the astroglial glutamate transporter EAAT2 is implicated in excitotoxic degeneration of motor neurons in amyotrophic lateral sclerosis (ALS). We previously reported that EAAT2 (a.k.a. GLT-1) is cleaved by caspase-3 at its cytosolic carboxy-terminus domain. This cleavage results in impaired glutamate transport activity and generates a proteolytic fragment (CTE) that we found to be post-translationally conjugated by

SUMO1. We show here that this sumoylated CTE fragment accumulates in the nucleus of spinal cord astrocytes of the SOD1-G93A mouse model of ALS at symptomatic stages of disease. Astrocytic expression of CTE, artificially tagged with SUMO1 (CTE-SUMO1) to mimic the native sumoylated fragment, recapitulates the nuclear accumulation pattern of the endogenous EAAT2-derived proteolytic fragment. Moreover, in a co-culture binary system, expression of CTE-SUMO1 in spinal cord astrocytes initiates extrinsic toxicity by inducing caspase-3 activation in motor neuron-derived NSC-34 cells or axonal growth impairment in primary motor neurons. Interestingly, prolonged nuclear accumulation of CTE-SUMO1 is intrinsically toxic to spinal cord astrocytes, although this gliotoxic effect of CTE-SUMO1 occurs later than the indirect, noncell autonomous toxic effect on motor neurons. As more evidence on the implication of SUMO substrates in neurodegenerative diseases emerges, our observations strongly suggest that the nuclear accumulation in spinal cord astrocytes of a sumoylated proteolytic fragment of the astroglial glutamate transporter EAAT2 could participate to the pathogenesis of ALS and suggest a novel, unconventional role for EAAT2 in motor neuron degeneration.

4.964 Isolation and Culture of Postnatal Spinal Motoneurons
Milligan, C. and Difondorwa, D.
Methods in Mol. Biol., ***793***, *77-85 (2011)*

Neuronal cultures, including motoneuron (MN) cultures, are established from embryonic animals. These approaches have provided novel insights into developmental and possibly disease mechanisms mediating cell survival or death. Motoneurons isolated from mouse models of disease, such as the SOD1G93A mouse, demonstrate subtle abnormalities that may contribute to pathology. Nonetheless, in the animal model, pathological events become more prominent as the animal matures, but the ability to isolate individual cells to investigate these events is limited. Here, we describe a protocol derived and modified from previously published protocols to isolate motoneurons from mature animals. While the yield of cells is low, the ability to examine mature motoneurons provides a new platform to investigate pathological changes associated with motoneuron disease.

4.965 Proteomic Analysis of Anaplasma phagocytophilum during Infection of Human Myeloid Cells Identifies a Protein That Is Pronouncedly Upregulated on the Infectious Dense-Cored Cell
Troese, M.J., Kahlon, A., Ragland, S.A., Ottens, A.K., Ojogun, N., Nelson, K.T., Walker, N.J., Borjesson, D.L. and Carlyon, J.A.
Infect. Immun., ***79(11)***, *4696-4707 (2011)*

Anaplasma phagocytophilum is an obligate intracellular bacterium that invades neutrophils to cause the emerging infectious disease human granulocytic anaplasmosis. *A. phagocytophilum* undergoes a biphasic developmental cycle, transitioning between an infectious dense-cored cell (DC) and a noninfectious reticulate cell (RC). To gain insights into the organism's biology and pathogenesis during human myeloid cell infection, we conducted proteomic analyses on *A. phagocytophilum* organisms purified from HL-60 cells. A total of 324 proteins were unambiguously identified, thereby verifying 23.7% of the predicted *A. phagocytophilum* proteome. Fifty-three identified proteins had been previously annotated as hypothetical or conserved hypothetical. The second most abundant gene product, after the well-studied major surface protein 2 (P44), was the hitherto hypothetical protein APH_1235. APH_1235 homologs are found in other *Anaplasma* and *Ehrlichia* species but not in other bacteria. The *aph_1235* RNA level is increased 70-fold in the DC form relative to that in the RC form. Transcriptional upregulation of and our ability to detect APH_1235 correlate with RC to DC transition, DC exit from host cells, and subsequent DC binding and entry during the next round of infection. Immunoelectron microscopy pronouncedly detects APH_1235 on DC organisms, while detection on RC bacteria minimally, at best, exceeds background. This work represents an extensive study of the *A. phagocytophilum* proteome, discerns the complement of proteins that is generated during survival within human myeloid cells, and identifies APH_1235 as the first known protein that is pronouncedly upregulated on the infectious DC form.

4.966 Vasoactive intestinal peptide reduces oxidative stress in pancreatic acinar cells through the inhibition of NADPH oxidase
Fujimori, N., Oono, T., Igarashi, H., Ito, T., Nakamura, T., Uchida, M., Coy, D.H., Jensen, R.T. and Takayanagi, R.
Peptides, ***32***, *2067-2076 (2011)*

Vasoactive intestinal peptide (VIP) attenuates experimental acute pancreatitis (AP) by inhibition of cytokine production from inflammatory cells. It has been suggested that reactive oxygen species (ROS) as

well as cytokines play pivotal roles in the early pathophysiology of AP. This study aimed to clarify the effect of VIP on the oxidative condition in pancreas, especially pancreatic acinar cells (acini). Hydrogen peroxide (H_2O_2)-induced intracellular ROS, assessed with CM-H_2DCFDA, increased time- and dose-dependently in acini isolated from rats. Cell viability due to ROS-induced cellular damage, evaluated by MTS assay, was decreased with ≥100 μmol/L H_2O_2. VIP significantly inhibited ROS production from acini and increased cell viability in a dose-dependent manner. Expression of antioxidants including catalase, glutathione reductase, superoxide dismutase (SOD) 1 and glutathione peroxidase was not altered by VIP except for SOD2. Furthermore, Nox1 and Nox2, major components of NADPH oxidase, were expressed in pancreatic acini, and significantly increased after H_2O_2 treatment. Also, NADPH oxidase activity was provoked by H_2O_2. VIP decreased NADPH oxidase activity, which was abolished by PKA inhibitor H89. These results suggested that VIP affected the mechanism of ROS production including NADPH oxidase through induction of a cAMP/PKA pathway. In conclusion, VIP reduces oxidative stress in acini through the inhibition of NADPH oxidase. These results combined with findings of our previous study suggest that VIP exerts its protective effect in pancreatic damage, not only through an inhibition of cytokine production, but also through a reduction of the injury caused by oxidative stress.

4.967 A Double-Inactivated Severe Acute Respiratory Syndrome Coronavirus Vaccine Provides Incomplete Protection in Mice and Induces Increased Eosinophilic Proinflammatory Pulmonary Response upon Challenge

Bolles, M., Deming, D., Long, K., Agnihothram, S., Whitmore, A., Ferris, M., Funkhouser, W., Gralinski, L., Totura, A., Heise, M. and Baric, R.S.

J. Virol., ***85(23)***, *12201-12215 (2011)*

Severe acute respiratory syndrome coronavirus (SARS-CoV) is an important emerging virus that is highly pathogenic in aged populations and is maintained with great diversity in zoonotic reservoirs. While a variety of vaccine platforms have shown efficacy in young-animal models and against homologous viral strains, vaccine efficacy has not been thoroughly evaluated using highly pathogenic variants that replicate the acute end stage lung disease phenotypes seen during the human epidemic. Using an adjuvanted and an unadjuvanted double-inactivated SARS-CoV (DIV) vaccine, we demonstrate an eosinophilic immunopathology in aged mice comparable to that seen in mice immunized with the SARS nucleocapsid protein, and poor protection against a nonlethal heterologous challenge. In young and 1-year-old animals, we demonstrate that adjuvanted DIV vaccine provides protection against lethal disease in young animals following homologous and heterologous challenge, although enhanced immune pathology and eosinophilia are evident following heterologous challenge. In the absence of alum, DIV vaccine performed poorly in young animals challenged with lethal homologous or heterologous strains. In contrast, DIV vaccines (both adjuvanted and unadjuvanted) performed poorly in aged-animal models. Importantly, aged animals displayed increased eosinophilic immune pathology in the lungs and were not protected against significant virus replication. These data raise significant concerns regarding DIV vaccine safety and highlight the need for additional studies of the molecular mechanisms governing DIV-induced eosinophilia and vaccine failure, especially in the more vulnerable aged-animal models of human disease.

4.968 Immune Adjuvant Efficacy of CpG Oligonucleotide in Cancer Treatment Is Founded Specifically upon TLR9 Function in Plasmacytoid Dendritic Cells

Nierkens, S., den Brok, M.H., Garcia, Z. et al

Cancer Res., ***71(20)***, *6428-6437 (2011)*

The differences in function, location, and migratory pattern of conventional dendritic cells (cDC) and plasmacytoid DCs (pDC) not only point to specialized roles in immune responses but also signify additive and interdependent relationships required to clear pathogens. We studied the *in vivo* requirement of cross-talk between cDCs and pDCs for eliciting antitumor immunity against *in situ* released tumor antigens in the absence or presence of the Toll-like receptor (TLR) 9 agonist CpG. Previous data indicated that CpG boosted tumor-specific T-cell responses after *in vivo* tumor destruction and increased survival after tumor rechallenges. The present study shows that cDCs are indispensable for cross-presentation of ablation-released tumor antigens and for the induction of long-term antitumor immunity. Depletion of pDCs or applying this model in type I IFN receptor–deficient mice abrogated CpG-mediated responses. $CD8\alpha^+$ cDCs and the recently identified merocytic cDCs were dependent on pDCs for CpG-induced upregulation of CD80. Moreover, DC transfer studies revealed that merocytic cDCs and $CD8\alpha^+$ cDCs were most susceptible to pDC help and subsequently promoted tumor-free survival in a therapeutic setting. By transferring wild-type pDCs into TLR9-deficient mice, we finally showed that TLR9 expression in pDCs is sufficient to benefit from CpG as an adjuvant. These studies indicate that the efficacy of CpG in cancer

immunotherapy is dependent on cross-talk between pDCs and specific subsets of cDCs.

4.969 Low-avidity anti-HPA-1a alloantibodies are capable of antigen-positive platelet destruction in the NOD/SCID mouse model of alloimmune thrombocytopenia

Backchoul, T., Kubiak, S., Krautwurst, A., Roderfeld, M., Siebert, H.C., Bein, G., Sachs, U.J. and Santosa, S.

*Transfusion, **51(11)**, 2455-2461 82011)*

BACKGROUND: Neonatal alloimmune thrombocytopenia (NAIT) is mostly caused by maternal antibodies against human platelet antigen 1a (HPA-1a) expressed on glycoprotein (GP) IIb/IIIa. Accumulated evidence indicated that anti-HPA-1a could be overlooked by standard methods due to low avidity. Low-avidity HPA-1a antibodies were shown to be detectable by surface plasmon resonance (SPR). We sought to investigate the frequency and in vivo relevance of low-avidity anti-HPA-1a.
STUDY DESIGN AND METHODS: A retrospective cohort consisting of 82 HPA-1bb mothers of HPA-1ab newborns with thrombocytopenia was analyzed using standard serologic methods. Maternal immunoglobulin (Ig)G fractions were investigated for low-avidity antibodies in SPR using purified GPIIb/IIIa (HPA-1a or -1b). The capability of HPA-1a antibodies to clear platelets (PLTs) in vivo was analyzed using the NOD/SCID mouse model of alloimmune thrombocytopenia.
RESULTS: HPA antibodies were detectable in sera from 68 of 82 (83%) mothers using standard serologic methods and undetectable in 14 of 82 sera. In SPR, IgG fractions of sera reacting positive in monoclonal antibody immobilization of PLT antigen (MAIPA) assay showed specific binding to an HPA-1a flow cell (mean, 87 ± 21 resonance units [RU]). When MAIPA-negative sera were tested in SPR, binding with low avidity was observed in 7 of 14 to HPA-1a (mean, 31 ± 5 RU), but not to HPA-1b flow cell (mean, 5 ± 2 RU). In vivo, low-avidity antibodies were capable of clearing HPA-1ab PLTs but not HPA-1bb PLTs in a NOD/SCID mouse model. Elimination kinetics were slower than observed with MAIPA-positive antibodies.
CONCLUSIONS: Low-avidity HPA-1a antibodies are present in a significant number of NAIT cases and, although they can escape detection by standard serology, they harbor the capability of PLT destruction in vivo.

4.970 Ginger Phenylpropanoids Inhibit IL-1β and Prostanoid Secretion and Disrupt Arachidonate-Phospholipid Remodeling by Targeting Phospholipases A2

Nievergelt, A., Marazzi, J., Schoop, r., Altmann, K-H. and Gertsch, J.

*J. Immunol., **187(8)**, 4140-4150 (2011)*

The rhizome of ginger (*Zingiber officinale*) is employed in Asian traditional medicine to treat mild forms of rheumatoid arthritis and fever. We have profiled ginger constituents for robust effects on proinflammatory signaling and cytokine expression in a validated assay using human whole blood. Independent of the stimulus used (LPS, PMA, anti-CD28 Ab, anti-CD3 Ab, and thapsigargin), ginger constituents potently and specifically inhibited IL-1β expression in monocytes/macrophages. Both the calcium-independent phospholipase A_2 ($iPLA_2$)-triggered maturation and the cytosolic phospholipase A_2 ($cPLA_2$)-dependent secretion of IL-1β from isolated human monocytes were inhibited. In a fluorescence-coupled PLA_2 assay, most major ginger phenylpropanoids directly inhibited $i/cPLA_2$ from U937 macrophages, but not hog pancreas secretory phospholipase A_2. The effects of the ginger constituents were additive and the potency comparable to the mechanism-based inhibitor bromoenol lactone for $iPLA_2$ and methyl arachidonyl fluorophosphonate for $cPLA_2$, with 10-gingerol/-shogaol being most effective. Furthermore, a ginger extract (2 μg/ml) and 10-shogaol (2 μM) potently inhibited the release of PGE_2 and thromboxane B2 (>50%) and partially also leukotriene B_4 in LPS-stimulated macrophages. Intriguingly, the total cellular arachidonic acid was increased 2- to 3-fold in U937 cells under all experimental conditions. Our data show that the concurrent inhibition of $iPLA_2$ and prostanoid production causes an accumulation of free intracellular arachidonic acid by disrupting the phospholipid deacylation-reacylation cycle. The inhibition of $i/cPLA_2$, the resulting attenuation of IL-1β secretion, and the simultaneous inhibition of prostanoid production by common ginger phenylpropanoids uncover a new anti-inflammatory molecular mechanism of dietary ginger that may be exploited therapeutically.

4.971 Helicobacter pylori DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates dextran sodium sulphate-induced colitis

Luther, J., Owyang, S.Y., takeuchi, T., Cole, T.S., Zhang, M., Liu, M., Erb-Downward, J., Rubenstein, J.H., Chen, C-C., Pierzchala, A.V., Paul, J.A. and Kao, J.Y.

*Gut, **60**, 1479-1486 (2011)*

Background and aims Epidemiological data have recently emerged to suggest *Helicobacter pylori* may protect against certain chronic inflammatory diseases such as inflammatory bowel disease (IBD). However, the mechanism for the observed inverse association between *H pylori* and IBD has not been described.
Methods The frequency of immunoregulatory (IRS) to immunostimulatory (ISS) sequences within the genome of various bacteria was calculated using MacVector software. The induction of type I IFN and IL-12 responses by DNA-pulsed murine bone marrow-derived dendritic cells (BMDC) and human plasmacytoid dendritic cells (DC) was analysed by cytokine production. The effect of *H pylori* DNA on *Escherichia coli* DNA production of type I IFN and IL-12 was assessed. The in-vivo significance of *H pylori* DNA suppression was assessed in a dextran sodium sulphate (DSS) model of colitis. The systemic levels of type I IFN were assessed in *H pylori*-colonised and non-colonised patients.
Results *H pylori* DNA has a significantly elevated IRS:ISS ratio. In-vitro experiments revealed the inability of *H pylori* DNA to stimulate type I IFN or IL-12 production from mouse BMDC or human plasmacytoid DC. *H pylori* DNA was also able to suppress *E coli* DNA production of type I IFN and IL-12. The administration of *H pylori* DNA before the induction of DSS colitis significantly ameliorated the severity of colitis compared with *E coli* DNA or vehicle control in both an acute and chronic model. Finally, the systemic levels of type I IFN were found to be lower in *H pylori*-colonised patients than non-colonised controls.
Conclusions This study indicates that *H pylori* DNA has the ability to downregulate pro-inflammatory responses from DC and this may partly explain the inverse association between *H pylori* and IBD.

4.972 Mitochondrial calcium and its regulation in neurodegeneration induced by oxidative stress
Barsukova, A.G., Bourdette, D. and Forte, M.
Eur. J. Neurosci., ***34(3)***, *437-447 (2011)*

A proposed mechanism of neuronal death associated with a variety of neurodegenerative diseases is the response of neurons to oxidative stress and consequent cytosolic Ca^{2+} overload. One hypothesis is that cytosolic Ca^{2+} overload leads to mitochondrial Ca^{2+} overload and prolonged opening of the permeability transition pore (PTP), resulting in mitochondrial dysfunction. Elimination of cyclophilin D (CyPD), a key regulator of the PTP, results in neuroprotection in a number of murine models of neurodegeneration in which oxidative stress and high cytosolic Ca^{2+} have been implicated. However, the effects of oxidative stress on the interplay between cytosolic and mitochondrial Ca^{2+} in adult neurons and the role of the CyPD-dependent PTP in these dynamic processes have not been examined. Here, using primary cultured cerebral cortical neurons from adult wild-type (WT) mice and mice missing cyclophilin D (CyPD-KO), we directly assess cytosolic and mitochondrial Ca^{2+}, as well as ATP levels, during oxidative stress. Our data demonstrate that during acute oxidative stress mitochondria contribute to neuronal Ca^{2+} overload by release of their Ca^{2+} stores. This result contrasts with the prevailing view of mitochondria as a buffer of cytosolic Ca^{2+} under stress conditions. In addition, we show that CyPD deficiency reverses the release of mitochondrial Ca^{2+}, leading to lower of cytosolic Ca^{2+} levels, attenuation of the decrease in cytosolic and mitochondrial ATP, and a significantly higher viability of adult CyPD-knockout neurons following exposure of neurons oxidative stress. The study offers a first insight into the mechanism underlying CyPD-dependent neuroprotection during oxidative stress.

4.973 Mechanism for antioxidative effects of thiazolidinediones in pancreatic β-cells
Chung, S.S., Kim, M., Lee, J.S., Ahn, B.Y., Jung, H.S., Lee, H.M. and Park, K.S.
Am. J. Physiol. Endocrinol. Metab., ***301***, *E912-E921 (2011)*

Thiazolidinediones (TZDs) are synthetic ligands of peroxisome proliferator-activated receptor-γ (PPARγ), a member of the nuclear receptor superfamily. TZDs are known to increase insulin sensitivity and also to have an antioxidative effect. In this study, we tested whether TZDs protect pancreatic β-cells from oxidative stress, and we investigated the mechanism involved in this process. To generate oxidative stress in pancreatic β-cells (INS-1 and βTC3) or isolated islets, glucose oxidase was added to the media. The extracellular and intracellular reactive oxygen species (ROS) were measured to directly determine the antioxidant effect of TZDs. The phosphorylation of JNK/MAPK after oxidative stress was detected by Western blot analysis, and glucose-stimulated insulin secretion and cell viability were also measured. TZDs significantly reduced the ROS levels that were increased by glucose oxidase, and they effectively prevented β-cell dysfunction. The antioxidative effect of TZDs was abolished in the presence of a PPARγ

antagonist, GW9662. Real-time PCR was used to investigate the expression levels of antioxidant genes. The expression of catalase, an antioxidant enzyme, was increased by TZDs in pancreatic β-cells, and the knockdown of catalase significantly inhibited the antioxidant effect of TZDs. These results suggest that TZDs effectively protect pancreatic β-cells from oxidative stress, and this effect is dependent largely on PPARγ. In addition, the expression of catalase is increased by TZDs, and catalase, at least in part, mediates the antioxidant effect of TZDs in pancreatic β-cells.

4.974 Loss of CFTR Affects Biliary Epithelium Innate Immunity and Causes TLR4–NF-κB—Mediated Inflammatory Response in Mice

Fiorotto, R., Scirpo, R., Trauner, M., Fabris, L., Hoque, R., Spirli, C. and Strazzabosco, M.
*Gastroenterology, **141(4)**, 1498-1508 (2011)*

Background & Aims
Loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR) in the biliary epithelium reduces bile flow and alkalinization in patients with cystic fibrosis (CF). Liver damage is believed to result from ductal cholestasis, but only 30% of patients with CF develop liver defects, indicating that another factor is involved. We studied the effects of CFTR deficiency on Toll-like receptor 4 (TLR4)-mediated responses of the biliary epithelium to endotoxins.
Methods
Dextran sodium sulfate (DSS) was used to induce colitis in C57BL/6J-*Cftr*^{tm1Unc} (Cftr-KO) mice and their wild-type littermates. Ductular reaction and portal inflammation were quantified by keratin-19 and CD45 immunolabeling. Cholangiocytes isolated from wild-type and Cftr-KO mice were challenged with lipopolysaccharide (LPS); cytokine secretion was quantified. Activation of nuclear factor κB (NF-κB), phosphorylation of TLR4, and activity of Src were determined. HEK-293 that expressed the secreted alkaline phosphatase reporter and human *TLR4* were transfected with CFTR complementary DNAs.
Results
DSS-induced colitis caused biliary damage and portal inflammation only in Cftr-KO mice. Biliary damage and inflammation were not attenuated by restoring biliary secretion with 24-nor-ursodeoxycholic acid but were significantly reduced by oral neomycin and polymyxin B, indicating a pathogenetic role of gut-derived bacterial products. Cftr-KO cholangiocytes incubated with LPS secreted significantly higher levels of cytokines regulated by TLR4 and NF-κB. LPS-mediated activation of NF-κB was blocked by the TLR4 inhibitor TAK-242. TLR4 phosphorylation by Src was significantly increased in Cftr-KO cholangiocytes. Expression of wild-type CFTR in the HEK293 cells stimulated with LPS reduced activation of NF-κB.
Conclusions
CFTR deficiency alters the innate immunity of the biliary epithelium and reduces its tolerance to endotoxin, resulting in an Src-dependent inflammatory response mediated by TLR4 and NF-κB. These findings might be used to develop therapies for CF-associated cholangiopathy.

4.975 Preferential Expression of Integrin αvβ8 Promotes Generation of Regulatory T Cells by Mouse CD103+ Dendritic Cells

Paidassi, H., Acharya, M., Zhang, A., Mukhopadhyay, S., Kwon, M., Chow, C., Stuart, L.M., Savill, J. and Lacy-Hulbert, A.
*Gastroenterology, **141(5)**, 1813-1820 (2011)*

Background & Aims
Immune responses in the intestine are controlled by regulatory T cells (Tregs), which prevent inflammation in response to commensal bacteria. A specific population of intestinal dendritic cells (DCs), marked by expression of CD103, generate Tregs more efficiently than other DC populations through mechanisms that involve retinoic acid and transforming growth factor (TGF)-β. However, it is not clear how CD103$^+$ DCs are specialized for this function. We investigated the ability of CD103$^+$ DCs to promote Treg generation through activation of TGF-β and the role of integrins with the αv subunit in this process.
Methods
Naïve T cells were cultured with purified DCs from mesenteric lymph nodes (MLNs) or intestines of wild-type and αv conditional knockout mice to assess generation of Tregs. Antigens were administered orally to mice, and antigen-specific generation of Tregs was measured in intestinal tissues. Expression of the integrin αv subunit was measured in purified subpopulations of DCs by quantitative polymerase chain reaction and immunoblot analyses.
Results
In vitro, CD103$^+$ DCs generated more Tregs in the presence of latent TGF-β than other MLN DCs. Efficient generation of Tregs required expression of the integrin αv subunit by DCs; mice that lacked αv in

immune cells did not convert naïve T cells to intestinal Tregs in response to oral antigen. $CD103^+$ DCs derived from the MLNs selectively expressed high levels of integrin αvβ8 compared with other populations of DCs.
Conclusions
Expression of αvβ8 is required for $CD103^+$ DCs to become specialized and activate latent TGF-β and generate Tregs during the induction of tolerance to intestinal antigens in mice.

4.976 Increased Expression of the Transient Receptor Potential Melastatin 7 Channel Is Critically Involved in Lipopolysaccharide-Induced Reactive Oxygen Species-Mediated Neuronal Death
Nunez-Villena, F., Becerra, A., Echeverria, C., Briceno, N., Porras, O., Armisen, R., Varela, D., Montorfano, I., Sarmiento, D. and Simon, F.
*Antioxidants & Redox Signaling, **15(9)**, 2425-2438 (2011)*

Aims: To assess the mechanisms involved in lipopolysaccharide (LPS)-induced neuronal cell death, we examined the cellular consequences of LPS exposure in differentiated PC12 neurons and primary hippocampal neurons. ***Results:*** Our data show that LPS is able to induce PC12 neuronal cell death without the participation of glial cells. Neuronal cell death was mediated by an increase in cellular reactive oxygen species (ROS) levels. Considering the prevalent role of specific ion channels in mediating the deleterious effect of ROS, we assessed their contribution to this process. Neurons exposed to LPS showed a significant intracellular Ca^{2+} overload, and nonselective cationic channel blockers inhibited LPS-induced neuronal death. In particular, we observed that both LPS and hydrogen peroxide exposure strongly increased the expression of the transient receptor protein melastatin 7 (TRPM7), which is an ion channel directly implicated in neuronal cell death. Further, both LPS-induced TRPM7 overexpression and LPS-induced neuronal cell death were decreased with dithiothreitol, diphenyliodonium, and apocynin. Finally, knockdown of TRPM7 expression using small interference RNA technology protected primary hippocampal neurons and differentiated PC12 neurons from the LPS challenge. ***Innovation:*** This is the first report showing that TRPM7 is a key protein involved in neuronal death after LPS challenge. ***Conclusion:*** We conclude that LPS promotes an abnormal ROS-dependent TRPM7 overexpression, which plays a crucial role in pathologic events, thus leading to neuronal dysfunction and death.

4.977 Ineffective CD8+ T-Cell Immunity to Adeno-Associated Virus Can Result in Prolonged Liver Injury and Fibrogenesis
Spahn, J., Pierce, R.H. and Crispe, I.N.
*Am. J. Pathol., **179(5)**, 2370-2381 (2011)*

Chronic viral hepatitis depends on the inability of the T-cell immune response to eradicate antigen. This results in a sustained immune response accompanied by tissue injury and fibrogenesis. We have created a mouse model that reproduces these effects, based on the response of $CD8^+$ T cells to hepatocellular antigen delivered by an adeno-associated virus (AAV) vector. Ten thousand antigen-specific $CD8^+$ T cells undergo slow expansion in the liver and can precipitate a subacute inflammatory hepatitis with stellate cell activation and fibrosis. Over time, antigen-specific $CD8^+$ T cells show signs of exhaustion, including high expression of PD-1, and eventually both inflammation and fibrosis resolve. This model allows the investigation of both chronic liver immunopathology and its resolution.

4.978 Steady state pig dendritic cells migrating in skin draining pseudo-afferent lymph are semi-mature
Bertho, N., marquet, F., Pascale, F., Kang, C., Bonneau, M. and Schwartz-Cornil, I.
*Vet. Immunol. Immunopathol., **144**, 430-436 (2011)*

Dendritic cells (DC) in peripheral tissues are considered as immature cells that mature and migrate towards lymph nodes upon stimulation with pathogens. This commonly accepted paradigm is challenged by the fact that tolerance to peripheral self antigen is controlled by mature DC and that DC collected from afferent lymph draining different tissues from several species, in the absence of pathogen signaling, were inconsistently found to be either at a mature or semi-mature state. In order to better define the maturation state of DC that migrate in lymph in absence of pathogen stimulation, we compared skin lymph DC to resident and LPS (lipopolysaccharide)-activated skin DC thanks to the establishment of a mini-pig model of lymph duct cannulation. Based on their co-stimulatory molecules expression and endocytotic capacities, pig lymph skin DC were found at an intermediate state of maturation between resident and LPS-activated skin DC and were fully capable of allogeneic T cell stimulation. Furthermore, lymph skin DC could be further matured by LPS or influenza stimulation. Thus, using the pig skin model which is relevant to human, we show that skin-derived DC constantly migrate at an intermediate state of maturation that can be

further enhanced upon appropriate stimulation.

4.979 Sperm selection using single layer centrifugation prior to cryopreservation can increase thawed sperm quality in stallions
Hoogewijs, M., Morrell, J., Van Soom, A., Govaere, J., Johannisson, A., Piepers, S., De Schauwer, C., De Kruif, A. and De Vliegher, S.
*Equine Vet. J., **43** 8Suppl 40), 35-41 (2011)*

Reasons for performing study: The increasing use of modern reproductive techniques in human medicine has led to a higher demand for isolation of motile sperm. Several of these isolation techniques have been adapted for veterinary use and can be applied for the selection of a superior sperm sample from stallion semen. Until recently a major disadvantage of such isolation techniques was the limitation in sperm volume that could be handled. Androcoll-E had been shown to be successful for processing large volumes of equine semen but there are few data to substantiate the potential beneficial effect of freezing an Androcoll-E selected equine sperm sample to obtain higher quality following thawing.

Objectives and methods: In this study, the effect of Androcoll-E treatment of sperm prior to cryopreservation was compared with cushioned centrifugation using ejaculates from 8 different stallions selected because they were known to have semen of differing quality following freezing.

Results: Androcoll-E treatment increased measures of semen quality prior to freezing. However, Androcoll-E treatment reduced the yield of sperm following centrifugation when compared with the cushion centrifuged control group (50.9 ± 14.2% vs. 97.1 ± 9.0%, respectively). Quality analysis following thawing showed an overall improved sperm quality for Androcoll-E treated samples and average post thaw progressive motility (PM) was 41.6% compared with 30.5% for the cushion centrifuged group.

Conclusions and potential relevance: Androcoll-E can be used with good results to select a superior sperm population prior to cryopreservation, in order to produce good-quality frozen thawed semen.

4.980 Islet isolation from adult designated pathogen-free pigs: use of the newer bovine nervous tissue–free enzymes and a revised donor selection strategy would improve the islet graft function
Jin, S-M., Shin, J.S., Kim, K.S., Gong, C-H., park, S.K., Kim, J-S., Yeom, S-C., Hwang, E.S., Lee, C.T., Kim, S-J., and Park, C-G.
*Xenotransplantation, **18**, 369-379 (2011)*

Background: In clinical trials using adult porcine islet products, islets should be isolated from the designated pathogen-free (DPF) pigs under the current good manufacturing practice (GMP) regulations. Our previous studies suggested that male DPF pigs are better donors than retired breeder pigs and histomorphometrical parameters of donor pancreas predict the porcine islet quality. We aimed to investigate whether the use of the newer bovine nervous tissue–free enzymes and a revised donor selection strategy could improve the islet graft function in the context of islet isolation with DPF pigs.
Methods: Using 30 DPF pigs within a closed herd, we compared the islet yield of porcine islets isolated with Liberase PI (n = 11, as a historical control group), Liberase MTF C/T, which is a GMP-grade enzyme (n = 12), and CIzyme collagenase MA/BP protease (n = 7). We analyzed the relationship between the diabetes reversal rate of recipient NOD/SCID mice (n = 75) and histomorphometric parameters of each donor pancreas as well as donor characteristics.
Results: Proportion of islets larger than 200 μm from the biopsied donor pancreas (P = 0.006) better predicted islet yield than age (P = 0.760) or body weight (P = 0.371) of donor. The proportion of islets larger than 200 μm from the biopsied donor pancreas was not related to the sex of the donor miniature pig (P = 0.358). The islet yield obtained with the three enzymes did not differ, even after stratification of the donor with the histomorphometric parameters of the biopsied donor pancreas and the sex of donor. The use of the newer bovine nervous tissue–free enzymes (P < 0.001), a higher proportion of large islets in donor pancreas (P = 0.006), and a male sex of the donor (P = 0.025) were independent predictors of earlier diabetes reversal.
Conclusions: Use of the newer bovine nervous tissue–free enzymes including a GMP-grade enzyme resulted in better islet quality than that of islet isolated using Liberase PI. To obtain high-quality islet from DPF pigs, the donor should be male pig and histomorphometrical parameters from donor pancreas should be considered.

4.981 Lung CD103+ Dendritic Cells Efficiently Transport Influenza Virus to the Lymph Node and Load

Viral Antigen onto MHC Class I for Presentation to CD8 T Cells
Ho, A.W.S., Prabhu, N., Betts, R.J., Ge, M.Q., Dai, X., Hutchinson, P.E., Lew, F.C., Wong, K.L., Hanson, B.J., macary, P.A. and Kemeny, D.M.
J. Immunol., ***187(11)***, *6011-6021 (2011)*

The uptake, transport, and presentation of Ags by lung dendritic cells (DCs) are central to the initiation of CD8 T cell responses against respiratory viruses. Although several studies have demonstrated a critical role of $CD11b^{low/neg}CD103^{+}$ DCs for the initiation of cytotoxic T cell responses against the influenza virus, the underlying mechanisms for its potent ability to prime CD8 T cells remain poorly understood. Using a novel approach of fluorescent lipophilic dye-labeled influenza virus, we demonstrate that $CD11b^{low/neg}CD103^{+}$ DCs are the dominant lung DC population transporting influenza virus to the posterior mediastinal lymph node as early as 20 h postinfection. By contrast, $CD11b^{high}CD103^{neg}$ DCs, although more efficient for taking up the virus within the lung, migrate poorly to the lymph node and remain in the lung to produce proinflammatory cytokines instead. $CD11b^{low/neg}CD103^{+}$ DCs efficiently load viral peptide onto MHC class I complexes and therefore uniquely possess the capacity to potently induce proliferation of naive CD8 T cells. In addition, the peptide transporters TAP1 and TAP2 are constitutively expressed at higher levels in $CD11b^{low/neg}CD103^{+}$ DCs, providing, to our knowledge, the first evidence of a distinct regulation of the Ag-processing pathway in these cells. Collectively, these results show that $CD11b^{low/neg}CD103^{+}$ DCs are functionally specialized for the transport of Ag from the lung to the lymph node and also for efficient processing and presentation of viral Ags to CD8 T cells.

4.982 Small molecule tyrosine kinase inhibitors for the treatment of intestinal inflammation
Sidhu, M., Cotoner, C.A., Guleng, B., Arihiro, S., Chang, S., Duncan, K.W., Ajami, A.M., Chau, M. and Reinecker, H-C.
Inflamm. Bowel Dis., ***17(12)***, *2416-2426 (2011)*

Background:
We developed a series of dendritic cell autoimmune modulators (DCAMs) based on small molecule Flt3 receptor tyrosine kinase inhibitors (TKIs) for the inhibition of intestinal inflammation and oral delivery.
Methods:
DCAMs were administered orally during and after induction of dextran sodium sulfate (DSS)-induced colitis. Dendritic cell recruitment and inflammatory responses were determined in the mucosal immune system during acute intestinal inflammatory responses and mucosal recovery. Bone marrow-derived macrophages were utilized to define the mechanisms by which DCAMs can modify responses to microbial signals.
Results:
Oral doses of DCAMs prevented severe weight loss and mucosal inflammation associated with DSS colitis in mice. The presence of DCAMs increased the number of $CD11c^{+}PDCA1^{+}$ dendritic cells, induced interleukin (IL)-10 expression, and reduced inflammatory cytokine expression in the mucosal immune system. Surprisingly, DCAMs regulated innate immune responses in macrophages resulting in the inhibition of tumor necrosis factor alpha (TNF-α) production and the induction of IL-10 expression during Toll-like receptor-mediated signaling.
Conclusions:
We identified two new imidazoacridinone derivatives that protect mice from severe colitis and promote mucosal recovery by enhancing protective cytokine production while inhibiting proinflammatory stimuli during microbial recognition. These compounds are promising candidates for further development into potent orally available drugs for the prevention of colitis and promotion of mucosal recovery.

4.983 Successful Isolation and Transplantation of Nonhuman Primate Islets Using a Novel Purified Enzyme Blend
Abouaish, J., Graham, M., Bansal-Pakala, P., Loganathan, G., Soltani, S.M., Tiwari, M., Yusa, T., papas, K.K., Sutherland, D.E.R., Mccarthy, R.C., Hering, B.J. andBalamurugan, A.N.
Transplantation, ***92(8)***, *e40-e42 (2011)*

No abstract available.

4.984 Natural killer (NK)–dendritic cell interactions generate MHC class II-dressed NK cells that regulate CD4+ T cells
Nakayama, M., Takeda, K., Kawano, M., takai, T., Ishii, N. and Ogasawara, K.
PNAS, ***108(45)***, *18360-18365 (2011)*

Natural killer (NK) cells contribute to not only innate but also to adaptive immunity by interacting with dendritic cells (DCs) and T cells. All activated human NK cells express HLA-DR and can initiate MHCII-dependent $CD4^+$ T-cell proliferation; however, the expression of MHCII by mouse NK cells and its functional significance are controversial. In this study, we show that NK–DC interactions result in the emergence of MHCII-positive NK cells. Upon in vitro or in vivo activation, mouse conventional NK cells did not induce MHCII transcripts, but rapidly acquired MHCII protein from DCs. MHCII *H2-Ab1*–deficient NK cells turned $I\text{-}A^b$-positive when adoptively transferred into wild-type mice or when cultured with WT splenic DCs. NK acquisition of MHCII was mediated by intercellular membrane transfer called "trogocytosis," but not upon DAP10/12- and MHCI-binding NK cell receptor signaling. MHCII-dressed NK cells concurrently acquired costimulatory molecules such as CD80 and CD86 from DCs; however, their expression did not reach functional levels. Therefore, MHCII-dressed NK cells inhibited DC-induced $CD4^+$ T-cell responses rather than activated $CD4^+$ T cells by competitive antigen presentation. In a mouse model for delayed-type hypersensitivity, adoptive transfer of MHCII-dressed NK cells attenuated footpad swelling. These results suggest that MHCII-dressed NK cells generated through NK–DC interactions regulate T cell-mediated immune responses.

4.985 In situ induction of dendritic cell–based T cell tolerance in humanized mice and nonhuman primates
Jung, K.C. et al
J. Exp. Med., ***208(12)***, *2477-2488 (2011)*

Induction of antigen-specific T cell tolerance would aid treatment of diverse immunological disorders and help prevent allograft rejection and graft versus host disease. In this study, we establish a method of inducing antigen-specific T cell tolerance in situ in diabetic humanized mice and Rhesus monkeys receiving porcine islet xenografts. Antigen-specific T cell tolerance is induced by administration of an antibody ligating a particular epitope on ICAM-1 (intercellular adhesion molecule 1). Antibody-mediated ligation of ICAM-1 on dendritic cells (DCs) led to the arrest of DCs in a semimature stage in vitro and in vivo. Ablation of DCs from mice completely abrogated anti–ICAM-1–induced antigen-specific T cell tolerance. T cell responses to unrelated antigens remained unaffected. In situ induction of DC-mediated T cell tolerance using this method may represent a potent therapeutic tool for preventing graft rejection.

4.986 An Effective Method to Release Human Islets From Surrounding Acinar Cells With Agitation in High Osmolality Solution
Shimoda, M., Itoh, T., Sugimoto, K., Takita, M., Chujo, D., Iwahashi, S., SoRelle, J.A., Naziruddin, B., Levy, M.F., Grayburn, P.A. and Matsumoto, S.
Transplantation Proceedings, ***43***, *3161-3166 (2011)*

Introduction
Islet purification is mainly performed by the density gradient method. However, purification of the embedded islets that are surrounded by exocrine tissue should be difficult, because their density is similar to exocrine tissue. In this study, we performed chart review to assess the relationship between the ratio of embedded islets and efficacy of purification. Then, we tested several conditions of a new method to free the islets from surrounded exocrine tissues using high osmolality solution with gentle agitation.
Materials and Methods
First, we performed chart review of our human islet isolation. Second, embedded islet-enriched human islet fractions (embedded islets >50%) were suspended in University of Wisconsin (UW) solution (UW group, 320 mOsm/kg/H_20) or osmolality-adjusted UW solution (400, 500, and 600 mOsm/kg/H_20; 400 group, 500 group, and 600 group, respectively). Each tube was gently shaken at 4°C. The tissue samples were taken before shaking and after 15, 30, and 60 minutes. Islet yield, percentage of embedded islets, and viabilities were assessed.
Results
The chart review revealed that high ratio of embedded islets deteriorated the efficacy of islet purification. The islet yield in all groups except for the 600 group did not change at 15 minutes, but it decreased in all groups at 60 minutes. The average percentage of embedded islets before shaking was 62.6%. Although percentage of embedded islets were decreasing in all groups, it was < 20% at 15 minutes in the 500 and 600 groups whereas it was >44% in the UW group, which indicated that higher osmolality would have a greater effect. Viability was >95% in all groups at 30 minutes.
Conclusions
The embedded islets deteriorated the efficacy of islet purification. Gentle agitation of embedded islets in high osmolality (500 mOsm/kg/H_2O, 15 minutes) could release islets from surrounded exocrine tissue.

4.987 Usefulness of the Secretory Unit of Islet Transplant Objects (SUITO) Index for Evaluation of Clinical Autologous Islet Transplantation

Matsumoto, S., Takita, M., Shimoda, M., Itoh, T., Iwahashi, S., Chujo, D., SoRelle, J.A., Tamura, Y., Tahman, A., Purcell, K., Onaca, N., Naziruddin, B. and Levy, M.F.
*Transplantation Proceedings, **43**, 3246-3249 (2011)*

Background
Assessing the engrafted islet mass is important in evaluating the efficacy of islet transplantation. We previously demonstrated that the average secretory unit of islet transplant objects (SUITO) index within 1 month of allogeneic islet transplantation was an excellent predictor of insulin independence. However, the usefulness of the SUITO index for evaluating autologous islet transplantation has not been explored. The purpose of the present study was to assess the relationship between the SUITO index and clinical outcomes after total pancreatectomy followed by autologous islet transplantation.
Methods
We performed 27 total pancreatectomies followed by autologous islet transplantation from October 2006 to January 2011. Cases were divided into an insulin-independent group (IIG; n = 12) and an insulin-dependent group (lDG; n = 15). The SUITO index was calculated by the formula [fasting C-peptide (ng/mL)/fasting glucose (mg/dL) −63] × 1,500. The average SUITO index within the first month of transplantation except for days 0, 1, and 2, maximum SUITO index, and most recent SUITO index were calculated in each case, and values were compared between the IIG and the IDG.
Results
The average SUITO index within 1 month was significantly higher in the IIG than in the IDG (24.6 ± 3.4 vs 14.9 ± 2.0, respectively; $P < .02$). The maximum SUITO indices were 45.7 ± 7.7 in the IIG and 30.1 ± 8.1 in the IDG (not significant), and the recent SUITO indices were 36.9 ± 6.7 in the IIG and 22.8 ± 6.1 in the IDG (not significant).
Conclusions
The average SUITO index within 1 month was an excellent predictor of insulin independence after total pancreatectomy followed by autologous islet transplantation.

4.988 Human Alveolar Epithelial Cell Injury Induced by Cigarette Smoke

Kosmider, B., Messier, E.M., Chu, H.W. and Mason, R.J.
*PloS One, **6(12)**, e26059 (2011)*

Background
Cigarette smoke (CS) is a highly complex mixture and many of its components are known carcinogens, mutagens, and other toxic substances. CS induces oxidative stress and cell death, and this cell toxicity plays a key role in the pathogenesis of several pulmonary diseases.
Methodology/Principal Findings
We studied the effect of cigarette smoke extract (CSE) in human alveolar epithelial type I-like (ATI-like) cells. These are isolated type II cells that are differentiating toward the type I cell phenotype *in vitro* and have lost many type II cell markers and express type I cell markers. ATI-like cells were more sensitive to CSE than alveolar type II cells, which maintained their differentiated phenotype *in vitro*. We observed disruption of mitochondrial membrane potential, apoptosis and necrosis that were detected by double staining with acridine orange and ethidium bromide or Hoechst 33342 and propidium iodide and TUNEL assay after treatment with CSE. We also detected caspase 3 and caspase 7 activities and lipid peroxidation. CSE induced nuclear translocation of Nrf2 and increased expression of Nrf2, HO-1, Hsp70 and Fra1. Moreover, we found that Nrf2 knockdown sensitized ATI-like cells to CSE and Nrf2 overexpression provided protection against CSE-induced cell death. We also observed that two antioxidant compounds N-acetylcysteine and trolox protected ATI-like cells against injury by CSE.
Conclusions
Our study indicates that Nrf2 activation is a major factor in cellular defense of the human alveolar epithelium against CSE-induced toxicity and oxidative stress. Therefore, antioxidant agents that modulate Nrf2 would be expected to restore antioxidant and detoxifying enzymes and to prevent CS-related lung injury and perhaps lessen the development of emphysema.

4.989 Association Between the Secretory Unit of Islet Transplant Objects Index and Satisfaction With Insulin Therapy Among Insulin-Dependent Islet Recipients

Takita, M., Matsumoto, S., Shimoda, M., Chujo, D.,Itoh, T., Iwaaaahashi, S., SoRelle, J.A., Onaca, N., Nazxiruddin, B. and Levy, M.F.

*Transplantation Proceedings, **43**, 3250-3255 (2011)*

Introduction
When patients do not become insulin independent after islet cell transplantation (ICT), another aim is to eliminate severe hypoglycemia. Previously we reported that a secretory unit of islet transplant objects (SUITO) index score >10 was associated with a reduction of severe hypoglycemia. In this study, we assessed patients' satisfaction with their insulin therapy based on the SUITO index.
Methods
The study involved 11 islet recipients with type 1 diabetes who underwent ICT but still used insulin. From those patients, 41 Insulin Therapy Satisfaction Questionnaires (ITSQ) were collected. The SUITO index (fasting C-peptide [ng/mL] × 1500/blood glucose [mg/dL] − 63) was calculated at the same outpatient visits that the survey was administered. ITSQ scores were summarized using subscales and compared among 3 groups: the pre-ICT group, the low-SUITO group (SUITO index score <10 post-ICT), and the high-SUITO group (SUITO index score ≥10). Higher survey scores indicated better satisfaction.
Results
Significant trend relationships across the 3 groups were observed in the ITSQ total score ($P = .02$ with Jonckheere-Terpstra test) and subscale scores of glycemic control ($P < .001$), hypoglycemic control ($P = .01$), and inconvenience of regimen ($P = .004$). The pairwise comparisons between the 3 groups found significant differences: high SUITO versus both pre-ICT and low SUITO for the total ITSQ score ($P = .03$ and .005, respectively) and glycemic control score ($P = .008$ and .001, respectively), and high SUITO versus low SUITO for hypoglycemic control score ($P = .04$) and inconvenience of regimen score ($P = .008$).
Conclusion
Islet recipients with a SUITO index ≥10 experienced higher satisfaction with insulin injection therapy compared with the pre-ICT group, even though they were insulin dependent. A SUITO index ≥10 is a reasonable benchmark for successful ICT.

4.990 Autologous islet cell transplantation to prevent surgical diabetes
Matsumoto, S.
*J. Diabetes, **3(4)**, 328-336 (2011)*

Autologous islet transplantation (AIT) is performed to prevent surgical diabetes after total or semi-total pancreatectomy for the treatment of chronic pancreatitis with severe abdominal pain. In addition, AIT is used in cases of benign pancreatic tumors and pancreatic trauma. It has been shown that AIT results in better outcomes in terms of glycemic control compared with allogeneic islet transplantation. The reasons for the favorable outcomes of AIT are thought to be: (i) patients have no autoimmune diseases; (ii) the transplanted islets do not suffer allogeneic rejection; (iii) diabetogenic antirejection drugs are not required; (iv) pancreata do not undergo a cytokine storm as a result of periods of brain death; (v) the period of cold preservation of retrieved pancreata is short; (vi) the isolated islets are immediately transplanted without culture; and (vii) pancreata with pancreatitis may contain more progenitor cells. Further research into AIT would help improve the results of allogeneic islet transplantation. Conversely, the technical difficulties associated with islet isolation appear to be the largest hurdle for AIT; therefore, remote center islet isolation may prove to be key in the promotion of this treatment.

4.991 Insulin independence by supplemental islet transplantation 5 years after initial islet transplantation
Matsumoto, S., Takita, M., Shimoda, M., Chujo, D., Itoh, T., Iwaaaaaaaaaahashi, S., Sorelle, J.A., Tamura, Y., Rahman, A., Purcell, K., Naziruddin, B., Onaca, N. and Levy, M.F.
*J. Diabetes, **3(4)**, 353-355 (2011)*

The Edmonton protocol, which proved islet cell transplantation could make Type 1 diabetic patients insulin independent, was introduced in 2000.1 However, in 2005, the same Edmonton group demonstrated that <10% of recipients could maintain insulin independence. 2 Therefore, >90% of patients who received islet transplantation experienced decreased islet function and resumed exogenous insulin injections. Most patients could maintain better glycemic control after resuming insulin injections,2 but still hoped to again be insulin independent. Here we report a case of supplemental islet transplantation in a patient wh received initial islet transplantation >5 years earlier. The patient had achieved temporary insulin independence and maintained some islet function before the supplemental islet infusion. Now, the patient has again become insulin independent.

4.992 Velocity Effect on Aptamer-Based Circulating Tumor Cell Isolation in Microfluidic Devices
Wan, Y., Tan, J., Asghar, W.,. Kim, Y-t., Liu, Y. and Iqbal, S.M.
J. Phys. Chem. B., ***115(47)***, *13891-13896 (2011)*

The isolation and detection of rare circulating tumor cells (CTCs) has been one of the focuses of intense research recently. In a microfluidic device, a number of factors can influence the enrichment capability of surface-bound probe molecules. This article analyzes the important factor of flow velocity in a microfluidic channel. The competition of surface-grafted anti-EGFR aptamers to bind the overexpressed EGFR on cell membranes against the drag force from the fluid flow is an important efficiency determining factor. The flow rate variations are applied both in experiments and in simulation models to study their effects on CTC capture efficiency. A mixture of mononuclear cells and human Glioblastoma cells is used to isolate cancer cells from the cellular flow. The results show interdependence between the adhesion probability, isolation efficiency, and flow rate. This work can help in designing flow-through lab-on-chip devices that use surface-bound probe affinities against overexpressed biomarkers for cell isolation. This work demonstrates that microfluidic based approaches have strong potential applications in CTC detection and isolation.

4.993 Mechanisms of Slower Nitric Oxide Uptake by Red Blood Cells and Other Hemoglobin-containing Vesicles
Azarov, I., Liu, C., Reynolds, H., Tsekouras, Z., Lee, J.S., Gladwin, M.T. and Kim-Shapiro, D.B.
J. Biol. Chem., ***286(39)***, *33567-33579 (2011)*

Nitric oxide (NO) acts as a smooth muscle relaxation factor and plays a crucial role in maintaining vascular homeostasis. NO is scavenged rapidly by hemoglobin (Hb). However, under normal physiological conditions, the encapsulation of Hb inside red blood cells (RBCs) significantly retards NO scavenging, permitting NO to reach the smooth muscle. The rate-limiting factors (diffusion of NO to the RBC surface, through the RBC membrane or inside of the RBC) responsible for this retardation have been the subject of much debate. Knowing the relative contribution of each of these factors is important for several reasons including optimization of the development of blood substitutes where Hb is contained within phospholipid vesicles. We have thus performed experiments of NO uptake by erythrocytes and microparticles derived from erythrocytes and conducted simulations of these data as well as that of others. We have included extracellular diffusion (that is, diffusion of the NO to the membrane) and membrane permeability, in addition to intracellular diffusion of NO, in our computational models. We find that all these mechanisms may modulate NO uptake by membrane-encapsulated Hb and that extracellular diffusion is the main rate-limiting factor for phospholipid vesicles and erythrocytes. In the case of red cell microparticles, we find a major role for membrane permeability. These results are consistent with prior studies indicating that extracellular diffusion of several gas ligands is also rate-limiting for erythrocytes, with some contribution of a low membrane permeability.

4.994 Histopaque provides optimal mouse islet purification kinetics: Comparison study with Ficoll, iodixanol and dextran
McCall, M.D., Maciver, A.H., Pawlick, R., Edgar, R. and Shapiro, A,M.J.
Islets, ***3(4)***, *144-149 (2011)*

Islet transplantation has become a very promising treatment for type 1 diabetes. To facilitate further clinical improvements in this exciting field, rodent islets are used to evaluate new strategies and modifications. One method to purify islets is on a density gradient, although the optimal gradient component can be debated. N=6 separate mouse islet isolations were used and the resulting islets were separated and purified on either a Ficoll, Histopaque, Dextran or Iodixanol gradient. Islets were assessed for recovery, viability, purity and in vitro functionality. Aliquots were transplanted into diabetic mice to assess in vivo functionality and survival. There was no difference in the number of islets recovered across groups nor in the size of recovered islets. Use of a Ficoll or Histopaque gradient led to the most pure and viable islets in comparison to Dextran and Iodixanol. Functionally, islets isolated on a Ficoll gradient had the highest glucose-stimulated insulin release in vitro while performing equally to Histopaque and Dextran gradients in vivo. Using a Ficoll gradient, however, comes at a higher monetary cost. We recommend using a Histopaque gradient, which led to the isolation of viable and functional islets with a reduced cost as compared to a Ficoll gradient.

4.995 Improving Efficacy of Clinical Islet Transplantation with Iodixanol Based Islet Purification, Thymoglobulin Induction and Blockage of IL-1-beta and TNF-alpha

Matsumoto, S., Takita, M., Chaussabel, D., Noguchi, H., Shimoda, M., Sugimoto, K., Itoh, T., Chujo, D., Sorelle, J., Onaca, N., Naziruddin, B. and Levy, M.F.
Cell Transplant, xxxxxx (2011)

Background: Poor efficacy is one of the issues for clinical islet transplantation. Recently, we demonstrated that pancreatic ductal preservation significantly improved the success rate of islet isolation; however, two transplants were necessary to achieve insulin independence. In this study, we introduced iodixanol based purification, thymoglobulin induction and double blockage of IL-1 beta and TNF-alpha as well as sirolimus free immunosuppression to improve the efficacy of clinical islet transplantation. Methods: Nine clinical grade human pancreata were procured. Pancreatic ductal preservation was performed using ET-Kyoto solution in all cases. When the isolated islets met the clinical criteria, they were transplanted. We utilized two methods of immunosuppression and anti-inflammation. The first protocol prescribed daclizumab for induction, then sirolimus and tacrolimus to maintain immunosuppression. The second protocol used thymoglobulin for induction and tacrolimus and mycophenolate mofetil to maintain immunosuppression. Eternacept and anakinra were administered as anti-inflammatory drugs. The total amount of insulin required, HbA1c, and the SUITO index were determined to analyze and compare the results of transplantation. Results: All isolated islet preparations (9/9) met the criteria for clinical transplantation, and they were transplanted into 6 type 1 diabetic patients. All patients achieved insulin independence with normal HbA1c levels; however, the first protocol required two islet infusions (N=3) and the second protocol only required a single infusion (N=3).The average SUITO index, at one month after a single-donor islet transplantation, was significantly higher in the second protocol (49.6±8.3 vs. 19.3±6.3, $p<0.05$). Conclusions: Pancreatic ductal preservation, iodixanol based purification combined with thymoglobulin induction and blockage of IL-1 beta and TNF-alpha as well as sirolimus free immunosuppression dramatically improved the efficacy of clinical islet transplantations. This protocol enabled us to perform successful single-donor islet transplantations. Further large scale studies are necessary to confirm these results and clarify the mechanism of each component.

4.996 Diapocynin and apocynin administration fails to significantly extend survival in G93A SOD1 ALS mice

Trumbull, K.A., McAllister, D.,Gandelman, M.M., Fung, W.Y., lew, T., Brennan, L., Lopez, N., Morre, J., Kalyanaraman, B. and Beckman, J.S.
*Neurobiol. of Dis., **45**, 137-144 (2012)*

NADPH oxidase has recently been identified as a promising new therapeutic target in ALS. Genetic deletion of NADPH oxidase (Nox2) in the transgenic $SOD1^{G93A}$ mutant mouse model of ALS was reported to increase survival remarkably by 97 days. Furthermore, apocynin, a widely used inhibitor of NADPH oxidase, was observed to dramatically extend the survival of the $SOD1^{G93A}$ ALS mice even longer to 113 days (Harraz et al. J Clin Invest 118: 474, 2008). Diapocynin, the covalent dimer of apocynin, has been reported to be a more potent inhibitor of NADPH oxidase. We compared the protection of diapocynin to apocynin in primary cultures of $SOD1^{G93A}$-expressing motor neurons against nitric oxide-mediated death. Diapocynin, 10 μM, provided significantly greater protection compared to apocynin, 200 μM, at the lowest statistically significant concentrations. However, administration of diapocynin starting at 21 days of age in the $SOD1^{G93A}$-ALS mouse model did not extend lifespan. Repeated parallel experiments with apocynin failed to yield protection greater than a 5-day life extension in multiple trials conducted at two separate institutions. The maximum protection observed was an 8-day extension in survival when diapocynin was administered at 100 days of age at disease onset. HPLC with selective ion monitoring by mass spectrometry revealed that both apocynin and diapocynin accumulated in the brain and spinal cord tissue to low micromolar concentrations. Diapocynin was also detected in the CNS of apocynin-treated mice. The failure to achieve significant protection with either apocynin or diapocynin raises questions about the utility for treating ALS patients.

4.997 Case Study of Processing and Insemination Techniques: Attempts to Improve Fertility of an Aged Stallion with Dilute Semen of Poor Quality

Blanchard, T.L., Brinsko, S.P., Love, C.C., Vest, D.D., Berezowski, C.B., Wendt, K.M., Stich, K. and Varner, D.D.
*J. Equine Vet. Sci., **32**, 5-11 (2012)*

The objective of this case study was to investigate whether semen centrifugation and low-dose

insemination techniques would improve fertility of an aged subfertile Quarter Horse stallion with low sperm concentration, motility, and morphology in ejaculates. Forty-five mares were bred by one of five treatments (n = 9 per group) using the entire ejaculate as follows: (1) Group Body: body insemination with ejaculate diluted 1:1 in TAMU extender; (2) Group Body-Cent: body insemination after centrifugation and re-suspension of sperm pellet to 1 mL in TAMU extender; (3) Group Horn-Cent: deep horn insemination after centrifugation and re-suspension of sperm pellet to 1 mL in TAMU extender; (4) Group Cent-Hys: hysteroscopic insemination onto the uterotubal papilla after centrifugation and re-suspension of sperm pellet to 200 μL in Kenney-Modified Tyrode's extender; and (5) Group Dens-Hys: hysteroscopic insemination onto the uterotubal papilla after discontinuous density gradient centrifugation and re-suspension of the sperm pellet in 200-μL Kenney-Modified Tyrode's extender. Pregnancy rates did not differ among treatment groups ($P = .77$). Semen centrifugation for low dose insemination did not appear to improve fertility of this subfertile stallion, despite use of entire ejaculates for each individual insemination dose.

4.998 Toll-like receptor 2-mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors

Hösel, M., Broxtermann, M., Janicki, H., Esser, K., Arzberger, S., Hartmann, P., Gillen, S., Kleeff, J., Stabenow, D., Odenthal, M., Knolle, P., Hallek, M., Protzer, U. and Büning, H.
Hepatology, ***55(1)***, *287-297 (2012)*

Adeno-associated viral vectors (rAAV) are frequently used in gene therapy trials. Although rAAV vectors are of low immunogenicity, humoral as well as T cell responses may be induced. While the former limits vector reapplication, the expansion of cytotoxic T cells correlates with liver inflammation and loss of transduced hepatocytes. Because adaptive immune responses are a consequence of recognition by the innate immune system, we aimed to characterize cell autonomous immune responses elicited by rAAV in primary human hepatocytes and nonparenchymal liver cells. Surprisingly, Kupffer cells, but also liver sinusoidal endothelial cells, mounted responses to rAAV, whereas neither rAAV2 nor rAAV8 were recognized by hepatocytes. Viral capsids were sensed at the cell surface as pathogen-associated molecular patterns by Toll-like receptor 2. In contrast to the Toll-like receptor 9–mediated recognition observed in plasmacytoid dendritic cells, immune recognition of rAAV in primary human liver cells did not induce a type I interferon response, but up-regulated inflammatory cytokines through activation of nuclear factor κB. *Conclusion*: Using primary human liver cells, we identified a novel mechanism of rAAV recognition in the liver, demonstrating that alternative means of sensing rAAV particles have evolved. Minimizing this recognition will be key to improving rAAV-mediated gene transfer and reducing side effects in clinical trials due to immune responses against rAAV.

4.999 Unraveling features of the natural MHC class II peptidome of skin-migrated dendritic cells

Muixi, L., Contreras, V., Collado, J.A., Alexandre, Y., Ballingall, K., Bonneau, M., Jaraquemada, D. and Schwartz-Cornil, I.
Int. Immunol., ***24(1)***, *59-69 (2012)*

Dendritic cells (DCs) migrating from peripheral tissues at steady state are considered the most efficient antigen-presenting cells (APCs) involved in the induction of peripheral T-cell tolerance via self-antigen presentation on MHC class II molecules. However, difficulties in obtaining sufficient numbers of such DCs have precluded previous analyses of their natural MHC class II peptidome in laboratory animals or humans. Here, we overcome this difficulty by collecting the large quantities of sheep DCs that migrate from the skin via the afferent lymphatics at steady state to the draining lymph node. We compared the repertoire of MHC class II-bound peptides from afferent lymph DCs with autologous APCs derived from peripheral blood. A large fraction of the MHC class II peptidome from skin DCs was derived from membrane-recycling proteins (59%) and from proteins of the antigen presentation machinery (50%), whereas these types of peptides constituted a more limited fraction in blood APCs (21 and 11%, respectively). One sheep cytokeratin peptide was identified in the skin DC peptidome indicating active processing of epithelium-derived antigens. Conversely, peptides derived from cytosolic and soluble antigens of the extracellular milieu were more represented in blood APCs than skin DCs. The biased peptidome of skin-migrated DCs indicates that these cells express a peptide repertoire for the generation of self-reactive and/or regulatory T cells mainly directed toward DC molecules from internal and external membranes and to a lesser extent toward antigens of the extracellular milieu, including some tissue-specific peptides.

4.1000 Mitochondrial Dynamics and Bioenergetic Dysfunction Is Associated with Synaptic Alterations in Mutant SOD1 Motor Neurons

Magrane, J., Sahaweh, M.A., Przedborski, S., Estevez, A.G. and Manfredi, G.
J. Neurosci., ***32(1)***, *229-242 (2012)*

Mutations in Cu,Zn superoxide dismutase (SOD1) cause familial amyotrophic lateral sclerosis (FALS), a rapidly fatal motor neuron disease. Mutant SOD1 has pleiotropic toxic effects on motor neurons, among which mitochondrial dysfunction has been proposed as one of the contributing factors in motor neuron demise. Mitochondria are highly dynamic in neurons; they are constantly reshaped by fusion and move along neurites to localize at sites of high-energy utilization, such as synapses. The finding of abnormal mitochondria accumulation in neuromuscular junctions, where the SOD1-FALS degenerative process is though to initiate, suggests that impaired mitochondrial dynamics in motor neurons may be involved in pathogenesis. We addressed this hypothesis by live imaging microscopy of photo-switchable fluorescent mitoDendra in transgenic rat motor neurons expressing mutant or wild-type human SOD1. We demonstrate that mutant SOD1 motor neurons have impaired mitochondrial fusion in axons and cell bodies. Mitochondria also display selective impairment of retrograde axonal transport, with reduced frequency and velocity of movements. Fusion and transport defects are associated with smaller mitochondrial size, decreased mitochondrial density, and defective mitochondrial membrane potential. Furthermore, mislocalization of mitochondria at synapses among motor neurons, *in vitro*, correlates with abnormal synaptic number, structure, and function. Dynamics abnormalities are specific to mutant SOD1 motor neuron mitochondria, since they are absent in wild-type SOD1 motor neurons, they do not involve other organelles, and they are not found in cortical neurons. Together, these results suggest that impaired mitochondrial dynamics may contribute to the selective degeneration of motor neurons in SOD1-FALS

4.1001 Stella TUM: current consensus and discussion on pancreatic stellate cell research

Erkan, M., Adler, GX. Apte, M.V. et al
Gut, ***61***, *172-178 (2012)*

The field of pancreatic stellate cell (PSC) biology is very young, as the essential in-vitro tools to study these cells (ie, methods to isolate and culture PSC) were only developed as recently as in 1998. Nonetheless, there has been an exponential increase in research output in this field over the past decade, with numerous research groups around the world focusing their energies into elucidating the biology and function of these cells. It is now well established that PSC are responsible for producing the stromal reaction (fibrosis) of two major diseases of the pancreas—chronic pancreatitis and pancreatic cancer. Despite exponentially increasing data, the methods for studying PSC remain variable. Although within individual laboratories methods are consistent, different methodologies used by various research groups make it difficult to compare results and conclusions. This article is not a review article on the functions of PSC. Instead, members of the Pancreatic Star Alliance (http://www.pancreaticstaralliance.com) discuss here and consolidate current knowledge, to outline and delineate areas of consensus or otherwise (eg, with regard to methodological approaches) and, more importantly, to identify essential directions for future research.

4.1002 Isolation and Culture of Spinal Cord Astrocytes

Kerstetter, A. and Miller, R.H.
Methods in Mol. Biol., ***814***, *93-104 (2012)*

Astrocytes are possibly the most numerous cells of the vertebrate central nervous system, yet a detailed characterization of their functions is still missing. One potential reason for the obscurity of astrocytic function is that they represent a diverse population of cells that all share some critical characteristics. In the CNS, astrocytes have been proposed to perform many functions. For example, they are supportive cells that provide guidance to newly formed migrating neurons and axons. They regulate the functions of endothelial cells at the blood brain barrier, provide nutrients, and maintain homeostasis including ionic balance within the CNS. More recently, dissecting the central role of astrocytes in mediating injury responses in the CNS, particularly the spinal cord, has become an area of considerable importance. The ability to culture-enriched populations of astrocytes has facilitated a detailed dissection of their potential roles in the developing and adult, normal, and injured brain and spinal cord. Most importantly, in vitro models have defi ned molecular signals that may mediate or regulate astrocyte functions and the capacity to modulate these signals may provide new opportunities for therapeutic intervention after spinal cord injury and other neural insults.

4.1003 Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease
Koyama, M. et al
Nature Med., ***18(1)****, 135-142 (2012)*

The presentation pathways by which allogeneic peptides induce graft-versus-host disease (GVHD) are unclear. We developed a bone marrow transplant (BMT) system in mice whereby presentation of a processed recipient peptide within major histocompatibility complex (MHC) class II molecules could be spatially and temporally quantified. Whereas donor antigen presenting cells (APCs) could induce lethal acute GVHD via MHC class II, recipient APCs were 100–1,000 times more potent in this regard. After myeloablative irradiation, T cell activation and memory differentiation occurred in lymphoid organs independently of alloantigen. Unexpectedly, professional hematopoietic-derived recipient APCs within lymphoid organs had only a limited capacity to induce GVHD, and dendritic cells were not required. In contrast, nonhematopoietic recipient APCs within target organs induced universal GVHD mortality and promoted marked alloreactive donor T cell expansion within the gastrointestinal tract and inflammatory cytokine generation. These data challenge current paradigms, suggesting that experimental lethal acute GVHD can be induced by nonhematopoietic recipient APCs.

4.1004 Insulin Protects Pancreatic Acinar Cells from Cytosolic Calcium Overload and Inhibition of Plasma Membrane Calcium Pump
Mmandad, P., James, A., Siriwardena, A.K., Elliott, A.C. and Bruce, J.I.E.
J. Biol. Chem., ***287(3)****, 1823-1836 (2012)*

Acute pancreatitis is a serious and sometimes fatal inflammatory disease of the pancreas without any reliable treatment or imminent cure. In recent years, impaired metabolism and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) overload in pancreatic acinar cells have been implicated as the cardinal pathological events common to most forms of pancreatitis, regardless of the precise causative factor. Therefore, restoration of metabolism and protection against cytosolic Ca^{2+} overload likely represent key therapeutic untapped strategies for the treatment of this disease. The plasma membrane Ca^{2+}-ATPase (PMCA) provides a final common path for cells to "defend" $[Ca^{2+}]_i$ during cellular injury. In this paper, we use fluorescence imaging to show for the first time that insulin treatment, which is protective in animal models and clinical studies of human pancreatitis, directly protects pancreatic acinar cells from oxidant-induced cytosolic Ca^{2+} overload and inhibition of the PMCA. This protection was independent of oxidative stress or mitochondrial membrane potential but appeared to involve the activation of Akt and an acute metabolic switch from mitochondrial to predominantly glycolytic metabolism. This switch to glycolysis appeared to be sufficient to maintain cellular ATP and thus PMCA activity, thereby preventing Ca^{2+} overload, even in the face of impaired mitochondrial function.

4.1005 Proteomic Analysis of Fractionated Toxoplasma Oocysts Reveals Clues to Their Environmental Resistance
Fritz, H.M., Bowyer, P.W., Bogyo, M., Conrad, P.A. and Boothroyd, J.C.
PloS One, 7(1), e29955 (2012)

Toxoplasma gondii is an obligate intracellular parasite that is unique in its ability to infect a broad range of birds and mammals, including humans, leading to an extremely high worldwide prevalence and distribution. This work focuses on the environmentally resistant oocyst, which is the product of sexual replication in felids and an important source of human infection. Due to the difficulty in producing and working with oocysts, relatively little is known about how this stage is able to resist extreme environmental stresses and how they initiate a new infection, once ingested. To fill this gap, the proteome of the wall and sporocyst/sporozoite fractions of mature, sporulated oocysts were characterized using one-dimensional gel electrophoresis followed by LC-MS/MS on trypsin-digested peptides. A combined total of 1021 non-redundant T. gondii proteins were identified in the sporocyst/sporozoite fraction and 226 were identified in the oocyst wall fraction. Significantly, 172 of the identified proteins have not previously been identified in Toxoplasma proteomic studies. Among these are several of interest for their likely role in conferring environmental resistance including a family of small, tyrosine-rich proteins present in the oocyst wall fractions and late embryogenesis abundant domain-containing (LEA) proteins in the cytosolic fractions. The latter are known from other systems to be key to enabling survival against desiccation.

4.1006 Biochemical properties of bull spermatozoa separated in iodixanol density solution
Beer-Ljubic, B., Aladrovic, J., Marenjak, T.S:, Majic-Balic. I., Laskaj, R. and Milinkovic-Tur, S.
Res.Vet. Sci., ***92****, 292-294 (2012)*

Bull spermatozoa samples contain variable portion of motile and normal morphology spermatozoa along with spermatozoa incapable of fertilization due to their pathologic changes. As semen quality is influenced by biochemical and morphological characteristics of all spermatozoa, the aim of the study was to separate spermatozoa in discontinuous iodixanol density gradient solution and to determine their cholesterol, phospholipid, triacylglycerol and lipid peroxide concentrations and creatine kinase activity. The study was performed in winter and included seven Simmental bulls aged 1.5–3.5 years. Semen samples were collected by use of artificial vagina. Upon evaluation of semen quality (volume, concentration and progressive sperm motility), the samples were centrifuged in iodixanol density solution to obtain two sperm fractions. The two fractions included sperms with progressive motility greater than 90% and less than 20%, respectively. A statistically significantly higher lipid peroxide concentration was determined in sperm fraction with <20% progressive motility. Different sperm subpopulations can be obtained by separating bull spermatozoa in different iodixanol density gradient solutions, while monitoring their biochemical properties can help assess the sperm quality.

4.1007 Homing and Long-Term Engraftment of Long- and Short-Term Renewal Hematopoietic Stem Cells
Liu, L., Papa, E.F., Dooner, M.S., Machan, J.T., Johnson, K.W., Goldberg, L.R., Qusenberry, P.J. and Covin, G.A.
PloS One, ***7(2)****, e31300 (2012)*

Long-term hematopoietic stem cells (LT-HSC) and short-term hematopoietic stem cells (ST-HSC) have been characterized as having markedly different *in vivo* repopulation, but similar *in vitro* growth in liquid culture. These differences could be due to differences in marrow homing. We evaluated this by comparing results when purified ST-HSC and LT-HSC were administered to irradiated mice by three different routes: intravenous, intraperitoneal, and directly into the femur. Purified stem cells derived from B6.SJL mice were competed with marrow cells from C57BL/6J mice into lethally irradiated C57BL/6J mice. Serial transplants into secondary recipients were also carried out. We found no advantage for ST-HSC engraftment when the cells were administered intraperitoneally or directly into femur. However, to our surprise, we found that the purified ST-HSC were not short-term in nature but rather gave long-term multilineage engraftment out to 387 days, albeit at a lower level than the LT-HSC. The ST-HSC also gave secondary engraftment. These observations challenge current models of the stem cell hierarchy and suggest that stem cells are in a continuum of change.

4.1008 Infection of bone marrow cells by dengue virus in vivo
Noisakran, S., Onlamoon, N., Hsiao, H-M., Clark, K.B., Villinger, F., Ansari, A.A. and Perng, G.C.
Exp. Hematol., ***240****, 250-259 (2012)*

Abnormal bone marrow (BM) suppression is one of the hallmarks of dengue virus (DENV) infection in patients. Although the etiology remains unclear, direct viral targeting of the BM has been reasoned to be a contributing factor. The present studies were carried out in an effort to determine the potential effect of DENV infection on the cellularity of BM using a previously established nonhuman primate model of DENV-induced coagulopathy. BM aspirates were collected at various times from the infected nonhuman primate and cells were phenotypically defined and isolated using standard flow cytometry (fluorescence-activated cell sorting). These isolated cells were subjected to detection of DENV utilizing quantitative real-time reverse transcription polymerase chain reaction, electron microscopy, and immunostaining techniques. DENV RNA was detectable by quantitative real-time reverse transcription polymerase chain reaction in BM specimens and the presence of DENV-like particles within platelet was confirmed by electron microscopy. Enumeration of BM cells revealed a transient surge in cellularity at day 1, followed by a gradual decline from days 2 to 10 post infection. Detailed phenotypic studies showed similar kinetics in the frequencies of $CD41^+CD61^+$ cells, regardless of CD34 and CD45 expression. The $CD61^+$ cells were not only the predominant cells that stained for DENV antigen but fluorescence-activated cell sorting–assisted isolation of $CD61^+$ cells from the BM were shown to contain infectious DENV by coculture with Vero cells. These data support the view that intravenous infection of nonhuman primate with DENV leads to direct infection of the BM, which is likely to be a contributing factor for transient cell suppression in the peripheral blood characteristic of acute DENV infection.

4.1009 Oral administration of Salmonella enterica serovar Typhimurium expressing swine interleukin-18 induces Th1-biased protective immunity against inactivated vaccine of pseudorabies virus
Kim, S.B., Kim, S.J., Lee, B.M., Han, Y.W., Rahman, M.M., Uyangaa, E., Kim, J.H., Choi, J.Y., Yoo, D.J., Kim, K. and Eo, S.K.
Vet. Microbiol., ***155****, 172-182 (2012)*

Enhancing and/or modulating innate and adaptive immunity by cytokines appears to be greatly useful to provide effective protective immunity against infectious diseases. However, an effective delivery system for mass administration in livestock industry is needed because of limitations such as cost, labor, time, and protein stability. Here the immunomodulatory functions of swine interleukine-18 (swIL-18), known as IFN-γ-inducing factor (IGIF), were evaluated in a vaccination model of pseudorabies virus (PrV) using attenuated *Salmonella enterica* serovar Typhimurium as the oral delivery system. The oral administration of *S. enterica* serovar Typhimurium expressing swIL-18 prior to vaccination with inactivated PrV vaccine induced enhanced levels of serum PrV-specific IgG and its IgG2 isotype, compared to administration of *S. enterica* serovar Typhimurium harboring the empty vector. Furthermore, *S. enterica* serovar Typhimurium expressing swIL-18 mounted Th1-biased cellular immune responses against PrV antigen, as evaluated by the production of IFN-γ and IL-4 from peripheral blood mononuclear cells of piglets. Subsequently, Th1-biased immunity induced by *S. enterica* serovar Typhimurium expressing swIL-18 showed rapid response and rendered piglets displayed more alleviated clinical signs following the virulent PrV challenge. Also, this alleviation of clinical signs was further confirmed by the reduction of nasal excretion of PrV after challenge. The present study demonstrates the extended use of immunomodulatory functions of swIL-18 orally delivered by attenuated *S. enterica* serovar Typhimurium.

4.1010 A Francisella tularensis Live Vaccine Strain That Improves Stimulation of Antigen-Presenting Cells Does Not Enhance Vaccine Efficacy
Schmitt, D.M., O'Dee, D.M., Horzempa, J., Carlson, P.E., Russo, B.C., Bales, J.M., Brown, M.J. and Nau, G.J.
PloS One, ***7(2)****, e31172 (2012)*

Vaccination is a proven strategy to mitigate morbidity and mortality of infectious diseases. The methodology of identifying and testing new vaccine candidates could be improved with rational design and *in vitro* testing prior to animal experimentation. The tularemia vaccine, *Francisella tularensis* live vaccine strain (LVS), does not elicit complete protection against lethal challenge with a virulent type A *Francisella* strain. One factor that may contribute to this poor performance is limited stimulation of antigen-presenting cells. In this study, we examined whether the interaction of genetically modified LVS strains with human antigen-presenting cells correlated with effectiveness as tularemia vaccine candidates. Human dendritic cells infected with wild-type LVS secrete low levels of proinflammatory cytokines, fail to upregulate costimulatory molecules, and activate human T cells poorly *in vitro*. One LVS mutant, strain 13B47, stimulated higher levels of proinflammatory cytokines from dendritic cells and macrophages and increased costimulatory molecule expression on dendritic cells compared to wild type. Additionally, 13B47-infected dendritic cells activated T cells more efficiently than LVS-infected cells. A deletion allele of the same gene in LVS displayed similar *in vitro* characteristics, but vaccination with this strain did not improve survival after challenge with a virulent *Francisella* strain. *In vivo*, this mutant was attenuated for growth and did not stimulate T cell responses in the lung comparable to wild type. Therefore, stimulation of antigen-presenting cells *in vitro* was improved by genetic modification of LVS, but did not correlate with efficacy against challenge *in vivo* within this model system.

4.1011 Surfactant Protein A Integrates Activation Signal Strength To Differentially Modulate T Cell Proliferation
Mukherjee, S., Giamberardino, C., Thomas, J., Evans, K., Goto, H., Ledford, J.G., Hsia, B., Pastva, A.M. and Wright, J.R.
J. Immunol., ***188(3)****, 957-967 (2012)*

Pulmonary surfactant lipoproteins lower the surface tension at the alveolar–airway interface of the lung and participate in host defense. Previous studies reported that surfactant protein A (SP-A) inhibits lymphocyte proliferation. We hypothesized that SP-A–mediated modulation of T cell activation depends upon the strength, duration, and type of lymphocyte activating signals. Modulation of T cell signal strength imparted by different activating agents ex vivo and in vivo in different mouse models and in vitro with

human T cells shows a strong correlation between strength of signal (SoS) and functional effects of SP-A interactions. T cell proliferation is enhanced in the presence of SP-A at low SoS imparted by exogenous mitogens, specific Abs, APCs, or in homeostatic proliferation. Proliferation is inhibited at higher SoS imparted by different doses of the same T cell mitogens or indirect stimuli such as LPS. Importantly, reconstitution with exogenous SP-A into the lungs of SP-$A^{-/-}$ mice stimulated with a strong signal also resulted in suppression of T cell proliferation while elevating baseline proliferation in unstimulated T cells. These signal strength and SP-A–dependent effects are mediated by changes in intracellular Ca^{2+} levels over time, involving extrinsic Ca^{2+}-activated channels late during activation. These effects are intrinsic to the global T cell population and are manifested in vivo in naive as well as memory phenotype T cells. Thus, SP-A appears to integrate signal thresholds to control T cell proliferation.

4.1012 RANKL Induces Organized Lymph Node Growth by Stromal Cell Proliferation
Hess, E., Duheron, V., Decossas, M., Lezot, F., Berdal, A., Chea, S., Golub, R., Bosisio, M.R., Bridal, S.L., Choi, Y., Yagita, H. and Mueller, C.G.
J. Immunol., ***188(3)***, *1245-1254 (2012)*

RANK and its ligand RANKL play important roles in the development and regulation of the immune system. We show that mice transgenic for *Rank* in hair follicles display massive postnatal growth of skin-draining lymph nodes. The proportions of hematopoietic and nonhematopoietic stromal cells and their organization are maintained, with the exception of an increase in B cell follicles. The hematopoietic cells are not activated and respond to immunization by foreign Ag and adjuvant. We demonstrate that soluble RANKL is overproduced from the transgenic hair follicles and that its neutralization normalizes lymph node size, inclusive area, and numbers of B cell follicles. Reticular fibroblastic and vascular stromal cells, important for secondary lymphoid organ formation and organization, express RANK and undergo hyperproliferation, which is abrogated by RANKL neutralization. In addition, they express higher levels of CXCL13 and CCL19 chemokines, as well as MAdCAM-1 and VCAM-1 cell-adhesion molecules. These findings highlight the importance of tissue-derived cues for secondary lymphoid organ homeostasis and identify RANKL as a key molecule for controlling the plasticity of the immune system.

4.1013 CD64 Expression Distinguishes Monocyte-Derived and Conventional Dendritic Cells and Reveals Their Distinct Role during Intramuscular Immunization
Llanglet, C., Tamoutounour, S., Henri, S., Luche, H., Ardouin, L., Gregoire, C., Malissen, B. and Guilliams, M.
J. Immunol., ***188(4)***, *1751-1760 (2012)*

Although most vaccines are administered i.m., little is known about the dendritic cells (DCs) that are present within skeletal muscles. In this article, we show that expression of CD64, the high-affinity IgG receptor FcγRI, distinguishes conventional DCs from monocyte-derived DCs (Mo-DCs). By using such a discriminatory marker, we defined the distinct DC subsets that reside in skeletal muscles and identified their migratory counterparts in draining lymph nodes (LNs). We further used this capability to analyze the functional specialization that exists among muscle DCs. After i.m. administration of Ag adsorbed to alum, we showed that alum-injected muscles contained large numbers of conventional DCs that belong to the $CD8\alpha^{+}$- and $CD11b^{+}$-type DCs. Both conventional DC types were capable of capturing Ag and of migrating to draining LNs, where they efficiently activated naive T cells. In alum-injected muscles, Mo-DCs were as numerous as conventional DCs, but only a small fraction migrated to draining LNs. Therefore, alum by itself poorly induces Mo-DCs to migrate to draining LNs. We showed that addition of small amounts of LPS to alum enhanced Mo-DC migration. Considering that migratory Mo-DCs had, on a per cell basis, a higher capacity to induce IFN-γ–producing T cells than conventional DCs, the addition of LPS to alum enhanced the overall immunogenicity of Ags presented by muscle-derived DCs. Therefore, a full understanding of the role of adjuvants during i.m. vaccination needs to take into account the heterogeneous migratory and functional behavior of muscle DCs and Mo-DCs revealed in this study.

4.1014 A novel neuroprotective therapy for Parkinson's disease using a viral noncoding RNA that protects mitochondrial Complex I activity
Kuan, W-L., Poole, E., Fletcher, M., KArniely, S., Tyers, P., Wills, M., Barker, R.A. and Sinclair, J.H.
J. Exp. Med., ***209(1)***, *1-10 (2012)*

Parkinson's disease (PD) is a neurodegenerative disorder that results in the loss of nigrostriatal dopamine neurons. The etiology of this cell loss is unknown, but it involves abnormalities in mitochondrial function. In this study, we have demonstrated that the administration of a novel noncoding p137 RNA, derived from

the human cytomegaloviral β2.7 transcript, can prevent and rescue dopaminergic cell death in vitro and in animal models of PD by protecting mitochondrial Complex I activity. Furthermore, as this p137 RNA is fused to a rabies virus glycoprotein peptide that facilitates delivery of RNA across the blood–brain barrier, such protection can be achieved through a peripheral intravenous administration of this agent after the initiation of a dopaminergic lesion. This approach has major implications for the potential treatment of PD, especially given that this novel agent could have the same protective effect on all diseased neurons affected as part of this disease process, not just the dopaminergic nigrostriatal pathway.

4.1015 Effect of centrifugal fractionation protocols on quality and recovery rate of equine sperm
Edmond, A.J., Brinsko, S.P., Love, C.C., Blanchard, T.L., Teague, S.R. and Varner, D.D.
*Theriogenology, **77**, 959-966 (2012)*

Centrifugal fractionation of semen is commonly done to improve quality of human semen in assisted-reproduction laboratories, allowing sperm separation based on their isopycnic points. Sperm with morphologic abnormalities are often more buoyant, promoting their retention above defined density media, with structurally normal sperm passing through the media following centrifugation. Three experiments were conducted to evaluate the effects of density-medium type, centrifuge-tube size, sperm number, and density-medium volume (column height) on stallion sperm quality and recovery rate in sperm pellets following centrifugation. In all three experiments, equine semen was initially centrifuged to increase sperm concentration. In Experiment 1, semen was layered over continuous or discontinuous gradients. For Experiment 2, semen was layered over three column heights of continuous gradients in 15- or 50-ml conical-bottom tubes. For Experiment 3, increasing sperm numbers were layered over continuous gradient in 15- or 50-ml conical-bottom tubes. Following centrifugation, sperm pellets were evaluated for sperm morphologic quality, motility, DNA integrity, and recovery rate. Centrifugal fractionation improved ($P < 0.05$) sperm morphology, motility, and DNA integrity, as compared to controls. The continuous gradient increased ($P < 0.05$) sperm recovery rate relative to the discontinuous gradient, whereas sperm processed in 15-ml tubes yielded higher velocity and higher recovery rates ($P < 0.05$ for each) than that processed in 50-ml tubes. Sperm recovery rate was not affected ($P > 0.05$) by column height of gradient. Increasing sperm number subjected to gradient centrifugation decreased ($P < 0.05$) sperm recovery rate when 15-ml tubes were used.

4.1016 Evaluation of the qualitative and quantitative effectiveness of three media of centrifugation (Maxifreeze, Cushion Fluid Equine, and PureSperm 100) in preparation of fresh or frozen-thawed brown bear spermatozoa
Nicolas, M., Alvarez, M., Borragan, S., Martinez-Pastor, F., Chamorro, C.A., Alvarez-Rodriguez, M., de Paz, P. and Anel, L.
*Theriogenology, **77**, 1119-1128 (2012)*

Centrifugation is a crucial procedure in sperm cryopreservation protocols of brown bear (*Ursus arctos*), because the semen must be processed to increase sperm concentration and/or clean urine-contaminated samples. The efficacy of three media for centrifugation (Maxifreeze [IMV technologies, L'Aigle, France], Cushion Fluid Equine (Minitübe, Tiefenbach, Germany), and PureSperm [Nidacon, Gothenburg, Sweden]) on the quality of bear spermatozoa was evaluated. In experiment one, two cushioned media used for protecting against mechanical stress during centrifugation were analyzed. In experiment two, a density gradient based on PureSperm was assessed in relation to the maximum retrieval and the quality of fresh spermatozoa, and the freezability of the spermatozoa selected in this density gradient was studied in experiment three. Finally, the selection of frozen-thawed sperm using PureSperm was analyzed in experiment four. Our results indicate that the use of dense isotonic cushion solutions (Maxifreeze, Cushion Fluid Equine) in centrifugation did not improve the quality of recovered spermatozoa compared with standard centrifugation. However, a density gradient prepared with PureSperm improved the quality of spermatozoa in fresh semen and frozen-thawed semen, but the spermatozoa selected from the fresh sample with this density gradient did not show a better resistance to freezing with this density gradient in comparison with the control sample.

4.1017 The unfolded protein response in models of human mutant G93A amyotrophic lateral sclerosis
Prell, T., Lautenschläger, J., Witte, O.W., Carri, M.T. and Grosskreutz, J.
*Eur. J. Neurosci., **35**, 652-660 82012)*

Recent studies indicate that endoplasmic reticulum (ER) stress is involved in the pathogenesis of familial and sporadic amyotrophic lateral sclerosis (ALS). ER stress occurs when the ER–mitochondria calcium

cycle (ERMCC) is disturbed and misfolded proteins accumulate in the ER. To cope with ER stress, the cell engages the unfolded protein response (UPR). While activation of the UPR has been shown in some ALS models and tissues, ER stress elements have not been studied directly in motor neurons. Here we investigated the expression of XBP1 and ATF6α and phosphorylation of eIF2α, and their modulation, in mutated $SOD1^{G93A}$ NSC34 and animal model of ALS. Expression of XBP1 and ATF6α mRNA and protein was enhanced in $SOD1^{G93A}$ NSC34 cells. Activation of ATF6α and XBP1 and phosphorylation of eIF2α were detectable in mutated $SOD1^{G93A}$ motor but not in wild-type motor neurons. Treatment with the ER stressor thapsigargin enhanced phosphorylation of eIF2α and activated proteolysis of ATF6α and splicing of XBP1 in NSC34 and motor neurons in a time-dependent manner. The present study thus provides direct evidence of activated UPR in motor neurons which overexpress human pathogenic mutant $SOD1^{G93A}$, providing evidence that ER stress plays a major role in ALS.

4.1018 Effects of Exercise on microRNA Expression in Young Males Peripheral Blood Mononuclear Cells
Radom-Aizik, S., Zaldivar, F., Leu, S-Y., Adams, G.R., Oliver, S. and Cooper, D.M.
*Clin. Trans. Sci., **5**, 32-38 (2012)*

MicroRNAs are increasingly seen as targets of drug discovery because they influence gene function acting both to silence and subtly modulate protein translation. Little is known about effects of dynamic physiological states on microRNA regulation in humans. We hypothesized that microRNA expression in peripheral blood mononuclear cells (PBMCs) would be affected by brief exercise. Twelve young men performed brief bouts of heavy exercise. PBMC microRNA was analyzed before and immediately after exercise using the Agilent Human microRNA V2 Microarray. Exercise altered expression level of 34 microRNAs (FDR < 0.05). Many of them play roles in inflammatory processes (e.g., miR-125b[↓], down-regulated by proinflammatory factor LPS; and miR-132[↑], 125b[↓] and let-7e[↓] involved inTLR4 signaling). Using previous exercise data in PBMCs, we linked the microRNA changes to specific gene pathways. This analysis identified 12 pathways including the TGF-β and MAPK signaling. We also compared exercise-associated microRNA changes in PBMCs with the exercise-associated microRNAs previously identified in neutrophils. Nine microRNAs were affected in both PBMCs and neutrophils, but only six changed in the same direction. A commonly occurring physiologic perturbation, brief heavy exercise, changes microRNA profiles in PBMCs, many of which are related to inflammatory processes. The pattern of change suggests that exercise differentially influences microRNAs in leukocyte subtypes

4.1019 ER stress activates the NLRP3 inflammasome via an UPR-independent pathway
Menu, P., Mayor, A., Zhou, R., Tardivel, A., Ichijo, H., Mori, K. and Tschopp, J.
*Cell Death and Differentiation, **3**, e261, (2012)*

Uncontrolled endoplasmic reticulum (ER) stress responses are proposed to contribute to the pathology of chronic inflammatory diseases such as type 2 diabetes or atherosclerosis. However, the connection between ER stress and inflammation remains largely unexplored. Here, we show that ER stress causes activation of the NLRP3 inflammasome, with subsequent release of the pro-inflammatory cytokine interleukin-1*β*. This ER-triggered proinflammatory signal shares the same requirement for reactive oxygen species production and potassium efflux compared with other known NLRP3 inflammasome activators, but is independent of the classical unfolded protein response (UPR). We thus propose that the NLRP3 inflammasome senses and responds to ER stress downstream of a previously uncharacterized ER stress response signaling pathway distinct from the UPR, thus providing mechanistic insight to the link between ER stress and chronic inflammatory diseases.

4.1020 Stromal TIMP3 Regulates Liver Lymphocyte Populations and Provides Protection against Th1 T Cell-Driven Autoimmune Hepatitis
Murthy, A., Washington Shao, Y., Defamie, V., Wedeles, C., Smookler, D. and Khokha, R.
*J. Immunol., **188**, 2876-2883 (2012)*

Lymphocyte infiltration into epithelial tissues and proinflammatory cytokine release are key steps in autoimmune disease. Although cell-autonomous roles of lymphocytes are well studied in autoimmunity, much less is understood about the stromal factors that dictate immune cell function. Tissue inhibitor of metalloproteinases 3 (TIMP3) controls systemic cytokine bioavailability and signaling by inhibiting the ectodomain shedding of cytokines and their receptors. The role of TIMP3 in cytokine biology is emerging; however, its contribution to cellular immunology remains unknown. In this study, we show that TIMP3 produced by the hepatic stroma regulates the basal lymphocyte populations in the liver and prevents autoimmune hepatitis. TIMP3 deficiency in mice led to spontaneous accumulation and activation of

hepatic $CD4^+$, $CD8^+$, and NKT cells. Treatment with Con A in a model of polyclonal T lymphocyte activation resulted in a greatly enhanced Th1 cytokine response and acute liver failure, which mechanistically depended on TNF signaling. Bone marrow chimeras demonstrated that TIMP3 derived from the stromal rather than hematopoietic compartment provided protection against autoimmunity. Finally, we identified hepatocytes as the major source of *Timp3* in a resting liver, whereas significant *Timp3* gene transcription was induced by hepatic stellate cells in the inflamed liver. These results uncover metalloproteinase inhibitors as critical stromal factors in regulating cellular immunity during autoimmune hepatitis.

4.1021 Gastroprotective effect of anti-cancer compound rohitukine: possible role of gastrin antagonism and H+ K+-ATPase inhibition

Singh, N., Singh, P., Shrivastva, S., Mishra, S.K., Lakshmi, V., Sharma, R. and Palit, G.
Naunyn-Schmiederberg's Arch. Pharmacol., ***385(3)***, *277-286 (2012)*

The present study was designed to evaluate the anti-ulcerogenic properties of an alkaloid chromane, rohitukine from *Dysoxylum binectariferum*. Anti-ulcer potential of rohitukine was assessed in cold restrained, pyloric ligated and ethanol induced ulcers in rats. In addition, rohitukine was tested in vitro for H^+ K^+-ATPase inhibitory activity in gastric microsomes. Moreover, we studied the role of rohitukine on the cytosolic concentration of Ca^{2+} in parietal cell-enriched cell suspension in order to ascertain its mechanism of action. Cytoprotective activity was evaluated through PGE_2 level. Rohitukine significantly attenuated the ulcers in cold restraint ulcer (CRU) model in a dose-related manner. Moreover, it significantly lowered the free acidity and pepsin activity in pyloric ligated rats while improved the depleted level of mucin. Furthermore, rohitukine significantly reversed the cold restrained-induced increase in gastrin level. Our in vitro study revealed that rohitukine moderately inhibited the microsomal H^+ K^+-ATPase activity with respect to positive control omeprazole. Furthermore, rohitukine potently antagonized the gastrin-elicited increase in cytosolic Ca^{2+} level in parietal cell-enriched suspension. In ethanol-induced gastric lesions in rats, rohitukine significantly inhibited the formation of erosions and increased PGE_2 content showing more potency than reference drug sucralfate. Our results thus suggest that rohitukine possess significant anti-ulcer and anti-gastrinic activity in rats. It is likely that gastro-protective influences of rohitukine are dependent partly on its acid-lowering potential and partly on cytoprotective property. The acid-reducing effect of rohitukine might be attributed to its lowering effect on gastrin production and/or antagonism of gastrin-evoked functional responses of parietal cells. Thus, rohitukine represent a useful agent in the treatment of peptic ulcer disease.

4.1022 Microfluidic Cytometer for the Characterization of Cell Lysis

SooHoo, J.R., Herr, J.K., Ramsey, J.M. and Walker, G.M.
Anal. Chem., ***84(5)***, *2195-2201 (2012)*

Blood cytometry and intercellular analysis typically requires lysis as a preparatory step, which can alter the results of downstream analyses. We fabricated a microfluidic cytometer to characterize erythrocyte lysis kinetics. Forward light scatter from erythrocytes was used for enumeration at specific locations on a microfluidic chip. Diffusive transport coupled with laminar flow was used to control the concentration and exposure time of the lysis reagent Zap-OGLOBIN II to erythrocytes. Standard clinical practice is to expose erythrocytes to lysis reagent for 10 min. Under optimum conditions, we achieved complete erythrocyte lysis of a blood sample in 0.7 s. A maximum lysis reaction rate of 1.55 s^{-1} was extrapolated from the data. Lysis began after 0.2 s and could be initiated with a lysis reagent concentration of 1.0% (68.5 mM). An equation that related lysis reagent concentration, [*A*], to erythrocyte lysis, [*B*], was determined to be $[B] = -0.77[A]^{0.29}t$.

4.1023 The impact of cushioned centrifugation protocols on semen quality of stallions

Bliss, S.B., Voge, J.L., Hayden, S.S., Teague, S.R., Brinsko, S.P., Love, C.C., Blanchard, T.L. and varner, D.D.
Theriogenology, ***77***, *1232-1239 (2012)*

The objective was to determine if decreased cushion-fluid volume and increased sperm number during centrifugation, or if sperm concentration of extended semen following centrifugation, affected stallion sperm quality. Three ejaculates from each of three stallions were subjected to cushioned centrifugation (1,000*g* for 20 min). Cushion-fluid volume was set at 1 or 3.5 ml, and sperm number per centrifuge tube was set 1 billion or 3 billion. Following centrifugation, sperm pellets were resuspended in semen extender containing 20% seminal plasma (v/v) with sperm concentrations of 25 or 250 million/mL. Sperm recovery

rate among centrifugation treatment groups was compared. Motion characteristics, plasma membrane intactness (SMI), and DNA quality (COMPαt) of sperm were compared among treatment groups and uncentrifuged controls immediately following centrifugation (Time 0 h) and following 24 h of cooled storage (Time 24 h). Centrifugation treatment did not affect sperm recovery rate ($P > 0.05$). At Time 0 h, no differences in experimental end points were detected between cushion-fluid volumes tested ($P > 0.05$). Values for percent total sperm motility, percent progressive sperm motility, and track straightness were similar between sperm-number treatments subjected to centrifugation ($P > 0.05$). At Time 24 h, values for all experimental endpoints were similar between centrifugation treatments for cushion volume per tube, and between centrifugation treatments for sperm number per tube ($P > 0.05$). Centrifugation treatments and control treatments were similar for five of six variables tested ($P > 0.05$). Sperm storage concentrations of 25×10^6 and 250×10^6/mL yielded similar values for percent total sperm motility, percent progressive sperm motility, percent SMI, and percent COMPαt ($P > 0.05$). A storage concentration of 250×10^6 sperm/ml yielded higher values for curvilinear velocity, and lower values for straightness, than all other groups ($P < 0.05$). In conclusion, centrifugation with as little as 1 ml of cushion fluid and a sperm number of up to 3×10^9 sperm in 50-ml conical-bottom centrifuge tubes had no detrimental effect on initial or cool-stored sperm quality. Additionally, storage of centrifuged sperm at a concentration of 250×10^6/mL with 20% seminal plasma (v/v) did not have a detrimental effect on percentages of motile or progressively motile sperm, or sperm DNA quality.

4.1024 High-Throughput Examination of Fluorescence Resonance Energy Transfer-Detected Metal-Ion Response in Mammalian Cells

Ma, H., Gibson, E.A., Dittmeer, P.J., Jimenez, R. and Palmer, A.E.
J. Am. Chem. Soc., ***134(5)***, *2488-2491 (2012)*

Fluorescence resonance energy transfer (FRET)-based genetically encoded metal-ion sensors are important tools for studying metal-ion dynamics in live cells. We present a time-resolved microfluidic flow cytometer capable of characterizing the FRET-based dynamic response of metal-ion sensors in mammalian cells at a throughput of 15 cells/s with a time window encompassing a few milliseconds to a few seconds after mixing of cells with exogenous ligands. We have used the instrument to examine the cellular heterogeneity of Zn^{2+} and Ca^{2+} sensor FRET response amplitudes and demonstrated that the cluster maps of the Zn^{2+} sensor FRET changes resolve multiple subpopulations. We have also measured the in vivo sensor response kinetics induced by changes in Zn^{2+} and Ca^{2+} concentrations. We observed an ~30 fold difference between the extracellular and intracellular sensors.

4.1025 Anti-Inflammatory Effects of Nicotinic Acid in Human Monocytes Are Mediated by GPR109A Dependent Mechanisms

Digby, J.E., Martinez, F., Jefferson, A., Ruparelia, N., Chai, J., Wamil, M., Greaves, D.RS. and Choudhury, R.P.
Arterioscler. Thromb. Vasc. Biol., ***32***, *669-676 (2012)*

Objective—Nicotinic acid (NA) treatment has been associated with benefits in atherosclerosis that are usually attributed to effects on plasma lipoproteins. The NA receptor GPR109A is expressed in monocytes and macrophages, suggesting a possible additional role for NA in modulating function of these immune cells. We hypothesize that NA has the potential to act directly on monocytes to alter mediators of inflammation that may contribute to its antiatherogenic effects in vivo.
Methods and Results—In human monocytes activated by Toll-like receptor (TLR)-4 agonist lipopolysaccharide, NA reduced secretion of proinflammatory mediators: TNF-α (by 49.2±4.5%); interleukin-6 (by 56.2±2.8%), and monocyte chemoattractant protein-1 (by 43.2±3.1%) (P<0.01). In TLR2 agonist, heat-killed Listeria monocytogenes-activated human monocytes, NA reduced secretion of TNF-α (by 48.6±7.1%), interleukin-6 (by 60.9±1.6%), and monocyte chemoattractant protein-1 (by 59.3±5.3%) (P<0.01; n=7). Knockdown of GPR109A by siRNA resulted in a loss of this anti-inflammatory effect in THP-1 monocytes. However, inhibition of prostaglandin D_2 receptor by MK0524 or COX2 by NS398 did not alter the anti-inflammatory effects of NA observed in activated human monocytes. Preincubation of THP-1 monocytes with NA 0.1 mmol/L reduced phosphorylated IKKβ by 42±2% (P<0.001) IKB-α by 54±14% (P<0.01). Accumulation of nuclear p65 NF-κB in response to lipopolysaccharide treatment was also profoundly inhibited, by 89±1.3% (n=4; P<0.01). NA potently inhibited monocyte adhesion to activated HUVEC, and VCAM, mediated by the integrin, very late antigen 4. Monocyte chemotaxis was also significantly reduced (by 45.7±1.2%; P<0.001).

Conclusion—NA displays a range of effects that are lipoprotein-independent and potentially antiatherogenic. These effects are mediated by GPR109A and are independent of prostaglandin pathways. They suggest a rationale for treatment with NA that is not dependent on levels of plasma cholesterol and possible applications beyond the treatment of dyslipidemia.

4.1026 Induction of Biogenic Magnetization and Redox Control by a Component of the Target of Rapamycin Complex 1 Signaling Pathway
Nishida, K. and Silver, P.A.
*PloS Biology, **10(2)**, e1001269 (2012)*

Author Summary Most organisms do not respond to magnetic fields. However, “magnetotactic” bacteria and migratory animals can sense geomagnetic fields and alter their behavior accordingly. These organisms often contain small magnetic particles that may be responsible for sensing magnetic fields. In magnetotactic bacteria, specific genes are crucial for the formation of these magnetic particles, but no such genes have yet been characterized in migratory animals. In humans, formation of magnetic particles can be observed in the neuronal tissue in neurodegenerative diseases. One explanation for the appearance of these magnetic particles is that they are the result of alterations in metabolism, which occur in neurodegenerative diseases. Here, we explore this hypothesis by inducing magnetism in yeast cells, which are not naturally magnetic and examine how changes in metabolism contribute to particle formation and magnetism. We find that yeast cells expressing a set of human proteins that sequester iron contain iron particles and become attracted by a magnet when grown with ferric citrate. Through physiological and genetic studies we show that target of rapamycin complex 1 (TORC1) signaling, which responds to nutritional signals, is important for the magnetization of these cells by altering the intracellular oxidation (or redox) state. We also show that genes involved in carbon metabolism affect magnetization. We propose that local redox control mediated by carbon metabolism and iron homeostasis, processes that exist in normal unmagnetized cells, are key for iron particle formation and magnetization. We conclude that magnetization of normal cells will be possible with these existing gene sets.

4.1027 Preparation of Adult Spinal Cord Motor Neuron Cultures Under Serum-Free Conditions
Montoya-Gacharna, J.V., Sutachan, J.J., Chan, W.S., Sideris, A., Blanck, T.J.J. and Recio-Pinto, E.
*Methods in Mol. Biol., **846**, 103-116 (2012)*

Spinal cord motor neuron cultures are an important tool for the study of mechanisms involved in motor neuron survival, degeneration and regeneration, volatile anesthetic-induced immobility, motor neuron disorders such as amyotrophic lateral sclerosis or spinal muscular atrophy as well as in spinal cord injury. Embryonic spinal cord motor neurons derived from rats have been successfully cultured; unfortunately, the culture of adult motor neurons has been problematic due to their short-term survival. Recently, by using a cocktail of target-derived factors, neurotrophins (brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor) and a permeable cyclic adenosine monophosphate analog, we have established a reproducible protocol for long-term cultures of healthy and functional adult motor neurons (Exp Neurol 220:303–315, 2009). Here, we now describe in detail the steps that we used for the optimization of the process of isolation and maintenance of adult rat ventral horn motor neurons in vitro.

4.1028 Effects of subacute oral warfarin administration on peripheral blood granulocytes in rats
Belij, S., Miljkovic, D., Popov, A., Subota, V., Timotijevic, G., Slavic, M., Mirkov, I., KAtaranovski, D. and Kataranovski, M.
Food Chem. Tox., 50, 1499-1507 (2012)

Warfarin affects mainly vitamin K dependent (VKD) processes, but the effects on some non-VKD-related activities such as tumor growth inhibition and mononuclear cell-mediated immune reactions were shown as well. In this study, the effect of subchronic (30 days) oral warfarin (0.35 mg/l and 3.5 mg/l) intake on peripheral blood granulocytes in rats was investigated. Increase in prothrombin and partial thromboplastin time at high warfarin dose reflected its basic activity. Priming effect for respiratory burst was noted at both warfarin doses, while only high warfarin dose resulted in priming for adhesion, the rise in intracellular myeloperoxidase content/release and stimulation of nitric oxide production. Differential effects of high warfarin dose were noted on granulocyte cytokines IL-6 (lack of the effect), TNF-α (decreased release and mRNA expression) and IL-12 (increase in mRNA for IL-12 subunits p35 and p40). Changes in granulocytes seems not to rely on mitogen activated kinases p38 and ERK. Warfarin intake was associated with an increase in circulating IL-6, fibrinogen and haptoglobin and with changes in the activity of erythrocyte antioxidant enzymes superoxide dismutase and catalase. The effects of oral warfarin intake on peripheral blood granulocytes demonstrated in this study might be relevant for oral anticoagulant therapy

strategies in humans.

4.1029 An integrated analytical approach for assessing the biological status of the soil microbial community
Pascaud, A., Soulas, M-L., Amellal, S. and Soulas, G.
*Eur. J. Soil Biol., **49,** 98-106 (2012)*

An integrated multicriteria analytical procedure for rapid, cost-effective characterisation of the biological status of soil bacterial community was developped. Commercially-available, light emission-based bioassays were selected for measuring cell density, activity, and diversity. All but Terminal Restriction Fragment Length Polymorphism (T-RFLP) were designed for multiwell-plate formats and high-throughput screening potential. Adenosine Tri Phosphate (ATP) was measured using a bioluminescence assay. Dehydrogenase activity (DHA) was measured on growing cells. Kinetic measurements of the formation of a coloured formazan derivative was used after nutrient broth addition to estimate initial cell concentrations by reference to *Escherichia coli* added as internal standard. Compared to conventional ATP and DHA determinations in soils, the procedures described here do not require extraction of ATP or formazan derivative from the soil matrix. Metabolic diversity was characterised using the Biolog™ system. T-RFLP was chosen for assessing bacterial community structure. The bioassays were performed on microbial preparations obtained after either direct dilution of soil suspensions or prior density-gradient separation of microbial cells from the soil matrix. Dilution maintains the original structure of native dominant microbial communities. Density-gradient separation of microbial cells is highly selective, drastically modifying metabolic (CLP Profiles) and species (T-RFLP patterns) diversity, as well as activity parameters.

4.1030 Differential changes in gene expression in rainbow trout hepatocytes exposed to extracts of oil sands process-affected water and the Athabasca River
Gagne, F., Douville, M., Andre, C., Debenest, T., talbot, A., Sherry, J., Hewitt, L.M., Frank, R.A., McMaster, M.E., Parrott, J. and Bickerton, G.
*Comp. Biochem. Physiol. B, **155**, 551-559 (2012)*

The oil sands region of northern Alberta represents the world's largest reserves of bitumen, and the accelerated pace of industrial extraction activity has raised concern about the possible impacts on the Athabasca River and its tributaries. An ecotoxicogenomic study was undertaken on *Oncorhynchus mykiss* trout hepatocytes exposed to extracts of water samples near the oil sand development area, as well as to oil sands process-affected water (OSPW) extracts using the quantitative reverse transcriptase polymerase chain reaction technique. The expression of the following genes (mRNA) was monitored to track changes in xenobiotic biotransformation (CYP1A1, CYP3A4, glutathione S-transferase, multi-drug resistance transporter), estrogenicity (estrogen receptor and vitellogenin), oxidative stress (superoxide dismutase and metallothionein) and DNA repair activity (DNA ligase). The extent of DNA-aromatic hydrocarbon adducts was also determined in cells by immuno-staining. A comparative analysis of gene expression between the river/lake and OSPW samples revealed that CYP3A4, metallothioneins, DNA ligase and GST genes, were specifically expressed by OSPW. Cells exposed to OSPW, commercial naphthenic acids, and benzo(a)pyrene showed increased polyaromatic hydrocarbon DNA-adducts, as determined by cell immunofluorescence analysis. Other genes were induced by all types of water samples, although the induction potential was stronger in OSPW most of the time (e.g., VTG gene was expressed nearly 15-fold by surface waters from the lake and river samples but increased to a maximum of 31-fold in OSPW). A multivariate discriminant function analysis revealed that the lake and river water samples were well discriminated from the OSPW. The CYP3A4 gene was the most highly expressed gene in cells exposed to OSPW and responded less to the lake or river water in the Athabasca River area. This study identified a suite of gene targets that responded specifically to OSPW extracts, which could serve as toxicogenomic fingerprints of OSPW contamination.

4.1031 Olesoxime delays muscle denervation, astrogliosis, microglial activation and motoneuron death in an ALS mouse model
Sunyach, C., Michaud, M., Arnoux, T., Bernard-Marissal, N., Aebisvher, J., Latyszenok, V., Gouarne, C., Raoul, C., Pruss, R.M., Bordet, st. and Pettmann, B.
*Neuropharmacol., **62**, 2345-2352 (2012)*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. The pathology is mimicked to a striking degree in transgenic mice carrying familial ALS-linked *SOD1* gene mutations. Olesoxime (TRO19622), a novel neuroprotective and reparative compound identified in a high-throughput screen

based on motoneuron (MN) survival, delays disease onset and improves survival in mutant *SOD1G93A* mice, a model for ALS. The present study further analyses the cellular basis for the protection provided by olesoxime at the neuromuscular junctions (NMJ) and the spinal cord. Studies were carried out at two disease stages, 60 days, presymptomatic and 104 days, symptomatic. Cohorts of wild type and *SOD1G93A* mice were randomized to receive olesoxime-charged food pellets or normal diet from day 21 onward. Analysis showed that olesoxime initially reduced denervation from 60 to 30% compared to *SOD1G93A* mice fed with control food pellets while at the symptomatic stage only a few NMJs were still preserved. Immunostaining of cryostat sections of the lumbar spinal cord with VAChT to visualize MNs, GFAP for astrocytes and Iba1 for microglial cells showed that olesoxime strongly reduced astrogliosis and microglial activation and prevented MN loss. These studies suggest that olesoxime exerts its protective effect on multiple cell types implicated in the disease process in *SOD1G93A* mice, slowing down muscle denervation, astrogliosis, microglial activation and MN death. A Phase 3 clinical study in ALS patients will determine whether olesoxime could be beneficial for the treatment of ALS.

4.1032 A new cannabinoid CB2 receptor agonist HU-910 attenuates oxidative stress, inflammation and cell death associated with hepatic ischaemia/reperfusion injury

Horvath, B., Magid, L., Mukhopadhyay, P., Batkai, S., Rajesh, M., Park, O., Tanchian, G., Gao, R.Y., Goodfellow, C.E., Glass, M., Mechoulam, R. and Pacher, P.

*Br. J. Pharmacol., **165(8)**, 2462-2478 (2012)*

BACKGROUND AND PURPOSE Cannabinoid CB_2 receptor activation has been reported to attenuate myocardial, cerebral and hepatic ischaemia-reperfusion (I/R) injury.
EXPERIMENTAL APPROACH We have investigated the effects of a novel CB_2 receptor agonist ((1S,4R)-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-7,7-dimethylbicyclo[2.2.1]hept-2-en-1-yl)methanol (HU-910) on liver injury induced by 1 h of ischaemia followed by 2, 6 or 24 h of reperfusion, using a well-established mouse model of segmental hepatic I/R.
KEY RESULTS Displacement of [^{3}H]CP55940 by HU-910 from specific binding sites in CHO cell membranes transfected with human CB_2 or CB_1 receptors ($hCB_{1/2}$) yielded K_i values of 6 nM and 1.4 μM respectively. HU-910 inhibited forskolin-stimulated cyclic AMP production by hCB_2 CHO cells (EC_{50}= 162 nM) and yielded EC_{50} of 26.4 nM in [^{35}S]GTPγS binding assays using hCB_2 expressing CHO membranes. HU-910 given before ischaemia significantly attenuated levels of I/R-induced hepatic pro-inflammatory chemokines (CCL3 and CXCL2), TNF-α, inter-cellular adhesion molecule-1, neutrophil infiltration, oxidative stress and cell death. Some of the beneficial effect of HU-910 also persisted when given at the beginning of the reperfusion or 1 h after the ischaemic episode. Furthermore, HU-910 attenuated the bacterial endotoxin-triggered TNF-α production in isolated Kupffer cells and expression of adhesion molecules in primary human liver sinusoidal endothelial cells stimulated with TNF-α. Pretreatment with a CB_2 receptor antagonist attenuated the protective effects of HU-910, while pretreatment with a CB_1 antagonist tended to enhance them.
CONCLUSION AND IMPLICATIONS HU-910 is a potent CB_2 receptor agonist which may exert protective effects in various diseases associated with inflammation and tissue injury.

4.1033 Modulation of Astrocytic Mitochondrial Function by Dichloroacetate Improves Survival and Motor Performance in Inherited Amyotrophic Lateral Sclerosis

Miquel, E., Cassina, A., Martinez-Palma, L., Bolatto, C., Trias, e., Gandelman, M., Radi, R., Barbeito, L. and Cassina, P.

*PloS One, **7(4)**, e34776 (2012)*

Mitochondrial dysfunction is one of the pathogenic mechanisms that lead to neurodegeneration in Amyotrophic Lateral Sclerosis (ALS). Astrocytes expressing the ALS-linked $SOD1^{G93A}$ mutation display a decreased mitochondrial respiratory capacity associated to phenotypic changes that cause them to induce motor neuron death. Astrocyte-mediated toxicity can be prevented by mitochondria-targeted antioxidants, indicating a critical role of mitochondria in the neurotoxic phenotype. However, it is presently unknown whether drugs currently used to stimulate mitochondrial metabolism can also modulate ALS progression. Here, we tested the disease-modifying effect of dichloroacetate (DCA), an orphan drug that improves the functional status of mitochondria through the stimulation of the pyruvate dehydrogenase complex activity (PDH). Applied to astrocyte cultures isolated from rats expressing the $SOD1^{G93A}$ mutation, DCA reduced phosphorylation of PDH and improved mitochondrial coupling as expressed by the respiratory control ratio (RCR). Notably, DCA completely prevented the toxicity of $SOD1^{G93A}$ astrocytes to motor neurons in coculture conditions. Chronic administration of DCA (500 mg/L) in the drinking water of mice expressing the $SOD1^{G93A}$ mutation increased survival by 2 weeks compared to untreated mice. Systemic DCA also

normalized the reduced RCR value measured in lumbar spinal cord tissue of diseased $SOD1^{G93A}$ mice. A remarkable effect of DCA was the improvement of grip strength performance at the end stage of the disease, which correlated with a recovery of the neuromuscular junction area in extensor digitorum longus muscles. Systemic DCA also decreased astrocyte reactivity and prevented motor neuron loss in $SOD1^{G93A}$ mice. Taken together, our results indicate that improvement of the mitochondrial redox status by DCA leads to a disease-modifying effect, further supporting the therapeutic potential of mitochondria-targeted drugs in ALS.

4.1034 Prevention of Trinitrobenzene Sulfonic Acid-Induced Experimental Colitis by Oral Administration of a Poly(lactic-coglycolic Acid) Microsphere Containing Prostaglandin E2 Receptor Subtype 4 Agonist

Okamoto, T., Uemoto, S. and Tabata, Y.
J. Pharmacol. Exp. Ther., ***341(2)***, *340-349 (2012)*

Prostaglandin E_2 receptor subtype 4 (EP4) agonists are known to reduce intestinal inflammation and enhance epithelium regeneration. We explored the possibility of colonic delivery of an EP4 agonist, 2-[(4-{[2-((1R,2R,3R)-3-hydroxy-2-{(1E,3S)-3-hydroxy-4-[3-(methoxymethyl)phenyl]but-1-enyl}-5-oxocyclopentyl)ethyl]sulfanyl}butanoyl)oxy]ethyl nonanoate (ONO-AE2-724), using poly(lactic-coglycolic acid) (PLGA) microspheres. Colitis was induced in mice by the intrarectal administration of trinitrobenzene sulfonic acid (TNBS). ONO-AE2-724-PLGA microspheres (EP4-MS) were prepared by the standard technique. Drug distributions after oral administration of EP4-MS were determined by liquid chromatography-tandem mass spectrometry analysis. To evaluate the protective effect of EP4-MS, animals were orally treated by gavage with single doses of EP4-MS 24 h before TNBS instillation. The changes in body weight, histopathology, immunohistochemistry, and expression of inflammatory cytokines were evaluated. Oral administration of EP4-MS enhanced colonic tissue drug concentration without any increase in the serum concentration during the 48 h after intake. EP4-MS pretreatment, but not unloaded ONO-AE2-724, significantly attenuated TNBS-induced colitis and diminished colonic mRNA expression levels of proinflammatory cytokines. In addition, a significant increase in the expression of CD25 and FoxP3 was found in isolated lamina propria $CD4^+$ T cells of EP4-MS-treated mice. Immunohistochemical analysis of Ki-67 and single-stranded DNA revealed that EP4-MS pretreatment significantly suppressed apoptosis of colonic cells and promoted epithelial cell proliferation. These results suggest that EP4-MS protect mice from TNBS-induced colitis by intestinal local ONO-AE2-724 delivery. The EP4-MS may offer a promising new therapeutic strategy to treat inflammatory bowel diseases.

4.1035 Epigenetic Modulation of Homer1a Transcription Regulation in Amygdala and Hippocampus with Pavlovian Fear Conditioning

Mahan, A.L., Mou, L., Shah, N., Hu, J-H., Worley, P.F. and Ressler, K.J.
J. Neurosci., ***32(13)***, *4651-4659 (2012)*

The consolidation of conditioned fear involves upregulation of genes necessary for long-term memory formation. An important question remains as to whether this results in part from epigenetic regulation and chromatin modulation. We examined whether Homer1a, which is required for memory formation, is necessary for Pavlovian cued fear conditioning, whether it is downstream of BDNF-TrkB activation, and whether this pathway utilizes histone modifications for activity-dependent transcriptional regulation. We initially found that Homer1a knock-out mice exhibited deficits in cued fear conditioning (5 tone–shock presentations with 70 dB, 6 kHz tones and 0.5 s, 0.6 mA footshocks). We then demonstrated that: (1) Homer1a mRNA increases after fear conditioning *in vivo* within both amygdala and hippocampus of wild-type mice; (2) it increases after BDNF application to primary hippocampal and amygdala cultures *in vitro*; and (3) these increases are dependent on transcription and MAPK signaling. Furthermore, using chromatin immunoprecipitation we found that both *in vitro* and *in vivo* manipulations result in decreases in Homer1 promoter H3K9 methylation in amygdala cells but increases in Homer1 promoter H3 acetylation in hippocampal cells. However, no changes were observed in H4 acetylation or H3K27 dimethylation. Inhibition of histone deacetylation by sodium butyrate enhanced contextual but not cued fear conditioning and enhanced Homer1 H3 acetylation in the hippocampus. These data provide evidence for dynamic epigenetic regulation of Homer1a following BDNF-induced plasticity and during a BDNF-dependent learning process. Furthermore, upregulation of this gene may be regulated through distinct epigenetic modifications in the hippocampus and amygdala.

4.1036 Actin polymerization stabilizes α4β1 integrin anchors that mediate monocyte adhesion

Rullo, J., Becker, H., Hyduk, S.J., Wong, J.C., Digby, G., Arora, P.D., Cano, A.P., HArtwig, J.,

McCulloch, C.A. ansd Cybulsky, M.I.
J. Cell Biol., ***197(1)***, *115-129 (2012)*

Leukocytes arrested on inflamed endothelium via integrins are subjected to force imparted by flowing blood. How leukocytes respond to this force and resist detachment is poorly understood. Live-cell imaging with Lifeact-transfected U937 cells revealed that force triggers actin polymerization at upstream α4β1 integrin adhesion sites and the adjacent cortical cytoskeleton. Scanning electron microscopy revealed that this culminates in the formation of structures that anchor monocyte adhesion. Inhibition of actin polymerization resulted in cell deformation, displacement, and detachment. Transfection of dominant-negative constructs and inhibition of function or expression revealed key signaling steps required for upstream actin polymerization and adhesion stabilization. These included activation of Rap1, phosphoinositide 3-kinase γ isoform, and Rac but not Cdc42. Thus, rapid signaling and structural adaptations enable leukocytes to stabilize adhesion and resist detachment forces.

4.1037 **Reduced Calreticulin Levels Link Endoplasmic Reticulum Stress and Fas-Triggered Cell Death in Motoneurons Vulnerable to ALS**
Bernard-Marissal, N., Moumen, A., Sunyach, C., Pellegrino, C., Dudley, K., Henderson, C.E., Raoul, C. and Pettmann, B.
J. Neurosci., ***32(14)***, *4901-4912 (2012)*

Cellular responses to protein misfolding are thought to play key roles in triggering neurodegeneration. In the mutant superoxide dismutase (mSOD1) model of amyotrophic lateral sclerosis (ALS), subsets of motoneurons are selectively vulnerable to degeneration. Fast fatigable motoneurons selectively activate an endoplasmic reticulum (ER) stress response that drives their early degeneration while a subset of mSOD1 motoneurons show exacerbated sensitivity to activation of the motoneuron-specific Fas/NO pathway. However, the links between the two mechanisms and the molecular basis of their cellular specificity remained unclear. We show that Fas activation leads, specifically in mSOD1 motoneurons, to reductions in levels of calreticulin (CRT), a calcium-binding ER chaperone. Decreased expression of CRT is both necessary and sufficient to trigger $SOD1^{G93A}$ motoneuron death through the Fas/NO pathway. In $SOD1^{G93A}$ mice *in vivo*, reductions in CRT precede muscle denervation and are restricted to vulnerable motor pools. *In vitro*, both reduced CRT and Fas activation trigger an ER stress response that is restricted to, and required for death of, vulnerable $SOD1^{G93A}$ motoneurons. Our data reveal CRT as a critical link between a motoneuron-specific death pathway and the ER stress response and point to a role of CRT levels in modulating motoneuron vulnerability to ALS.

4.1038 **Endogenous GFAP-Positive Neural Stem/Progenitor Cells in the Postnatal Mouse Cortex Are Activated following Traumatic Brain Injury**
Ahmed, A.I., Shtaya, A.B., Zaben, M.J., Owens, E.V., Kiecker, C. and Gray, W.P.
J. Neurotrauma, ***29(5)***, *828-842 (2012)*

Interest in promoting regeneration of the injured nervous system has recently turned toward the use of endogenous stem cells. Elucidating cues involved in driving these precursor cells out of quiescence following injury, and the signals that drive them toward neuronal and glial lineages, will help to harness these cells for repair. Using a biomechanically validated *in vitro* organotypic stretch injury model, cortico-hippocampal slices from postnatal mice were cultured and a stretch injury equivalent to a severe traumatic brain injury (TBI) applied. In uninjured cortex, proliferative potential under *in vitro* conditions is virtually absent in older slices (equivalent postnatal day 15 compared to 8). However, following a severe stretch injury, this potential is restored in injured outer cortex. Using slices from mice expressing a fluorescent reporter on the human glial fibrillary acidic protein (GFAP) promoter, we show that GFAP+ cells account for the majority of proliferating neurospheres formed, and that these cells are likely to arise from the cortical parenchyma and not from the subventricular zone. Moreover, we provide evidence for a correlation between upregulation of sonic hedgehog signaling, a pathway known to regulate stem cell proliferation, and this restoration of regenerative potential following TBI. Our results indicate that a source of quiescent endogenous stem cells residing in the cortex and subcortical tissue proliferate *in vitro* following TBI. Moreover, these proliferating cells are multipotent and are derived mostly from GFAP-expressing cells. This raises the possibility of using this endogenous source of stem cells for repair following TBI.

4.1039 A New Enzyme Mixture to Increase the Yield and Transplant Rate of Autologous and Allogeneic Human Islet Products

Balamurugan, A.N., Loganathan, G., Bellin, M.D., Wilhelm, J.J., Harmon, J., Anazawa, t., Soltani, S.M., Radosevich, D.M., Yuasa, T., Tiwari, M., Papas, K.K., McCarthy, sr., Sutherland, D.E.R. and Hering, B.J.
*Transplantation, **93(7)**, 693-702 (2012)*

Background. The optimal enzyme blend that maximizes human islet yield for transplantation remains to be determined. In this study, we evaluated eight different enzyme combinations (ECs) in an attempt to improve islet yield. The ECs consisted of purified, intact or truncated class 1 (C1) and class 2 (C2) collagenases from *Clostridium histolyticum (Ch*), and neutral protease (NP) from *Bacillus thermoproteolyticus rokko* (thermolysin) or *Ch* (ChNP).

Methods. We report the results of 249 human islet isolations, including 99 deceased donors (research n=57, clinical n=42) and 150 chronic pancreatitis pancreases. We prepared a new enzyme mixture (NEM) composed of intact C1 and C2 collagenases and ChNP in place of thermolysin. The NEM was first tested in split pancreas (n=5) experiments and then used for islet autologous (n=21) and allogeneic transplantation (n=10). Islet isolation outcomes from eight different ECs were statistically compared using multivariate analysis.

Results. The NEM consistently achieved higher islet yields from pancreatitis (P<0.003) and deceased donor pancreases (P<0.001) than other standard ECs. Using the NEM, islet products met release criteria for transplantation from 8 of 10 consecutive pancreases, averaging 6510±2150 islet equivalent number/gram (IEQ/g) pancreas and 694,681±147,356 total IEQ/transplantation. In autologous isolation, the NEM yielded more than 200,000 IEQ from 19 of 21 pancreases (averaging 422,893±181,329 total IEQ and 5979±1469 IEQ/kg recipient body weight) regardless of the severity of fibrosis.

Conclusions. A NEM composed of ChNP with CIzyme high intact C1 collagenase recovers higher islet yield from deceased and pancreatitis pancreases while retaining islet quality and function.

4.1040 Effect of daily semen centrifugation and resuspension on the longevity of equine sperm quality following cooled storage

Love, C.C., Blanchard, T.L., varner, D.D., Brinsko, S.P., Voge, J., Sudderth, K., Teague, S. and LaCaze, K.
*Theriogenology, **77**, 1911-1917 (2012)*

An experiment was conducted to determine whether cooled semen quality could be maintained for a longer interval by conducting daily centrifugation of extended semen, with resuspension of the sperm pellet in fresh extender. Semen treatments included SP10NC and SP50NC which contained 10 and 50% seminal plasma, respectively, were not centrifuged (NC), and were stored at 4 to 7 °C for 96 h. Treatments SP10C and SP50C contained 10 and 50% seminal plasma, respectively, but were centrifuged (C) after 24, 48, and 72 h of cooled storage, with daily resuspension in fresh extender containing 10% seminal plasma. Percent total sperm motility (TMOT) and progressively motile (PMOT) was reduced ($P < 0.05$) in the SP50NC treatment after 24, 48, 72, and 96 h of storage, and TMOT did not differ ($P > 0.05$) in the SP10C, SP50C, SP10NC groups after the same storage periods. The % COMP-α_t did not differ ($P > 0.05$) among treatments at any time period. Percent membrane intact sperm (SMI) was reduced in SP50NC, as compared to SP10C at 48, 72, and 96 h ($P < 0.05$). Daily centrifugation and resuspension of sperm exposed to 50% seminal plasma for the first 24 h (SP50C) yielded similar TMOT, PMOT, VCL, SMI, % COMP-α_t ($P > 0.05$) to Groups SP10NC and SP10C after 96 h of storage. Daily centrifugation and resuspension of cool-stored equine semen in fresh extender may be a method to increase sperm longevity.

4.1041 Loss of Sialic Acid Binding Domain Redirects Protein σ1 to Enhance M Cell-Directed Vaccination

Zlotkowska, D., Maddaloni, M., Riccardi, C., Walters, N., Holderness, K., CaLLIS, g., Rynda-Apple, A. and Pascual, D.W.
*PloS One, **7(4)**, e36182 (2012)*

Ovalbumin (OVA) genetically fused to protein sigma 1 (pσ1) results in tolerance to both OVA and pσ1. Pσ1 binds in a multi-step fashion, involving both protein- and carbohydrate-based receptors. To assess the relative pσ1 components responsible for inducing tolerance and the importance of its sialic binding domain (SABD) for immunization, modified OVA-pσ1, termed OVA-pσ1(short), was deleted of its SABD, but with its M cell targeting moiety intact, and was found to be immunostimulatory and enhanced $CD4^+$ and $CD8^+$ T cell proliferation. When used to nasally immunize mice given with and without cholera toxin (CT) adjuvant, elevated SIgA and serum IgG responses were induced, and OVA-pσ1(s) was more efficient for immunization than native OVA+CT. The immune antibodies (Abs) were derived from elevated Ab-forming cells in the upper respiratory tissues and submaxillary glands and were supported by mixed Th

cell responses. Thus, these studies show that pσ1(s) can be fused to vaccines to effectively elicit improved SIgA responses.

4.1042 P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice

Hoque, R., Sohail, M.A., Salhanick, S.,, Malik, A.F., Ghani, A., Robson, S.C. and Mehal, W.Z.
Am. J. Physiol. Gastrointest. Liver Physiol., ***302***, *G1171-G1179 (2012)*

Inflammation contributes to liver injury in acetaminophen (APAP) hepatotoxicity in mice and is triggered by stimulation of immune cells. The purinergic receptor P2X7 is upstream of the nod-like receptor family, pryin domain containing-3 (NLRP3) inflammasome in immune cells and is activated by ATP and NAD that serve as damage-associated molecular patterns. APAP hepatotoxicity was assessed in mice genetically deficient in *P2X7*, the key inflammatory receptor for nucleotides (*P2X7−/−*), and in wild-type mice. *P2X7−/−* mice had significantly decreased APAP-induced liver necrosis. In addition, APAP-poisoned mice were treated with the specific P2X7 antagonist A438079 or etheno-NAD, a competitive antagonist of NAD. Pre- or posttreatment with A438079 significantly decreased APAP-induced necrosis and hemorrhage in APAP liver injury in wild-type but not *P2X7−/−* mice. Pretreatment with etheno-NAD also significantly decreased APAP-induced necrosis and hemorrhage in APAP liver injury. In addition, APAP toxicity in mice lacking the plasma membrane ecto-NTPDase *CD39* (*CD39−/−*) that metabolizes ATP was examined in parallel with the use of soluble apyrase to deplete extracellular ATP in wild-type mice. *CD39−/−* mice had increased APAP-induced hemorrhage and mortality, whereas apyrase also decreased APAP-induced mortality. Kupffer cells were treated with extracellular ATP to assess P2X7-dependent inflammasome activation. P2X7 was required for ATP-stimulated IL-1β release. In conclusion, P2X7 and exposure to the ligands ATP and NAD are required for manifestations of APAP-induced hepatotoxicity.

4.1043 Outbreak of Equine Herpesvirus Myeloencephalopathy in France: a Clinical and Molecular Investigation

Pronost, S., Legrand, L., Pitel, P-H., Wegge, B., Lissens, J., Freymuth, F., Richard, E. and Fortier, G.
Transboundary and Emerging Dis., ***59(3)***, *256-263 (2012)*

Equid herpesvirus 1 (EHV-1)-associated myeloencephalopathy (EHM) is a disease affecting the central nervous system of horses. Despite the constantly increasing interest about this syndrome, epidemiological data are limited especially when related to the description of large outbreaks. The aim of this article is to describe clinical, virological and molecular data obtained throughout a severe outbreak of EHM, with emphasis on laboratory diagnostic methods. The epidemic disease concerned a riding school in France where 7/66 horses aged 12–22 years developed signs of neurological disease in July 2009. Diagnosis of EHM was supported by EHV-1 detection using both real-time PCR and virus culture, and SNP-PCR test for viral strain characterization. EHM morbidity was 10.6% (7/66), mortality was 7.5% (5/66) and case fatality rate was 71.4% (5/7). Clinical presentation of the disease was characterized by the fact that fever was systematically present within 2 days before the severe neurological signs were noted. EHV-1 was detected by PCR in each available blood and nasal swab samples. Neuropathogenic strain only (G_{2254}) was isolated during the current outbreak; C_t values, used as an indicative level of the viral load, ranged 26.0–37.0 among the six sampled horses. The amount of virus in biological samples was not systematically related to the intensity of the clinical signs being observed. In conclusion, this article described a severe outbreak of EHM while limited in time and restricted to one premise. Molecular data strongly suggested taking into account any low viral load as being a potential risk factor for neurological manifestations.

4.1044 Isolation of Porcine Pancreatic Islets for Xenotransplantation

Ulrichs, K., Eber, S., Schneiker, b., Gahn, S., Strauss, A., Moskalenko, V.a dn Chodnevskaja, I.
Methods in Mol. Biol., ***885***, *213-232 (2012)*

This chapter deals with a technique for isolating intact islets of Langerhans from the pig pancreas based on our experience performing approximately 750 isolations. The procedure we describe involves identi fi cation of an optimal donor pancreas, puri fi cation and in vitro culture of islets, diabetes induction in recipients, and transplantation of islets and their immunomodulation. Besides the sophistication of the technical equipment employed, the major factors in fl uencing the isolation outcome are the pig breed, the number and morphology of the islets in the donor pancreas, the quality of the collagenase/neutral protease, and the skill of the team members.

4.1045 Heparin-binding epidermal growth factor-like growth factor suppresses experimental liver fibrosis in mice

Huang, G., Besner, G.E. and Brigstock, D.R.

Lab. Invest., ***92(5)***, *703-712 (2012)*

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a cytoprotective agent in several organ systems but its roles in liver fibrosis are unclear. We studied the roles of HB-EGF in experimental liver fibrosis in mice and during hepatic stellate cell (HSC) activation. Thioacetamide (TAA; 100 mg/kg) was administered by intraperitoneal injection three times a week for 4 weeks to wild-type HB-EGF$^{+/+}$ or HB-EGF-null (HB-EGF$^{-/-}$) male mice. Livers were examined for histology and expression of key fibrotic markers. Primary cultured HSCs isolated from untreated HB-EGF$^{+/+}$ or HB-EGF$^{-/-}$ mice were examined for fibrotic markers and/or cell migration either during culture-induced activation or after exogenous HB-EGF (100 ng/ml) treatment. TAA induced liver fibrosis in both HB-EGF$^{+/+}$ and HB-EGF$^{-/-}$ mice. Hepatic HB-EGF expression was decreased in TAA-treated HB-EGF$^{+/+}$ mice by 37.6% (P<0.05) as compared with animals receiving saline alone. HB-EGF$^{-/-}$ mice treated with TAA showed increased hepatic α-smooth muscle actin-positive cells and collagen deposition, and, as compared with HB-EGF$^{+/+}$ mice, TAA-stimulated hepatic mRNA levels in HB-EGF$^{-/-}$ mice were, respectively, 2.1-, 1.7-, 1.8-, 2.2-, 1.2- or 3.3-fold greater for α-smooth muscle actin, α1 chain of collagen I or III (COL1A1 or COL3A1), transforming growth factor-β1, connective tissue growth factor or tissue inhibitor of metalloproteinase-1 (P<0.05). HB-EGF expression was detectable in primary cultured HSCs from HB-EGF$^{+/+}$ mice. Both endogenous and exogenous HB-EGF inhibited HSC activation in primary culture, and HB-EGF enhanced HSC migration. These findings suggest that HB-EGF gene knockout in mice increases susceptibility to chronic TAA-induced hepatic fibrosis and that HB-EGF expression or action is associated with suppression of fibrogenic pathways in HSCs.

4.1046 The Double-Stranded RNA Bluetongue Virus Induces Type I Interferon in Plasmacytoid Dendritic Cells via a MYD88-Dependent TLR7/8-Independent Signaling Pathway

Ruscanu, S. et al

J. Virol., ***86(10)***, *5817-5828 (2012)*

Dendritic cells (DCs), especially plasmacytoid DCs (pDCs), produce large amounts of alpha/beta interferon (IFN-α/β) upon infection with DNA or RNA viruses, which has impacts on the physiopathology of the viral infections and on the quality of the adaptive immunity. However, little is known about the IFN-α/β production by DCs during infections by double-stranded RNA (dsRNA) viruses. We present here novel information about the production of IFN-α/β induced by bluetongue virus (BTV), a vector-borne dsRNA Orbivirus of ruminants, in sheep primary DCs. We found that BTV induced IFN-α/β in skin lymph and in blood *in vivo*. Although BTV replicated in a substantial fraction of the conventional DCs (cDCs) and pDCs *in vitro*, only pDCs responded to BTV by producing a significant amount of IFN-α/β. BTV replication in pDCs was not mandatory for IFN-α/β production since it was still induced by UV-inactivated BTV (UV-BTV). Other inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-12p40, were also induced by UV-BTV in primary pDCs. The induction of IFN-α/β required endo-/lysosomal acidification and maturation. However, despite being an RNA virus, UV-BTV did not signal through Toll-like receptor 7 (TLR7) for IFN-α/β induction. In contrast, pathways involving the MyD88 adaptor and kinases dsRNA-activated protein kinase (PKR) and stress-activated protein kinase (SAPK)/Jun N-terminal protein kinase (JNK) were implicated. This work highlights the importance of pDCs for the production of innate immunity cytokines induced by a dsRNA virus, and it shows that a dsRNA virus can induce IFN-α/β in pDCs via a novel TLR-independent and Myd88-dependent pathway. These findings have implications for the design of efficient vaccines against dsRNA viruses.

4.1047 Controlled Synthesis of Cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics

Rossow, T., Heyman, J.A., Ehrlicher, A.J., Langhoff, A., Weitz, D.A., Haag, R. and Seiffert, S.

J. Am. Chem. Soc., ***134(10)***, *4983-4989 (2012)*

Micrometer-sized hydrogel particles that contain living cells can be fabricated with exquisite control

through the use of droplet-based microfluidics and bioinert polymers such as polyethyleneglycol (PEG) and hyperbranched polyglycerol (hPG). However, in existing techniques, the microgel gelation is often achieved through harmful reactions with free radicals. This is detrimental for the viability of the encapsulated cells. To overcome this limitation, we present a technique that combines droplet microfluidic templating with bio-orthogonal thiol–ene click reactions to fabricate monodisperse, cell-laden microgel particles. The gelation of these microgels is achieved via the nucleophilic Michael addition of dithiolated PEG macro-cross-linkers to acrylated hPG building blocks and does not require any initiator. We systematically vary the microgel properties through the use of PEG linkers with different molecular weights along with different concentrations of macromonomers to investigate the influence of these parameters on the viability and proliferation of encapsulated yeast cells. We also demonstrate the encapsulation of mammalian cells including fibroblasts and lymphoblasts.

4.1048 A Reversible Early Oxidized Redox State That Precedes Macromolecular ROS Damage in Aging Nontransgenic and 3xTg-AD Mouse Neurons

Ghosh,, D., LeVault, K.R., Barnett, A.J. and Brewer, G.J.

J. Neurosci., ***32(17)***, *5821-5832 (2012)*

The brain depends on redox electrons from nicotinamide adenine dinucleotide (reduced form; NADH) to produce ATP and oxyradicals (reactive oxygen species [ROS]). Because ROS damage and mitochondrial dysregulation are prominent in aging and Alzheimer's disease (AD) and their relationship to the redox state is unclear, we wanted to know whether an oxidative redox shift precedes these markers and leads to macromolecular damage in a mouse model of AD. We used the 3xTg-AD mouse model, which displays cognitive deficits beginning at 4 months. Hippocampal/cortical neurons were isolated across the age span and cultured in common nutrients to control for possible hormonal and vascular differences. We found an increase of NAD(P)H levels and redox state in nontransgenic (non-Tg) neurons until middle age, followed by a decline in old age. The 3xTg-AD neurons maintained much lower resting NAD(P)H and redox states after 4 months, but the NADH regenerating capacity continuously declined with age beginning at 2 months. These redox characteristics were partially reversible with nicotinamide, a biosynthetic precursor of NAD^+. Nicotinamide also protected against glutamate excitotoxicity. Compared with non-Tg neurons, 3xTg-AD neurons had more mitochondria/neuron and lower glutathione (GSH) levels that preceded age-related increases in ROS levels. These GSH deficits were again reversible with nicotinamide in 3xTg-AD neurons. Surprisingly, low macromolecular ROS damage was only elevated after 4 months in the 3xTg-AD neurons if antioxidants were removed. The present data suggest that a more oxidized redox state and a lower antioxidant GSH defense can be dissociated from neuronal ROS damage, changes that precede the onset of cognitive deficits in the 3xTg-AD model.

4.1049 Consumption of Rice Bran Increases Mucosal Immunoglobulin A Concentrations and Numbers of Intestinal Lactobacillus spp.

Henderson, A.J., Kumar, A., Barnett, B., Dow, S.W. and Ryan, E.P.

J. Med. Food, ***15(5)***, *469-475 (2012)*

Gut-associated lymphoid tissue maintains mucosal homeostasis by combating pathogens and inducing a state of hyporesponsiveness to food antigens and commensal bacteria. Dietary modulation of the intestinal immune environment represents a novel approach for enhancing protective responses against pathogens and inflammatory diseases. Dietary rice bran consists of bioactive components with disease-fighting properties. Therefore, we conducted a study to determine the effects of whole dietary rice bran intake on mucosal immune responses and beneficial gut microbes. Mice were fed a 10% rice bran diet for 28 days. Serum and fecal samples were collected throughout the study to assess total immunoglobulin A (IgA) concentrations. Tissue samples were collected for cellular immune phenotype analysis, and concentrations of native gut *Lactobacillus* spp. were enumerated in the fecal samples. We found that dietary rice bran induced an increase in total IgA locally and systemically. In addition, B lymphocytes in the Peyer's patches of mice fed rice bran displayed increased surface IgA expression compared with lymphocytes from control mice. Antigen-presenting cells were also influenced by rice bran, with a significant increase in myeloid dendritic cells residing in the lamina propria and mesenteric lymph nodes. Increased colonization of native *Lactobacillus* was observed in rice bran–fed mice compared with control mice. These findings suggest that rice bran–induced microbial changes may contribute to enhanced mucosal IgA responses, and we conclude that increased rice bran consumption represents a promising dietary intervention to modulate mucosal immunity for protection against enteric infections and induction of beneficial gut bacteria.

4.1050 Role of Lentivirus-Mediated Overexpression of Programmed Death-Ligand 1 on Corneal Allograft

Survival
Nosov, M., Wilk, M., Morcos, M., Cregg, M., O'Flynn, L., Treacy, O. and Ritter, T.
Am. J. Transplant., ***12(5)****, 1313-1322 (2012)*

To investigate the role of lentivirus-mediated overexpression of programmed death-ligand 1 (PD-L1) on rat corneal allograft survival. A fully allogeneic rat cornea transplant model was used for *in vivo* studies. Lentiviral (LV) vectors are efficient tools for *ex vivo* genetic modification of cultured corneas. LV vector encoding for PD-L1 (LV.PD-L1) and LV vector encoding for eGFP (LV.eGFP, as control) were constructed and tested. PD-L1 or eGFP expression was increased on corneal cells upon LV.PD-L1 and LV.eGFP transduction, respectively. Both allogeneic controls and allogeneic LV.eGFP transduced corneas were uniformly rejected (MST: 13.8 ± 1.7 days and 12.3 ± 1.9 days, respectively). In contrast, allogeneic LV.PD-L1 transduced corneas showed a high percentage (83%) of graft survival (MST > 30 days, n = 5, 15 days, n = 1). Graft opacity of PD-L1 transduced corneas was present but was significantly reduced compared to control or eGFP expressing corneas. Flow cytometric analysis revealed that percentages of $CD3^+CD8^+CD161^+$ and $CD3^+CD8^+CD161^-$ lymphocytes were decreased in animals receiving LV.PD-L1 transduced corneas compared to animals grafted with LV.eGFP transduced corneas. Moreover, reduced expression of proinflammatory cytokines (IFN-γ and IL-6) in PD-L1 transduced corneas compared to allogeneic controls was also observed. Local PD-L1 gene transfer in cultured corneas is a promising approach for the prolongation of corneal allograft survival and attenuation of graft rejection.

4.1051 Motoneuron programmed cell death in response to proBDNF
Taylor, A.R., Gifondorwa, D.J., Robinson, M.B., Strupe, J.L., Prevette, D., Johnson, J.E., Hemstead, B., Oppenheim, R.W. and Milligan, C.E.
Develop. Neurobiol., ***72(5)****, 699-712 (2012)*

Motoneurons (MN) as well as most neuronal populations undergo a temporally and spatially specific period of programmed cell death (PCD). Several factors have been considered to regulate the survival of MNs during this period, including availability of muscle-derived trophic support and activity. The possibility that target-derived factors may also negatively regulate MN survival has been considered, but not pursued. Neurotrophin precursors, through their interaction with $p75^{NTR}$ and sortilin receptors have been shown to induce cell death during development and following injury in the CNS. In this study, we find that muscle cells produce and secrete proBDNF. ProBDNF through its interaction with $p75^{NTR}$ and sortilin, promotes a caspase-dependent death of MNs in culture. We also provide data to suggest that proBDNF regulates MN PCD during development *in vivo.*

4.1052 High-frequency vagus nerve stimulation improves portal hypertension in cirrhotic rats
Bockx, I., Verdrengh, K., Vander Elst, I. et al
Gut, ***61****, 604-612 (2012)*

Objective The liver is innervated by the vagus nerve. Its efferent neurotransmitters acetylcholine (ACh) and vasoactive intestinal peptide (VIP) are both well-known vasodilators. A study was undertaken to determine whether electrical vagus nerve stimulation (STIM) influences portal vein pressure.
Methods The left vagus nerve upstream of the hepatic branch was stimulated at 5 Hz (ACh release) and 10 Hz (VIP release) in normal and cirrhotic rats.
Results STIM at both frequencies decreased portal pressure in normal rats while, in cirrhotic rats, only 10 Hz STIM resulted in long-lasting reduction of portal pressure. Hepatic branch vagotomy prevented the STIM-induced decrease in pressure, proving that the effect is a direct hepatic effect. Deafferentation of the left vagus nerve by pretreatment with capsaicin did not change the effect of STIM, showing that the vagus efferents and not the afferents are responsible for the decrease in portal pressure. Injecting microspheres before and after STIM showed that STIM did not lead to redistribution of systemic blood flow but decreased portal pressure by lowering intrahepatic resistance. Using in situ liver perfusion to evaluate the intrahepatic effect of ACh and VIP, both neurotransmitters significantly decreased the perfusion pressure in normal rats. VIP also decreased portal pressure in cirrhotic rats, confirming the results of STIM. This VIP-induced decrease in pressure could be prevented by a VIP receptor 2 antagonist. L-NAME did not inhibit the VIP effect in cirrhotic rats, indicating that VIP does not act via nitric oxide.
Conclusion High-frequency electrical vagus stimulation improves portal hypertension in cirrhotic rats, most likely through release of VIP, binding to VIP receptor 2. As the technology is already in use for other applications, vagus nerve stimulation might be an important new strategy in the treatment of portal hypertension.

4.1053 Tissue engineering the monosynaptic circuit of the stretch reflex arc with co-culture of embryonic motoneurons and proprioceptive sensory neurons
Guo, X., Ayala, J.E., Gonzales, M., Stancescu, M., Lambert, S. and Hickman, J.J.
*Biomaterials, **33**, 5723-5731 (2012)*

The sensory circuit of the stretch reflex arc is composed of intrafusal muscle fibers and their innervating proprioceptive neurons that convert mechanical information regarding muscle length and tension into action potentials that synapse onto the homonymous motoneurons in the ventral spinal cord which innervate the extrafusal fibers of the same muscle. To date, the in vitro synaptic connection between proprioceptive sensory neurons and spinal motoneurons has not been demonstrated. A functional in vitro system demonstrating this connection would enable the understanding of feedback by the integration of sensory input into the spinal reflex arc. Here we report a co-culture of rat embryonic motoneurons and proprioceptive sensory neurons from dorsal root ganglia (DRG) in a defined serum-free medium on a synthetic silane substrate (DETA). Furthermore, we have demonstrated functional synapse formation in the co-culture by immunocytochemistry and electrophysiological analysis. This work will be valuable for enabling in vitro model systems for the study of spinal motor control and related pathologies such as spinal cord injury, muscular dystrophy and spasticity by improving our understanding of the integration of the mechanosensitive feedback mechanism.

4.1054 Endothelial Progenitor Cell Cotransplantation Enhances Islet Engraftment by Rapid Revascularization
Kang, S., Park, H.S., Jo, A., Hong, S.H., Lee, H.N., Lee, Y.Y., Park, J.S., Jung, H.S., Chung, S.S. and Park, K.S.
*Diabetes, **61**, 866-876 (2012)*

Impaired revascularization of transplanted islets is a critical problem that leads to progressive islet loss. Since endothelial progenitor cells (EPCs) are known to aid neovascularization, we aimed to enhance islet engraftment by cotransplanting EPCs with islets. Porcine islets, with (islet-EPC group) or without (islet-only group) human cord blood–derived EPCs, were transplanted into diabetic nude mice. The islet-EPC group reached euglycemia by ∼11 days posttransplantation, whereas the islet-only group did not. Also, the islet-EPC group had a higher serum porcine insulin level than the islet-only group. Islets from the islet-EPC group were more rapidly revascularized at the early period of transplantation without increment of final capillary density at the fully revascularized graft. Enhanced revascularization rate in the islet-EPC group was mainly attributed to stimulating vascular endothelial growth factor-A production from the graft. The rapid revascularization by EPC cotransplantation led to better graft perfusion and recovery from hypoxia. EPC cotransplantation was also associated with greater β-cell proliferation, probably by more basement membrane production and hepatocyte growth factor secretion. In conclusion, cotransplantation of EPCs and islets induces better islet engraftment by enhancing the rate of graft revascularization. These findings might provide a directly applicable tool to enhance the efficacy of islet transplantation in clinical practice.

4.1055 PMO-126 The role of vascular-adhesion-protein 1 (vap-1) in mediating monocyte migration across inflamed hepatic sinusoidal endothelium
Zimmermann, H., Weston, C.J., Curbishley, S.M. and Adams, D.H.
*Gut, **61**, A124 (2012)*

Introduction There is compelling evidence that accumulating monocyte-derived macrophages are pivotally involved in driving liver fibrogenesis. It remains unclear which molecules mediate transmigration of these cells across hepatic sinusoidal endothelial cells (HSEC). VAP-1 is an atypical adhesion molecule with enzymatic monoamine oxidase activity that is predominantly expressed in the liver microvasculature. It possesses key function in the recruitment of various lymphocyte subsets. The aim of this study was to decipher VAP-1 contribution to monocytic transendothelial transmigration.
Methods Primary human HSEC were isolated from explanted and grown to confluence in flow chambers. After activation with TNF-α/IFN-γ for 24h HSEC were treated with VAP-1 antibody and enzyme inhibitors. Monocytes were enriched from peripheral blood by using OptiPrep gradient. Monocyte subsets ($CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$, $CD14^{+}CD16^{++}$) were isolated by FACS-sorting. Isolated monocytes were perfused over HSEC monolayers under constant flow simulating physiological shear stress (0.05 Pa). Adhesion and transmigration was studied using phase contrast microscopy. Transwell assays were used to study the phenotype of transmigrated monocytes by flowcytometry.
Results HSEC pretreatment with VAP-1 antibody (TK8-14) or enzyme inhibitor Semicarbizide equally

reduced monocyte transmigration by ~50%. VCAM-1 blockade had a similar but redundant effect whereas CLEVER-1-antibody or LOX-inhibitor (β-APN) did not alter monocyte transmigration. VAP-1 antibody acted in a time-dependent manner with influence on monocyte adhesion only after short-term application (15 min). Inhibiting VAP-1 led to profound reduction of proinflammatory *nonclassical* $CD14^{+}CD16^{++}$ monocyte transmigration but also affected *classical* $CD14^{++}CD16^{-}$ whereas the *intermediate* $CD14^{++}CD16^{+}$ subtype was not affected. Under static conditions VAP-1 enzymatic or antibody inhibition was significantly blunted suggesting flow to be a mandatory prerequisite for the biological function of VAP-1 on monocytes. Increased expression of HLA-DR and the M2 macrophage marker CD206 on monocyte subsets after endothelial transmigration was not altered by VAP-1 inhibition.
Conclusion Endothelial VAP-1 differentially modulates monocyte recruitment under flow conditions in a time-dependent fashion and .favours transmigration of a proinflammatory monocyte subset. The critical role of VAP-1 enzyme function renders small molecules as a promising therapeutic approach in combating liver inflammation and subsequent fibrosis.

4.1056 **Antigen-specific effector CD8 T cells regulate allergic responses via IFN-γ and dendritic cell function**
Tang, Y., Guan, S.P., Chua, B.Y.L., Zhou, Q., Ho, A.W.S., Wong, K.H.S., Wong, K.L., Wong, W.S.F. and Kemeny, D.M.
J. Allergy Clin. Immunol., ***129****, 1611-1620 (2012)*

Background
Previous studies have shown that CD8 T cells can both prevent and cause allergic responses. However, the underlying mechanisms remain to be elucidated.
Objective
We aim to investigate the potential of CD8 T cells with different IFN-γ expressions to modulate the elicitation of allergic inflammation following ovalbumin (OVA) challenge and investigate the underlying mechanisms.
Methods
To study the role of IFN-γ in the effect of CD8 T cells, effector CD8 T cells from CD8 OVA transgenic (OT-I) mice and IFN-$\gamma^{-/-}$OT-I mice were transferred to OVA-sensitized mice the day before 3 challenges with OVA. The effect on lung dendritic cells (DCs) exerted by CD8 T cells was studied with *ex vivo* culture of sorted DCs from treatment mice with CD4 T cells.
Results
Effector OT-I, but not IFN-$\gamma^{-/-}$OT-I CD8 T cells, attenuated eosinophilia and mucus secretion in the lungs of sensitized mice in an antigen-specific manner. Effector IFN-$\gamma^{-/-}$OT-I CD8 T cells displayed a Tc2-/Tc17-biased phenotype with weaker cytotoxicity and were able to both induce and exacerbate eosinophilia as well as neutrophilia. OT-I CD8 T cells increased the ability of lung $CD11b^{+}CD103^{-}$ DCs to both prime the differentiation of naive OVA-specific CD4 T cells toward a T_H1 phenotype and enhance IFN-γ production by antigen-experienced lung CD4 T cells.
Conclusion
Effector CD8 T cells attenuate pulmonary inflammation and alter the ability of DCs within the allergic lung to polarize T cells to a T_H1 phenotype during a T_H2 response. In the absence of IFN-γ, CD8 T cells assume a Tc2-/Tc17-biased phenotype and potentiate inflammation.

4.1057 **Expression of Transient Receptor Potential Ankyrin 1 (TRPA1) and Its Role in Insulin Release from Rat Pancreatic Beta Cells**
Cao, D-S., Zhong, L., Hsieh, T-h., Abooj, M., Bishnoi, M., Hughes, L. and Premkumar, L.S.
PloS One, ***7(5)****, e38005 (2012)*

Objective
Several transient receptor potential (TRP) channels are expressed in pancreatic beta cells and have been proposed to be involved in insulin secretion. However, the endogenous ligands for these channels are far from clear. Here, we demonstrate the expression of the transient receptor potential ankyrin 1 (TRPA1) ion channel in the pancreatic beta cells and its role in insulin release. TRPA1 is an attractive candidate for inducing insulin release because it is calcium permeable and is activated by molecules that are produced during oxidative glycolysis.
Methods

Immunohistochemistry, RT-PCR, and Western blot techniques were used to determine the expression of TRPA1 channel. Ca^{2+} fluorescence imaging and electrophysiology (voltage- and current-clamp) techniques were used to study the channel properties. TRPA1-mediated insulin release was determined using ELISA.
Results
TRPA1 is abundantly expressed in a rat pancreatic beta cell line and freshly isolated rat pancreatic beta cells, but not in pancreatic alpha cells. Activation of TRPA1 by allyl isothiocyanate (AITC), hydrogen peroxide (H_2O_2), 4-hydroxynonenal (4-HNE), and cyclopentenone prostaglandins (PGJ_2) and a novel agonist methylglyoxal (MG) induces membrane current, depolarization, and Ca^{2+} influx leading to generation of action potentials in a pancreatic beta cell line and primary cultured pancreatic beta cells. Activation of TRPA1 by agonists stimulates insulin release in pancreatic beta cells that can be inhibited by TRPA1 antagonists such as HC030031 or AP-18 and by RNA interference. TRPA1-mediated insulin release is also observed in conditions of voltage-gated Na^+ and Ca^{2+} channel blockade as well as ATP sensitive potassium (K_{ATP}) channel activation.
Conclusions
We propose that endogenous and exogenous ligands of TRPA1 cause Ca^{2+} influx and induce basal insulin release and that TRPA1-mediated depolarization acts synergistically with K_{ATP} channel blockade to facilitate insulin release.

4.1058 miR-132 Enhances Dendritic Morphogenesis, Spine Density, Synaptic Integration, and Survival of Newborn Olfactory Bulb Neurons
Pathania, M., Torres-Reveron, J., Yan, L., Kimura, T., Lin, T.V., Gordon, V., Teng, Z-Q., Zhao, X., Fulga, T.A., Van Vactor, D. and Borsdey, A.
*PloS One, **7(5)**, e38174 (2012)*

An array of signals regulating the early stages of postnatal subventricular zone (SVZ) neurogenesis has been identified, but much less is known regarding the molecules controlling late stages. Here, we investigated the function of the activity-dependent and morphogenic microRNA miR-132 on the synaptic integration and survival of olfactory bulb (OB) neurons born in the neonatal SVZ. *In situ* hybridization revealed that miR-132 expression occurs at the onset of synaptic integration in the OB. Using *in vivo* electroporation we found that sequestration of miR-132 using a sponge-based strategy led to a reduced dendritic complexity and spine density while overexpression had the opposite effects. These effects were mirrored with respective changes in the frequency of GABAergic and glutamatergic synaptic inputs reflecting altered synaptic integration. In addition, timely directed overexpression of miR-132 at the onset of synaptic integration using an inducible approach led to a significant increase in the survival of newborn neurons. These data suggest that miR-132 forms the basis of a structural plasticity program seen in SVZ-OB postnatal neurogenesis. miR-132 overexpression in transplanted neurons may thus hold promise for enhancing neuronal survival and improving the outcome of transplant therapies.

4.1059 CD70–CD27 Interaction Augments CD8+ T-Cell Activation by Human Epidermal Langerhans Cells
Polak, M.E., Newell, L., Taraban, V.Y., Pickard, C., Healy, E., Friedmann, P.S., Al-Shamkhani, A. and Ardern-Jones, M.R.
*J. Invest. Dermatol., **132(6)**, 1636-1644 (2012)*

Human cutaneous dendritic cells (DCs) from epidermal and dermal compartments exhibit functional differences in their induction of CD4+ T-cell and humoral immune responses; however, differences in the regulation of memory CD8+ T-cell responses by human skin DCs remain poorly characterized. We tested the capacity of human Langerhans cells (LCs) and dermal dendritic cells (DDCs) to induce antigen-specific cytokine production and proliferation of memory CD8+ cells. Although tumor necrosis factor-α-matured human DCs from both epidermal and dermal compartments showed efficient potential to activate CD8+ cells, LCs were constitutively more efficient than DDCs in cross-presenting CD8+ epitopes, as well as direct presentation of viral antigen to Epstein–Barr virus-specific CD8+ T cells. LCs showed greater expression of CD70, and blockade of CD70–CD27 signaling demonstrated that superiority of CD8+ activation by epidermal LC is CD70 dependent. This CD70-related activation of CD8+ cells by LCs denotes a central role of LCs in CD8+ immunity in skin, and suggests that regulation of LC CD70 expression is important in enhancing immunity against cutaneous epithelial pathogens and cancer.

4.1060 Elucidating the Mechanism By Which Monocytes Can Inhibit Hypoxic PA-SMC Proliferation
Parmar, S., Thompson, A.A.R., Higgins, K.R., Sabroe, I., Parker, L.C., Lawrie, A., Arnold, J., Walker, S., Elliot, C.A., Conddliffe, R., Kiely, D.G., Whyte, M.K. and Walmsley, S.R.
*Am. J. Respir. Crit. Care Med., **185**, A5660 (2012)*

Introduction Pulmonary arterial hypertension (PAH) is a devastating disease characterised by the narrowing and occlusion of the small pulmonary arteries leading to increased right ventricular pressures and eventual failure. The triggers for the vascular remodelling events are still poorly understood. Understanding the pathogenesis of PAH is key to developing novel therapies. Interestingly, inflammation and local tissue hypoxia have been linked to the process. Using co-culture models we investigated the role of hypoxia monocyte PASMC interactions. **Methods** Peripheral blood mononuclear cells from healthy volunteers, patients with idiopathic PAH (IPAH) and patients with systemic sclerosis associated PAH (SScPAH) were isolated by Optiprep and CD14+ monocytes purified by negative selection. Monocytes were then cultured with hPA-SMCs (1:5) in normoxia (19 kPa) and hypoxia (3 kPa) for 24 hours +/- 10 ng/ml LPS and cell counts were. **Results** Monocytes undergo significantly less apoptosis in 24 hr hypoxia (3kPa) (32.88% +/- 3.09) than in normoxia (13kPa) (63.51% +/- 14.44) (*P< 0.05, n=4). Total PA-SMC counts increased significantly in hypoxia to normoxia (36420 +/- 6792 to 21167 +/- 1113, **P<0.01, n=8).This increase was abrogated with addition of monocytes (Normoxia: 26936 +/- 4269, hypoxia 24787 +/- 1859, n=10). Supernatant transfer reduced total cell numbers to co-culture values (Normoxia: 28276.67 +/- 1508.645 Hypoxia: 26578.0 +/- 908.847 n=4). Monocytes could not inhibit platelet derived growth factor induced proliferation (3 ng/ml PDGF monoculture: 31616.4 +/- 1395.0, co-culture: 30732.7 +/- 1276.1, ***P<0.001, n=6). Exdogenous IL-1ra could inhibit hypoxia induced proliferation to the extent of co-cultures (3 ng/ml IL-1ra hypoxic monoculture: 29846.7 +/- 1039.3, normoxic monoculture: 25830.0 +/- 994.9, n=6). We saw no inhibition of hypoxic PASMC proliferation by IPAH monocytes in contrast to monocytes from healthy controls or from patients with SSc-PAH (IPAH: 64307.5 +/- 12566.4, controls: 27935 +/- 672.5, SScPAH: 36517 +/- 3571 n=3, n=6 and n=6 respectively). Monocytes from IPAH patients are unable to suppress hypoxic PA-SMC proliferation Smooth muscle cells were seeded at 25,000 cells /well and cultured in normoxia (room air) (open bars) and hypoxia (3 kPa) (closed bars) for 24 hours before serum starvation. Plates were cultured for further 24 hours +/- monocytes. Total cell counts were carried out by Coulter Counter. (bars represent mean +/- SEM , n=6 control, n=4 SScPAH, n=3 IPAH *P<0.05, **P<0.01, ***P<0.001) Results are also expressed in terms of percentage change from normoxic monoculture **Conclusions** Both monocytes and hPASMCs demonstrate a pro-survival and proliferative phenotype in hypoxia. Monocytes inhibit the hypoxia induced increase in hPA-SMC cell counts. Supernatant transfers suggest this is independent of monocyte hypoxia and direct cell contact and is selective for hypoxia. Interestingly, IL-1ra was able to inhibit hypoxic proliferation, although we were unable to detect IL-1ra, IL-1 beta or IL-1 alpha in our culture supernatants. Importantly we show that monocytes from patients with IPAH cannot inhibit hypoxic proliferation compared to monocytes from SSc-PAH or controls. **Acknowledgements** SP: NIHR CVBRU funded PhD studentship SRW: Wellcome Intermediate fellowship.

4.1061 Cytotoxic activity to acute myeloid leukemia cells by Antp-TPR hybrid peptide targeting Hsp90

Horibe, T., Kawamoto, M., Kohno, M. and Kawakami, K.

*J. Bioscience and Bioengineering, **114(1)**, 96-103 (2012)*

We previously reported that Antp-TPR hybrid peptide inhibited the interaction of Hsp90 with TPR2A and had selective cytotoxic activity discriminating between normal and cancer cells to induce cancer cell death. In this study, we investigated the cytotoxic activity of Antp-TPR peptide toward acute myeloid leukemia (AML) cells. It was demonstrated that Antp-TPR peptide induced AML cell death in cell lines such as U937, K562, THP-1, and HL-60 via activation of caspases 3 and 7, and disruption of mitochondrial membrane potential. Conversely, Antp-TPR peptide did not reduce the viability of normal cells including peripheral blood mononuclear cells (PBMCs), although both geldanamycin and 17-AAG, small-molecule inhibitors of Hsp90, mediated cytotoxicity to these normal cells at low concentrations. In addition, mutation analysis of TPR peptide demonstrated that the highly conserved amino acids Lys and Arg were critical to the cytotoxic activity. These results indicated that Antp-TPR hybrid peptide would provide potent and selective therapeutic options in the treatment of AML.

4.1062 PD-1 Blockage Reverses Immune Dysfunction and Hepatitis B Viral Persistence in a Mouse Animal Model

Tzeng, H-T.,Tsai, H-F., Liao, H-J., Lin, Y-J., Chen, L., Chen, P-J.and Hsu, P-N.

*PloS One, **7(6)**, e39179 (2012)*

Persistent hepatitis B viral (HBV) infection results in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Recent studies in animal models of viral infection indicate that the interaction between the inhibitory receptor, programmed death (PD)-1, on lymphocytes and its ligand (PD-L1) play a critical

role in T-cell exhaustion by inducing T-cell inactivation. High PD-1 expression levels by peripheral T-lymphocytes and the possibility of improving T-cell function by blocking PD-1-mediated signaling confirm the importance of this inhibitory pathway in inducing T-cell exhaustion. We studied T-cell exhaustion and the effects of PD-1 and PD-L1 blockade on intrahepatic infiltrating T-cells in our recently developed mouse model of HBV persistence. In this mouse animal model, we demonstrated that there were increased intrahepatic PD-1-expressing CD8+ and CD4+ T cells in mice with HBV persistence, but PD-1 upregulation was resolved in mice which had cleared HBV. The Intrahepatic CD8+ T-cells expressed higher levels of PD-1 and lower levels of CD127 in mice with HBV persistence. Blockade of PD-1/PD-L1 interactions increased HBcAg-specific interferon (IFN)-γ production in intrahepatic T lymphocytes. Furthermore, blocking the interaction of PD-1 with PD-L1 by an anti-PD-1 monoclonal antibody (mAb) reversed the exhausted phenotype in intrahepatic T lymphocytes and viral persistence to clearance of HBV *in vivo*. Our results indicated that PD-1 blockage reverses immune dysfunction and viral persistence of HBV infection in a mouse animal model, suggesting that the anti-PD-1 mAb might be a good therapeutic candidate for chronic HBV infection.

4.1063 Role of Differentiation of Liver Sinusoidal Endothelial Cells in Progression and Regression of Hepatic Fibrosis in Rats

Xie, G., Wang, X., Wang, L., Wang, L., Atkinson, R.D., Kanel, G.C., Gaarde, W.A. and Deleve, L.D.
*Gastroenterology, **142(4)**, 918-927 (2012)*

Background & Aims
Capillarization, characterized by loss of differentiation of liver sinusoidal endothelial cells (LSECs), precedes the onset of hepatic fibrosis. We investigated whether restoration of LSEC differentiation would normalize crosstalk with activated hepatic stellate cells (HSC) and thereby promote quiescence of HSC and regression of fibrosis.
Methods
Rat LSECs were cultured with inhibitors and/or agonists and examined by scanning electron microscopy for fenestrae in sieve plates. Cirrhosis was induced in rats using thioacetamide, followed by administration of BAY 60-2770, an activator of soluble guanylate cyclase (sGC). Fibrosis was assessed by Sirius red staining; expression of α-smooth muscle actin was measured by immunoblot analysis.
Results
Maintenance of LSEC differentiation requires vascular endothelial growth factor-A stimulation of nitric oxide–dependent signaling (via sGC and cyclic guanosine monophosphate) and nitric oxide–independent signaling. In rats with thioacetamide-induced cirrhosis, BAY 60-2770 accelerated the complete reversal of capillarization (restored differentiation of LSECs) without directly affecting activation of HSCs or fibrosis. Restoration of differentiation to LSECs led to quiescence of HSCs and regression of fibrosis in the absence of further exposure to BAY 60-2770. Activation of sGC with BAY 60-2770 prevented progression of cirrhosis, despite continued administration of thioacetamide.
Conclusions
The state of LSEC differentiation plays a pivotal role in HSC activation and the fibrotic process.

4.1064 Application of antioxidants and centrifugation for cryopreservation of boar spermatozoa

Zhang, W., Yi, K., Chen, C., Hou, X. and Zhou, X.
*Animal Reprod. Sci., **132**, 123-128 (2012)*

Although cryopreserved boar semen has been available since 1975, a major breakthrough in commercial application has not yet occurred due to the high susceptibility of boar spermatozoa to damage during cryopreservation and the complicated process required for deep freezing. In recent years, the application of antioxidants during the cryopreservation of boar semen has been the subject of considerable research aimed at improving the quality of post-thaw semen. Centrifugation is necessary before using cryopreservation protocols for freezing boar spermatozoa. Studies of the effect of different centrifugation regimens on boar sperm recovery, yield and cryosurvival have made significant contributions. Therefore this review elucidates results of recent applications of various antioxidants and centrifugation regimens used in efforts to improve cryopreservation of boar spermatozoa. This review is intended to enhance understanding of the roles of these antioxidants and centrifugation regimens with respect to mechanisms that increase resistance to cryodamage of boar spermatozoa. In addition, the discussion addresses the need for developing an objective evaluation of effectiveness and estimating the prospect of application of new techniques for the cryopreservation of boar semen and its use in artificial insemination.

4.1065 Delayed cryopreservation of stallion sperm: effect of iodixanol density gradient centrifugation
Heutelbeck, A., Oldenhof, H., Henke, S., Martinsson, G. and Sieme, H.
*J. Equine Vet. Sci., **32**, 488-489 (2012)*

The infrastructure needed for cryopreservation of stallion sperm is not always available. In these cases, diluted semen needs to be shipped to a facility where cryopreservation can be done typically after one day storage at 4°C. Centrifugation processing of semen, including density centrifugation, is generally used in the selection of high quality sperm for artificial insemination. The aim of this study was to evaluate if selection of sperm via two-layer iodixanol density centrifugation improves cryosurvival after delayed cryopreservation, as compared to the standard centrifugation processing. Moreover, centrifugation processing directly after semen collection and storage of selected sperm was compared with centrifugation of stored sperm just prior to cryopreservation. For each processing method, twelve ejaculates were tested from stallions of the Hannoverian warmblood breed (two ejaculates from each of six stallions). Sperm motility was evaluated using computer assisted sperm analysis. Sperm stained with both propidium iodide and Sybr-14 was analyzed by flow cytometry to discriminate between membrane damaged and intact cells, respectively. Fractions of normal double stranded and denatured single-stranded DNA were determined after acid treatment and acridine orange staining, as a measure for sperm chromatin stability and integrity. Two-layer iodixanol density centrifugation resulted in a 5% increase in the percentage of plasma membrane intact sperm, as compared to diluted sperm or sperm processed using ordinary centrifugation. In addition, chromatin stability was found to be increased for sperm obtained after iodixanol density centrifugation. Centrifugation processing at the day of collection or after storage resulted in similar percentages of plasma membrane intact sperm after one day storage at 4°C, whereas progressive motility of sperm processed after storage was slightly decreased. Iodixanol density centrifugation resulted in increased cryosurvival rates as compared to ordinary centrifugation. Highest survival rates after freezing and thawing were obtained when centrifugation processing and cryopreservation were performed at the day of collection. In case of delayed cryopreservation, survival rates could not be increased by centrifugation processing. Post-freeze plasma membrane integrity tended to be higher when sperm was selected via centrifugation processing directly after semen collection as compared to sperm processed just prior to cryopreservation, both for ordinary as well as for iodixanol density centrifugation. Taken together, survival after delayed cryopreservation can be increased when two-layer iodixanol density centrifugation is applied directly after semen collection.

4.1066 Arachidonic acid stimulates TNFα production in Kupffer cells via a reactive oxygen species-pERK1/2-Egr1-dependent mechanism
Cubero, F.J. and Nieto, N.
*Am. J. Physiol. Gastrointest. Liver Physiol., **303**, G228-G239 (2012)*

Kupffer cells are a key source of mediators of alcohol-induced liver damage such as reactive oxygen species, chemokines, growth factors, and eicosanoids. Since diets rich in polyunsaturated fatty acids are a requirement for the development of alcoholic liver disease, we hypothesized that polyunsaturated fatty acids could synergize with ethanol to promote Kupffer cell activation and TNFα production, hence, contributing to liver injury. Primary Kupffer cells from control and from ethanol-fed rats incubated with arachidonic acid showed similar proliferation rates than nontreated cells; however, arachidonic acid induced phenotypic changes, lipid peroxidation, hydroperoxides, and superoxide radical generation. Similar effects occurred in human Kupffer cells. These events were greater in Kupffer cells from ethanol-fed rats, and antioxidants and inhibitors of arachidonic acid metabolism prevented them. Arachidonic acid treatment increased NADPH oxidase activity. Inhibitors of NADPH oxidase and of arachidonic acid metabolism partially prevented the increase in oxidant stress. Upon arachidonic acid stimulation, there was a rapid and sustained increase in TNFα, which was greater in Kupffer cells from ethanol-fed rats than in Kupffer cells from control rats. Arachidonic acid induced ERK1/2 phosphorylation and nuclear translocation of early growth response-1 (Egr1), and ethanol synergized with arachidonic acid to promote this effect. PD98059, a mitogen extracellular kinase 1/2 inhibitor, and curcumin, an Egr1 inhibitor, blocked the arachidonic acid-mediated upregulation of TNFα in Kupffer cells. This study unveils the mechanism whereby arachidonic acid and ethanol increase TNFα production in Kupffer cells, thus contributing to alcoholic liver disease.

4.1067 Surface Proteome Analysis and Characterization of Surface Cell Antigen (Sca) or Autotransporter Family of Rickettsia typhi
Sears, K.T., Ceraul, S.M., Gillespie, J.J., Allen Jr., E.D., Popov, V.L., Ammerman, N.C., Rahman, M.S. and Azad, A.F.

*PloS Pathogens, **8(8)**, e1002856 (2012)*

Surface proteins of the obligate intracellular bacterium *Rickettsia typhi*, the agent of murine or endemic typhus fever, comprise an important interface for host-pathogen interactions including adherence, invasion and survival in the host cytoplasm. In this report, we present analyses of the surface exposed proteins of *R. typhi* based on a suite of predictive algorithms complemented by experimental surface-labeling with thiol-cleavable sulfo-NHS-SS-biotin and identification of labeled peptides by LC MS/MS. Further, we focus on proteins belonging to the surface cell antigen (Sca) autotransporter (AT) family which are known to be involved in rickettsial infection of mammalian cells. Each species of *Rickettsia* has a different complement of *sca* genes in various states; *R. typhi*, has genes *sca1* thru *sca5*. *In silico* analyses indicate divergence of the Sca paralogs across the four *Rickettsia* groups and concur with previous evidence of positive selection. Transcripts for each *sca* were detected during infection of L929 cells and four of the five Sca proteins were detected in the surface proteome analysis. We observed that each *R. typhi* Sca protein is expressed during *in vitro* infections and selected Sca proteins were expressed during *in vivo* infections. Using biotin-affinity pull down assays, negative staining electron microscopy, and flow cytometry, we demonstrate that the Sca proteins in *R. typhi* are localized to the surface of the bacteria. All Scas were detected during infection of L929 cells by immunogold electron microscopy. Immunofluorescence assays demonstrate that Scas 1–3 and 5 are expressed in the spleens of infected Sprague-Dawley rats and Scas 3, 4 and 5 are expressed in cat fleas (*Ctenocephalides felis*). Sca proteins may be crucial in the recognition and invasion of different host cell types. In short, continuous expression of all Scas may ensure that rickettsiae are primed i) to infect mammalian cells should the flea bite a host, ii) to remain infectious when extracellular and iii) to infect the flea midgut when ingested with a blood meal. Each Sca protein may be important for survival of *R. typhi* and the lack of host restricted expression may indicate a strategy of preparedness for infection of a new host.

4.1068 Gene Profile of Chemokines on Hepatic Stellate Cells of Schistosome-Infected Mice and Antifibrotic Roles of CXCL9/10 on Liver Non-Parenchymal Cells
Liang, Y-j., Luo, J., Lu, Q., Zhou, Y., Wu, H-w., Zheng, D., Ren, Y-y., Sun, K-y., Wang, Y. and Zhang, Z-s.
*PloS One, **7(8)**, e42490 (2012)*

Hepatic stellate cells (HSCs) play a key role in the development of liver fibrosis caused by schistosomiasis. Chemokines were widely expressed and involved in cellular activation, proliferation and migration in inflammatory and infectious diseases. However, little is known about the expressions of chemokines on HSCs in the schistosoma infection. In addition, the roles of chemokines in pathogenesis of liver fibrosis are not totally clear. In our study, we used microarray to analyze the temporal gene expressions of primary HSCs isolated from mice with both acute and chronic schistosomiasis. Our microarray data showed that most of the chemokines expressed on HSCs were upregulated at 3 weeks post-infection (*p.i*) when the egg granulomatous response was not obviously evoked in the liver. However, some of them like CXCL9, CXCL10 and CXCL11 were subsequently decreased at 6 weeks *p.i* when the granulomatous response reached the peak. In the chronic stage, most of the differentially expressed chemokines maintained persistent high-abundances. Furthermore, several chemokines including CCR2, CCR5, CCR7, CXCR3, CXCR4, CCL2, CCL5, CCL21, CXCL9 and CXCL10 were expressed by HCSs and the abundances of them were changed following the praziquantel treatment in the chronic stage, indicating that chemokines were possibly necessary for the persistence of the chronic stage. In vitro experiments, hepatic non-parenchymal cells, primary HSCs and human HSCs line LX-2 were stimulated by chemokines. The results showed that CXCL9 and CXCL10, but not CXCL11 or CXCL4, significantly inhibited the gene expressions of Col1α1, Col3α1 and α-SMA, indicating the potential anti-fibrosis effect of CXCL9 and CXCL10 in schistosomiasis. More interestingly, soluble egg antigen (SEA) of *Schistosoma japonicum* was able to inhibit transcriptional expressions of some chemokines by LX-2 cells, suggesting that SEA was capable of regulating the expression pattern of chemokine family and modulating the hepatic immune microenvironment in schistosomiasis.

4.1069 EF24 suppresses maturation and inflammatory response in dendritic cells
Vilekar, P., Awasthi, S., Natarajan, A., Anant, S. and Awasthi, V.
*Int. Immunol., **24(7)**, 455-464 (2012)*

Synthetic curcuminoid EF24 was studied for its effect on the maturation and inflammatory response in murine bone marrow derived immortalized JAWS II dendritic cells (DCs). EF24 reduced the expression of LPS-induced MHC class II, CD80 and CD86 molecules. It also abrogated the appearance of dendrites, a

typical characteristic of mature DCs. These effects were accompanied by the inhibition of LPS-induced activation of transcription factor nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB). Simultaneous reduction of pro-inflammatory cytokines [tumor necrosis factor (TNF)-α, IL-6] both at the mRNA and secreted levels was also observed. To investigate the dependency of LPS effects on MyD88 adaptor protein, we transfected JAWS II DCs with dominant negative MyD88 plasmid construct (MyD88-DN). EF24 reduced NF-κB activity and TNF-α secretion in a MyD88-dependent manner. These results suggest that EF24 modulates DCs by suppressing their maturation and reducing the secretion of inflammatory cytokines. Further, it appears that EF24 acts at or upstream of MyD88 in the LPS-TLR4/MyD88/NF-κB pathway.

4.1070 **Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice**
Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B. and Chang, E.B.
Nature, ***487***, *104-109 (2012)*

The composite human microbiome of Western populations has probably changed over the past century, brought on by new environmental triggers that often have a negative impact on human health[1]. Here we show that consumption of a diet high in saturated (milk-derived) fat, but not polyunsaturated (safflower oil) fat, changes the conditions for microbial assemblage and promotes the expansion of a low-abundance, sulphite-reducing pathobiont, *Bilophila wadsworthia*[2]. This was associated with a pro-inflammatory T helper type 1 (T_H1) immune response and increased incidence of colitis in genetically susceptible *Il10*$^{-/-}$, but not wild-type mice. These effects are mediated by milk-derived-fat-promoted taurine conjugation of hepatic bile acids, which increases the availability of organic sulphur used by sulphite-reducing microorganisms like *B. wadsworthia*. When mice were fed a low-fat diet supplemented with taurocholic acid, but not with glycocholic acid, for example, a bloom of *B. wadsworthia* and development of colitis were observed in *Il10*$^{-/-}$ mice. Together these data show that dietary fats, by promoting changes in host bile acid composition, can markedly alter conditions for gut microbial assemblage, resulting in dysbiosis that can perturb immune homeostasis. The data provide a plausible mechanistic basis by which Western-type diets high in certain saturated fats might increase the prevalence of complex immune-mediated diseases like inflammatory bowel disease in genetically susceptible hosts.

4.1071 **Co-administration of live attenuated Salmonella enterica serovar Typhimurium expressing swine interleukin-18 and interferon-α provides enhanced Th1-biased protective immunity against inactivated vaccine of pseudorabies virus**
Kim, J.S., Kim, S.B., Han, Y.W., uyangaa, E., Kim, J.H., Choi, J.Y., Kim, K. and Eo, S.K.
Microbiol. Immunol., ***56(8)***, *529-540 (2012)*

The co-administration of two or more cytokines may generate additive or synergistic effects for controlling infectious diseases. However, the practical use of cytokine combinations for the modulation of immune responses against inactivated vaccine has not been demonstrated in livestock yet, primarily due to protein stability, production, and costs associated with mass administration. In light of the current situation, we evaluated the immunomodulatory functions of the combined administration of swine interleukin-18 (swIL-18) and interferon-α (swIFN-α) against an inactivated PrV vaccine using attenuated *Salmonella enterica* serovar Typhimurium as a cytokine delivery system. Co-administration of *S. enterica* serovar Typhimurium expressing swIL-18 and swIFN-α produced enhanced Th1-biased humoral and cellular immune responses against the inactivated PrV vaccine, when compared to single administration of *S. enterica* serovar Typhimurium expressing either swIL-18 or swIFN-α. Also, enhanced immune responses in co-administered piglets occurred rapidly after virulent PrV challenge, and piglets that received co-administration of *S. enterica* serovar Typhimurium expressing swIL-18 and swIFN-α displayed a greater alleviation of clinical severity following the virulent PrV challenge, as determined by clinical scores and cumulative daily weight gain. Furthermore, this enhancement was confirmed by reduced nasal shedding of PrV following viral challenge. Therefore, these results suggest that oral co-administration of *S. enterica* serovar Typhimurium expressing swIL-18 and swIFN-α provide enhanced Th1-biased immunity against inactivated PrV vaccine to alleviate clinical signs caused by PrV challenge.

4.1072 **Interferon-γ, tumor necrosis factor, and interleukin-18 cooperate to control growth of Mycobacterium tuberculosis in human macrophages**
Robinson, C.M., Jung, J-Y. and Nau, G.J.
Cytokine, ***60***, *233-241 (2012)*

Mycobacterium tuberculosis (MTB) remains a leading infectious threat to human health. Macrophages are

the cells targeted for infection by the bacterium as well as key effector cells for clearance of the pathogen. Interleukin (IL)-27 opposes macrophage-mediated control of MTB because supplying IL-12 and blocking the activity of IL-27 limits bacterial growth in primary human macrophages. The purpose of this study was to determine the immunological regulators of this macrophage mechanism to restrict MTB growth. Interferon (IFN)-γ, TNF-α, and IL-18 were all demonstrated to be important to the environment that limits bacterial growth when IL-12 is supplied and IL-27 is neutralized. We find IL-18 works in conjunction with IL-12 to achieve optimal IFN-γ production in this system. We also demonstrate novel interactions between these cytokines to influence the expression or responsiveness to one another. Quantitative assays show that IFN-γ enhances expression of the IL-18 receptor signaling chain, as well as TNF expression and secretion. In turn, TNF-α augments expression of the receptor for IFN-γ, the amount at the cell surface, and the extent of IFN-γ -induced signaling. We further define how the cytokine environment supports an enhanced state of classical macrophage activation. Collectively, these results describe novel immunological mechanisms that provide additional insights into the effects of IL-12 and IL-27 on macrophage regulation during MTB infection.

4.1073 Iodixanol density gradient centrifugation for selecting stallion sperm for cold storage and cryopreservation

Stuhtmann, G., Oldenhof, H., peters, P., Klewitz, J., Martinsson, G. and Sieme, H.
Animal Reprod. Sci., ***133(3-4)***, *184-190 (2012)*

Density gradient centrifugation can be used for selection of sperm of superior quality and removal of seminal plasma for use in artificial insemination. In this study, the use of two-layer iodixanol density gradient centrifugation was evaluated for processing of stallion semen. The protocol includes centrifugation through a 16% iodixanol top layer of 1.090 g mL^{-1} and collection of motile and intact sperm on a 30% iodixanol bottom layer of 1.165 g mL^{-1}. Sperm recovery and effects on sperm quality were determined during cold storage as well as after cryopreservation and compared with ordinary dilution and centrifugation. Two-layer iodixanol density gradient centrifugation allows for selection of greater percentages of morphologically normal and progressively motile sperm compared to ordinary centrifugation. This likely results from collecting sperm on the bottom layer that functions as cushion fluid, which prevents mechanical forces as occur when sperm are packed in a pellet. In addition, percentages of membrane and chromatin integrity are increased when cells are selected based on their density via centrifugation through the top and bottom layers. Removal of seminal plasma and increased initial percentages of motile and membrane intact sperm after iodixanol density gradient centrifugation also result in greater percentages of progressively motile and membrane intact sperm during cold storage as well as after freezing and thawing. In conclusion, the two-layer iodixanol density gradient centrifugation protocol described in this manuscript allows for selection of stallion sperm with greater survival rates for cold storage and cryopreservation.

4.1074 Increased Responsiveness of Peripheral Blood Mononuclear Cells to In Vitro TLR 2, 4 and 7 Ligand Stimulation in Chronic Pain Patients

Kwok, Y.H., Hutchinson, M.R., Gentgall, M.G. and Rolan, P.E.
PloS One, ***7(8)***, *e44232 (2012)*

Glial activation via Toll-like receptor (TLR) signaling has been shown in animals to play an important role in the initiation and establishment of chronic pain. However, our ability to assess this central immune reactivity in clinical pain populations is currently lacking. Peripheral blood mononuclear cells (PBMCs) are an accessible source of TLR expressing cells that may mirror similarities in TLR responsiveness of the central nervous system. The aim of this study was to characterize the IL-1β response to various TLR agonists in isolated PBMCs from chronic pain sufferers (on and not on opioids) and pain-free controls. Venous blood was collected from 11 chronic pain sufferers on opioids (≥ 20 mg of morphine / day), 8 chronic pain sufferers not on opioids and 11 pain-free controls. PBMCs were isolated and stimulated *in vitro* with a TLR2 (Pam3CSK4), TLR4 (LPS) or TLR7 (imiquimod) agonist. IL-1β released into the supernatant was measured with ELISA. Significantly increased IL-1β expression was found in PBMCs from chronic pain sufferers (on and not on opioids) compared with pain-free controls for TLR2 ($F_{(6, 277)}$ = 15, P<0.0001), TLR4 ($F_{(8, 263)}$ = 3, P = 0.002) and TLR7 ($F_{(2,201)}$ = 5, P = 0.005) agonists. These data demonstrate that PBMCs from chronic pain sufferers were more responsive to TLR agonists compared with controls, suggesting peripheral cells may have the potential to become a source of biomarkers for

chronic pain.

4.1075 Enriching the captive elephant population genetic pool through artificial insemination with frozen-thawed semen collected in the wild
Hildebrandt, T.B., Hermes, R., Saragusty, J., Potier, R., Schwammer, H.M., Balfanz, F., Vielgrader, H., Baker, B., Bartels, P. and Göritz, F.
*Theriogenology, **78**, 1398-1404 (2012)*

The first successful AI in an elephant was reported in 1998, using fresh semen. Since then almost 40 calves have been produced through AI in both Asian and African elephants worldwide. Following these successes, with the objective of enriching the captive population with genetic material from the wild, we evaluated the possibility of using frozen-thawed semen collected from wild bulls for AI in captivity. Semen, collected from a 36-yr-old wild African savanna elephant (*Loxodonta africana*) in South Africa was frozen using the directional freezing technique. This frozen-thawed semen was used for four inseminations over two consecutive days, two before and two after ovulation, in a 26-yr-old female African savanna elephant in Austria. Insemination dose of 1200×10^6 cells per AI with 61% motility resulted in pregnancy, which was confirmed through ultrasound examination 75, 110 and 141 days after the AI procedure. This represents the first successful AI using wild bull frozen-thawed semen in elephants. The incorporation of AI with frozen-thawed semen into the assisted reproduction toolbox opens the way to preserve and transport semen between distant individuals in captivity or, as was done in this study, between wild and captive populations, without the need to transport stressed or potentially disease-carrying animals or to remove animals from the wild. In addition, cryopreserved spermatozoa, in combination with AI, are useful methods to extend the reproductive lifespan of individuals beyond their biological lifespan and an important tool for genetic diversity management and phenotype selection in these endangered mammals.

4.1076 3′poly-G-Tailed ODNs Inhibit F-spondin to Induce Cell Death and Neurite Retraction in Rat Embryonic Neurons
Cheng, Y-C., Chen, T-A., Chen, C-Y., Liang, C-M. and Liang, S-M.
*Mol. Neurobiol., **45(3)**, 536-549 (2012)*

The effects and mechanism of action of oligodeoxyribonucleotides containing CpG motif (CpG-ODNs) on neuron cells are largely unexamined. Here, we found that CpG-A ODNs but not other types of CpG-ODNs induced neurite retraction and cell apoptosis of rat embryonic neurons in a TLR9-independent manner. These effects of CpG-A ODNs were primarily due to the poly-guanosine at the 3′ terminus (3′G-ODNs). Pull-down analysis showed that 3′G-ODNs associated with transcription factor Y-BOX1 (YB-1) to facilitate the translocation of YB-1 into the nucleus via the nuclear localizing sequence of YB-1. YB-1 then interacted with the promoter of F-spondin directly at −45 and −1,375 sites as demonstrated by chromatin immunoprecipitation (ChIP) analysis. Binding of YB-1 to F-spondin promoter resulted in downregulation of F-spondin expression. Overexpression of F-spondin rescued the cell death and neurite retraction induced by 3′G-ODNs in embryonic neuron cells. Taken together, these findings suggest that 3′G-ODNs enhance nucleus YB-1 to inhibit F-spondin leading to cell death and neurite retraction of embryonic neuron cells

4.1077 Focal Increases of Axoplasmic Ca2+, Aggregation of Sodium–Calcium Exchanger, N-type Ca2+ Channel, and Actin Define the Sites of Spheroids in Axons Undergoing Oxidative Stress
Barsukova, A.G., Forte, M. and Bourdette, D.
*J. Neurosci., **32(35)**, 12028-12037 (2012)*

The excitotoxic effects of kainic acid (KA) in the mouse hippocampus is strain dependent. Following KA administration, the large majority of hippocampal pyramidal cells die in the FVB/N (FVB) mouse, while the pyramidal cells of the C57BL/6 (B6) strain are largely spared. We generated aggregation chimeras between the sensitive FVB and the resistant B6 strains to investigate whether intrinsic or extrinsic features of a neuron confer cell vulnerability or resistance to KA. The constitutive expression of transgenic green fluorescence protein (GFP) or β-galactosidase expressed from the ROSA26 locus was used to mark cells in FVB or B6 mice, respectively. These makers enable the identification of cells from each parental genotype while TUNEL (terminal deoxynucleotidyl transferase-mediated biotinylated dUTP nick end labeling)-staining labeled dying cells. The analysis of the percentage of dying cells in FVB-GFP ↔ B6-ROSA chimeras yielded an intriguing mix of both intrinsic and extrinsic factors in the readout of cell phenotype. Thus, normally resistant B6-ROSA pyramidal neurons demonstrated an increasing sensitivity to KA, in a linear fashion, when the percentage of FVB-GFP cells was increased, either across chimeras or in different

regions of the same chimera. However, the death of B6-ROSA pyramidal cells never exceeded ~70% of the total amount of B6 neurons regardless of the amount of FVB cells in the chimeric hippocampus. In a similar manner, FVB-GFP cells show lower amounts of cell death in chimeras that are colonized by B6-ROSA cells, but again, are never fully rescued. These data indicate that both intrinsic and extrinsic factors modulate the sensitivity of hippocampal pyramidal cells to kainic acid.

4.1078 Neuroprotective effects of phenolic antioxidant tBHQ associate with inhibition of FoxO3a nuclear translocation and activity

Bahia, P.K., Pugh, V., Hoyland, K., Hensley, V., Rattray, M. and Williams, R.J.
J. Neurochem., ***123(1)***, *182-191(2012)*

The Forkhead transcription factor, FoxO3a induces genomic death responses in neurones following translocation from the cytosol to the nucleus. Nuclear translocation of FoxO3a is triggered by trophic factor withdrawal, oxidative stress and the stimulation of extrasynaptic NMDA receptors. Receptor activation of phosphatidylinositol 3-kinase (PI3K)–Akt signalling pathways retains FoxO3a in the cytoplasm, thereby inhibiting the transcriptional activation of death-promoting genes. We hypothesized that phenolic antioxidants such as tert-Butylhydroquinone (tBHQ), which is known to stimulate PI3K–Akt signalling, would inhibit FoxO3a translocation and activity. Treatment of cultured cortical neurones with NMDA increased the nuclear localization of FoxO3a, reduced the phosphorylation of FoxO3a, increased caspase activity and up-regulated Fas ligand expression. In contrast the phenolic antioxidant, tBHQ, caused retention of FoxO3a in the cytosol coincident with enhanced PI3K- dependent phosphorylation of FoxO3a. tBHQ-induced nuclear exclusion of FoxO3a was associated with reduced FoxO-mediated transcriptional activity. Exposure of neurones to tBHQ inhibited NMDA-induced nuclear translocation of FoxO3a, prevented NMDA-induced up-regulation of FoxO-mediated transcriptional activity, blocked caspase activation and protected neurones from NMDA-induced excitotoxic death. Collectively, these data suggest that phenolic antioxidants such as tBHQ oppose stress-induced activation of FoxO3a and therefore have potential neuroprotective utility in neurodegeneration.

4.1079 Histone methyltransferase ASH1 orchestrates fibrogenic gene transcription during myofibroblast transdifferentiation

Perugorria, M.J., Wilson, C.L., Zeybel, M., Walsh, M., Amin, S., Robinson, S., White, S.A., Burt, A.D., Oakley, F., Tsukamoto, H., Mann, D.A. and Mann, J.
Hepatology, ***56(3)***, *1129-1139 (2012)*

Transdifferentiation of hepatic stellate cells (HSCs) to a myofibroblast-like phenotype is the pivotal event in liver fibrosis. The dramatic change in phenotype associated with transdifferentiation is underpinned by a global change in gene expression. Orchestrated changes in gene expression take place at the level of chromatin packaging which is regulated by enzymatic activity of epigenetic regulators that in turn affect histone modifications. Using expression profiling of epigenetic regulators in quiescent and activated primary HSCs we found a number of histone methyltranferases including MLL1, MLL5, Set1 and ASH1 to be highly up-regulated during transdifferentiation of HSCs. All of these histone methyltranferases regulate methylation of lysine 4 of histone H3, which is a signature of actively transcribed genes. We therefore postulated that one or more of these enzymes may be involved in positively influencing expression of profibrogenic genes. *Conclusion:* We find that ASH1 directly binds to the regulatory regions of alpha smooth muscle actin (αSMA), collagen I, tissue inhibitor of metalloproteinase-1 (TIMP1) and transforming growth factor beta1 (TGFβ1) in activated HSCs while depletion of ASH1 caused broad suppression of fibrogenic gene expression. We also discovered that MeCP2 positively regulates ASH1 expression and therefore identify ASH1 as a key transcriptional activator component of the MeCP2 epigenetic relay pathway that orchestrates coordinated induction of multiple profibrogenic genes.

4.1080 Wiskott–Aldrich Syndrome Protein Deficiency in Innate Immune Cells Leads to Mucosal Immune Dysregulation and Colitis in Mice

Ngyen, D.D. et al
Gastroenterology, ***143(3)***, *719-729 (2012)*

Background & Aims
Immunodeficiency and autoimmune sequelae, including colitis, develop in patients and mice deficient in Wiskott–Aldrich syndrome protein (WASP), a hematopoietic cell-specific intracellular signaling molecule that regulates the actin cytoskeleton. Development of colitis in WASP-deficient mice requires lymphocytes; transfer of T cells is sufficient to induce colitis in immunodeficient mice. We investigated

the interactions between innate and adaptive immune cells in mucosal regulation during development of T cell–mediated colitis in mice with WASP-deficient cells of the innate immune system.
Methods
Naïve and/or regulatory $CD4^+$ T cells were transferred from 129 SvEv mice into RAG-2–deficient (RAG-2 KO) mice or mice lacking WASP and RAG-2 (WRDKO). Animals were observed for the development of colitis; effector and regulatory functions of innate immune and T cells were analyzed with in vivo and in vitro assays.
Results
Transfer of unfractionated $CD4^+$ T cells induced severe colitis in WRDKO, but not RAG-2 KO, mice. Naïve wild-type T cells had higher levels of effector activity and regulatory T cells had reduced suppressive function when transferred into WRDKO mice compared with RAG-2 KO mice. Regulatory T-cell proliferation, generation, and maintenance of FoxP3 expression were reduced in WRDKO recipients and associated with reduced numbers of $CD103^+$ tolerogenic dendritic cells and levels of interleukin-10. Administration of interleukin-10 prevented induction of colitis following transfer of T cells into WRDKO mice.
Conclusions
Defective interactions between WASP-deficient innate immune cells and normal T cells disrupt mucosal regulation, potentially by altering the functions of tolerogenic dendritic cells, production of interleukin-10, and homeostasis of regulatory T cells.

4.1081 Isolation and Identification of Red Cell Progenitor Subsets from Whole Blood, Mobilized Peripheral Blood and Cord Blood

He, J., Wang, F. and Zhu, F.
*Transfusion, **52(s3)**, 185A (2012)*

Background/Case Studies: Red blood cell (RBC) transfusion is an essential therapeutic act and the need for blood is thereby widespread. However there is a major imbalance between demand and supply of donors, so that
there is mounting research to develop suitable surrogates for human donated blood. Functional RBCs have already been generated from a variety of cellular progenitors. To investigate a similar population of cells that can be also isolated from the whole blood, mobilized peripheral blood and cord blood, we purified and identified a population of 'immature' red cells that are phenotypically similar to red cell progenitor subsets.
Study Design/Methods:
The whole blood, mobilized peripheral blood and cord blood were mixed with 6% polysucrose400 respectively, incubated at 37 C for 1 hour. Leukocyte rich supernatant above the sedimented red cells was collected and
washed. Suspended with PBS cells were layered onto the erythrocyte and leukocyte Ficoll-Optiprep gradient (the three densities in one tube up to down were 1.091, 1.109, 1.117). After centrifugation the isolated fraction were harvested to analyze the phenotype using cell surface markers CD45+/ CD34+/CD133+, CD45+/CD71+/ CD235a+, CD45+/C14+/CD66b+ by FACS. CD34+133+ cells were isolated from interfaces A, CD71+cells and CD71−cells were separated from the depletion of CD34+133+ cells population from interfaces A by MACS. Five fractions included above three separated population, interfaces B and C were expanded to assess BFU-E, CFU-E, BFU-GM, BFU-GEMM in methylcellulose medium. The results of phenotype and colony
assay from three kinds of samples were compared.
Results/Findings: After centrifugation the sample showed A, B and C bands up to down. Large number of red cells and white cells held at interface A giving poorly separated bands; thin bands of red cells were isolated from interface B and C. Greater enrichment for CD45+/CD34+/CD133+ and CD45+/CD14+/CD66b+ could be
found in interface A and greater enrichment for CD45+/CD71+/CD235a+ could be found in interfaces B and C. CD45+/CD71+/CD235a+ population from interfaces B and C of UCB cells are signifi cantly rich compared to
interfaces B and C of the whole blood and mobilized peripheral blood. The result of red cell colony-forming unit of CD34+133+, CD71+, CD71−, B and C cell populations from three kinds of samples were 0.35 ± 0.15%, 66.16 ± 5.19%, 20 ± 3.87%, 83.33 ± 1.24%, 85.45 ± 2.31% respectively. Almost all the red cell colony-forming potential was within the B and C fractions from three kinds of samples. **Conclusion:** Using 6% polysucrose400 sedimentation and erthrocte-Ficoll-Optiprep gradient can isolate immature red cells from the leukocyte fraction. Cord blood is the best source for red cell progenitor subsets which are suitable for the generation of mature and functional RBCs.

4.1082 **Nilotinib protects the murine liver from ischemia/reperfusion injury**
Ocuin, L.M., Cavnar, M.J., Sorenson, E.C., Bamboat, Z.M., Greer, J.B., Kim, T.S., Popow, R. and DeMatteo, R.P.
J. Hepatol., ***57***, *766-773 (2012)*

Background & aims
The mitogen-activated protein kinases (MAPKs), c-Jun N-terminal kinase (JNK), and p38, mediate liver ischemia/reperfusion (I/R) injury via cell death and inflammatory cytokine expression, respectively. Nilotinib is an orally available receptor tyrosine kinase inhibitor used for chronic myelogenous leukemia that also has *in vitro* activity against JNK and p38. In this study, we examine its therapeutic potential against hepatic I/R injury.
Methods
The effects of nilotinib on liver I/R injury were tested using a murine model of warm, segmental liver I/R. Serum ALT was measured and livers were analyzed by histology, RT-PCR, Western blot, and flow cytometry. The *in vitro* effects of nilotinib on hepatocyte and non-parenchymal cell (NPC) MAPK activation and cytokine production were also tested.
Results
Mice receiving nilotinib had markedly lower serum ALT levels and less histologic injury and apoptosis following liver I/R. Nilotinib did not inhibit its known receptor tyrosine kinases. Nilotinib lowered intrahepatic expression of IL-1β, IL-6, MCP-1, and MIP-2 and systemic levels of IL-6, MCP-1, and TNF. Nilotinib reduced NPC activation of p38 MAPK signaling and decreased the recruitment of inflammatory monocytes and their production of TNF. Nilotinib attenuated JNK phosphorylation and hepatocellular apoptosis. *In vitro*, nilotinib demonstrated direct inhibition of JNK activation in isolated hepatocytes cultured under hypoxic conditions, and blocked activation of p38 MAPK and cytokine production by stimulated NPCs.
Conclusions
Nilotinib lowers both liver JNK activation and NPC p38 MAPK activation and may be useful for ameliorating liver I/R injury in humans.

4.1083 **Progenitor/Stem Cell Fate Determination: Interactive Dynamics of Cell Cycle and Microvesicles**
Aliotta, J.M., Lee, D., Puente, N., Faradyan, S., Sears, E.H., Amaral, A., Goldberg, L., Dooner, M.S., Pereira, M. and Quesenberry, P.J.
Stem Cells and Development, ***21(10)***, *1627-1638 (2012)*

We have shown that hematopoietic stem/progenitor cell phenotype and differentiative potential change throughout cell cycle. Lung-derived microvesicles (LDMVs) also change marrow cell phenotype by inducing them to express pulmonary epithelial cell-specific mRNA and protein. These changes are accentuated when microvesicles isolated from injured lung. We wish to determine if microvesicle-treated stem/progenitor cell phenotype is linked to cell cycle and to the injury status of the lung providing microvesicles. Lineage depleted, Sca-1+ (Lin-/Sca-1+) marrow isolated from mice were cultured with interleukin 3 (IL-3), IL-6, IL-11, and stem cell factor (cytokine-cultured cells), removed at hours zero (cell cycle phase G0/G1), 24 (late G1/early S), and 48 (late S/early G2/M), and cocultured with lung tissue, lung conditioned media (LCM), or LDMV from irradiated or nonirradiated mice. Alternatively, Lin-/Sca-1+ cells not exposed to exogenous cytokines were separated into G0/G1 and S/G2/M cell cycle phase populations by fluorescence-activated cell sorting (FACS) and used in coculture. Separately, LDMV from irradiated and nonirradiated mice were analyzed for the presence of adhesion proteins. Peak pulmonary epithelial cell-specific mRNA expression was seen in G0/G1 cytokine-cultured cells cocultured with irradiated lung and in late G1/early S cells cocultured with nonirradiated lung. The same pattern was seen in cytokine-cultured Lin-/Sca-1 cells cocultured with LCM and LDMV and when FACS-separated Lin-/Sca-1 cells unexposed to exogenous cytokines were used in coculture. Cells and LDMV expressed adhesion proteins whose levels differed based on cycle status (cells) or radiation injury (LDMV), suggesting a mechanism for microvesicle entry. These data demonstrate that microvesicle modification of progenitor/stem cells is influenced by cell cycle and the treatment of the originator lung tissue.

4.1084 **In vivo reprogramming of Sox9+ cells in the liver to insulin-secreting ducts**
Banga, A., Akinci, E., Greder, L.V., Dutton, J.R. and Slack, J.M.W.
PNAS, ***109(38)***, *15336-15341 (2012)*

In embryonic development, the pancreas and liver share developmental history up to the stage of bud formation. Therefore, we postulated that direct reprogramming of liver to pancreatic cells can occur when

suitable transcription factors are overexpressed. Using a polycistronic vector we misexpress *Pdx1*, *Ngn3*, and *MafA* in the livers of NOD-SCID mice rendered diabetic by treatment with streptozotocin (STZ). The diabetes is relieved long term. Many ectopic duct-like structures appear that express a variety of β-cell markers, including dense core granules visible by electron microscopy (EM). Use of a vector also expressing GFP shows that the ducts persist long after the viral gene expression has ceased, indicating that this is a true irreversible cell reprogramming event. We have recovered the insulin$^+$ cells by cell sorting and shown that they display glucose-sensitive insulin secretion. The early formed insulin$^+$ cells can be seen to coexpress SOX9 and are also labeled in mice lineage labeled for *Sox9* expression. SOX9$^+$ cells are normally found associated with small bile ducts in the periportal region, indicating that the duct-like structures arise from this source. This work confirms that developmentally related cells can be reprogrammed by suitable transcription factors and also suggests a unique therapy for diabetes.

4.1085 **Circumventing Antivector Immunity by Using Adenovirus-Infected Blood Cells for Repeated Application of Adenovirus-Vectored Vaccines: Proof of Concept in Rhesus Macaques**
Sun, C., Feng, L., Zhang, Y., Xiao, L., Pan, W., Li, C., Zhang, L. and Chen, L.
J. Virol., ***86(20)***, *11031-11042 (2012)*

Adenovirus has been extensively exploited as a vector platform for delivering vaccines. However, preexisting antiadenovirus immunity is the major stumbling block for application of adenovirus-vectored vaccines. In this study, we found that freshly isolated peripheral blood mononuclear cells (PBMCs), mostly CD14$^+$ cells, from adenovirus serotype 5 (Ad5)-seropositive primates (humans and rhesus macaques) can be efficiently infected with Ad5 *in vitro*. On the basis of this observation, a novel strategy based on adenoviral vector-infected PBMC (AVIP) immunization was explored to circumvent antivector immunity. Autologous infusion of Ad5-SIVgag-infected PBMCs elicited a strong Gag-specific cellular immune response but induced weaker Ad5-neutralizing antibody (NAb) in Ad5-seronegative macaques than in macaques intramuscularly injected with Ad5-SIVgag. Moreover, Ad5-seropositive macaques receiving multiple AVIP immunizations with Ad5-SIVenv, Ad5-SIVgag, and Ad5-SIVpol vaccines elicited escalated Env-, Gag-, and Pol-specific immune responses after each immunization that were significantly greater than those in macaques intramuscularly injected with these Ad5-SIV vaccines. After challenged intravenously with a highly pathogenic SIVmac239 virus, macaques receiving AVIP immunization demonstrated a significant reduction in viral load at both the peak time and set-point period compared with macaques without Ad5-SIV vaccines. Our study warranted further research and development of the AVIP immunization as a platform for repeated applications of adenovirus-vectored vaccines.

4.1086 **Dendritic Cells Are Central Coordinators of the Host Immune Response to *Staphylococcus aureus* Bloodstream Infection**
Schindler, D., Gutierrez, M.O., Beineke, A., Rauter, Y., Rohde, M., Foster, S., Goldmann, O. and Medina, E.
Am. J. Pathol., ***181(4)***, *1327-1337 (2012)*

Dendritic cells (DCs) play an important role in integration of the immune responses induced by pathogens. The purpose of this study was to determine the importance of DCs in host defense against *Staphylococcus aureus* bacteremia. Using a murine infection model, we demonstrated that DCs are rapidly recruited into infected tissue after intravenous inoculation with *S. aureus*. The recruited DCs were fully functional and in a more advanced stage of maturation than those isolated from uninfected mice. Depletion of DCs in CD11c-DTR transgenic mice resulted in substantial worsening of infection, as indicated by increased bacterial loads in kidneys and lungs, accelerated mortality, and more severe pathology. Furthermore, DC depletion completely abolished IL-12 production in response to infection. The beneficial effect afforded by DCs during *S. aureus* infection was not mediated by their contribution to direct bacterial killing, nor by increased neutrophil recruitment. Instead, neutrophil influx (along with expression of CXC chemokines) was significantly enhanced in infected tissue after depletion of DCs. We also found that the bactericidal capacity of the recruited neutrophils was significantly impaired in DC-depleted mice. More importantly, the detrimental effect of DC depletion was practically reversed by treatment with exogenous recombinant mouse IL-12. Our results demonstrated that DCs, probably through their production of IL-12, play an important role in coordinating the inflammatory response during *S. aureus* infection.

4.1087 **Elevated levels of serum-soluble triggering receptor expressed on myeloid cells-1 in patients with IBD do not correlate with intestinal TREM-1 mRNA expression and endoscopic disease activity**
Saurer, L., Rihs, S., Birrer, M., Saxer-Seculic, N., RAdsak, M. and Mueller, C.
J. Chron's and Colitis, ***6****, 913-923 (2012)*

Background & aims
Triggering receptor expressed on myeloid cells-1 (TREM-1) is a potent amplifier of pro-inflammatory responses. We have previously demonstrated a substantial increase in TREM-1-expressing macrophages in the inflamed intestinal mucosa of patients with inflammatory bowel diseases (IBD). TREM-1 is also produced as a soluble receptor (sTREM-1). Here, we aimed to determine whether serum sTREM-1 could be used as a surrogate marker of disease activity in patients with IBD.
Methods
Intestinal biopsies and concurrently collected sera from patients with Crohn's disease (CD) and Ulcerative colitis (UC) enrolled in the Swiss IBD cohort study were analyzed for intestinal TREM-1 mRNA and serum sTREM-1 expression. TREM-1 mRNA and sTREM-1 were correlated with the endoscopically determined disease activity. Serum sTREM-1 and TREM-1 mRNA expression levels were further determined in sera and colonic tissues collected at various time-points post disease induction in an experimental mouse model of colitis and correlated with disease activity.
Results
Expression of TREM-1 mRNA was upregulated in intestinal biopsies from patients with active disease but not in patients with quiescent disease. Serum sTREM-1 was elevated in IBD patients compared to normal controls. No substantial differences in sTREM-1 expression levels were found in patients with active versus quiescent disease. In colitic mice, colonic TREM-1 mRNA and serum sTREM-1 were also upregulated. While colonic TREM-1 mRNA expression levels correlated with disease activity, augmented serum sTREM-1 in fact associated with a milder course of disease.
Conclusions
Analysis of sTREM-1 as a surrogate marker of disease activity in patients with IBD warrants caution.

4.1088 Improvement of Porcine Islet Isolation by Inhibition of Trypsin Activity During Pancreas Preservation and Digestion Using α1-Antitrypsin
Shimoda, M., Noguchi, H., Fujita, Y., Takita, M., Ikemoto, TS., Chujo, D., Naziruddin, B., Levy, M.F., Kobayashi, N., Grayburn, P.A. and Matsumoto, S.
*Cell Transplant., **21(2-3)**, 465-471 (2012)*

Porcine islets are considered to be a promising resource for xenotransplantation. However, it is difficult to isolate porcine islets because of the marked fragility and rapid dissociation. Endogenous trypsin is one of the main factors to damage islets during the isolation procedure. Recent studies have suggested that trypsin inhibitors during the preservation of pancreas or the collagenase digestion can improve the result of islet isolation. In this study, we examined whether α1-antitrypsin (Aralast^{TM}), which inhibits several endogenous proteases and has immunomodulatory properties, can protect islets from the proteases and improve the results of porcine islet isolation. Twelve porcine pancreata were divided into three groups: without Aralast group (standard, $n = 5$), preserved with Aralast using the ductal injection (DI) method (DI, $n = 3$), and with Aralast using the DI method and in the collagenase solution (DI+C, $n = 4$). Efficacy of islet isolation was assessed by islet yields, purity, and viability. The trypsin activity of the preservation and the digestion solution during the isolation procedure was measured. During islet isolation, the trypsin activity in DI+C group was significantly inhibited compared to the standard group, whereas DI group showed less effect than DI+C group. The average of postpurification islet equivalents (IEQ) per pancreas weight in the DI+C group was significantly higher than the standard group (standard: 3516 ± 497 IEQ/g, DI: 4607 ± 1090 IEQ/g, DI+C: 7097 ± 995 IEQ/g; $p = 0.017$ between standard and DI+C). In the DI+C group, stimulation index was higher than in other groups, although there was no significant difference. The presence of Aralast in both DI solution and collagenase solution markedly inhibited trypsin activity during pancreas digestion procedure and improved the porcine islet isolation. Inhibition of trypsin activity by Aralast could improve porcine islet isolation.

4.1089 Evaluation of Osmolality of Density Gradient for Human Islet Purification
Noguchi, H., Naziruddin, B., Shimmoda, M., Fujita, Y., Chujo, D., Takita, M., Peng, H., Sugimoto, K., Itoh, T., Kobayashi, N., Onaca, N., levy, M.F. and Matsumoto, S.
Cell Transplant., 21(2-3), 493-500 (2012)

For pancreatic islet transplantation, the most common method of islet purification is density gradient centrifugation because of the differences in density between islets and acinar tissue. The density of islets/acinar tissue depends on several conditions, such as osmolality of purification solution. In this study, we evaluated the osmolality of iodixanol-controlled density gradients (400, 450, and 500 mOsm/kg) on the islet purification step. The density of the purification solutions was controlled by changing the volumetric ratio of iodixanol and the purification solutions (iodixanol-Kyoto solutions; IK solutions). The osmolality

of density gradients was controlled by addition of 10× Hanks balanced salt solution (HBSS) solution. Density of both islets and acinar tissue increased relative to increase of the osmolality of purification solutions. There were no significant differences among the three groups on islet yield after density-adjusted purification and the rate of postpurification recovery. In vitro and in vivo assays suggest that the quality of islets was similar among the three groups. Our data suggest that efficacy of purification and quality of isolated islets is similar when the osmolality of purification solutions is between 400 and 500 mOsm/kg and density adjustment is applied. Since the density of islet and acinar tissue is changed according to osmolality, the density adjustment is important when using several osmolality solutions.

4.1090 Islet Purification Method Using Large Bottles Effectively Achieves High Islet Yield from Pig Pancreas

Shimoda, M., Noguchi, H., Fujita, Y., Takita, M., Ikemoto, T., Chujo, D., Naziruddin, B., Levy, M.F., Kobayashi, N., Grayburn, P.A. and Matsumoto, S.
*Cell Transplant, **21 (2-3)**, 501-508 (2012)*

Porcine islets are a promising resource for xenotransplantation. However, low efficacy of islet isolation because of their marked fragility remains a problem. Recently we found that the standard purification method using COBE 2991 cell processor (COBE) with Ficoll density gradient solution damaged islets mechanically by high shearing force. In this study, we evaluated our new purification method using large plastic bottles for the efficacy of islet purification. Ten porcine pancreata were used. The average warm ischemic time was over 40 min; therefore, these pancreata were considered to be in a marginal condition. After digestion, the digested tissue was divided into three groups. Each group was purified using either top loading method with bottle (top group) or bottom loading method with bottle (bottom group) or standard COBE method (COBE group). Islet yield per pancreas weight (IEQ/g) and the rate of postpurification recovery in the top group were significantly higher than the COBE group (top: 8060 ± 1652 IEQ/g, bottom: 4572 ± 614 IE/g, COBE: 3900 ± 734 IE/g. $p < 0.02$ in top vs. COBE; top percentage of recovery: 99.3 ± 12.3%, bottom: 62.6 ± 8.8%, COBE: 49.5 ± 6.7%, $p < 0.02$ in top vs. bottom and COBE). The average sizes of purified islets in the top and bottom groups were significantly larger than COBE group (Average diameter top: 156 ± 8 μm, bottom: 147 ± 6 μm, COBE: 119 ± 6 μm, $p < 0.01$ in top vs. COBE and in bottom vs. COBE), which indicated that bottle method can reduce shear force during purification. Our new purification using top loading bottle method enabled us to obtain a high yield of porcine islets from marginal pancreata.

4.1091 Comparison of Ulinastatin, Gabexate Mesilate, and Nafamostat Mesilate in Preservation Solution for Islet Isolation

Noguchi, H., Naziruddin, B., Jackson, A., Shimoda, M., Fujita, Y., Chujo, D., Takita, M., Peng, H., Sugimoto, K., Itoh, T., Kobayashi, N., Ueda, M., Okitsu, T., Iwanaga, Y., Nagata, H., Liu, X., Kamiya, H., Onaca, N., Levy, M.F. and Matsumoto, S.
*Cell Transplant., **21(2-3)**, 509-516 (2012)*

For islet transplantation, maintaining organ viability after pancreas procurement is critically important for optimal graft function and survival. We recently reported that islet yield was significantly higher in the modified ET-Kyoto (MK) solution, which includes a trypsin inhibitor (ulinastatin), compared with the UW solution, and that the advantages of MK solution are trypsin inhibition and less collagenase inhibition. In this study, we compared ulinastatin with other trypsin inhibitors, gabexate mesilate, and nafamostat mesilate, in preservation solution for islet isolation. Ulinastatin was easily dissolved in ET-Kyoto solution, while ET-Kyoto with gabexate mesilate and nafamostat mesilate became cloudy immediately after addition. Although there were no significant differences in islet yield among the three groups, viability was significantly higher for the MK group than for the GK group or the NK group. The stimulation index was significantly higher for the MK group than for the GK group. In summary, there are no other trypsin inhibitors that are more effective than ulinastatin. Based on these data, we now use ET-Kyoto solution with ulinastatin for clinical islet transplantation.

4.1092 Fresh Islets Are More Effective for Islet Transplantation Than Cultured Islets

Noguchi, H., Naziruddin, B., Jackson, A., Shimoda, M., Ikemoto, T., Fujita, Y., Chujo, D., Takita, M., Peng, H., Sugimoto, K., Itoh, T., Kobayashi, N., Onaca, N., Levy, M.L. and Matsumoto, S.
*Cell Transplant., **21(2-3)**, 517-523 (2012)*

For clinical islet transplantation, isolated islets deteriorate rapidly in culture, although culturing islets prior to transplantation provides flexibility for evaluation of isolated islets and pretreatment of patients. In the present study, we compared human fresh islets to cultured islets with in vitro and in vivo assays. After culture for 24, 48, and 72 h, islet yield significantly decreased from 2,000 to 1,738 ± 26 (13% loss), 1,525 ± 30 (24% loss), or 1,298 ± 18 IEQ (35% loss), respectively. The ATP contents were significantly higher in the 6-h cultured group (near fresh group) than in 48-h culture groups. The stimulation index was relatively higher in the 6-h cultured group than in 48-h cultured group. Human islets with or without culture were transplanted into diabetic nude mice. The attainability of posttransplantation normoglycemia was significantly higher in fresh group than in the culture groups. Intraperitoneal glucose tolerance testing (IPGTT) showed that the blood glucose levels of mice transplanted with fresh islets were significantly lower than with cultured islets at 30, 60, 90, and 120 min after injection. These data suggest that human islet transplantation without culture could avoid the deterioration of islets during culture and improve the outcome of islet transplantation. Based on these data, we have transplanted fresh islets without culture for our current clinical islet transplantation protocol.

4.1093 Adverse Events in Clinical Islet Transplantation: One Institutional Experience

Takita, M., Matsumoto, S., Noguchi, H., Shimoda, M., Ikemoto, T., Chujo, D., Tamura, Y., Olsen, G.S., Naziruddin, B., Purcell, K., Onaca, N. and Levy, M.F.

Cell Transplant., ***21(2-3)****, 547-551 (2012)*

Islet transplantation is one of the most promising treatments for an unstable form of type 1 diabetes. However, islet transplantation still has some obstacles, such as low success rate of islet isolation, difficulty to obtain long-term insulin freedom, and adverse events related to transplant protocol. We describe the adverse events of current clinical islet transplantation at our institute in this report. Nine type 1 diabetic patients received 17 islet infusions from March 2005 to October 2008. The islet infusion procedure and immunosuppression regimen were based on a modified Edmonton protocol. Severe adverse events (SAEs) were defined as events that were more than grade 3 according to the Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation, version 4.1 (Collaborative Islet Transplant Registry, CITR). Sixteen events were reported as SAEs and among them 12 events were probably or definitely related to transplant protocols; all occurred within 1 year after infusion except for one. Five adverse events (31%) occurred within 10 days after transplantation and were related to infusion procedures. Seven events (44%) occurred after 50 days and were related to immunosuppressive therapy. SAEs related to the protocol included three events of elevated liver enzymes, two of hemorrhage into gall bladder or peritoneal cavity, two of neutropenia, two of infection, one of vomiting, one of diarrhea, and one of renal dysfunction. All events were grade 3, except for one case that was grade 4 of neutropenia. All SAEs resolved with no sequelae. Neoplasms and deaths were not observed in our study. The present study suggests need to improve both infusion procedure and immunosuppressive strategy from the view of preventing SAEs.

4.1094 Atorvastatin inhibits proliferation and apoptosis, but induces senescence in hepatic myofibroblasts and thereby attenuates hepatic fibrosis in rats

Klein, S., Klösel, J., Schierwagen, R., Körner, C., Granzow, M., Huss, S., Gretchen, I., Mazar, R., Weber, S., van den Ven, P.F.M., Pieper-Fürst, U., Fürst, D.O., Nattermann, J., Lammert, F., Sauerbruch, T. and Trebicka, J.

Lab. Invest., ***92(10)****, 1440-1450 (2012)*

Hepatic myofibroblasts (MFB) show increased proliferation, migration and collagen production, which are crucial for hepatic fibrogenesis. Atorvastatin treatment inhibits proliferation, apoptosis and cytokine production of MFB in bile duct-ligated (BDL) rats *in vivo*. Here, we have further investigated the underlying mechanisms. Primary rat hepatic stellate cells (HSC) were isolated and culture-activated to hepatic MFB. Following 3 days of incubation with atorvastatin (10^{-4}, 10^{-5} and 10^{-6} M), transcription levels of profibrotic cytokines (transforming growth factor-β1, connective tissue growth factor and TIMP1) and procollagen Ia were analyzed by real time PCR. Proliferation was investigated by 5'-bromo-2'-deoxyuridine assays. α-Smooth muscle actin protein expression was examined by western blotting. Fluorescence-activated cell sorting analysis of Annexin V and propidium iodide were used to measure apoptosis. Furthermore, p21 western blotting and β-galactosidase staining were investigated in MFB as senescence markers. Subsequently, hepatic expression of desmin and senescence markers were analyzed in the livers of rats receiving atorvastatin (15 mg/kg*d) for 1 week starting 3 and 5 weeks after BDL. Atorvastatin inhibited the activation of HSC to MFB and decreased cytokine and collagen production in MFB *in vitro*. In addition, proliferation, cytokine and collagen production of MFB were reduced by

atorvastatin. Atorvastatin initiated apoptosis at 10^{-4} M and attenuated it at 10^{-5} M. Atorvastatin induced p21 protein expression and β-galactosidase staining of MFB *in vitro* and *in vivo*. Atorvastatin elicits similiar effects on MFB as previously seen *in vivo*: it decreases MFB turnover and fibrogenesis. We suggest that a further mechanism explaining these effects is senescence of cells.

4.1095 TRIM50 Protein Regulates Vesicular Trafficking for Acid Secretion in Gastric Parietal Cells
Nishi, M., Aoyama, F., Kisa, F., Zhu, H., Sun, M., Lin, P., Ohta, H., Van, B., Yamamoto, S., Kakizawa, S., Sakai, H., Ma, J., Sawaguchi, A. and Takeshima, H.
J. Biol. Chem., ***287(40)****, 333523-33532 (2012)*

Of the TRIM/RBCC family proteins taking part in a variety of cellular processes, TRIM50 is a stomach-specific member with no defined biological function. Our biochemical data demonstrated that TRIM50 is specifically expressed in gastric parietal cells and is predominantly localized in the tubulovesicular and canalicular membranes. In cultured cells ectopically expressing GFP-TRIM50, confocal microscopic imaging revealed dynamic movement of TRIM50-associated vesicles in a phosphoinositide 3-kinase-dependent manner. A protein overlay assay detected preferential binding of the PRY-SPRY domain from the TRIM50 C-terminal region to phosphatidylinositol species, suggesting that TRIM50 is involved in vesicular dynamics by sensing the phosphorylated state of phosphoinositol lipids. *Trim50* knock-out mice retained normal histology in the gastric mucosa but exhibited impaired secretion of gastric acid. In response to histamine, *Trim50* knock-out parietal cells generated deranged canaliculi, swollen microvilli lacking actin filaments, and excess multilamellar membrane complexes. Therefore, TRIM50 seems to play an essential role in tubulovesicular dynamics, promoting the formation of sophisticated canaliculi and microvilli during acid secretion in parietal cells.

4.1096 CD8 T cell - Dendritic Cell crosstalk up-regulates CD40L expression and IL-12p70 production
Tay, Q. W., Ho, L.C., Low, P.Y. and Kemeny, D.M.
Immunology, ***137*** *(Supp 1), P0874, 185-772 (2012)*

Purpose/Objective: CD8 T cells have been shown to skew the immune response by stimulating dendritic cells (DC) to produce IL-12p70. The purpose of our study was to investigate the effect on CD8 T cell expression of CD40L.
Materials and methods: Ovalbumin (OVA)-specific T cell receptor transgenic mouse CD8 T cells (OT-I) were isolated by positive selection using anti-CD8 coated MACS beads and co-cultured splenic DC isolated using Optiprep and by positive selection with anti-CD11c coated MACS beads, and cultured in complete medium (RPMI 1640, 10% Fetal calf serum (FCS), antibiotics and non-essential amino acids and 0.5% 2 mercaptoethanol). CD40L expression was measured by Flow cytometry, IL-12p70 was determined by ELISA (R&D systems). CD8 T cells were activated with PMA (10 ng/ml) and Ionomycin (400 ng/ml) to induce CD44high effector memory cells. CD11c+ splenic DCs were pulsed with 1 lg/ml of peptide (typically SIINFEKL) for 1 h before co-culture with pre-activated CD8 T cells at a ratio of 1 DC to 3 T cells. Altered SIINFEKL peptides (SAINFEKL, EIINFEKL, SIIRFEKL, SIINYEKL) were purchased from AnaSpec Inc (USA).
Results: Co-culture of effector memory CD8 T cells with DC up regulated CD8 T cell expression of CD40L that reached a maximum after 6 h. IL-12p70 levels in the culture reached 80% of its maximum value after 8 h. Altered peptide ligands SAINFEKL and SIINYEKL induced intermediate levels of CD40L while EINFEKYL and SIIRFEKL failed to induce CD40L on CD8 T cells. There was a corresponding reduction in IL-12p70 in the medium. Using CD8 T cells stimulated with anti-CD3 and CD28 addition of IL-12p70 enhanced CD40L expression on CD8s and addition of IL-12p70 neutralising antibody reduced CD40L expression.
Conclusions: CD8 T cell receptor dependent signals stimulate DCs to secrete IL-12p70 that up-regulates CD40L expression on CD8 T cells.

4.1097 Developmental rates of IVP bovine embryos using minigradient of Percoll, Isolate and Optiprep
Vianna, L.L., pradiee, J., Santos, E.C., Gonc, A., Pfeifer, L.F.M., Dode, M.A., Vieira, A.D., De Lima, V.F.H., Correal, M.N. and pegoraro, L.M.C.
Reproducion in Domestic Animals, ***47(s4),*** *416-613 (2012)*

The sperm selection method is one important step of embryo IVP systems and may influence the embryonic development rates. The objective of this study was to compare the efficiency of different sperm selection methods used in bovine IVP systems in terms of embryo development. A total of 2455 oocytes obtained from ovaries of Bos taurus cattle collected in a slaughterhouse were used in this experiment

(n = 11 replicates). After in vitro maturation oocytes were distributed and inseminated according to four treatments: (i) Conventional Percoll group (90 and 45%) – 4 ml, centrifuged at 700· g for 20 min, (ii) Minipercoll group (90 and 45%) – 800 lL, centrifuged at 700· g for 5 min, (iii) Miniisolate group (90 and 45%) – 800 lL, centrifuged at 700· g for 5 min; and (iv) Minioptiprep group (30.28 and 26%) – 1.2 ml, centrifuged at 900· g for 15 min. Developmental rates at D2 (cleaved/oocytes inseminated) and at D8 (blastocyst/ oocytes inseminated) of culture were compared among treatments. Data were analysed by using chi-squre test. The cleavage rates were similar among the Conventional Percoll_ and Miniisolate_ groups (70.4% vs. 67.1%; $p > 0.05$). Minipercoll_ and Minioptiprep_ groups had lower cleavage rate than Conventional Percoll_ (65.8%, 57.9% vs. 70.4%; $p < 0.05$). At Day 8 blastocyst rate for Minipercoll, Minioptiprep and Conventional Percoll were 16.1%, 16.9%, and 18.4%, respectively ($p > 0.05$). Among the minigradientes, the Miniisolate had higher blastocyst yield than Minipercoll and Minioptiprep (21.1% vs. 16.1% and 16.9%, respectively; $p < 0.05$). In conclusion, the minigradients, in the concentration and volume used in our experiment, reached similar results to the Percoll Conventional group. Therefore, they can be use in the routine of IVP bovine embryos as alternatives with same viability and lower time consuming and production cost.

4.1098

4.1099 **The Peroxisome Proliferator-activated Receptor {gamma} (PPAR{gamma}) Controls Natural Protective Mechanisms against Lipid Peroxidation in Amyotrophic Lateral Sclerosis**
Benedusi, V., Martorana, F., Brambilla, L., Maggi, A. and Rossi, D.
J. Biol. Chem., ***287(43)***, *35899-35911 (2012)*

Recent evidence highlights the peroxisome proliferator-activated receptors (PPARs) as critical neuroprotective factors in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). To gain new mechanistic insights into the role of these receptors in the context of ALS, here we investigated how PPAR transcriptional activity varies in $hSOD1^{G93A}$ ALS transgenic mice. We demonstrate that PPARγ-driven transcription selectively increases in the spinal cord of symptomatic $hSOD1^{G93A}$ mice. This phenomenon correlates with the up-regulation of target genes, such as lipoprotein lipase and glutathione *S*-transferase α-2, which are implicated in scavenging lipid peroxidation by-products. Such events are associated with enhanced PPARγ immunoreactivity within motor neuronal nuclei. This observation, and the fact that PPARγ displays increased responsiveness in cultured $hSOD1^{G93A}$ motor neurons, points to a role for this receptor in neutralizing deleterious lipoperoxidation derivatives within the motor cells. Consistently, in both motor neuron-like cultures and animal models, we report that PPARγ is activated by lipid peroxidation end products, such as 4-hydroxynonenal, whose levels are elevated in the cerebrospinal fluid and spinal cord from ALS patients. We propose that the accumulation of critical concentrations of lipid peroxidation adducts during ALS progression leads to the activation of PPARγ in motor neurons. This in turn triggers self-protective mechanisms that involve the up-regulation of lipid detoxification enzymes, such as lipoprotein lipase and glutathione *S*-transferase α-2. Our findings indicate that anticipating natural protective reactions by pharmacologically modulating PPARγ transcriptional activity may attenuate neurodegeneration by limiting the damage induced by lipid peroxidation derivatives.

4.1100 **Impact of Tissue Volume and Purification on Clinical Autologous Islet Transplantation for the Treatment of Chronic Pancreatitis**
Matsumoto, S., Takita, M., AShimoda, M., Sugimoto, K., Itoh, T., Chujo, D., SoRelle, J.A., Tamura, Y., Rahman, A.M., Onaca, N., Naziruddin, B. and Levy, M.F.
Cell Transplantation, ***21(4)***, *625-632 (2012)*

Autologous islet transplantation after total pancreatectomy is an excellent treatment for painful chronic pancreatitis. Traditionally, islets have been isolated without purification; however, purification is applied when the tissue volume is large. Nevertheless, the impact of tissue volume and islet purification on clinical outcomes of autologous islet transplantation has not been well examined. We analyzed 27 cases of autologous islet transplantation performed from October 2006 to January 2011. After examining the relationship between tissue volume and portal pressure at various time points, we compared islet characteristics and clinical outcomes between cases with complications (complication group) and without (noncomplication group), as well as cases with purification (purification group) and without (nonpurification group). Tissue volume significantly correlated with maximum ($R = 0.61$), final ($R = 0.53$), and delta (i.e., difference between base and maximum; $R = 0.71$) portal pressure. The complication group had a significantly higher body mass index, tissue volume, islet yield, and portal pressure (maximum, final, delta), suggesting that complications were associated with high tissue volume and high portal pressure.

Only one of four patients (25%) in the complication group became insulin free, whereas 11 of 23 patients (49%) in the noncomplication group became insulin free with smaller islet yields. The purification group had a higher islet yield and insulin independence rate but had similar final tissue volume, portal pressure, and complication rates compared with the nonpurification group. In conclusion, high tissue volume was associated with high portal pressure and complications in autologous islet transplantation. Islet purification effectively reduced tissue volume and had no negative impact on islet characteristics. Therefore, islet purification can reduce the risk of complications and may improve clinical outcome for autologous islet transplantation when tissue volume is large.

4.1101 Correlation of Released HMGB1 Levels with the Degree of Islet Damage in Mice and Humans and With the Outcomes of Islet Transplantation in Mice
Itoh, T., Takita, M., SoRelle, J.A., Shimoda, M., Sugimoto, K., Chujo, D., Qin, H., Naziruddin, B., Levy, M.F. and Matsumoto, S.
Cell Transplantation, ***21(7)***, *1371-1381 (2012)*

Establishing reliable islet potency assay is a critical and unmet issue for clinical islet transplantation. Recently, we reported that islets contained high levels of high mobility group box 1 (HMGB1) and damaged islets released HMGB1 in a mouse model. In this study, we hypothesized that the amount of released HMGB1 could reflect the degree of islet damage, and could predict the outcome of islet transplantation. Four groups of damaged mouse islets and three groups of damaged human islets were generated by hypoxic conditions. These islets were assessed by in vivo (transplantation) and in vitro (released HMGB1 levels, released C-peptide levels, PI staining, TUNEL staining, ATP/DNA, and glucose-stimulated insulin release test) assays. In addition, the ability of each assay to distinguish between noncured (n = 13) and cured (n = 7) mice was assessed. The curative rates of STZ-diabetic mice after receiving control, hypoxia-3h, hypoxia-6h, and hypoxia-24h mouse islets were 100%, 40%, 0%, and 0%, respectively. Only amounts of released HMGB1 and ratio of PI staining significant increased according to the degree of damages in both human and mouse islets. In terms of predictability of curing diabetic mice, amounts of released HMGB1 showed the best sensitivity (100%), specificity (100%), positive (100%), and negative predictive values (100%) among all the assays. The amount of released HMGB1 reflected the degree of islet damage and correlated with the outcome of islet transplantation in mice. Hence, released HMGB1 levels from islets should be a useful marker to evaluate the potency of isolated islets.

4.1102 Antagonism of sphingosine 1-phosphate receptor 2 causes a selective reduction of portal vein pressure in bile duct-ligated rodents
Kageyama, Y. et al
Hepatology, ***56(4)***, *1427-1438 (2012)*

Sinusoidal vasoconstriction, in which hepatic stellate cells operate as contractile machinery, has been suggested to play a pivotal role in the pathophysiology of portal hypertension. We investigated whether sphingosine 1-phosphate (S1P) stimulates contractility of those cells and enhances portal vein pressure in isolated perfused rat livers with Rho activation by way of S1P receptor 2 ($S1P_2$). Rho and its effector, Rho kinase, reportedly contribute to the pathophysiology of portal hypertension. Thus, a potential effect of $S1P_2$ antagonism on portal hypertension was examined. Intravenous infusion of the $S1P_2$ antagonist, JTE-013, at 1 mg/kg body weight reduced portal vein pressure by 24% without affecting mean arterial pressure in cirrhotic rats induced by bile duct ligation at 4 weeks after the operation, whereas the same amount of $S1P_2$ antagonist did not alter portal vein pressure and mean arterial pressure in control sham-operated rats. Rho kinase activity in the livers was enhanced in bile duct-ligated rats compared to sham-operated rats, and this enhanced Rho kinase activity in bile duct-ligated livers was reduced after infusion of the $S1P_2$ antagonist. $S1P_2$ messenger RNA (mRNA) expression, but not $S1P_1$ or $S1P_3$, was increased in bile duct-ligated livers of rats and mice and also in culture-activated rat hepatic stellate cells. $S1P_2$ expression, determined in *S1P* $_2^{LacZ/+}$ mice, was highly increased in hepatic stellate cells of bile duct-ligated livers. Furthermore, the increase of Rho kinase activity in bile duct-ligated livers was observed as early as 7 days after the operation in wildtype mice, but was less in *S1P*$_2^{-/-}$ mice. *Conclusion*: S1P may play an important role in the pathophysiology of portal hypertension with Rho kinase activation by way of $S1P_2$. The $S1P_2$ antagonist merits consideration as a novel therapeutic agent for portal hypertension.

4.1103 Velocimetry of red blood cells in microvessels by the dual-slit method: Effect of velocity gradients
Roman, S., Lorthois, S., Duru, P. and Risso, F.
Microvascular Res., ***84***, *249-261 (2012)*

The dual-slit is a photometric technique used for the measurement of red blood cell (RBC) velocity in microvessels. Two photometric windows (slits) are positioned along the vessel. Because the light is modulated by the RBCs flowing through the microvessel, a time dependent signal is captured for each window. A time delay between the two signals is obtained by temporal cross correlation, and is used to deduce a velocity, knowing the distance between the two slits. Despite its wide use in the field of microvascular research, the velocity actually measured by this technique has not yet been unambiguously related to a relevant velocity scale of the flow (*e.g.* mean or maximal velocity) or to the blood flow rate. This is due to a lack of fundamental understanding of the measurement and also because such a relationship is crucially dependent on the non-uniform velocity distribution of RBCs in the direction parallel to the light beam, which is generally unknown.
The aim of the present work is to clarify the physical significance of the velocity measured by the dual-slit technique. For that purpose, dual-slit measurements were performed on computer-generated image sequences of RBCs flowing in microvessels, which allowed all the parameters related to this technique to be precisely controlled. A parametric study determined the range of optimal parameters for the implementation of the dual-slit technique. In this range, it was shown that, whatever the parameters governing the flow, the measured velocity was the maximal RBC velocity found in the direction parallel to the light beam.
This finding was then verified by working with image sequences of flowing RBCs acquired in PDMS micro-systems *in vitro*. Besides confirming the results and physical understanding gained from the study with computer generated images, this *in vitro* study showed that the profile of RBC maximal velocity across the channel was blunter than a parabolic profile, and exhibited a non-zero sliding velocity at the channel walls.
Overall, the present work demonstrates the robustness and high accuracy of the optimized dual-slit technique in various flow conditions, especially at high hematocrit, and discusses its potential for applications *in vivo*.

4.1104 Upregulation of the Tim-3/Galectin-9 Pathway of T Cell Exhaustion in Chronic Hepatitis B Virus Infection

Nebbia, G., Peppa, D., Schurich, A., Khanna, P., Singh, H.D., Cheng, Y., Rosenberg, W., Dusheiko, G., Gilson, R., ChinAleong, J., Kennedy, P. and Maini, M.K.
*PloS One, **7(10)**, e7648 (2012)*

The S-type lectin galectin-9 binds to the negative regulatory molecule Tim-3 on T cells and induces their apoptotic deletion or functional inactivation. We investigated whether galectin-9/Tim-3 interactions contribute to the deletion and exhaustion of the antiviral T cell response in chronic hepatitis B virus infection (CHB). We found Tim-3 to be expressed on a higher percentage of CD4 and CD8 T cells from patients with CHB than healthy controls ($p<0.0001$) and to be enriched on activated T cells and those infiltrating the HBV-infected liver. Direct *ex vivo* examination of virus-specific CD8 T cells binding HLA-A2/peptide multimers revealed that Tim-3 was more highly upregulated on HBV-specific CD8 T cells than CMV-specific CD8 T cells or the global CD8 T cell population in patients with CHB ($p<0.001$) or than on HBV-specific CD8 after resolution of infection. T cells expressing Tim-3 had an impaired ability to produce IFN-γ and TNF-α upon recognition of HBV-peptides and were susceptible to galectin-9-triggered cell death *in vitro*. Galectin-9 was detectable at increased concentrations in the sera of patients with active CHB-related liver inflammation ($p = 0.02$) and was strongly expressed by Kupffer cells within the liver sinusoidal network. Tim-3 blockade resulted in enhanced expansion of HBV-specific CD8 T cells able to produce cytokines and mediate cytotoxicity in vitro. Blocking PD-1 in combination with Tim-3 enhanced the number of patients from whom functional antiviral responses could be recovered and/or the strength of responses, indicating that these co-inhibitory molecules play a non-redundant role in driving T cell exhaustion in CHB. Patients taking antivirals able to potently suppress HBV viraemia continued to express Tim-3 on their T cells and respond to Tim-3 blockade. In summary, both Tim-3 and galectin-9 are increased in CHB and may contribute to the inhibition and deletion of T cells as they infiltrate the HBV-infected liver.

4.1105 Neurotrophic factor-secreting autologous muscle stem cell therapy for the treatment of laryngeal denervation injury

Halum, S.L., McRae, B., Bijangi-Vishehsaraei, K. and Hiatt, K.
*The Laryngoscope, **122(11)**, 2482-2496 (2012)*

Objectives/Hypothesis:

To determine if the spontaneous reinnervation that characteristically ensues after recurrent laryngeal nerve (RLN) injury could be selectively promoted and directed to certain laryngeal muscles with the use of neurotrophic factor (NF)-secreting muscle stem cell (MSC) vectors while antagonistic reinnervation is inhibited with vincristine (VNC).
Study Design:
Basic science investigation involving primary cell cultures, gene cloning/transfer, and animal experiments.
Methods:
MSC survival assays were used to test multiple individual NFs in vitro. Motoneuron outgrowth assays assessed the trophic effects of identified NF on cranial nerve X (CNX)-derived motoneurons in vitro. Therapeutic NF was cloned into a lentiviral vector, and MSCs were tranduced to secrete NF. Sixty rats underwent left RLN transection injury, and at 3 weeks received injections of either MSCs (n = 24), MSCs secreting NF (n = 24), or saline (n = 12) into the left thyroarytenoid muscle complex; half of the animals in the MSC groups simultaneously received left posterior cricoarytenoid injections of VNC, whereas half of the animals received saline.
Results:
Ciliary neurotrophic factor (CNTF) had the greatest survival-promoting effect on MSCs in culture. The addition of CNTF (50 ng/mL) to CNX motoneuron cultures resulted in enhanced neurite outgrowth and branching. In the animal model, the injected MSCs fused with the denervated myofibers, immunohistochemistry demonstrated enhanced reinnervation based on motor endplate to nerve contact, and reverse transcriptase-polymerase chain reaction confirmed stable CNTF expression at longest follow-up (4 months) in the CNTF-secreting MSC treated groups.
Conclusions:
MSC therapy may have a future role in selectively promoting and directing laryngeal reinnervation after RLN injury.

4.1106 Interplay of foot-and-mouth disease virus, antibodies and plasmacytoid dendritic cells: virus opsonization under non-neutralizing conditions results in enhanced interferon-alpha responses
Lannes, N., Python, S. and Summerfield, A.
Vet. Res., ***43***, *64-72 (2012)*

Foot-and-mouth disease virus (FMDV) is a highly infectious member of the *Picornaviridae* inducing an acute disease of cloven-hoofed species. Vaccine-induced immune protection correlates with the presence of high levels of neutralizing antibodies but also opsonising antibodies have been proposed as an important mechanism of the immune response contributing to virus clearance by macrophages and leading to the production of type-I interferon (IFN) by plasmacytoid dendritic cells (pDC). The present study demonstrates that the opsonising antibody titres mediating enhanced IFN-α responses in pDC were similar to neutralizing titres, when antigenically related viruses from the same serotype were employed. However, sera cross-reacted also with non-neutralized isolates of multiple serotypes, when tested in this assay. Both uncomplexed virus and immune complexed virus stimulated pDC *via* Toll-like receptor 7. An additional finding of potential importance for strain-specific differences in virulence and/or immunogenicity was that pDC activation by FMDV strongly differed between viral isolates. Altogether, our results indicate that opsonising antibodies can have a broader reactivity than neutralizing antibodies and may contribute to antiviral responses induced against antigenically distant viruses.

4.1107 Human Melanoma Metastasis in NSG Mice Correlates with Clinical Outcome in Patients
Quintana, E., Piskounova, E., Shackleton, M., Weinberg, D., Eskiocak, U., Fullen, D.R., Johnson, T.M. and Morrison, S.J.
Science Translational Med., ***4(159)***, *159ra149 (2012)*

Studies of human cancer metastasis have been limited by a lack of experimental assays in which cancer cells from patients metastasize in vivo in a way that correlates with clinical outcome. This makes it impossible to study intrinsic differences in the metastatic properties of cancers from different patients. We recently developed an assay in which human melanomas readily engraft in nonobese diabetic/severe combined immunodeficient interleukin-2 receptor-γ chain null (NSG) mice. We show that melanomas from 25 patients exhibited reproducible differences in the rate of spontaneous metastasis after transplantation into NSG mice and that these differences correlated with clinical outcome in the patients. Stage IIIB/C melanomas that formed distant metastases within 22 months in patients also formed tumors that metastasized widely in NSG mice, whereas stage IIIB/C melanomas that did not form distant metastases within 22 to 50 months in patients metastasized more slowly in NSG mice. These differences in the efficiency of metastasis correlated with the presence of circulating melanoma cells in the blood of NSG

mice, suggesting that the rate of entry into the blood is one factor that limits the rate of metastasis. The study of NSG mice can therefore yield information about the metastasis of human melanomas in vivo, in this case revealing intrinsic differences among stage III melanomas in their ability to circulate/survive in the blood and to metastasize.

4.1108 Miscellaneous Pathogens
Austin, B. and Austin, D.A.
Bacterial Fish Pathogens, 413-441 (2012)

Pseudoalteromonas piscicida, Pseudoalteromonas undina, Shewanella putrefaciens, Arcobacter cryaerophilus, Halomonas (=Deleya) cupida, Acinetobacter sp., *Moraxella* sp., *Moritella marina, Moritella viscosa, Mycoplasma mobile, Myxococcus piscicola, Aquaspirillum* sp., *Janthinobacterium lividum, Pasteurella skyensis, Piscirickettsia salmonis*, *Rickettsia*-like organisms, *Streptobacillus*, '*Candidatus* Arthromitus', '*Candidatus* Branchiomonas cysticola', '*Candidatus* Clavochlamydia salmonicola', '*Candidatus* Piscichlamydia salmonis' and '*Candidatus* Renichlamydia lutjani' have been associated with fish diseases. *Moritella viscosa* has been recovered from winter ulcer disease (= skin lesions) in Atlantic salmon with pathogenicity mechanisms reflecting the presence of extracellular products. Protection has been achieved with an adjuvanted formalin inactivated whole cell vaccine. *Piscirickettsia salmonis* is an obligate parasite, which has been associated with coho salmon syndrome, Huito disease and salmonid rickettsial septicaemia. Good protection was recorded by use of a formalised whole cell suspension. *Candidatus* are uncultured organisms, which may be visualised in pathological material.

4.1109 3.03 – Cell Separation, Perfusion from Tissue, Organelle Fractionation: A Comparison of the Methods Used for Porcine Islet Isolation for Transplantation as a Treatment for Type 1 Diabetes Mellitus
Rafati, S., Le, C., Rajotte, R.V. and Rayat, G.R.
*Comp. Sampling and Sample Preparation, **3**, 33-51 (2012)*

Diabetes mellitus is a chronic condition of disordered glucose metabolism characterized by high blood glucose levels. Its prevalence has rapidly increased in recent years and has been predicted to continue to increase in the years to come. Type 1 diabetes mellitus is commonly treated by insulin therapy; however, secondary complications of diabetes still develop. The replacement of damaged beta cells in the islets could be accomplished by whole pancreas or islet transplantation. However, transplantation is currently challenged by the increasing demand for organs. The transplantation of porcine islets could potentially overcome the shortage of human organ donors and is being considered as one of the most feasible alternative treatments for type 1 diabetes mellitus. The methods for adult porcine islet isolation have dramatically improved since the 1970s and have lead to the enhancement of the quality of adult porcine islet preparations. Neonatal porcine islets with simpler isolation technique and differentiation capacity are becoming more attractive for preclinical large animal studies. The optimization of enzymatic digestion methods, culture conditions, quality control, and improvement of immunosuppressive therapies to overcome rejection are among the challenges that need to be met before porcine islet xenotransplantation can be applied to patients with type 1 diabetes mellitus.

4.1110 Safety and tolerability of the T-cell depletion protocol coupled with anakinra and etanercept for clinical islet cell transplantation
Takita, M., Matsumoto, S., Shimoda, M., Chujo, D., Itoh, T., SoRelle, J.A., Purcell, K., Onaca, N., Naziruddin, B. and Levy, M.F.
*Clin. Transplant., **26**, E471-E484 (2012)*

Background
Islet cell transplantation (ICT) is a promising approach to cure patients with type 1 diabetes. We have implemented a new immunosuppression protocol with antithymoglobulin plus anti-inflammatory agents of anakinra and eternacept for induction and tacrolimus plus mycophenolate mofetil for maintenance [T-cell depletion with anti-inflammatory (TCD-AI) protocol], resulting in successful single-donor ICT.
Methods
Eight islet recipients with type 1 diabetes reported adverse events (AEs) monthly. AEs were compared between three groups: first infusion with the TCD-AI protocol (TCD-AI-1st) and first and second infusion with the Edmonton-type protocol (Edmonton-1st and Edmonton-2nd).
Results

The incidence of symptomatic AEs within the initial three months in the TCD-AI-1st group was less than in the Edmonton-1st and Edmonton-2nd groups, with a marginally significant difference (mean ± SE: 5.5 ± 0.3, 7.5 ± 0.5, and 8.3 ± 1.3, respectively; $p = 0.07$). A significant reduction in liver enzyme elevation after ICT was found in the TCD-AI-1st group compared with the Edmonton-1st and Edmonton-2nd groups ($p < 0.05$). Because of AEs, all patients in the Edmonton protocol eventually converted to the TCD-AI protocol, whereas all patients tolerated the TCD-AI protocol.

Conclusions

TCD-AI protocol can be tolerated for successful ICT, although this study includes small cohort, and large population trial should be taken.

4.1111 Gamma Interferon (IFN-γ) Receptor Restricts Systemic Dengue Virus Replication and Prevents Paralysis in IFN-α/β Receptor-Deficient Mice

Prestwood, T.R., Morar, M.M., Zellweger, R.M., Miller, R., May, M.M., Yauch, L.E., Lada, S.M. and Shresta, S.

J. Virol., ***86(23)***, *12561-12570 (2012)*

We previously reported that mice lacking alpha/beta and gamma interferon receptors (IFN-α/βR and -γR) uniformly exhibit paralysis following infection with the dengue virus (DENV) clinical isolate PL046, while only a subset of mice lacking the IFN-γR alone and virtually no mice lacking the IFN-α/βR alone develop paralysis. Here, using a mouse-passaged variant of PL046, strain S221, we show that in the absence of the IFN-α/βR, signaling through the IFN-γR confers approximately 140-fold greater resistance against systemic vascular leakage-associated dengue disease and virtually complete protection from dengue-induced paralysis. Viral replication in the spleen was assessed by immunohistochemistry and flow cytometry, which revealed a reduction in the number of infected cells due to IFN-γR signaling by 2 days after infection, coincident with elevated levels of IFN-γ in the spleen and serum. By 4 days after infection, IFN-γR signaling was found to restrict DENV replication systemically. Clearance of DENV, on the other hand, occurred in the absence of IFN-γR, except in the central nervous system (CNS) (brain and spinal cord), where clearance relied on IFN-γ from $CD8^+$ T cells. These results demonstrate the roles of IFN-γR signaling in protection from initial systemic and subsequent CNS disease following DENV infection and demonstrate the importance of $CD8^+$ T cells in preventing DENV-induced CNS disease.

4.1112 Nucleoside salvage pathway kinases regulate hematopoiesis by linking nucleotide metabolism with replication stress

Austin, W.R., Armijo, A.L., Campbell, D.O., Singh, A.S., Hsieh, T., Nathanson, D., Herschman, H.R., Phelps, M.E., Witte, O.N., Czermin, J. and Radu, C.G.

J. Exp. Med., ***209(12)***, *2215-2228 (2012)*

Nucleotide deficiency causes replication stress (RS) and DNA damage in dividing cells. How nucleotide metabolism is regulated in vivo to prevent these deleterious effects remains unknown. In this study, we investigate a functional link between nucleotide deficiency, RS, and the nucleoside salvage pathway (NSP) enzymes deoxycytidine kinase (dCK) and thymidine kinase (TK1). We show that inactivation of dCK in mice depletes deoxycytidine triphosphate (dCTP) pools and induces RS, early S-phase arrest, and DNA damage in erythroid, B lymphoid, and T lymphoid lineages. *TK1*$^{-/-}$ erythroid and B lymphoid lineages also experience nucleotide deficiency but, unlike their *dCK*$^{-/-}$ counterparts, they still sustain DNA replication. Intriguingly, dCTP pool depletion, RS, and hematopoietic defects induced by dCK inactivation are almost completely reversed in a newly generated *dCK/TK1* double-knockout (DKO) mouse model. Using NSP-deficient DKO hematopoietic cells, we identify a previously unrecognized biological activity of endogenous thymidine as a strong inducer of RS in vivo through TK1-mediated dCTP pool depletion. We propose a model that explains how TK1 and dCK "tune" dCTP pools to both trigger and resolve RS in vivo. This new model may be exploited therapeutically to induce synthetic sickness/lethality in hematological malignancies, and possibly in other cancers.

4.1113 Accumulation of Activated Invariant Natural Killer T Cells in the Tumor Microenvironment after α-Galactosylceramide-Pulsed Antigen Presenting Cells

Nagato, K., Motohashi, S., Ishibashi, F., Okita, K., Yamasaki, K., Moriya, Y., Hoshino, H., Yoshida, S., Hanaoka, H., Fujii, S-i., Taniguchi, M., Yoshino, I. and Nakayama, T.

J. Clin. Immunol., ***32(5)***, *1071-1081 (2012)*

Purpose

The intravenous administration of α-Galactosylceramide (α-GalCer)-pulsed antigen presenting cells

(APCs) is well tolerated and the increased IFN-γ producing cells in the peripheral blood after the treatment appeared to be associated with prolonged survival. An exploratory study protocol was designed with the preoperative administration of α-GalCer-pulsed APCs to clarify the mechanisms of these findings, while especially focusing on the precise tumor site.

Methods

Patients with operable advanced lung cancer received an intravenous injection of α-GalCer-pulsed APCs before surgery. The resected lung and tumor infiltrating lymphocytes (TILs) as well as peripheral blood mononuclear cells were collected and the invariant NKT (iNKT) cell-specific immune responses were analyzed.

Results

Four patients completed the study protocol. We observed a significant increase in iNKT cell numbers in the TILs and augmented IFN-γ production by the α-GalCer-stimulated TILs.

Conclusion

The administration of α-GalCer-pulsed APCs successfully induced the dramatic infiltration and activation of iNKT cells in the tumor microenvironment.

4.1114 Blockade of Myeloid-Derived Suppressor Cells after Induction of Lymphopenia Improves Adoptive T Cell Therapy in a Murine Model of Melanoma

Kodumudi, K.N., Weber, A., Sarnaik, A.A. and Pilon-Thomas, S.
*J. Immunol., **189(11)**, 5147-5154 (2012)*

Administration of nonmyeloablative chemotherapeutic agents or total body irradiation (TBI) prior to adoptive transfer of tumor-specific T cells may reduce or eliminate immunosuppressive populations such as T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSC). Little is known about these populations during immune reconstitution. This study was designed to understand the reconstitution rate and function of these populations post TBI in melanoma tumor-bearing mice. Reconstitution rate and suppressive activity of $CD4^+CD25^+Foxp3^+$ Tregs and $CD11b^+Gr1^+$ MDSC following TBI-induced lymphopenia was measured in B16 melanoma tumor-bearing mice. To ablate the rapid reconstitution of suppressive populations, we treated mice with docetaxel, a known chemotherapeutic agent that targets MDSC, in combination with adoptive T cell transfer and dendritic cell immunotherapy. Both Treg and MDSC populations exhibited rapid reconstitution after TBI-induced lymphopenia. Although reconstituted Tregs were just as suppressive as Tregs from untreated mice, MDSC demonstrated enhanced suppressive activity of $CD8^+$ T cell proliferation compared with endogenous MDSC from tumor-bearing mice. TBI-induced lymphopenia followed by docetaxel treatment improved the efficacy of adoptive T cell transfer and dendritic cell immunotherapy in melanoma-bearing mice, inducing a significant reduction in tumor growth and enhancing survival. Tumor regression correlated with increased CTL activity and persistence of adoptively transferred T cells. Overall, these findings suggest that TBI-induced MDSC are highly immunosuppressive and blocking their rapid reconstitution may improve the efficacy of vaccination strategies and adoptive immunotherapy.

4.1115 Hepatic Vascular Endothelial Growth Factor Regulates Recruitment of Rat Liver Sinusoidal Endothelial Cell Progenitor Cells

Wang, L., Wang, X., Wang, L., Chiu, J.D., Van De Ven, G., Gaarde, W.A. and Deleve, L.D:
*Gastroenterology, **143(6)**, 1555-1563 (2012)*

Background & Aims

After liver injury, bone marrow–derived liver sinusoidal endothelial cell progenitor cells (BM SPCs) repopulate the sinusoid as liver sinusoidal endothelial cells (LSECs). After partial hepatectomy, BM SPCs provide hepatocyte growth factor, promote hepatocyte proliferation, and are necessary for normal liver regeneration. We examined how hepatic vascular endothelial growth factor (VEGF) regulates recruitment of BM SPCs and their effects on liver injury.

Methods

Rats were given injections of dimethylnitrosamine to induce liver injury, which was assessed by histology and transaminase assays. Recruitment of SPCs was analyzed by examining BM SPC proliferation, mobilization to the circulation, engraftment in liver, and development of fenestration (differentiation).

Results

Dimethylnitrosamine caused extensive denudation of LSECs at 24 hours, followed by centrilobular hemorrhagic necrosis at 48 hours. Proliferation of BM SPCs, the number of SPCs in the bone marrow, and mobilization of BM SPCs to the circulation increased 2- to 4-fold by 24 hours after injection of dimethylnitrosamine; within 5 days, 40% of all LSECs came from engrafted BM SPCs. Allogeneic

resident SPCs, infused 24 hours after injection of dimethylnitrosamine, repopulated the sinusoid as LSECs and reduced liver injury. Expression of hepatic VEGF messenger RNA and protein increased 5-fold by 24 hours after dimethylnitrosamine injection. Knockdown of hepatic VEGF with antisense oligonucleotides completely prevented dimethylnitrosamine-induced proliferation of BM SPCs and their mobilization to the circulation, reduced their engraftment by 46%, completely prevented formation of fenestration after engraftment as LSECs, and exacerbated dimethylnitrosamine injury.
Conclusions
BM SPC recruitment is a repair response to dimethylnitrosamine liver injury in rats. Hepatic VEGF regulates recruitment of BM SPCs to liver and reduces this form of liver injury.

4.1116 The Role of Interferon-γ Inducible Protein-10 in a Mouse Model of Acute Liver Injury Post Induced Pluripotent Stem Cells Transplantation

Chan, C-C., Cheng, L-Y., Lu, J., Huang, Y-H., Chiou, S-H., Tsai, P-H., Huo, T-l., Lin, H-C. and Lee, F-Y.
*PloS One, **7(12)**, e50577 (2012)*

BackgroundLiver injuries are important medical problems that require effective therapy. Stem cell or hepatocyte transplantation has the potential to restore function of the damaged liver and ameliorate injury. However, the regulatory factors crucial for the repair and regeneration after cell transplantation have not been fully characterized. Our study investigated the effects and the expression of the regulatory factors in mouse models of acute liver injury either transplanted with the induced pluripotent stem cells (iPS) or the hepatocytes that differentiated from iPS cells (iHL).Methods/Principal FindingsMice received CCl4 injection and were randomized to receive vehicle, iPS, or iHL transfusions vial tail veins and were observed for 24, 48 or 72 hours. The group of mice with iPS transplantation performed better than the group of mice receiving iHL in reducing the serum alanine aminotransferase, aspartate aminotransferase, and liver necrosis areas at 24 hours after CCl4 injury. Moreover, iPS significantly increased the numbers of proliferating hepatocytes at 48 hours. Cytokine array identified that chemokine IP-10 could be the potential regulatory factor that ameliorates liver injury. Further studies revealed that iPS secreted IP-10 in vitro and transfusion of iPS increased IP-10 protein and mRNA expressions in the injured livers in vivo. The primary hepatocytes and non-parenchyma cells were isolated from normal and injured livers. Hepatocytes from injured livers that received iPS treatment expressed more IP-10 mRNA than their non-hepatocyte counter-parts. In addition, animal studies revealed that administration of recombinant IP-10 (rIP-10) effectively reduced liver injuries while IP-10-neutralizing antibody attenuated the protective effects of iPS and decreased hepatocyte proliferation. Both iPS and rIP-10 significantly reduced the 72-hour mortality rate in mice that received multiple CCl4-injuries.Conclusions/SignificanceThese findings suggested that IP-10 may have an important regulatory role in facilitating the repair and regeneration of injured liver after iPS transplantation.

4.1117 β-Glucan Curdlan Induces IL-10–Producing CD4+ T Cells and Inhibits Allergic Airway Inflammation

Kawashima, S., Hirose, K., Iwata, A., Takahashi, K., Ohkubo, A., Tamachi, T., Ikeda, K., Kagami, S-i. and Nakajima, H.
*J. Immunol., **189(12)**, 5713-5721 (2012)*

A number of studies have suggested a correlation between a decreased incidence in infectious diseases and an increased incidence of allergic diseases, including asthma. Although several pathogen-derived products have been shown to possess therapeutic potential for allergic diseases, it remains largely unknown whether β-glucan, a cell wall component of a variety of fungi, yeasts, and bacteria, has a regulatory potential for allergic diseases. In this study, we examined the effect of curdlan, a linear β-(1-3)-glucan, on the development of allergic airway inflammation. We found that i.p. injection of curdlan significantly inhibited Ag-induced eosinophil recruitment and Th2 cytokine production in the airways. The activation of $CD4^+$ T cells in the presence of curdlan induced IL-10–producing $CD4^+$ T cells with high levels of c-Maf expression. Curdlan-induced development of IL-10–producing $CD4^+$ T cells required the presence of APCs and ICOS/ICOS ligand interaction. Curdlan-induced development of IL-10–producing $CD4^+$ T cells also required intrinsic expression of STAT6. Furthermore, the transfer of Ag-specific $CD4^+$ T cells that were stimulated in the presence of curdlan inhibited Ag-induced eosinophil recruitment into the airways. Taken together, these results suggest that curdlan is capable of inducing IL-10–producing $CD4^+$ T cells and inhibiting the development of eosinohilic airway inflammation, underscoring the therapeutic potential of curdlan for allergic diseases.

4.1118 Role of Galectin-3 in Classical and Alternative Macrophage Activation in the Liver following

Acetaminophen Intoxication
Docan Dragomir, A-C., Sun, R., Choi, H., Laskin, J.D. and Laskin, D.L.
J. Immunol., ***189(12)***, *5934-5941 (2012)*

Inflammatory macrophages have been implicated in hepatotoxicity induced by the analgesic acetaminophen (APAP). In these studies, we characterized the phenotype of macrophages accumulating in the liver following APAP intoxication and evaluated the role of galectin-3 (Gal-3) in macrophage activation. Administration of APAP (300 mg/kg, i.p.) to wild-type mice resulted in the appearance of two distinct subpopulations of $CD11b^+$ cells in the liver, which expressed high or low levels of the monocyte/macrophage activation marker Ly6C. Whereas $CD11b^+/Ly6C^{hi}$ macrophages exhibited a classically activated proinflammatory phenotype characterized by increased expression of TNF-α, inducible NO synthase, and CCR2, $CD11b^+/Ly6C^{lo}$ macrophages were alternatively activated, expressing high levels of the anti-inflammatory cytokine IL-10. APAP intoxication was also associated with an accumulation of $Gal\text{-}3^+$ macrophages in the liver; the majority of these cells were $Ly6C^{hi}$. APAP-induced increases in $CD11b^+/Ly6C^{hi}$ macrophages were significantly reduced in $Gal\text{-}3^{-/-}$ mice. This reduction was evident 72 h post APAP and was correlated with decreased expression of the classical macrophage activation markers, inducible NO synthase, IL-12, and TNF-α, as well as the proinflammatory chemokines CCL2 and CCL3, and chemokine receptors CCR1 and CCR2. Conversely, numbers of $CD11b^+/Ly6C^{lo}$ macrophages increased in livers of APAP-treated $Gal\text{-}3^{-/-}$ mice; this was associated with increased expression of the alternative macrophage activation markers Ym1 and Fizz1, increased liver repair, and reduced hepatotoxicity. These data demonstrate that both classically and alternatively activated macrophages accumulate in the liver following APAP intoxication; moreover, Gal-3 plays a role in promoting a persistent proinflammatory macrophage phenotype.

4.1119 Diesel Exhaust Particle Exposure In Vitro Alters Monocyte Differentiation and Function
Chaudhuri, N., Jary, H., Lea, S., Khan, N., Piddock, K.C., Dockrell, D.H., Donaldson, K., Duffin, R., Singh, D., Parker, L.C. and Sabroe, I.
PloS One, ***7(12)***, *e51107 (2012)*

Air pollution by diesel exhaust particles is associated with elevated mortality and increased hospital admissions in individuals with respiratory diseases such as asthma and chronic obstructive pulmonary disease. During active inflammation monocytes are recruited to the airways and can replace resident alveolar macrophages. We therefore investigated whether chronic fourteen day exposure to low concentrations of diesel exhaust particles can alter the phenotype and function of monocytes from healthy individuals and those with chronic obstructive pulmonary disease. Monocytes were purified from the blood of healthy individuals and people with a diagnosis of chronic obstructive pulmonary disease. Monocyte-derived macrophages were generated in the presence or absence of diesel exhaust particles and their phenotypes studied through investigation of their lifespan, cytokine generation in response to Toll like receptor agonists and heat killed bacteria, and expression of surface markers. Chronic fourteen day exposure of monocyte-derived macrophages to concentrations of diesel exhaust particles >10 μg/ml caused mitochondrial and lysosomal dysfunction, and a gradual loss of cells over time both in healthy and chronic obstructive pulmonary disease individuals. Chronic exposure to lower concentrations of diesel exhaust particles impaired CXCL8 cytokine responses to lipopolysaccharide and heat killed *E. coli*, and this phenotype was associated with a reduction in CD14 and CD11b expression. Chronic diesel exhaust particle exposure may therefore alter both numbers and function of lung macrophages differentiating from locally recruited monocytes in the lungs of healthy people and patients with chronic obstructive pulmonary disease.

4.1120 Canine Recombinant Adenovirus Vector Induces an Immunogenicity-Related Gene Expression Profile in Skin-Migrated CD11b+ -Type DCs
Contreras, V., Urien, C., Jouneau, L., Bourge, M., Bouet-Cararo, C., Bonneau, M., Zientara, S., Klonjkowski, B., Schwartz-Cornil, I.
PloS One, ***7(12)***, *e52513 (2012)*

Gene expression profiling of the blood cell response induced early after vaccination has previously been demonstrated to predict the immunogenicity of vaccines. In this study, we evaluated whether the analysis of the gene expression profile of skin-migrated dendritic cells (DCs) could be informative for the *in vitro* prediction of immunogenicity of vaccine, using canine adenovirus serotype 2 (CAV2) as vaccine vector. CAV2 has been shown to induce immunity to transgenes in several species including sheep and is an interesting alternative to human adenovirus-based vectors, based on the safety records of the parental strain

in dogs and the lack of pre-existing immunity in non-host species. Skin-migrated DCs were collected from pseudo-afferent lymph in sheep. Both the $CD11b^+$ -type and $CD103^+$ -type skin-migrated DCs were transduced by CAV2. An analysis of the global gene response to CAV2 in the two skin DC subsets showed that the gene response in $CD11b^+$ -type DCs was far higher and broader than in the $CD103^+$ -type DCs. A newly released integrative analytic tool from Ingenuity systems revealed that the CAV2-modulated genes in the $CD11b^+$ -type DCs clustered in several activated immunogenicity-related functions, such as immune response, immune cell trafficking and inflammation. Thus gene profiling in skin-migrated DC *in vitro* indicates that the $CD11b^+$ DC type is more responsive to CAV2 than the $CD103^+$ DC type, and provides valuable information to help in evaluating and possibly improving viral vector vaccine effectiveness.

4.1121 LAT Region Factors Mediating Differential Neuronal Tropism of HSV-1 and HSV-2 Do Not Act in Trans

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*PloS One, **7(12)**, e53281 (2012)*

After HSV infection, some trigeminal ganglion neurons support productive cycle gene expression, while in other neurons the virus establishes a latent infection. We previously demonstrated that HSV-1 and HSV-2 preferentially establish latent infection in A5+ and KH10+ sensory neurons, respectively, and that exchanging the latency-associated transcript (LAT) between HSV-1 and HSV-2 also exchanges the neuronal preference. Since many viral genes besides the LAT are functionally interchangeable between HSV-1 and HSV-2, we co-infected HSV-1 and HSV-2, both *in vivo* and *in vitro*, to determine if *trans*-acting viral factors regulate whether HSV infection follows a productive or latent pattern of gene expression in sensory neurons. The pattern of HSV-1 and HSV-2 latent infection in trigeminal neurons was no different following co-infection than with either virus alone, consistent with the hypothesis that a *trans*-acting viral factor is not responsible for the different patterns of latent infection of HSV-1 and HSV-2 in A5+ and KH10+ neurons. Since exchanging the LAT regions between the viruses also exchanges neuronal preferences, we infected transgenic mice that constitutively express 2.8 kb of the LAT region with the heterologous viral serotype. Endogenous expression of LAT did not alter the pattern of latent infection after inoculation with the heterologous serotype virus, demonstrating that the LAT region does not act in *trans* to direct preferential establishment of latency of HSV-1 and HSV-2. Using HSV1-RFP and HSV2-GFP in adult trigeminal ganglion neurons *in vitro*, we determined that HSV-1 and HSV-2 do not exert *trans*-acting effects during acute infection to regulate neuron specificity. Although some neurons were productively infected with both HSV-1 and HSV-2, no A5+ or KH10+ neurons were productively infected with both viruses. Thus, *trans*-acting viral factors do not regulate preferential permissiveness of A5+ and KH10+ neurons for productive HSV infection and preferential establishment of latent infection.

4.1122 Molecular Characterization of the Regenerative Response Induced by Intrarenal Transplantation of Selected Renal Cells in a Rodent Model of Chronic Kidney Disease

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*Cell Tissues Organs, **196(4)**, 374-384 (2012)*

Dedifferentiation and proliferation of resident tubular epithelial cells is a mechanism of action potentially contributing to repair and regeneration in kidneys presenting with ischemic or chronic disease. To more efficiently develop cell and tissue engineering technologies for the kidney, we have developed molecular assays to evaluate the acquisition of a pluripotent state associated with stem/progenitor cell phenotype during induction of a regenerative response within the kidneys of rats with chronic kidney disease (CKD) following therapeutic intervention. Intrarenal delivery of selected bioactive renal cells leads to significant upregulation of pluripotency-associated SOX2 mRNA within the diseased kidney tissue from 1 to 24 weeks after treatment. The overall regenerative response index was assessed by quantitative composite expression of CD24, NODAL and LEFTY1 proteins, which were induced within 1 week of cell treatment and peaked at 12 weeks after treatment, reaching statistical significance ($p < 0.05$) compared to untreated CKD controls. Molecular assays that incorporate the assessment of SOX2 and the regenerative response index may prove to be valuable tools for the detection and monitoring of the tissue response after the delivery of regenerative treatments for CKD, thereby significantly shortening the developmental timelines associated with such therapies.

4.1123 Butyrate increases IL-23 production by stimulated dendritic cells

Berndt, B.E., Zhang, M., Owyang, S.Y., Cole, T.S., Wang, T.W., Luther, J., Veniaminova, N.A., Merchant,

J.L., Chen, C-C., Huffnagle, G.B. and Kao, J.Y.
*Am. J. Physiol. Gastrointest. Liver Physiol., **303**, G1384-G1392 (2012)*

The gut microbiota is essential for the maintenance of intestinal immune homeostasis and is responsible for breaking down dietary fiber into short-chain fatty acids (SCFAs). Butyrate, the most abundant bioactive SCFA in the gut, is a histone deacetylase inhibitor (HDACi), a class of drug that has potent immunomodulatory properties. This characteristic of butyrate, along with our previous discovery that conventional dendritic cells (DCs) are required for the development of experimental colitis, led us to speculate that butyrate may modulate DC function to regulate gut mucosal homeostasis. We found that butyrate, in addition to suppressing LPS-induced bone marrow-derived DC maturation and inhibiting DC IL-12 production, significantly induced IL-23 expression. The upregulation of mRNA subunit IL-23p19 at the pretranslational level was consistent with the role of HDACi on the epigenetic modification of gene expression. Furthermore, the mechanism of IL-23p19 upregulation was independent of Stat3 and ZBP89. Coculture of splenocytes with LPS-stimulated DCs pretreated with or without butyrate was performed and showed a significant induction of IL-17 and IL-10. We demonstrated further the effect of butyrate in vivo using dextran sulfate sodium (DSS)-induced colitis and found that the addition of butyrate in the drinking water of mice worsened DSS-colitis. This is in contrast to the daily intraperitoneal butyrate injection of DSS-treated mice, which mildly improved disease severity. Our study highlights a novel effect of butyrate in upregulating IL-23 production of activated DCs and demonstrates a difference in the host response to the oral vs. systemic route of butyrate administration.

4.1124 Characterization of coagulation factor synthesis in nine human primary cell types
Dashty, M:, Akbarkhanzadeh, V., Zeebregts, C.J., Spek, C.A., Sijbrands, E.J., Pepplenbosch, M.P. and Rezaee, F.
*Scientific Reports, **2:787**, DOI: 10.1038/srep00787 (2012)*

The coagulation/fibrinolysis system is essential for wound healing after vascular injury. According to the standard paradigm, the synthesis of most coagulation factors is restricted to liver, platelets and endothelium. We challenged this interpretation by measuring coagulation factors in nine human primary cell types. FX mRNA was expressed by fibroblasts, visceral preadipocytes/adipocytes and hepatocytes, but not in macrophages or other cells. All cells expressed FVIII except endothelial cells. Fibroblasts, endothelial cells and macrophages produced thrombomodulin but not FV. Interestingly, vascular-related cells (platelets/monocytes) that expressed FV did not express FX and *vice versa.* Monocytes expressed FV, FVIII and FXIIIA, which are positive regulators of clot formation, but these cells also contained thrombomodulin, a negative regulator of coagulation. Our data show that the expression of coagulation factors is much more complex than previously thought, and we speculate that this intricate regulation of coagulation factor expression is necessary for correct fine-tuning of fibrinogenesis *versus* fibrinolysis.

4.1125 Suppressors of Cytokine Signaling Promote Fas-Induced Apoptosis through Downregulation of NF-κB and Mitochondrial Bfl-1 in Leukemic T Cells
Oh, J., Kim, S-H., Ahn, S. and Lee, C-E.
*J. Immunol., **189(12)**, 5561-5571 (2012)*

Suppressors of cytokine signaling (SOCS) are known as negative regulators of cytokine- and growth factor–induced signal transduction. Recently they have emerged as multifunctional proteins with regulatory roles in inflammation, autoimmunity, and cancer. We have recently reported that SOCS1 has antiapoptotic functions against the TNF-α– and the hydrogen peroxide–induced T cell apoptosis through the induction of thioredoxin, which protects protein tyrosine phosphatases and attenuates Jaks. In this study, we report that SOCS, on the contrary, promote death receptor Fas-mediated T cell apoptosis. The proapoptotic effect of SOCS1 was manifested with increases in Fas-induced caspase-8 activation, truncated Bid production, and mitochondrial dysfunctions. Both caspase-8 inhibitor c-Flip and mitochondrial antiapoptotic factor Bfl-1 were significantly reduced by SOCS1. These proapoptotic responses were not associated with changes in Jak or p38/Jnk activities but were accompanied with downregulation of NF-κB and NF-κB–dependent reporter gene expression. Indeed, p65 degradation via ubiquitination was accelerated in SOCS1 overexpressing cells, whereas it was attenuated in SOCS1 knockdown cells. With high NF-κB levels, the SOCS1-ablated cells displayed resistance against Fas-induced apoptosis, which was abrogated upon siBfl-1 transfection. The results indicate that the suppression of NF-κB–dependent induction of prosurvival factors, such as Bfl-1 and c-Flip, may serve as a mechanism for SOCS action to promote Fas-mediated T cell apoptosis. SOCS3 exhibited a similar proapoptotic function. Because both SOCS1 and SOCS3 are induced upon TCR stimulation, SOCS would play a role in

activation-induced cell death by sensitizing activated T cells toward Fas-mediated apoptosis to maintain T cell homeostasis.

An effective purification method using large bottles for human pancreatic islet isolation
Shimoda, M., Itoh, T., Iwahashi, S., Takita, M., Sugimoto, K., Kanak, M.A., Chujo, D., Naziruddin, B., Levy, M.F., Grayburn, P.A. and Matsumoto, S.
Islets, ***4(6)****, 1-7 (2012)*

The purification process is one of the most difficult procedures in pancreatic islet isolation. It was demonstrated that the standard purification method using a COBE 2991 cell processor with Ficoll density gradient solution harmed islets mechanically by high shear force. We reported that purification using large bottles with a lower viscosity gradient solution could improve the efficacy of porcine islet purification. In this study, we examined whether the new bottle purification method could improve the purification of human islets. Nine human pancreata from brain-dead donors were used. After pancreas digestion, the digested tissue was divided into three groups. Each group was purified by continuous density gradient using ET-Kyoto and iodixanol gradient solution with either the standard COBE method (COBE group) or the top loading (top group) or bottom loading (bottom group) bottle purification methods. Islet yield, purity, recovery rate after purification, and in vitro and in vivo viability were compared. Islet yield per pancreas weight (IE/g) and the recovery rate in the top group were significantly higher than in the COBE and bottom groups. Furthermore, the average size of purified islets in the top group was significantly larger than in the COBE group, which indicated that the bottle method could reduce the shear force to the islets. In vivo viability was also significantly higher in the top group compared with the COBE group. In conclusion, the top-loading bottle method could improve the quality and quantity of human islets after purification.

α-type-1 polarized dendritic cell-based vaccination in recurrent high-grade glioma: a phase I clinical trial
Akiyama, Y., Oshita, C., Kume, A., Lizuka, A., Miyata, H., Komiyama, M., Ashizawa, T., Yagoto, M., Abe, Y., Mitsuya, K., Watanabe, R., Sugino, T., Yamaguchi, K. and Nakasu, Y.
BMC Cancer, ***12****:623 (2012)*

Background
High-grade gliomas including glioblastoma multiforme (GBM) are among the most malignant and aggressive of tumors, and have a very poor prognosis despite a temozolomide-based intensive treatment. Therefore, a novel therapeutic approach to controlling recurrence is needed. In the present study, we investigated the effect of activated dendritic cell (DC) (α-type-1 polarized DC)-based immunotherapy on high-grade glioma patients with the HLA-A2 or A24 genotype.
Methods
Nine patients with recurrent high-grade gliomas including 7 with GBMs who fulfilled eligibility criteria were enrolled into a phase I study of monocyte-derived DC-based immunotherapy. HLA-genotyping revealed 1 case of HLA-A*0201 and 8 cases of A*2402. Enriched monocytes obtained using OptiPrep™ from leukapheresis products on day1, were incubated with GM-CSF and IL-4 in a closed serum-free system, and activated on day6 with TNF-α, IL-1β, IFN-α, IFN-γ, and poly I/C. After pulsing with a cocktail of 5 synthetic peptides (WT-1, HER2, MAGE-A3, and MAGE-A1 or gp100) restricted to HLA-A2 or A24 and KLH, cells were cryopreserved until used. Thawed DCs were injected intradermally in the posterior neck at a dose per cohort of 1.0, 2.0 and 5.0×10^7/body.
Results
The frequency of $CD14^+$ monocytes increased to 44.6% from 11.9% after gradient centrifugation. After a 7-day-incubation with cytokines, the mean percentage of DCs rated as $lin^-HLA\text{-}DR^+$ in patients was 56.2 ± 19.1%. Most DCs expressed high levels of maturation markers, co-stimulatory molecules and type-1 phenotype ($CD11c^+HLA\text{-}DR^+$) with a DC1/2 ratio of 35.6. The amount of IL-12 produced from activated DCs was 1025 ± 443 pg/ml per 10^5 cells. All 76 DC injections were well tolerated except for transient liver dysfunction with grade II. Six patients showed positive immunological responses to peptides in an ELISPOT assay, and positive skin tests to peptide-pulsed DC and KLH were recognized in 4 cases. The clinical response to DC injections was as follows :1 SD and 8 PD. Interestingly, the SD patient, given 24 DC injections, showed a long-term recurrence-free and immunological positive response period.
Conclusions
These results indicate peptide cocktail-treated activated α-type-1 DC-based immunotherapy to be a potential therapeutic tool against recurrent high-grade glioma with mainly HLA-A*2402.
Trial registration

Current non-randomized investigational trial UMIN-CTR UMIN ID: 000000914.

Isolation of Chlamydia trachomatis and membrane vesicles derived from host and bacteria
Frohlich, K., Hua, Z., Wang, J. and Shen, L.
*J. Microbiol. Methods, **91**, 222-230 (2012)*

The study of intracellular bacteria and nanometer-size membrane vesicles within infected host cells poses an important challenge as it is difficult to identify each distinct population in the context of the complex populations generated from active host-pathogen interactions. Here, suspension cultures of L929 cells infected with the prevalent obligate intracellular bacterium *Chlamydia trachomatis* strain F/Cal-IC-13 are utilized for the large scale preparation and isolation of natural membrane vesicles and bacterial forms. Cell lysis with nitrogen cavitation in combination with differential centrifugation, OptiPrep™ density gradient separation, and immunoenrichment using anti-chlamydial lipopolysaccharide antibodies and MagnaBind beads allows for the isolation of both productive and persistent bacterial forms, as well as membrane vesicles derived from the host and pathogen. We have evaluated these populations by electron microscopy and Western blot analysis for identification of biomarkers. In addition, purified persistent forms of *C. trachomatis* induced by ampicillin display adenosine-5′-triphosphate (ATP) transport activity, suggesting that ampicillin-induced persistent *C. trachomatis* organisms, at least in part, rely upon host ATP as an energy source. Importantly, several chlamydial cytotoxic and/or secreted proteins are demonstrated to be associated with these vesicles, supporting the idea that membrane vesicles are generated by *Chlamydia* as a means of carrying and delivering virulence factors necessary for pathogenesis. The ability to produce large-scale infections and generate distinct bacteria and host-derived populations for biochemical analysis, while reducing the burdens of time and cost have implications in all areas of chlamydiology. These protocols can be applied to other strains of *C. trachomatis* or other intracellular bacteria.

4.1126 Resveratrol mediates anti-atherogenic effects on cholesterol flux in human macrophages and endothelium via PPARγ and adenosine
Voloshyna, I., Hai, O., Littlefield, M:J., Carsons, S. and Reiss, A.
*Eur. J. Pharmacol., **698**, 299-309 (2013)*

Resveratrol is a bioactive molecule used in dietary supplements and herbal medicines and consumed worldwide. Known cardioprotective and anti-inflammatory properties of resveratrol have spurred investigation of the mechanisms involved. The present study explored potential atheroprotective actions of resveratrol on cholesterol metabolism in cells of the arterial wall, including human macrophages and arterial endothelium. Using QRT-PCR and Western blotting techniques, we measured expression of the proteins involved in reverse cholesterol transport (ABCA1, ABCG1 and SR-B1) and the scavenger receptors responsible for uptake of modified cholesterol (CD36, SR-A1 and LOX-1). We analyzed the effect of resveratrol on apoA-1-and HDL-mediated cholesterol efflux in human THP-1 macrophages. The effect of resveratrol on oxLDL internalization and foam cell formation were evaluated using confocal and light microscopy. Our data indicate that resveratrol regulates expression of major proteins involved in cholesterol transport, promotes apoA-1 and HDL-mediated efflux, downregulates oxLDL uptake and diminishes foam cell formation. Mechanistically, resveratrol effects were dependent upon PPAR-γ and adenosine 2A receptor pathways. For the first time we demonstrate that resveratrol regulates expression of the cholesterol metabolizing enzyme cytochrome P450 27-hydroxylase, providing efficient cholesterol elimination via formation of oxysterols. This study establishes that resveratrol attenuates lipid accumulation in cultured human macrophages via effects on cholesterol transport. Further in vivo studies are needed to determine whether resveratrol may be an additional resource available to reduce lipid deposition and atherosclerosis in humans.

4.1127 Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells
Carambia, A., Frenzel, C., Bruns, O.T., Schwinge, D., Reimer, R., Hohenberg, H., Huber, S., Tiegs, G., Schramm, C., Lohse, A.W. and Herkel, J.
*J. Hepatol., **58**, 112-118 (2013)*

Background & Aims
The liver can mitigate the inflammatory activity of infiltrating T cells by mechanisms that are not entirely clear. Here we investigated the role of liver sinusoidal endothelial cells (LSECs) in regulating the activity of inflammatory CD4 T cells.
Methods

Interactions between T helper (Th) 1 or Th17 cells and LSEC were studied by intravital microscopy and by *in vitro* stimulation assays.
Results
Circulating CD4 T cells established lasting and repeated interactions with liver endothelium *in vivo*.

Stimulation of Th1 and Th17 cells by LSEC greatly inhibited their capacity to secrete interferon-γ or interleukin-17 *in vitro*; in contrast, stimulation by dendritic cells (DCs) resulted in considerable secretion of both cytokines. Cytokine release by Th1 or Th17 cells seemed to be actively suppressed by LSEC, as indicated by the inhibition of cytokine secretion even in the presence of Th1- and Th17-promoting DC. This inhibition of CD4 T cell effector function seemed to depend on the dominance of inhibitory over activating co-stimulatory signals on LSEC, since (1) cytokine secretion could be restored by increased CD28 co-activation; (2) LSEC from interleukin-$10^{-/-}$ mice, which manifest increased activating signals, such as MHC II, and decreased inhibitory signals, such as PD-L1, failed to suppress cytokine secretion; and (3) cytokine secretion by Th1 or Th17 cells that lacked PD-1, the ligand for inhibitory PD-L1, could not be suppressed by LSEC.
Conclusions
LSEC inhibit inflammatory cytokine secretion of Th1 and Th17 effector CD4 T cells in dependence of interleukin-10 and PD-1.

4.1128 Macrophage-derived hedgehog ligands promotes fibrogenic and angiogenic responses in human schistosomiasis mansoni
Pereira, T.A., Xie, G., Choi, S.S., Syn, W-K., Voieta, I., Lu, J., Chan, I.S., Swiderska, M., Amaral, K.B., Antunes, C.M., Secor, W.E., Witek, R.P., Lambertucci, J.R., Pereira, F.L. and Diehl, A.M.
Liver Int., ***33(1)***, *149-161 (2013)*

Background
Schistosomiasis mansoni is a major cause of portal fibrosis and portal hypertension. The Hedgehog pathway regulates fibrogenic repair in some types of liver injury.
Aims
Determine if Hedgehog pathway activation occurs during fibrosis progression in schistosomiasis and to determine if macrophage-related mechanisms are involved.
Methods
Immunohistochemistry was used to characterize the cells that generate and respond to Hedgehog ligands in 28 liver biopsies from patients with different grades of schistosomiasis fibrosis staged by ultrasound. Cultured macrophages (RAW264.7 and primary rat Kupffer cells) and primary rat liver sinusoidal endothelial cells (LSEC) were treated with schistosome egg antigen (SEA) and evaluated using qRT-PCR. Inhibition of the Hedgehog pathway was used to investigate its role in alternative activation of macrophages (M2) and vascular tube formation.
Results
Patients with schistosomiasis expressed more ligands (Shh and Ihh) and target genes (Patched and Gli2) than healthy individuals. Activated LSEC and myofibroblasts were Hedgehog responsive [Gli2(+)] and accumulated in parallel with fibrosis stage ($P < 0.05$). Double IHC for Ihh/CD68 showed that Ihh(+) cells were macrophages. *In vitro* studies demonstrated that SEA-stimulated macrophages to express Ihh and Shh mRNA ($P < 0.05$). Conditioned media from such macrophages induced luciferase production by Shh-LightII cells ($P < 0.001$) and Hedgehog inhibitors blocked this effect ($P < 0.001$). SEA-treated macrophages also up-regulated their own expression of M2 markers, and Hh pathway inhibitors abrogated this response ($P < 0.01$). Inhibition of the Hedgehog pathway in LSEC blocked SEA-induced migration and tube formation.
Conclusion
SEA stimulates liver macrophages to produce Hh ligands, which promote alternative activation of macrophages, fibrogenesis and vascular remodelling in schistosomiasis.

4.1129 Changes in the properties of normal human red blood cells during in vivo aging
Franco, R.S., Puchulu-Campanella, M.E., Barber, L.A., Palascak, M.B., Joiner, C.H., Low, P.S. and Cohen, R.M.
Am. J. Hematol., ***88(1)***, *44-51 (2013)*

The changes in red blood cells (RBC) as they age and the mechanisms for their eventual removal have been of interest for many years. Proposed age-related changes include dehydration with increased density and decreased size, increased membrane IgG, loss of membrane phospholipid asymmetry, and decreased

activity of KCl cotransport. The biotin RBC label allows unambiguous identification of older cells and exploration of their properties as they age. Autologous normal human RBC were labeled ex vivo and, after reinfusion, compared with unlabeled RBC throughout their lifespan. RBC density increased with age, with most of the change in the first weeks. Near the end of their lifespan, RBC had increased surface IgG. However, there was no evidence for elevated external phosphatidylserine (PS) even though older RBC had significantly lower activity of aminophospholipid translocase (APLT). KCl cotransport activity persisted well past the reticulocyte stage, but eventually decreased as the RBC became older. These studies place limitations on the use of density fractionation for the study of older human RBC, and do not support loss of phospholipid asymmetry as a mechanism for human RBC senescence. However, increased levels of IgG were associated with older RBC, and may contribute to their removal from the circulation.

4.1130 Hedgehog signalling regulates liver sinusoidal endothelial cell capillarisation
Xie, G., Choi, S.S., Syn, W-K. et al
Gut, ***62***, *299-309 (2013)*

Objective Vascular remodelling during liver damage involves loss of healthy liver sinusoidal endothelial cell (LSEC) phenotype via capillarisation. Hedgehog (Hh) signalling regulates vascular development and increases during liver injury. This study therefore examined its role in capillarisation.
Design Primary LSEC were cultured for 5 days to induce capillarisation. Pharmacological, antibody-mediated and genetic approaches were used to manipulate Hh signalling. Effects on mRNA and protein expression of Hh-regulated genes and capillarisation markers were evaluated by quantitative reverse transcription PCR and immunoblot. Changes in LSEC function were assessed by migration and tube forming assay, and gain/loss of fenestrae was examined by electron microscopy. Mice with acute or chronic liver injury were treated with Hh inhibitors; effects on capillarisation were assessed by immunohistochemistry.
Results Freshly isolated LSEC expressed Hh ligands, Hh receptors and Hh ligand antagonist Hhip. Capillarisation was accompanied by repression of Hhip and increased expression of Hh-regulated genes. Treatment with Hh agonist further induced expression of Hh ligands and Hh-regulated genes, and upregulated capillarisation-associated genes; whereas Hh signalling antagonist or Hh ligand neutralising antibody each repressed expression of Hh target genes and capillarisation markers. LSEC isolated from *Smo*$^{loxP/loxP}$ transgenic mice that had been infected with adenovirus expressing Cre-recombinase to delete Smoothened showed over 75% knockdown of Smoothened. During culture, Smoothened-deficient LSEC had inhibited Hh signalling, less induction of capillarisation-associated genes and retention of fenestrae. In mice with injured livers, inhibiting Hh signalling prevented capillarisation.
Conclusions LSEC produce and respond to Hh ligands, and use Hh signalling to regulate complex phenotypic changes that occur during capillarisation.

4.1131 Activation of toll-like receptor 3 attenuates alcoholic liver injury by stimulating Kupffer cells and stellate cells to produce interleukin-10 in mice
Byun, J-S., Suh, Y-G., Yi, H-S., Lee, Y-S. and Jeong, W-I.
J. Hepatol., ***58***, *342-349 (2013)*

Background & Aims
The important function of toll-like receptor (TLR) 4 in Kupffer cells and hepatic stellate cells (HSCs) has been well documented in alcoholic liver injury. However, little is known about the role of TLR3. Thus, we tested whether TLR3 activation in HSCs and Kupffer cells could attenuate alcoholic liver injury *in vivo*, and investigated its possible mechanism *in vitro*.
Methods
Alcoholic liver injury was achieved by feeding wild type (WT), TLR3 knockout (*TLR3*$^{-/-}$) and interleukin (*IL)-10*$^{-/-}$ mice with high-fat diet plus binge ethanol drinking for 2 weeks. To activate TLR3, polyinosinic–polycytidylic acid (poly I:C) was injected into mice. For *in vitro* studies, HSCs and Kupffer cells were isolated and treated with poly I:C.
Results
In WT mice, poly I:C treatment reduced alcoholic liver injury and fat accumulation by suppressing nuclear factor-κB activation and sterol response element-binding protein 1c expression in the liver. In addition, freshly isolated HSCs and Kupffer cells from poly I:C-treated mice showed enhanced expression of IL-10 compared to controls. Infiltrated macrophage numbers and the expression of tumor necrosis factor-α, monocyte chemoattractant protein-1 and IL-6 on these cells were decreased after poly I:C treatment. *In*

vitro, poly I:C treatment enhanced the expression of IL-10 via a TLR3-dependent mechanism in HSCs and Kupffer cells. Finally, the protective effects of poly I:C on alcoholic liver injury were diminished in *TLR3*$^{-/-}$ and *IL-10*$^{-/-}$ mice.

Conclusions

TLR3 activation ameliorates alcoholic liver injury via the stimulation of IL-10 production in HSCs and Kupffer cells. TLR3 could be a novel therapeutic target for the treatment of alcoholic liver injury.

4.1132 Mutant SOD1G93A triggers mitochondrial fragmentation in spinal cord motor neurons: Neuroprotection by SIRT3 and PGC-1α

Song, W., Song, Y., Kincaid, B., Bossy, B. and Bossy-Wetzel, E.
*Neurobiology of Disease, **51**, 72-81 (2013)*

Mutations in the Cu/Zn Superoxide Dismutase (SOD1) gene cause an inherited form of ALS with upper and lower motor neuron loss. The mechanism underlying mutant SOD1-mediated motor neuron degeneration remains unclear. While defects in mitochondrial dynamics contribute to neurodegeneration, including ALS, previous reports remain conflicted. Here, we report an improved technique to isolate, transfect, and culture rat spinal cord motor neurons. Using this improved system, we demonstrate that mutant SOD1^{G93A} triggers a significant decrease in mitochondrial length and an accumulation of round fragmented mitochondria. The increase of fragmented mitochondria coincides with an arrest in both anterograde and retrograde axonal transport and increased cell death. In addition, mutant SOD1^{G93A} induces a reduction in neurite length and branching that is accompanied with an abnormal accumulation of round mitochondria in growth cones. Furthermore, restoration of the mitochondrial fission and fusion balance by dominant-negative dynamin-related protein 1 (DRP1) expression rescues the mutant SOD1^{G93A}-induced defects in mitochondrial morphology, dynamics, and cell viability. Interestingly, both SIRT3 and PGC-1α protect against mitochondrial fragmentation and neuronal cell death by mutant SOD1^{G93A}. This data suggests that impairment in mitochondrial dynamics participates in ALS and restoring this defect might provide protection against mutant SOD1^{G93A}-induced neuronal injury.

4.1133 IKKβ in Myeloid Cells Controls the Host Response to Lethal and Sublethal Francisella tularensis LVS Infection

Samaniego, S. and Marcu, K.B.
*PloS One, **8(1)**, e54124 (2013)*

Background

The NF-κB activating kinases, IKKα and IKKβ, are key regulators of inflammation and immunity in response to infection by a variety of pathogens. Both IKKα and IKKβ have been reported to modulate either pro- or anti- inflammatory programs, which may be specific to the infectious organism or the target tissue. Here, we analyzed the requirements for the IKKs in myeloid cells *in vivo* in response to *Francisella tularensis* Live Vaccine Strain (*Ft.* LVS) infection.

Methods and Principal Findings

In contrast to prior reports in which conditional deletion of IKKβ in the myeloid lineage promoted survival and conferred resistance to an *in vivo* group B streptococcus infection, we show that mice with a comparable conditional deletion (IKKβ cKO) succumb more rapidly to lethal *Ft.* LVS infection and are unable to control bacterial growth at sublethal doses. Flow cytometry analysis of hepatic non-parenchymal cells from infected mice reveals that IKKβ inhibits M1 classical macrophage activation two days post infection, which has the collateral effect of suppressing IFN-γ^+ CD8$^+$ T cells. Despite this early enhanced inflammation, IKKβ cKO mice are unable to control infection; and this coincides with a shift toward M2a polarized macrophages. In comparison, we find that myeloid IKKα is dispensable for survival and bacterial control. However, both IKKα and IKKβ have effects on hepatic granuloma development. IKKα cKO mice develop fewer, but well-contained granulomas that accumulate excess necrotic cells after 9 days of infection; while IKKβ cKO mice develop numerous micro-granulomas that are less well contained.

Conclusions

Taken together our findings reveal that unlike IKKα, IKKβ has multiple, contrasting roles in this bacterial infection model by acting in an anti-inflammatory capacity at early times towards sublethal *Ft.* LVS infection; but in spite of this, macrophage IKKβ is also a critical effector for host survival and efficient pathogen clearance.

4.1134 Significant Improvement in Islet Yield and Survival With Modified ET-Kyoto Solution: ET-Kyoto/Neutrophil Elastase Inhibitor

Machida, T., Tanemura, M., Ohmura, Y., tanida, T., Wada, H., Kobayashi, S., Marubashi, S., Eguchi, H., Ito, T., Nagano, H., Mori, M., Doki, Y. and Sawas, Y.
*Cell Transplantation, **22**, 159-173 (2013)*

Although islet transplantation can achieve insulin independence in patients with type 1 diabetes, sufficient number of islets derived from two or more donors is usually required to achieve normoglycemia. Activated neutrophils and neutrophil elastase (NE), which is released from these neutrophils, can directly cause injury in islet grafts. We hypothesized that inhibition of NE improves islet isolation and islet allograft survival. We tested our hypothesis by examining the effects of modified ET-Kyoto solution supplemented with sivelestat, a NE inhibitor (S-Kyoto solution), on islet yield and viability in islet isolation and the effect of intraperitoneally injected sivelestat on islet graft survival in a mouse allotransplant model. NE and proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 increased markedly at the end of warm digestion during islet isolation and exhibited direct cytotoxic activity against the islets causing their apoptosis. The use of S-Kyoto solution significantly improved islet yield and viability. Furthermore, treatment with sivelestat resulted in significant prolongation of islet allograft survival in recipient mice. Furthermore, serum levels of IL-6 and TNF-α at 1 and 2 weeks posttransplantation were significantly higher in islet recipients than before transplantation. Our results indicated that NE released from activated neutrophils negatively affects islet survival and that its suppression both in vitro and in vivo improved islet yield and prolonged islet graft survival. The results suggest that inhibition of NE activity could be potentially useful in islet transplantation for patients with type 1 diabetes mellitus.

4.1135 Circulatory Antigen Processing by Mucosal Dendritic Cells Controls CD8+ T Cell Activation

Chang, S-Y., Song, J-H., Guleng, B., Alonso, C., Arihiro, S., Zhao, Y., Chiang, H-S., O'Keeffe, M., Liao, G., Karp, C.L., Kweon, M-N., Sharpe, A.H., Bhan, A., Terhorst, C. and Reinecker, H-C.
*Immunity, **38(1)**, 153-165 (2013)*

Circulatory antigens transit through the small intestine via the fenestrated capillaries in the lamina propria prior to entering into the draining lymphatics. But whether or how this process controls mucosal immune responses remains unknown. Here we demonstrate that dendritic cells (DCs) of the lamina propria can sample and process both circulatory and luminal antigens. Surprisingly, antigen cross-presentation by resident $CX3CR1^+$ DCs induced differentiation of precursor cells into $CD8^+$ T cells that expressed interleukin-10 (IL-10), IL-13, and IL-9 and could migrate into adjacent compartments. We conclude that lamina propria $CX3CR1^+$ DCs facilitate the surveillance of circulatory antigens and act as a conduit for the processing of self- and intestinally absorbed antigens, leading to the induction of $CD8^+$ T cells, that partake in the control of T cell activation during mucosal immune responses.

4.1136 Hepatic Stellate Cells Preferentially Induce $Foxp3^+$ Regulatory T Cells by Production of Retinoic Acid

Dunham, R.M., Thapa, M., Velazquez, V.M., Elrod, E.J., Denning, T.L., Pulendran, B. and Grakoui, A.
*J. Immunol., **190**, 2009-2016 (2013)*

The liver has long been described as immunosuppressive, although the mechanisms underlying this phenomenon are incompletely understood. Hepatic stellate cells (HSCs), a population of liver nonparenchymal cells, are potent producers of the regulatory T cell (Treg)–polarizing molecules TGF-β1 and all-*trans* retinoic acid, particularly during states of inflammation. HSCs are activated during hepatitis C virus infection and may therefore play a role in the enrichment of Tregs during infection. We hypothesized that Ag presentation in the context of HSC activation will induce naive T cells to differentiate into $Foxp3^+$ Tregs. To test this hypothesis, we investigated the molecular interactions between murine HSCs, dendritic cells, and naive $CD4^+$ T cells. We found that HSCs alone do not present Ag to naive $CD4^+$ T cells, but in the presence of dendritic cells and TGF-β1, preferentially induce functional Tregs. This Treg induction was associated with retinoid metabolism by HSCs and was dependent on all-*trans* retinoic acid. Thus, we conclude that HSCs preferentially generate $Foxp3^+$ Tregs and, therefore, may play a role in the tolerogenic nature of the liver.

4.1137 Phagocytosis Is the Main CR3-Mediated Function Affected by the Lupus-Associated Variant of CD11b in Human Myeloid Cells

Fossati-Jimack, L., Ling, G.S., Cortini, A., Szajnaa, M., Malik, T.H., McDonald, J.U., Pickering, M.C.,

Cook, H.T., Taylor, P.R. and Botto, M.
*PloS One, **8(2)**, e57082 (2013)*

The CD11b/CD18 integrin (complement receptor 3, CR3) is a surface receptor on monocytes, neutrophils, macrophages and dendritic cells that plays a crucial role in several immunological processes including leukocyte extravasation and phagocytosis. The minor allele of a non-synonymous CR3 polymorphism (rs1143679, conversation of arginine to histidine at position 77: R77H) represents one of the strongest genetic risk factor in human systemic lupus erythematosus, with heterozygosity (77R/H) being the most common disease associated genotype. Homozygosity for the 77H allele has been reported to reduce adhesion and phagocytosis in human monocytes and monocyte-derived macrophages, respectively, without affecting surface expression of CD11b. Herein we comprehensively assessed the influence of R77H on different CR3-mediated activities in monocytes, neutrophils, macrophages and dendritic cells. R77H did not alter surface expression of CD11b including its active form in any of these cell types. Using two different iC3b-coated targets we found that the uptake by heterozygous 77R/H macrophages, monocytes and neutrophils was significantly reduced compared to 77R/R cells. Allele-specific transduced immortalized macrophage cell lines demonstrated that the minor allele, 77H, was responsible for the impaired phagocytosis. R77H did not affect neutrophil adhesion, neutrophil transmigration *in vivo* or Toll-like receptor 7/8-mediated cytokine release by monocytes or dendritic cells with or without CR3 pre-engagement by iC3b-coated targets. Our findings demonstrate that the reduction in CR3-mediated phagocytosis associated with the 77H CD11b variant is not macrophage-restricted but demonstrable in other CR3-expressing professional phagocytic cells. The association between 77H and susceptibility to systemic lupus erythematosus most likely relates to impaired waste disposal, a key component of lupus pathogenesis

4.1138 Mesothelial cells give rise to hepatic stellate cells and myofibroblasts via mesothelial–mesenchymal transition in liver injury
Li, Y., Wang, J. and Asahina, K.
*PNAS, **110(6)**, 2324-2329 (2013)*

In many organs, myofibroblasts play a major role in the scarring process in response to injury. In liver fibrogenesis, hepatic stellate cells (HSCs) are thought to transdifferentiate into myofibroblasts, but the origins of both HSCs and myofibroblasts remain elusive. In the developing liver, lung, and intestine, mesothelial cells (MCs) differentiate into specific mesenchymal cell types; however, the contribution of this differentiation to organ injury is unknown. In the present study, using mouse models, conditional cell lineage analysis has demonstrated that MCs expressing Wilms tumor 1 give rise to HSCs and myofibroblasts during liver fibrogenesis. Primary MCs, isolated from adult mouse liver using antibodies against glycoprotein M6a, undergo myofibroblastic transdifferentiation. Antagonism of TGF-β signaling suppresses transition of MCs to mesenchymal cells both in vitro and in vivo. These results indicate that MCs undergo mesothelial–mesenchymal transition and participate in liver injury via differentiation to HSCs and myofibroblasts.

4.1139 Langerin negative dendritic cells promote potent CD8+ T-cell priming by skin delivery of live adenovirus vaccine microneedle arrays
Bachy, V., Hervouet, C., Becker, P.D., Chorro, L., Carlin, L.M., Herath, S., Papagatsias, T., Barbaroux, J-B., Oh, S-J., Benlahrech, A., Athanasopolous, T., Dickson, G., Patterson, S., Kwon, S-Y., Geissmann, F. and Klavinskis, L.S.
*PNAS, **110(8)**, 3041-3046 (2013)*

Stabilization of virus protein structure and nucleic acid integrity is challenging yet essential to preserve the transcriptional competence of live recombinant viral vaccine vectors in the absence of a cold chain. When coupled with needle-free skin delivery, such a platform would address an unmet need in global vaccine coverage against HIV and other global pathogens. Herein, we show that a simple dissolvable microneedle array (MA) delivery system preserves the immunogenicity of vaccines encoded by live recombinant human adenovirus type 5 (rAdHu5). Specifically, dried rAdHu5 MA immunization induced $CD8^+$ T-cell expansion and multifunctional cytokine responses equipotent with conventional injectable routes of immunization. Intravital imaging demonstrated MA cargo distributed both in the epidermis and dermis, with acquisition by $CD11c^+$ dendritic cells (DCs) in the dermis. The MA immunizing properties were attributable to $CD11c^+$ $MHCII^{hi}$ $CD8\alpha^{neg}$ epithelial cell adhesion molecule ($EpCAM^{neg}$) $CD11b^+$ langerin (Lang; $CD207)^{neg}$ DCs, but neither Langerhans cells nor $Lang^+$ DCs were required for $CD8^+$ T-cell priming. This study demonstrates an important technical advance for viral vaccine vectors progressing to the clinic

and provides insights into the mechanism of $CD8^+$ T-cell priming by live rAdHu5 MAs.

4.1140 BRG1-mediated immune tolerance: facilitation of Treg activation and partial independence of chromatin remodeling
Chaiyachati, B.H., Jani, A., Wan, Y., Huang, H., Flavell, R. and chi, T.
EMBO J., ***32(3)***, *395-408 (2013)*

Treg activation in response to environmental cues is necessary for regulatory T cells (Tregs) to suppress inflammation, but little is known about the transcription mechanisms controlling Treg activation. We report that despite the known proinflammatory role of the chromatin-remodelling factor BRG1 in CD4 cells, deleting *Brg1* in all αβ T cell lineages led to fatal inflammation, which reflected essential roles of BRG1 in Tregs. *Brg1* deletion impaired Treg activation, concomitant with the onset of the inflammation. Remarkably, as the inflammation progressed, Tregs became increasingly activated, but the activation levels could not catch up with the severity of inflammation. *In vitro* assays indicate that BRG1 regulates a subset of TCR target genes including multiple chemokine receptor genes. Finally, using a method that can create littermates bearing either a tissue-specific point mutation or deletion, we found the BRG1 ATPase activity partially dispensable for BRG1 function. Collectively, these data suggest that BRG1 acts in part via remodelling-independent functions to sensitize Tregs to inflammatory cues, thus allowing Tregs to promptly and effectively suppress autoimmunity.

4.1141 Freezing African Elephant Semen as a New Population Management Tool
Hermes, R., Saragusty, J., Göritz, F., Bartels, P., Potier, R.., Baker, B., Streich, W.J. and Hildebrandt, T.B.
PloS One, ***8(3)***, *e57616 (2013)*

Background
The captive elephant population is not self-sustaining and with a limited number of breeding bulls, its genetic diversity is in decline. One way to overcome this is to import young and healthy animals from the wild. We introduce here a more sustainable alternative method - importation of semen from wild bulls without removing them from their natural habitat. Due to the logistics involved, the only practical option would be to transport cryopreserved sperm. Despite some early reports on African elephant semen cryopreservation, the utility of this new population management tool has not been evaluated.
Methodology/Principal Findings
Semen was collected by electroejaculation from 14 wild African savanna elephant (*Loxodonta africana*) bulls and cryopreserved using the directional freezing technique. Sperm treatments evaluated included the need for centrifugation, the use of hen or quail yolk, the concentration of glycerol (3%, 5% or 7%) in the extender, and maintenance of motility over time after thawing. Our results suggest that dilution in an extender containing hen yolk and 7% glycerol after centrifugation best preserved post-thaw sperm motility when compared to all other treatments ($P \leq 0.012$ for all). Using this approach we were able to achieve after thawing (mean ± SD) 54.6±3.9% motility, 85.3±2.4% acrosome integrity, and 86.8±4.6% normal morphology with no decrease in motility over 1 h incubation at 37°C. Sperm cryopreserved during this study has already lead to a pregnancy of a captive female elephant following artificial insemination.
Conclusions/Significance
With working techniques for artificial insemination and sperm cryopreservation of both African and Asian elephants in hand, population managers can now enrich captive or isolated wild elephant populations without removing valuable individuals from their natural habitat.

4.1142 Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC, and plasma cytokines contributes to oxidative stress in sickle cell disease
George, A., Pushkaran, S., Konstantinidis, D.G., Koochaki, S., Malik, P., Mohandas, N., Zheng, Y., Joiner, C.H. and Kalfa, T.A.
Blood, ***121(11)***, *2099-2107 (2013)*

Chronic inflammation has emerged as an important pathogenic mechanism in sickle cell disease (SCD). One component of this inflammatory response is oxidant stress mediated by reactive oxygen species (ROS) generated by leukocytes, endothelial cells, plasma enzymes, and sickle red blood cells (RBC). Sickle RBC ROS generation has been attributed to sickle hemoglobin auto-oxidation and Fenton chemistry reactions catalyzed by denatured heme moieties bound to the RBC membrane. In this study, we demonstrate that a significant part of ROS production in sickle cells is mediated enzymatically by NADPH oxidase, which is regulated by protein kinase C, Rac GTPase, and intracellular Ca^{2+} signaling within the sickle RBC. Moreover, plasma from patients with SCD and isolated cytokines, such as transforming

growth factor β1 and endothelin-1, enhance RBC NADPH oxidase activity and increase ROS generation. ROS-mediated damage to RBC membrane components is known to contribute to erythrocyte rigidity and fragility in SCD. Erythrocyte ROS generation, hemolysis, vaso-occlusion, and the inflammatory response to tissue damage may therefore act in a positive-feedback loop to drive the pathophysiology of sickle cell disease. These findings suggest a novel pathogenic mechanism in SCD and may offer new therapeutic targets to counteract inflammation and RBC rigidity and fragility in SCD.

4.1143 Monoacylglycerol Lipase Controls Endocannabinoid and Eicosanoid Signaling and Hepatic Injury in Mice

Cao, Z., Mulvihill, M.M., Mukhopadhyay, P., Xu, H., Erdelyi, K., hao, E., Holovac, E., Hasko, G., Cravatt, B.F., Nomura, D.K. and Pacher, P.

Gastroenterology, ***144***, *808-817 (2013)*

Background & Aims

The endocannabinoid and eicosanoid lipid signaling pathways have important roles in inflammatory syndromes. Monoacylglycerol lipase (MAGL) links these pathways, hydrolyzing the endocannabinoid 2-arachidonoylglycerol to generate the arachidonic acid precursor pool for prostaglandin production. We investigated whether blocking MAGL protects against inflammation and damage from hepatic ischemia/reperfusion (I/R) and other insults.

Methods

We analyzed the effects of hepatic I/R in mice given the selective MAGL inhibitor JZL184, in *Mgll*$^{-/-}$ mice, *fatty acid amide hydrolase*$^{-/-}$ mice, and in *cannabinoid receptor type 1*$^{-/-}$ (CB_1-/-) and *cannabinoid receptor type 2*$^{-/-}$ (CB_2-/-). Liver tissues were collected and analyzed, along with cultured hepatocytes and Kupffer cells. We measured endocannabinoids, eicosanoids, and markers of inflammation, oxidative stress, and cell death using molecular biology, biochemistry, and mass spectrometry analyses.

Results

Wild-type mice given JZL184 and *Mgll*$^{-/-}$ mice were protected from hepatic I/R injury by a mechanism that involved increased endocannabinoid signaling via CB_2 and reduced production of eicosanoids in the liver. JZL184 suppressed the inflammation and oxidative stress that mediate hepatic I/R injury. Hepatocytes were the major source of hepatic MAGL activity and endocannabinoid and eicosanoid production. JZL184 also protected from induction of liver injury by D-(+)-galactosamine and lipopolysaccharides or CCl_4.

Conclusions

MAGL modulates hepatic injury via endocannabinoid and eicosanoid signaling; blockade of this pathway protects mice from liver injury. MAGL inhibitors might be developed to treat conditions that expose the liver to oxidative stress and inflammatory damage.

4.1144 Secretory Leukocyte Protease Inhibitor Reverses Inhibition by CNS Myelin, Promotes Regeneration in the Optic Nerve, and Suppresses Expression of the Transforming Growth Factor-β Signaling Protein Smad2

Hannila, S.S., Siddiq, M.M., Carmel, J.B., Hou, J., Chaudhry, N., Bradley, P.M.J., Hillaire, M., Richman, E.L., Hart, R.P. and Filbin, M.T.

J. Neurosci., ***33(12)***, *5138-5151 (2013)*

After CNS injury, axonal regeneration is limited by myelin-associated inhibitors; however, this can be overcome through elevation of intracellular cyclic AMP (cAMP), as occurs with conditioning lesions of the sciatic nerve. This study reports that expression of secretory leukocyte protease inhibitor (SLPI) is strongly upregulated in response to elevation of cAMP. We also show that SLPI can overcome inhibition by CNS myelin and significantly enhance regeneration of transected retinal ganglion cell axons in rats. Furthermore, regeneration of dorsal column axons does not occur after a conditioning lesion in SLPI null mutant mice, indicating that expression of SLPI is required for the conditioning lesion effect. Mechanistically, we demonstrate that SLPI localizes to the nuclei of neurons, binds to the Smad2 promoter, and reduces levels of Smad2 protein. Adenoviral overexpression of Smad2 also blocked SLPI-induced axonal regeneration. SLPI and Smad2 may therefore represent new targets for therapeutic intervention in CNS injury.

4.1145 Regulation of ERα Protein Expression by 17β-Estradiol in Cultured Neurons of Hypothalamic Ventromedial Nucleus

Mallikov, V. and Madeira, M.D.

*Neurochem. Res., **38**, 82-89 (2013)*

The activation of the subtype α of estrogen receptors (ERα) in the hypothalamic ventromedial nucleus (VMNvl) is required to stimulate female sexual receptivity. Moreover, the hormone was found to govern the expression of the receptor. Its removal due to ovariectomy and subsequent substitution suggest that the hormone down-regulates the expression of ERα. In contrast, in normally cycling animals the expression of the receptor peaks at proestrus, the phase of highest concentration of 17β-estradiol in estrous cycle. Therefore, in this study we examined the influence of the hormone on ERα expression in primary dissociated cultures of neurons isolated from the VMNvl of young adult female rats. Measurements of ERα immunofluorescence revealed that both supraphysiological and physiological concentrations of 17β-estradiol increase the expression of ERα. Analyses with selective agonists showed that both nuclear ERs are able to mediate the action of the hormone. However, the activation of ERα had a stronger effect on the expression of its own receptor than the activation of ERβ. Simultaneous activation of both receptors attenuated the influence of ERα alone. Physiological concentrations of progesterone were found to revoke the effect of 17β-estradiol, whereas the expression of ERα is up-regulated by progesterone alone. These data indicate that the expression of ERα in VMNvl neurons is under the control of both types of nuclear ERs and, in addition, progesterone receptors (PRs). The particular contribution of the receptors is dependent on their level of expression and the hormonal context. In neurons expressing high quantity of ERα, ERβ attenuates the overall expression of the receptor, whereas in cells containing mostly ERβ it contributes to the up-regulation of ERα synthesis. Simultaneous activation of ERs and PRs reverses the influences of the receptors due to inter-inhibition of their transcriptional activities.

4.1146 Reciprocal Induction Between α-Synuclein and β-Amyloid in Adult Rat Neurons
Majd, S., Chegini, F., Chataway, T., Zhou, X-F. and Gai, W.
*Neurotox. Res., **23**, 69-78 (2013)*

In spite of definite roles for β-amyloid (Aβ) in familial Alzheimer's disease (AD), the cause of sporadic AD remains unknown. Amyloid senile plaques and Lewy body pathology frequently coexist in neocortical and hippocampal regions of AD and Parkinson's diseases. However, the relationship between Aβ and α-synuclein (α-Syn), the principle components in the pathological structures, in neuronal toxicity and the mechanisms of their interaction are not well studied. As Aβ and α-Syn accumulate in aging patients, the biological functions and toxicity of these polypeptides in the aging brain may be different from those in young brain. We examined the neurotoxicity influences of Aβ1-42 or α-Syn on mature neurons and the effects of Aβ1-42 or α-Syn on the production of endogenous α-Syn or Aβ1-40 reciprocally using a model of culture enriched with primary neurons from the hippocampus of adult rats. Treatment of neurons with high concentrations of Aβ1-42 or α-Syn caused significant apoptosis of neurons. Following Aβ1-42 treatment at sub apoptotic concentrations, both intra- and extra-cellular α-Syn levels were significantly increased. Reciprocally, the non-toxic levels of α-Syn treatment also increased intra- and extra-cellular Aβ1-40 levels. The phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002, suppressed α-Syn-induced Aβ1-40 elevation, as well as Aβ1-42-induced α-Syn elevation. Thus, high concentrations of Aβ1-42 and α-Syn exert toxic effects on mature neurons; however, non-toxic concentration treatment of these polypeptides induced the production of each other reciprocally with possible involvement of PI3K pathway.

4.1147 Isolation, Characterization, and Transplantation of Adult Liver Progenitor Cells
Yovchev, M.I., Dabeva, M.D. and Oertel, M.
*Methods in Mol. Biol., **976**, 37-51 (2013)*

Many chronic liver diseases are life-threatening. When the liver loses the ability to repair itself the only treatment currently available is liver transplant. However, there are not enough donors to treat all the patients. This requires the search of alternative therapies utilizing stem and progenitor cells for treatment of these patients and restoration of their normal liver function.
Hepatic progenitor cells can be isolated from livers at different developmental stages including adult liver. In the adult rat liver, there is clear evidence that progenitor cells (also called "oval cells") derive from precursors in the canals of Herring that are capable to differentiate into hepatocytes and bile duct cells. In experimental models, hepatic progenitor cells can be isolated and propagated in vitro and used for restoration of the diseased liver. The first step in utilization of progenitor cells is their identification in the liver, isolation of purified progenitor cell fractions, which are subsequently transplanted in the diseased liver for evaluation of liver repopulation by transplanted cells, and evaluation their potentials for clinical application.
The present protocol describes the isolation of non-parenchymal cells (NPCs) from wt $DPPIV^+$ F344 rats,

followed by purification of "oval cells", immunohistochemical staining techniques to characterize these cells, their transplantation into retrorsine-treated mutant $DPPIV^{-}$ rats, as well as the enzyme histochemical staining for DPPIV to detect transplanted cells in the host liver.

4.1148 Isolation of Urothelial Cells from Bladder Tissue
Sangha, N.
Methods in Mol. Biol., ***1001***, *21-33 (2013)*

Presented below is a methodology for the isolation, expansion, and maintenance of urothelial cells derived from human bladder. Such bladder-derived urothelial cells, taken together with bladder or alternately sourced smooth muscle cells, may be complexed with an appropriately shaped biodegradable scaffold to create regenerative constructs capable of seeding formation of new bladder or bladder-like neo-organs upon implantation in human cystectomy patients.

4.1149 Ex Vivo Culture and Separation of Functional Renal Cells
Bruce, A.T., Guthrie, K.I. and Kelley, R.
Methods in Mol. Biol., ***1001***, *53-64 (2013)*

The following methods outline the procedures for isolating primary renal cells from kidney tissue via enzymatic digestion, followed by their culture, harvest, and then fractionation of renal subpopulations from primary culture. The current methods describe procedures to sub-fractionate biologically active cells that have been used to treat and stabilize renal function in models of chronic kidney disease (Kelley et al. Am J Physiol Renal Physiol 299(5):F1026–F1039, 2010).

4.1150 Formulation of Selected Renal Cells for Implantation into a Kidney
Halberstadt, C., Robbins, N., McCoy, D.W., Guthrie, K.I., Bruce, A.T., Knight, T.A. and Payne, R.G.
Methods in Mol. Biol., ***1001***, *279-287 (2013)*

Delivery of cells to organs has primarily relied on formulating the cells in a nonviscous liquid carrier. We have developed a methodology to isolate selected renal cells (SRC) that have provided functional stability to damaged kidneys in preclinical models (Kelley et al. Poster presentation at 71st scientific sessions of American diabetes association, 2011; Kelley et al. Oral presentation given at Tissue Engineering and Regenerative Medicine International Society (TERMIS)—North America annual conference, 2010; Presnell et al. Tissue Eng Part C Methods 17:261–273, 2011; Kelley et al. Am J Physiol Renal Physiol 299:F1026–F1039, 2010). In order to facilitate SRC injection into the kidney of patients who have chronic kidney disease, we have developed a strategy to immobilize the cells in a hydrogel matrix. This hydrogel (gelatin) supports cells by maintaining them in a three-dimensional state during storage and shipment (both at cold temperatures) while facilitating the delivery of cells by liquefying when engrafting into the kidney. This chapter will define a method for the formulation of the kidney epithelial cells within a hydrogel.

4.1151 All-trans-retinoic acid ameliorates experimental allergic encephalomyelitis by affecting dendritic cell and monocyte development
Zhan, X-X., Liu, Y., Yang, J-F., Wang, G-Y., Mu, L., Zhang, T-S., Xie, X-L., Wang, J-H., Liu, Y-M., Kong, Q-F., Li, H-L. and Sun, B.
Immunology, ***138(4)***, *333-345 (2013)*

Experimental allergic encephalomyelitis (EAE) can be induced in animal models by injecting the $MOG_{35–55}$ peptide subcutaneously. Dendritic cells (DCs) that are located at the immunization site phagocytose the $MOG_{35–55}$ peptide. These DCs mature and migrate into the nearest draining lymph nodes (dLNs), then present antigen, resulting in the activation of naive T cells. T helper type 1 (Th1) and Th17 cells are the primary cells involved in EAE progression. All-*trans*-retinoic acid (AT-RA) has been shown to have beneficial effects on EAE progression; however, whether AT-RA influences DC maturation or mediates other functions is unclear. In the present study, we showed that AT-RA led to the down-regulation of MHC class II, CD80 (B7-1) and CD86 (B7-2) expressed on the surface of DCs that were isolated from dLNs or spleen 3 days post-immunization in an EAE model. Changes to DC function influenced Th1/Th17 subset polarization. Furthermore, the number of $CD44^{+}$ monocytes (which might trigger EAE progression) was also significantly decreased in dLNs, spleen, subarachnoid space and the spinal cord parenchyma after AT-RA treatment. These findings are the first to demonstrate that AT-RA impairs the antigen-presenting capacity of DCs, leading to down-regulation of pathogenic Th1 and Th17 inflammatory cell responses and reducing EAE severity.

4.1152 Droplets as Reaction Compartments for Protein Nanotechnology
Devenish, S.R.A., Kaltenbach, M., Fishlechner, M. and Hollfelder, F.
*Methods in Mol. Biol., **996**, 269-286 (2013)*

Extreme miniaturization of biological and chemical reactions in pico- to nanoliter microdroplets is emerging as an experimental paradigm that enables more experiments to be carried out with much lower sample consumption, paving the way for high-throughput experiments. This review provides the protein scientist with an experimental framework for (a) formation of polydisperse droplets by emulsification or, alternatively, of monodisperse droplets using microfluidic devices; (b) construction of experimental rigs and microfluidic chips for this purpose; and (c) handling and analysis of droplets.

4.1153 Platelets Recognize Brain-Specific Glycolipid Structures, Respond to Neurovascular Damage and Promote Neuroinflammation
Sotnikov, I., Veremeyko, T., Starossom, S.C., Barteneva, N., Weiner, H.L. and Ponomarev, E.D.
*PloS One, **8(3)**, e58979 (2013)*

Platelets respond to vascular damage and contribute to inflammation, but their role in the neurodegenerative diseases is unknown. We found that the systemic administration of brain lipid rafts induced a massive platelet activation and degranulation resulting in a life-threatening anaphylactic-like response in mice. Platelets were engaged by the sialated glycosphingolipids (gangliosides) integrated in the rigid structures of astroglial and neuronal lipid rafts. The brain-abundant gangliosides GT1b and GQ1b were specifically recognized by the platelets and this recognition involved multiple receptors with P-selectin (CD62P) playing the central role. During the neuroinflammation, platelets accumulated in the central nervous system parenchyma, acquired an activated phenotype and secreted proinflammatory factors, thereby triggering immune response cascades. This study determines a new role of platelets which directly recognize a neuronal damage and communicate with the cells of the immune system in the pathogenesis of neurodegenerative diseases

4.1154 Trolox contributes to Nrf2-mediated protection of human and murine primary alveolar type II cells from injury by cigarette smoke
Messier, E.M., Bahmed, K., Tuder, R.M., Chu, H.W., Bowler, R.P. and Kosmider, B.
*Cell Death and Disease, **4**, e573 (2013)*

Cigarette smoke (CS) is a main risk factor for chronic obstructive pulmonary disease (COPD). Oxidative stress induced by CS causes DNA and lung damage. Oxidant/antioxidant imbalance occurs in the distal air spaces of smokers and in patients with COPD. We studied the effect of oxidative stress generated by CS both *in vivo* and *in vitro* on murine primary alveolar type II (ATII) cells isolated from nuclear erythroid 2-related factor-2 $(Nrf2)^{-/-}$ mice. We determined human primary ATII cell injury by CS *in vitro*and analyzed ATII cells isolated from smoker and non-smoker lung donors*ex vivo*. We also studied whether trolox (water-soluble derivative of vitamin E) could protect murine and human ATII cells against CS-induced DNA damage and/or decrease injury. We analyzed oxidative stress by 4-hydroxynonenal expression, reactive oxygen species (ROS) generation by Amplex Red Hydrogen Peroxide Assay, Nrf2, heme oxygenase 1, p53 and P53-binding protein 1 (53BP1) expression by immonoblotting, Nrf2 nuclear translocation, Nrf2 and p53 DNA-binding activities, apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay and cytokine production by ELISA. We found that ATII cells isolated from $Nrf2^{-/-}$mice are more susceptible to CS-induced oxidative DNA damage mediated by p53/53BP1 both *in vivo* and *in vitro* compared with wild-type mice. Therefore, Nrf2 activation is a key factor to protect ATII cells against injury by CS. Moreover, trolox abolished human ATII cell injury and decreased DNA damage induced by CS *in vitro*. Furthermore, we found higher inflammation and p53 mRNA expression by RT-PCR in ATII cells isolated from smoker lung donors in comparison with non-smokers *ex vivo*. Our results indicate that the Nrf2 and p53 cross talk in ATII cells affect the susceptibility of these cells to injury by CS. Trolox can protect against oxidative stress, genotoxicity and inflammation induced by CS through ROS scavenging mechanism, and serve as a potential antioxidant prevention strategy against oxidative injury of ATII cells in CS-related lung diseases.

4.1155 Preparation of Kupffer cell enriched non-parenchymal liver cells with high yield and reduced damage of surface markers by a modified method for flow cytometry
Xu, F., Zhen, P., Zheng, Y., Lljuan, F., Aiting, Y., Min, C., Hong, Y. and Jidong, J.
*Cell Biol. Int., **37**, 284-291 (2013)*

The aim of this study was to optimise a collagenase perfusion protocol for the isolation of a liver non-parenchymal cell (NPC) suspension enriched for Kupffer cells that reduced damage to F4/80 antigen cell surface expression to allow analysis by flow cytometry.
Kupffer cell-enriched liver NPCs were isolated from C57BL/6 mice using different protocols. Flow cytometry was used to examine the effect of collagenase digestion on F4/80 expression on Kupffer cells, and results were represented by the percentage of F4/80 positive cells and by the F4/80 mean fluorescence intensity (MFI). The perfusion temperature, concentration of collagenase solution and total dosage of collagenase for liver perfusion influenced the effect of collagenase perfusion on the expression of F4/80 antigen on Kupffer cells. Collagenase perfusion at 28°C resulted in an increased percentage of F4/80 positive cells ($P = 0.001$) and MFI ($P = 0.005$) compared with 37°C. Perfusion with a total dose of 1.0 g/kg BW collagenase (using a 0.75 mg/mL solution) resulted in the highest percentage of F4/80 positive cells ($P = 0.001$) compared with 0.8 g/kg BW and 1.2 g/kg BW collagenase. Isolation of cells using the modified protocol resulted in a higher percentage of Kupffer cells ($P < 0.001$) and a higher MFI of F4/80 antigen ($P < 0.001$) compared with the common protocol.

4.1156 Characterization of the Two Intra-Individual Sequence Variants in the 18S rRNA Gene in the Plant Parasitic Nematode, Rotylenchulus reniformis
Nyaku, S.T., Sripathi, V.R., Kantety, R.V., Gu, Y.Q., Lawrence, K. and Sharma, G.C.
*PloS One, **8(4)**, e60891(2013)*

The *18S* rRNA gene is fundamental to cellular and organismal protein synthesis and because of its stable persistence through generations it is also used in phylogenetic analysis among taxa. Sequence variation in this gene within a single species is rare, but it has been observed in few metazoan organisms. More frequently it has mostly been reported in the non-transcribed spacer region. Here, we have identified two sequence variants within the near full coding region of *18S* rRNA gene from a single reniform nematode (RN) *Rotylenchulus reniformis* labeled as reniform nematode variant 1 (RN_VAR1) and variant 2 (RN_VAR2). All sequences from three of the four isolates had both RN variants in their sequences; however, isolate 13B had only RN variant 2 sequence. Specific variable base sites (96 or 5.5%) were found within the *18S* rRNA gene that can clearly distinguish the two *18S* rDNA variants of RN, in 11 (25.0%) and 33 (75.0%) of the 44 RN clones, for RN_VAR1 and RN_VAR2, respectively. Neighbor-joining trees show that the RN_VAR1 is very similar to the previously existing *R. reniformis* sequence in GenBank, while the RN_VAR2 sequence is more divergent. This is the first report of the identification of two major variants of the *18S* rRNA gene in the same single RN, and documents the specific base variation between the two variants, and hypothesizes on simultaneous co-existence of these two variants for this gene.

4.1157 Overexpression of human mutated G93A SOD1 changes dynamics of the ER mitochondria calcium cycle specifically in mouse embryonic motor neurons
Lautenschläger, J., Prell, T., Ruhmer, J., Weidemann, L., Witte, O.W. and Grosskreutz, J.
*Exp. Neurol., **247**, 91-100 (2013)*

Motor neurons vulnerable to the rapidly progressive deadly neurodegenerative disease amyotrophic lateral sclerosis (ALS) inherently express low amounts of calcium binding proteins (CaBP), likely to allow physiological motor neuron firing frequency modulation. At the same time motor neurons are susceptible to AMPA receptor mediated excitotoxicity and internal calcium deregulation which is not fully understood. We analysed ER mitochondria calcium cycle (ERMCC) dynamics with subsecond resolution in G93A hSOD1 overexpressing motor neurons as a model of ALS using fluorescent calcium imaging. When comparing vulnerable motor neurons and non-motor neurons from G93A hSOD1 mice and their non-transgenic littermates, we found a decelerated cytosolic calcium clearance in the presence of G93A hSOD1. While both non-transgenic as well as G93A hSOD1 motor neurons displayed large mitochondrial calcium uptake by the mitochondrial uniporter (mUP), the mitochondrial calcium extrusion system was altered in the presence of G93A hSOD1. In addition, ER calcium uptake by the sarco-/endoplasmic reticulum ATPase (SERCA) was increased in G93A hSOD1 motor neurons. In survival assays, blocking the mitochondrial sodium calcium exchanger (mNCE) by CGP37157 as well as inhibiting SERCA by cyclopiazonic acid showed protective effects against kainate induced excitotoxicity. Thus, our study shows for the first time that the functional consequence of G93A hSOD1 overexpression in intact motor neurons is indeed a disturbance of the ER mitochondria calcium cycle, and identified two promising targets for therapeutic intervention in the pathology of ALS.

4.1158 Up-regulation of immunoglobulin G gene expression in the hippocampus of rats subjected to acute immobilization stress

Wang, S., Huang, G., Wang, Y., Huang, Y., Lin, S. and Gu, J.
*J. Neuroimmunol.,**258**, 1-9 (2013)*

Immunoglobulin G (IgG) is thought to be produced by matured B lymphocytes, however, it was recently found to be synthesized in neurons of the brain, especially showing higher expression level in the hippocampus. To study the possible effects of IgG in the hippocampus, we examined IgG protein and mRNA expressions in rat hippocampal neurons with immunohistochemistry, immunofluorescence, in situ hybridization and laser microdissection-assisted RT-PCR. Increased IgG expressions at both protein and mRNA levels were detected in the hippocampus of an acute immobilization stress model of rat. No change was observed in the cortex or the thalamus. Furthermore, the microtubule-associated protein 2 (MAP2) and β III tubulin proteins did not show significant changes. Based on these findings, we hypothesize that hippocampal IgG may play a key role in adverse circumstances such as stress. The finding of increased IgG expression in the hippocampus following stress may also provide possibilities for developing antidepressant medication.

4.1159 Histone Deacetylase Inhibitors (HDACis) That Release the Positive Transcription Elongation Factor b (P-TEFb) from Its Inhibitory Complex Also Activate HIV Transcription

Bartholomeeusen, K., Fujinaga, K., Xiang, Y. and Peterlin, B.M.
*J. Biol. Chem., **288(20)**, 14400-14407 (2013)*

Numerous studies have looked at the effects of histone deacetylase inhibitors (HDACis) on HIV reactivation in established transformed cell lines and primary $CD4^+$ T cells. However, their findings remain confusing, and differences between effects of class I- and class II-specific HDACis persist. Because no clear picture emerged, we decided to determine how HDACis reactivate HIV in transformed cell lines and primary cells. We found that neither histone H3 nor tubulin acetylation correlated with HIV reactivation in Jurkat and HeLa cells. Rather, HDACis that could reactivate HIV in chromatin or on episomal plasmids also released free positive transcription elongation factor b (P-TEFb) from its inhibitory 7SK snRNP. In resting primary $CD4^+$ T cells, where levels of P-TEFb are vanishingly low, the most potent HDACi, suberoylanilide hydroxyamic acid (SAHA), had minimal effects. In contrast, when these cells were treated with a PKC agonist, bryostatin 1, which increased levels of P-TEFb, then SAHA once again reactivated HIV. We conclude that HDACis, which can reactivate HIV, work via the release of free P-TEFb from the 7SK snRNP.

4.1160 Comparison of cryoprotective effects of iodixanol, trehalose and cysteamine on ram semen

Cirit, U., Bagis, H., Demir, K., Agca, C., Pabuccuoglu, S., Varisli, Ö., Clifford-Rathert, C. and Agca, Y.
*Animal Reprod. Science, **139**, 38-44 (2013)*

This study was conducted to improve cryosurvival of electroejaculated (EE) ram semen in the presence of iodixanol (OptiPrep™), trehalose or cysteamine. A tris-based extender was used to prepare 12 extenders containing OptiPrep™ (Op), trehalose (Tr) or cysteamine (Cy) alone, or different combinations of these compounds. Extenders were designated as follows: Tris (control), Op1.25 (1.25% Op, v/v), Op2.5 (2.5% Op, v/v), Op5 (5% Op, v/v), Tr50 (50 mM Tr), Tr100 (100 mM Tr), Cy (5 mM Cy), OpTr (2.5% Op and 100 mM Tr), OpCy (2.5% Op and 5 mM Cy), TrCy (100 mM Tr and 5 mM Cy), OpTrCy1 (2.5% Op, 100 mM Tr and 5 mM Cy) and OpTrCy2 (1.25% Op, 50 mM Tr and 2.5 mM Cy).
A two-step dilution was used and glycerol was added at 5 °C in the second step. Diluted samples were equilibrated for 1 h, loaded in 0.25 mL straws and frozen in a programmable freezing machine. Supplementation of 5% OptiPrep™ significantly protected post-thaw progressive motility, membrane integrity, acrosomal integrity and morphological damages. Trehalose supplementation protected membrane integrity of ram sperm; however, it did not help post-thaw motility and morphology. Supplementation of 5 mM cysteamine had detrimental effect on cryosurvival of EE ram semen. These results demonstrate that the supplementation of iodixanol increases the cryosurvival of EE ram semen in a dose-dependent manner.

4.1161 Nilotinib induces apoptosis and autophagic cell death of activated hepatic stellate cells via inhibition of histone deacetylases

Shaker, M.E., Ghani, A., Shiha, G.E., Ibrahim, T.M. and Mehal, W.Z.
*Biochim. Biophys. Acta, **1833**, 1992-2003 (2013)*

Increasing hepatic stellate cell (HSC) death is a very attractive approach for limiting liver fibrosis. Tyrosine kinase inhibitors have been shown to have anti-fibrotic properties, but the mechanisms are poorly understood. Here, we identified the mechanism of action of the second-generation tyrosine kinase inhibitor nilotinib in inducing HSC death. Human HSC line (LX-2) and rat HSCs were treated with nilotinib and its predecessor, imatinib, in the absence or presence of various blockers, known to interfere with death signaling pathways. Nilotinib, but not imatinib, induced progressive cell death of activated, but not quiescent, HSCs in a dose-dependent manner. Activated HSCs died through apoptosis, as denoted by increased DNA fragmentation and caspase activation, and through autophagy, as indicated by the accumulation of autophagic markers, light chain (LC)3A-II and LC3B-II. Although inhibition of caspases with Z-VAD-FMK suppressed nilotinib-induced HSCs' apoptosis, there was no increase in HSCs' survival, because autophagy was exacerbated. However, blocking the mitochondrial permeability transition pore (mPTP) opening with cyclosporin A completely abolished both apoptosis and autophagy due to nilotinib. Moreover, nilotinib treatment decreased the protein expression of histone deacetylases 1, 2 and 4. Interestingly, pretreament with C646, a selective p300/CBP histone acetyl transferase inhibitor, resulted in diverting nilotinib-induced apoptosis and autophagy towards necrosis. In conclusion, the identification of mPTP as a target of nilotinib in activated HSCs suggests coordination with histone deacetylases inhibition to induce apoptosis and autophagy. Thus, our study provides novel insights into the anti-fibrotic effects of nilotinib.

4.1162 Aging Exacerbates Microvascular Endothelial Damage Induced by Circulating Factors Present in the Serum of Septic Patients

Tucsek, Z., Gautam, T., Sonntag, W.E., Toth, P., Saito, H., Salomao, R., Szabo, C., Csiszar, A. and Ungvari, Z.

J. Geronol. A Biol. Sci. Med. Sci., ***68(6)****, 652-660 (2013)*

The elderly patients show a significantly elevated mortality rate during sepsis than younger patients, due to their higher propensity to microvascular dysfunction and consequential multiorgan failure. We tested whether aging renders vascular endothelial cells more susceptible to damage induced by inflammatory factors present in the circulation during sepsis. Primary microvascular endothelial cells derived from young (3 months) and aged (24 months) Fischer 344 × Brown Norway rats were treated with sera obtained from sepsis patients and healthy controls. Oxidative stress (MitoSox fluorescence), death receptor activation (caspase 8 activity), and apoptotic cell death (caspase 3 activity) induced by treatment with septic sera were exacerbated in aged endothelial cells as compared with responses obtained in young cells. Induction of heme oxygenase-1 and thrombomodulin in response to treatment with septic sera was impaired in aged endothelial cells. Treatment with septic sera elicited greater increases in tumor necrosis factor-α expression in aged endothelial cells, as compared with young cells, whereas induction of inducible nitric oxide synthase, intercellular adhesion molecule-1, and vascular cell adhesion molecule did not differ between the two groups. Collectively, aging increases sensitivity of microvascular endothelial cells (MVECs) to oxidative stress and cellular damage induced by inflammatory factors present in the circulation during septicemia. We hypothesize that these responses may contribute to the increased vulnerability of elderly patients to multiorgan failure associated with sepsis.

4.1163 Treatment with the cytochrome P450 ω-hydroxylase inhibitor HET0016 attenuates cerebrovascular inflammation, oxidative stress and improves vasomotor function in spontaneously hypertensive rats

Toth, P., Csiszar, A., Sosnowska, D., Tucsek, Z., Cseplo, P., Springo, Z., Tarantini, S., Sonntag, W.E., Ungvari, Z. and Koller, A.

Br. J. Pharmacol., ***168(8)****, 1878-1888 (2013)*

Background and Purpose

Hypertension increases cerebrovascular oxidative stress and inflammation and impairs vasomotor function. These pathological alterations lead to dysregulation of cerebral blood flow and exacerbate atherogenesis, increasing the morbidity of ischaemic cerebrovascular diseases and promoting vascular cognitive impairment. We aimed to test the hypothesis that increased production of the arachidonic acid metabolite 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) contributes to hypertension-induced cerebrovascular alterations.

Experimental Approach

We treated male spontaneously hypertensive rats (SHR) with HET0016 (N-hydroxy-N′-(4-butyl-2-methylphenyl)-formamidine), an inhibitor of 20-HETE synthesis. In middle cerebral arteries (MCAs) of SHRs, we focused on vasomotor responses and end points that are highly relevant for cellular reactive oxygen species (ROS) production, inflammatory cytokine expression and NF-κB activation.

Key Results
SHRs treated with HET0016 remained hypertensive (SHR + HET0016: 149 ± 8 mmHg, Wistar-Kyoto rat: 115 ± 4 mmHg; $P < 0.05$.), although their systolic blood pressure was decreased compared to untreated SHRs (191 ± 6 mmHg). In MCAs of SHRs, flow-induced constriction was increased, whereas ACh- and ATP-induced dilations were impaired. This functional impairment was reversed by treatment with HET0016. Treatment with HET0016 also significantly decreased oxidative stress in MCAs of SHRs (as shown by dihydroethidium staining and analysis of vascular 5-nitrotyrosine, 4-hydroxynonenal and carbonyl content) and inhibited cerebrovascular inflammation (shown by the reduced mRNA expression of TNFα, IL-1β and IL-6). Treatment of SHRs with HET0016 also attenuated vascular NF-κB activation. *In vitro* treatment with 20-HETE significantly increased vascular production of ROS and promoted NF-κB activation in cultured cerebromicrovascular endothelial cells.
Conclusions and Implications
Taken together, treatment with HET0016 confers anti-oxidative and anti-inflammatory effects in the cerebral arteries of SHRs by disrupting 20-HETE-mediated autocrine/paracrine signalling pathways in the vascular wall. It is likely that HET0016-induced decreases in blood pressure also potentiate the cerebrovascular protective effects of the drug.

4.1164 Trypomastigotes and amastigotes of Trypanosoma cruzi induce apoptosis and STAT3 activation in cardiomyocytes in vitro
Stahl, P., Ruppert, V., Meyer, T., Schmidt, J., Campos, M.A., Gazzinelli, R.T., Maisch, B., Schwarz, R.T. and Debierre-Grockiego, F.
Apoptosis, ***18(6)***, *653-663 (2013)*

The haemoflagellate *Trypanosoma cruzi* is the causative agent of Chagas' disease that occurs in approximately 8 million people in Latin America. Patients infected with *T. cruzi* frequently suffer of cardiomegaly and may die of myocardial failure. Here we show that *T. cruzi* trypomastigotes (extracellular form) increased in vitro apoptosis of rat cardiomyocytes. Additionally, we demonstrated that amastigotes (intracellular form), for which a method for purification was established, were also able to induce cardiomyocyte apoptosis. Increase of apoptosis was associated with up-regulation of the apoptotic gene *bax* by trypomastigotes, while expression of the anti-apoptotic gene *bcl-2* was down-regulated by amastigotes. The transcription factor STAT3 but not STAT1 was activated in cardiomyocytes by trypomastigotes. In addition, *tlr7* gene expression was up-regulated in cardiomyocytes incubated with trypomastigotes, suggesting that this Toll-like receptor is involved in the intracellular recognition after host cell invasion by *T. cruzi*. Glycosylphosphatidylinositols purified from trypomastigotes did not induce cardiomyocyte apoptosis and STAT activation but down-regulated *tlr7* gene expression. In conclusion, cardiomyopathy observed in Chagas' disease might be in part due to apoptosis of cardiomyocytes induced directly by the parasite.

4.1165 Antifibrotic Effects of a Recombinant Adeno-Associated Virus Carrying Small Interfering RNA Targeting TIMP-1 in Rat Liver Fibrosis
Cong, M., Liu, T., Wang, P., Fan, X., yang, A., Bai, Y., Peng, Z., Wu, P., Tong, X., Chen, J., Li, H., Cong, R., Tang, S., Wang, B., Jia, J. and You, H.
Am. J. Pathol., ***182(5)***, *1607-1616 (2013)*

Elevated tissue inhibitor of metalloproteinase 1 (TIMP-1) expression contributes to excess production of extracellular matrix in liver fibrosis. Herein, we constructed a recombinant adeno-associated virus (rAAV) carrying siRNA of the *TIMP-1* gene (rAAV/siRNA–TIMP-1) and investigated its effects on liver fibrosis in rats. Two models of rat liver fibrosis, the carbon tetrachloride and bile duct ligation models, were treated with rAAV/siRNA–TIMP-1. In the carbon tetrachloride model, rAAV/siRNA–TIMP-1 administration attenuated fibrosis severity, as determined by histologic analysis of hepatic collagen accumulation, hydroxyproline content, and concentrations of types I and III collagen in livers and sera. Levels of mRNA and active matrix metalloproteinase (MMP) 13 were elevated, whereas levels of mRNA and active MMP-2 were decreased. Moreover, a marked decrease was noted in the expression of α-smooth muscle actin, a biomarker of activated hepatic stellate cells (HSCs), and transforming growth factor-β1, critical for the development of liver fibrosis. Similarly, rAAV/siRNA–TIMP-1 treatment significantly alleviated bile duct ligation–induced liver fibrosis. Furthermore, this treatment dramatically suppressed TIMP-1 expression in HSCs from both model rats. These data indicate that the administration of rAAV/siRNA–TIMP-1 attenuated liver fibrosis by directly elevating the function of MMP-13 and diminishing activated HSCs. It also resulted in indirect decreased expression of type I collagen, MMP-2, and transforming growth factor-β1. In conclusion, rAAV/siRNA–TIMP-1 may be an effective antifibrotic gene therapy agent.

4.1166 FACS Array Profiling Identifies Ecto-5′ Nucleotidase as a Striatopallidal Neuron-Specific Gene Involved in Striatal-Dependent Learning

Ena, S.L., De Backer, J-F., Schiffmann, S.N., and de Kerchove d'Exaerde, A.
J. Neurosci., ***33(20)***, *8794-8809 (2013)*

The striatopallidal (STP) and striatonigral (STN) neurons constitute the main neuronal populations of the striatum. Despite the increasing knowledge concerning their involvement in multiple tasks associated with the striatum, it is still challenging to understand the precise differential functions of these two neuronal populations and to identify and study new genes involved in these functions. Here, we describe a reliable approach, applied on adult mouse brain, to generate specific STP and STN neuron gene profiles. STP and STN neurons were identified in the same animal using the transgenic Adora2A-Cre × Z/EG mouse model combined with retrograde labeling, respectively. Gene profiling was generated from FACS-purified neurons leading to the identification of new STP and STN neuron-specific genes. Knock-down models based on Cre-dependent lentiviral vector were developed to investigate their function either in striatal or in STP neurons. Thereby, we demonstrate that ecto-5′-nucleotidase (NT5e) is specifically expressed in STP neurons and is at the origin of most of the extracellular adenosine produced in the striatum. Behavioral analysis of striatal and STP neuron knock-down mouse models as well as NT5e knock-out mice demonstrates the implication of this STP neuron enzyme in motor learning.

4.1167 Role of Fatty-Acid Synthesis in Dendritic Cell Generation and Function

Rehman, A., Hemmert, K.C., Ochi, A., Jamal, M., Henning, J.R., Barilla, R., Quesada, J.P., Zambirinis, C.P., Tang, K., Ego-Osuala, M., Rao, R.S., Greco, S., Deutsch, M., Narayan, S., Pachter, H.L., Graffeo, C.S., Acehan, D. and Miller, G.
J. Immunol., ***190(9)***, *4640-4649 (2013)*

Dendritic cells (DC) are professional APCs that regulate innate and adaptive immunity. The role of fatty-acid synthesis in DC development and function is uncertain. We found that blockade of fatty-acid synthesis markedly decreases dendropoiesis in the liver and in primary and secondary lymphoid organs in mice. Human DC development from PBMC precursors was also diminished by blockade of fatty-acid synthesis. This was associated with higher rates of apoptosis in precursor cells and increased expression of cleaved caspase-3 and BCL-xL and downregulation of cyclin B1. Further, blockade of fatty-acid synthesis decreased DC expression of MHC class II, ICAM-1, B7-1, and B7-2 but increased their production of selected proinflammatory cytokines including IL-12 and MCP-1. Accordingly, inhibition of fatty-acid synthesis enhanced DC capacity to activate allogeneic as well as Ag-restricted $CD4^+$ and $CD8^+$ T cells and induce CTL responses. Further, blockade of fatty-acid synthesis increased DC expression of Notch ligands and enhanced their ability to activate NK cell immune phenotype and IFN-γ production. Because endoplasmic reticulum (ER) stress can augment the immunogenic function of APC, we postulated that this may account for the higher DC immunogenicity. We found that inhibition of fatty-acid synthesis resulted in elevated expression of numerous markers of ER stress in humans and mice and was associated with increased MAPK and Akt signaling. Further, lowering ER stress by 4-phenylbutyrate mitigated the enhanced immune stimulation associated with fatty-acid synthesis blockade. Our findings elucidate the role of fatty-acid synthesis in DC development and function and have implications to the design of DC vaccines for immunotherapy.

4.1168 Single-cell analysis and sorting using droplet-based microfluidics

Mazutis, L., Gilbert, J., Ung, W.L., Weitz, D.A., Griffiths, A.D. and Heyman, J.A.
Nature Protocols, ***8(5)***, *870-891 (2013)*

We present a droplet-based microfluidics protocol for high-throughput analysis and sorting of single cells. Compartmentalization of single cells in droplets enables the analysis of proteins released from or secreted by cells, thereby overcoming one of the major limitations of traditional flow cytometry and fluorescence-activated cell sorting. As an example of this approach, we detail a binding assay for detecting antibodies secreted from single mouse hybridoma cells. Secreted antibodies are detected after only 15 min by co-compartmentalizing single mouse hybridoma cells, a fluorescent probe and single beads coated with anti-mouse IgG antibodies in 50-pl droplets. The beads capture the secreted antibodies and, when the captured antibodies bind to the probe, the fluorescence becomes localized on the beads, generating a clearly distinguishable fluorescence signal that enables droplet sorting at ∼200 Hz as well as cell enrichment. The microfluidic system described is easily adapted for screening other intracellular, cell-surface or secreted proteins and for quantifying catalytic or regulatory activities. In order to screen ∼1 million cells, the

microfluidic operations require 2–6 h; the entire process, including preparation of microfluidic devices and mammalian cells, requires 5–7 d.

4.1169 Role of host cell traversal by the malaria sporozoite during liver infection
Tavares, J., Formaglio, P., Thiberge, S., Mordelet, E., Van Rooijen, N., Medvinsky, A., Menard, R. and Amino, R.
J. Exp. Med., ***210(5)***, *905-915 (2013)*

Malaria infection starts when the sporozoite stage of the *Plasmodium* parasite is injected into the skin by a mosquito. Sporozoites are known to traverse host cells before finally invading a hepatocyte and multiplying into erythrocyte-infecting forms, but how sporozoites reach hepatocytes in the liver and the role of host cell traversal (CT) remain unclear. We report the first quantitative imaging study of sporozoite liver infection in rodents. We show that sporozoites can cross the liver sinusoidal barrier by multiple mechanisms, targeting Kupffer cells (KC) or endothelial cells and associated or not with the parasite CT activity. We also show that the primary role of CT is to inhibit sporozoite clearance by KC during locomotion inside the sinusoid lumen, before crossing the barrier. By being involved in multiple steps of the sporozoite journey from the skin to the final hepatocyte, the parasite proteins mediating host CT emerge as ideal antibody targets for vaccination against the parasite.

4.1170 Protoplast isolation optimization and regeneration of cell wall in Gracilaria gracilis (Gracilariales, Rhodophyta)
Huddy, S.M., Meyers, A.E. and Coyne, V.E.
J. Appl. Phycol., ***25(2)***, *433-443 (2013)*

This paper reports the first successful isolation and cell wall regeneration of Gracilaria gracilis (Stackhouse) Steentoft, Irvine et Farnham protoplasts. These results form an important foundation for the development of a successful tissue culture system for G. gracilis. Initially, an isolation protocol was optimized by investigation of the effects of the enzyme constituents and concentrations, the pre-treatment of thalli, the incubation period and temperature, and the pH of the enzymatic medium on protoplast yields. A pre-treatment of G. gracilis thalli with 1 % (w/v) papain for 30 min followed by a 3-h enzymatic digestion of thalli with an enzymatic mixture containing 2 % (w/v) cellulase Onozuka R-10, 1 % (w/v) macerozyme R-10, and 10 U mL−1 agarase at pH 6.15 was found to produce the highest yield of protoplasts at 22 °C. Reliably high yields (20–30 × 105 protoplasts g−1 f.wt) of protoplasts could be obtained from G. gracilis thalli when this optimized protocol was used. Cell wall re-synthesis by G. gracilis protoplasts, which constitutes the first step towards whole plant regeneration, was followed using calcoflour staining and scanning electron microscopy. Protoplasts were shown to complete the initial stages of cell wall re-synthesis within the first 24 h of culturing.

4.1171 Toll Like Receptor 3 Plays a Critical Role in the Progression and Severity of Acetaminophen-Induced Hepatotoxicity
Cavassani, K.A., Moreira, A.P., Habiel, D., Ito, T., Coelho, A.L., Allen, R.M., Hu, B., Raphelson, J., Carson IV, W.F., Schaller, M.A., Lukacs, N.W., Omary, M.B., Hogaboam, C.M. and Kunkel, S.L:
PloS One, ***8(6)***, *e65899 (2013)*

Toll-like receptor (TLR) activation has been implicated in acetaminophen (APAP)-induced hepatotoxicity. Herein, we hypothesize that TLR3 activation significantly contributed to APAP-induced liver injury. In fasted wildtype (WT) mice, APAP caused significant cellular necrosis, edema, and inflammation in the liver, and the *de novo* expression and activation of TLR3 was found to be necessary for APAP-induced liver failure. Specifically, liver tissues from similarly fasted TLR3-deficient (*tlr3*$^{-/-}$) mice exhibited significantly less histological and biochemical evidence of injury after APAP challenge. Similar protective effects were observed in WT mice in which TLR3 was targeted through immunoneutralization at 3 h post-APAP challenge. Among three important death ligands (i.e. TNFα, TRAIL, and FASL) known to promote hepatocyte death after APAP challenge, TNFα was the only ligand that was significantly reduced in APAP-challenged *tlr3*$^{-/-}$ mice compared with APAP-challenged WT controls. *In vivo* studies demonstrated that TLR3 activation contributed to TNFα production in the liver presumably via F4/80^{+} and CD11c^{+} immune cells. *In vitro* studies indicated that there was cooperation between TNFα and TLR3 in the activation of JNK signaling in isolated and cultured liver epithelial cells (i.e. nMuLi). Moreover, TLR3 activation enhanced the expression of phosphorylated JNK in APAP injured livers. Thus, the current study demonstrates that TLR3 activation contributes to APAP-induced hepatotoxicity.

4.1172 Efficient gene expression from integration-deficient lentiviral vectors in the spinal cord

Peluffo, H., Foster, E., Ahmed, S.G., lago, N., Hutson, T.H., Moon, L., Wanisch, K., Caraballo-miralles, V., Olmos, G., Llado, J., McMahon, S.B. and Yanez-Munoz, R.J.
Gene Therapy, ***20***, *645-657 (2013)*

Gene transfer to spinal cord cells may be crucial for therapy in spinal muscular atrophy, amyotrophic lateral sclerosis and spinal cord injury. Lentiviral vectors are efficient for transduction of a variety of cells, but like all integrating vectors they pose a risk of insertional mutagenesis. Integration-deficient lentiviral vectors (IDLVs) remain episomal but retain the transduction efficiency of standard integrating lentiviral vectors, particularly when the episomes are not diluted out through repeated cell division. We have now applied IDLVs for transduction of spinal cord *in vitro*, in explants and *in vivo*. Our results demonstrate similar efficiency of *eGFP* expression from integrating lentiviral vectors and IDLVs in most cell types analyzed, including motor neurons, interneurons, dorsal root ganglia (DRG) neurons and astroglia. IDLV-mediated expression of pro-glial-cell-derived neurotrophic factor (*Gdnf*) rescues motor neuron cultures from death caused by removal of exogenous trophic support. IDLVs also mediate efficient RNA interference in DRG neuron cultures. After intraparenchymal injection in the rat and mouse cervical and lumbar regions *in vivo*, transduction is mainly neuronal, with both motor neurons and interneurons being efficiently targeted. These results suggest that IDLVs could be efficient and safer tools for spinal cord transduction in future therapeutic strategies.

4.1173 Rapid Generation of Human-Like Neutralizing Monoclonal Antibodies in Urgent Preparedness for Influenza Pandemics and Virulent Infectious Diseases

Meng, W., Pan, W., Zhang, A.J.X., Li, Z., Wei, G., Feng, L., Dong, Z., Li, C., Hu, X., Sun, C., Luo, Q., Yuen, K-Y., Zhong, N. and Chen, L:
PloS One, ***8(6)***, *e66278 (2013)*

Background
The outbreaks of emerging infectious diseases caused by pathogens such as SARS coronavirus, H5N1, H1N1, and recently H7N9 influenza viruses, have been associated with significant mortality and morbidity in humans. Neutralizing antibodies from individuals who have recovered from an infection confer therapeutic protection to others infected with the same pathogen. However, survivors may not always be available for providing plasma or for the cloning of monoclonal antibodies (mAbs).
Methodology/Principal Findings
The genome and the immunoglobulin genes in rhesus macaques and humans are highly homologous; therefore, we investigated whether neutralizing mAbs that are highly homologous to those of humans (human-like) could be generated. Using the H5N1 influenza virus as a model, we first immunized rhesus macaques with recombinant adenoviruses carrying a synthetic gene encoding hemagglutinin (HA). Following screening an antibody phage display library derived from the B cells of immunized monkeys, we cloned selected macaque immunoglobulin heavy chain and light chain variable regions into the human IgG constant region, which generated human-macaque chimeric mAbs exhibiting over 97% homology to human antibodies. Selected mAbs demonstrated potent neutralizing activities against three clades (0, 1, 2) of the H5N1 influenza viruses. The *in vivo* protection experiments demonstrated that the mAbs effectively protected the mice even when administered up to 3 days after infection with H5N1 influenza virus. In particular, mAb 4E6 demonstrated sub-picomolar binding affinity to HA and superior *in vivo* protection efficacy without the loss of body weight and obvious lung damage. The analysis of the 4E6 escape mutants demonstrated that the 4E6 antibody bound to a conserved epitope region containing two amino acids on the globular head of HA.
Conclusions/Significance
Our study demonstrated the generation of neutralizing mAbs for potential application in humans in urgent preparedness against outbreaks of new influenza infections or other virulent infectious diseases.

4.1174 Separation of Penaeus vannamei haemocyte subpopulations by iodixanol density gradient centrifugation

Dantas-Lima, J.J., Tuan, V.V., Corteel, M., Grauwet, K., An, N.T.T., Sorgeloos, P. and Nauwynck, H.J.
Aquaculture, ***408-409***, *128-135 (2013)*

Methodologies for separation of immune cell subpopulations are essential tools in immunology studies. Up to date, only one methodology for separating crustacean haemocyte subpopulations using Percoll density gradient centrifugation has been described.
In the present work, a new methodology to separate *Penaeus vannamei* haemocyte subpopulations was

developed, using a two-step iodixanol density gradient centrifugation. *P. vannamei* haemolymph was collected with anticoagulant and centrifuged through a first gradient (densities from 1.063 to 1.109 g/ml) for 10 min at 2000 *g*. Three bands were formed: two bands with lower density close together, and a third band with higher density. The first two were collected together whilst the third band was collected separately. The volume fraction in-between these bands contained dispersed cells and was also collected. The suspension containing the mixture of the first two bands was centrifuged through a second gradient (densities from 1.047 to1.087 g/ml) for 15 min at 2000 *g*. Two bands were formed and collected individually. All the cell suspensions were used for *in vitro* culture (cell survival evaluation) and for evaluation of cell morphology by flow cytometry and light microscopy. Each of the three bands contained a major cell type with distinct morphology and behaviour. The dispersed cell fraction contained a mixture of two different cell types, which were distinct from the cell types in the bands. By order of appearance from the top of the gradient, the cell types were named: subpopulations (Sub) 1 (band 1), Sub 2 (band 2), Sub 3 + 4 (dispersed cells) and Sub 5 (band 3). The purity level (percentage of the major cell type) of Sub 1, 2 and 5 was 95.0 ± 1.0%, 97.7 ± 1.2% and 99.4 ± 0.8%, respectively. Cells of Sub 2 showed the best survival time *in vitro* (up to 96 h) followed by cells from Sub 1, Sub 3 + 4 and Sub 5. Phagocytic activity was detected in Sub 1 and 4.
This methodology allowed the separation and characterization of five morphologically distinct and physiologically active *P. vannamei* haemocyte subpopulations, from which three were isolated with a very high degree of purity. Therefore, we consider this methodology a valuable alternative for the traditional crustacean haemocyte separation procedure in Percoll.

4.1175 Microfluidic primary culture model of the lower motor neuron–neuromuscular junction circuit
Southan, K.A., King, A.E., Blizzard, C.A., McCormack, G.H. and Dickson, T.C.
*J. Neurosci. Methods, **218**, 164-169 (2013)*

Modelling the complex process of neuromuscular signalling is key to understanding not only normal circuit function but also importantly the mechanisms underpinning a range of degenerative diseases. We describe a novel *in vitro* model of the lower motor neuron–neuromuscular junction circuit, incorporating primary spinal motor neurons, supporting glia and skeletal muscle. This culture model is designed to spatially mimic the unique anatomical and cellular interactions of this circuit in compartmented microfluidic devices, such that the glial cells are located with motor neuron cell bodies in the cell body chamber and motor neuron axons extend to a distal chamber containing skeletal muscle cells whilst simultaneously allowing targeted intervention. This model is suitable for use in conjunction with a range of downstream experimental approaches and could also be modified to utilise other cellular sources including appropriate immortal cell lines, cells derived from transgenic models of disease and also patient derived stem cells.

4.1176 Examination of MARCO Activity on Dendritic Cell Phenotype and Function Using a Gene Knockout Mouse
Komine, H., Kuhn, L., Matsushita, N., Mule, J.J. and Pilon-Thomas, S.
*PloS One, **8(7)**, e67795 (2013)*

We have reported the upregulation of MARCO, a member of the class A scavenger receptor family, on the surface of murine and human dendritic cells (DCs) pulsed with tumor lysates. Exposure of murine tumor lysate-pulsed DCs to an anti-MARCO antibody led to loss of dendritic-like processes and enhanced migratory capacity. In this study, we have further examined the biological and therapeutic implications of MARCO expression by DCs. DCs generated from the bone marrow (bm) of MARCO knockout ($MARCO^{-/-}$) mice were phenotypically similar to DCs generated from the bm of wild-type mice and produced normal levels of IL-12 and TNF-α when exposed to LPS. $MARCO^{-/-}$ DCs demonstrated enhanced migratory capacity in response to CCL-21 *in vitro*. After subcutaneous injection into mice, $MARCO^{-/-}$ TP-DCs migrated more efficiently to the draining lymph node leading to enhanced generation of tumor-specific IFN-γ producing T cells and improved tumor regression and survival in B16 melanoma-bearing mice. These results support targeting MARCO on the surface of DCs to improve trafficking and induction of anti-tumor immunity.

4.1177 Low Immunogenicity of Neural Progenitor Cells Differentiated from Induced Pluripotent Stem Cells Derived from Less Immunogenic Somatic Cells
Liu, P., Chen, S., Li, X., Qin, L., Huang, K., Wang, L., Huang, W., Li, S., Jia, B., Zhong, M., Pan, G., Cai, J. and Pei, D.

*PloS One, **8(7)**, e69617 (2013)*

The groundbreaking discovery of induced pluripotent stem cells (iPS cells) provides a new source for cell therapy. However, whether the iPS derived functional lineages from different cell origins have different immunogenicity remains unknown. It had been known that the cells isolated from extra-embryonic tissues, such as umbilical cord mesenchymal cells (UMCs), are less immunogenic than other adult lineages such as skin fibroblasts (SFs). In this report, we differentiated iPS cells from human UMCs and SFs into neural progenitor cells (NPCs) and analyzed their immunogenicity. Through co-culture with allologous peripheral blood mononuclear cells (PBMCs), we showed that UMCs were indeed less immunogenic than skin cells to simulate proliferation of PBMCs. Surprisingly, we found that the NPCs differentiated from UMC-iPS cells retained low immunogenicity as the parental UMCs based on the PBMC proliferation assay. In cytotoxic expression assay, reactions in most kinds of immune effector cells showed more perforin and granzyme B expression with SF-NPCs stimulation than that with UMC-NPCs stimulation in PBMC co-culture system, in T cell co-culture system as well. Furthermore, through whole genome expression microarray analysis, we showed that over 70 immune genes, including all members of HLA-I, were expressed at lower levels in NPCs derived from UMC-iPS cells than that from SF-iPS cells. Our results demonstrated a phenomenon that the low immunogenicity of the less immunogenic cells could be retained after cell reprogramming and further differentiation, thus provide a new concept to generate functional lineages with lower immunogenicity for regenerative medicine.

4.1178 Alternative Immunomodulatory Strategies for Xenotransplantation: CD80/CD86-CTLA4 Pathway-Modified Immature Dendritic Cells Promote Xenograft Survival
Tian, M., Lv, Y., Zhai, C., Zhu, H., Yu, L. and Wang, B.
*PloS One, **8(7)**, e69640 (2013)*

Background
Xenotransplantation is a promising approach to circumventing the current organ shortage. However, T-cell-dependent anti-xenoresponses are a major challenge to successful xenografts. Given the advantages of the use of CTLA4-Ig in the survival of allografts, the purpose of the study was to investigate the therapeutic potential of CTLA4-IgG4 modified immature dendritic cells (imDCs) in the prevention of islets xenograft rejection.
Methods
CTLA4-IgG4 was constructed by the fusion of the extracellular regions of porcine CTLA4 to human the hIgG4 Fc region. The imDCs were induced and cultured from porcine peripheral blood mononuclear cells (PBMC). The CTLA4-IgG4 modified imDCs were delivered via the portal vein to the liver of diabetic mice (insulin-dependent diabetes mellitus) before islet xenografting, and mCTLA4-Ig was administered intravenously after xenotransplantation.
Results
The xenograft survival of mice receiving unmodified imDCs was approximately 30 days. However, following administration of CTLA4-IgG4 modified imDCs before grafting and mCTLA4-Ig after grafting, xenografts survived for more than 100 days. Flow cytometric analysis showed that the $CD4^{+}CD25^{+}Foxp3^{+}$ Treg population was increased in spleens. The efficacy of donor CTLA4-IgG4 modified imDCs correlated partially with the amplification of Tregs.
Conclusions
These results confirm that selective inhibition of the direct and indirect pathways of T-cell activation by donor CTLA4-IgG4 modified imDCs and receptor CTLA4-Ig is a highly effective strategy to promote survival of xenografts.

4.1179 High-quality RNA extraction from rat pancreatic islet
Kiba, T., Tanemura, M. and Yagyu, K.
*Cell Biol. Int. Reports, **20(1)**, 1-4 (2013)*

In recent years, increasing interest surrounding islet replacement therapies in human has provided the drive for advances in the methods used to isolate from humans as well as a host of animal research models. However, there has been no reports describing a technique that reliably improves the quality of RNA extracted from rat pancreatic islet. Male Sprague–Dawley rats, 10- to 12-week-old, were housed in a certified animal care facility. The rats were underwent bile duct cannulation with pancreatic inflation after clamping the distal common bile duct. The pancreas was excised and digested with ETK/Liberase TL solution at 37°C for 30 min without shaking. Cold ETK was added to stop digestion. The islets were purified by discontinuous iodixanol density gradients of 25, 23, 20 and 11% in a modified ETK/OptiPrep®

solution. After a 15 min centrifugation at 1,000g, islets were collected from the interface between the 20 and 11% layer. Immediately after purification, islets were used for RNA extraction. RNA was extracted from isolated rat pancreatic islet cells, using the commercially available kit. In the present study, we have described a technique that reliably improves the quality of RNA extracted from rat pancreatic islet using the perfusion technique in the bile duct. The islet cells that we isolated using this technique were suitable for high quality RNA extraction.

4.1180 Ultra-pure platelet isolation from canine whole blood

Trichler, S.A., Bulla, S.C., Thomason, J., Lunsford, K.V. and Bulla, C.
BMC Vet. Res., ***9****:144 (2013)*

Background
Several research applications involving platelets, such as proteomic and transcriptomic analysis, require samples with very low numbers of contaminating leukocytes, which have considerably higher RNA and protein content than platelets. We sought to develop a platelet purification protocol that would minimize contamination, involve minimal centrifugation steps, and yield highly pure platelet samples derived from low volume whole blood samples from healthy dogs.
Results
Using an optimized OptiPrep density gradient technique, platelet recovery was 51.56% with 99.99% platelet purity and leukocyte contamination of 100 leukocytes per 10^8 platelets, on average. Platelet samples were subjected to additional purification with CD45-labeled Dynabeads after density barrier centrifugation resulting in a 95-fold depletion of residual leukocytes. Platelets purified using these methods remained inactivated as assessed by Annexin V and P-selectin labeling with flow cytometry.
Conclusions
The use of OptiPrep density gradient is a quick method for obtaining highly purified platelet samples from low volumes of canine whole blood with minimal contamination. Additional depletion of residual leukocytes can be achieved using CD45-labeled beads. These platelet samples can then be used for many downstream applications that require ultra-pure platelet samples such as RNA and protein analysis.

4.1181 Dendritic Cell Subtypes from Lymph Nodes and Blood Show Contrasted Gene Expression Programs upon Bluetongue Virus Infection

Ruscanu, S., Jouneau, L., Urien, C., Bourge, M., Lecardonnel, J., Moroldo, M., Loup, B., Dalod, M., Elhmouzi-Younes, J., Bevilacqua, C. and Schwartz-Cornil, I.
J. Virol., ***87(16)****, 9333-9343 (2013)*

Human and animal hemorrhagic viruses initially target dendritic cells (DCs). It has been proposed, but not documented, that both plasmacytoid DCs (pDCs) and conventional DCs (cDCs) may participate in the cytokine storm encountered in these infections. In order to evaluate the contribution of DCs in hemorrhagic virus pathogenesis, we performed a genome-wide expression analysis during infection by Bluetongue virus (BTV), a double-stranded RNA virus that induces hemorrhagic fever in sheep and initially infects cDCs. Both pDCs and cDCs accumulated in regional lymph nodes and spleen during BTV infection. The gene response profiles were performed at the onset of the disease and markedly differed with the DC subtypes and their lymphoid organ location. An integrative knowledge-based analysis revealed that blood pDCs displayed a gene signature related to activation of systemic inflammation and permeability of vasculature. In contrast, the gene profile of pDCs and cDCs in lymph nodes was oriented to inhibition of inflammation, whereas spleen cDCs did not show a clear functional orientation. These analyses indicate that tissue location and DC subtype affect the functional gene expression program induced by BTV and suggest the involvement of blood pDCs in the inflammation and plasma leakage/hemorrhage during BTV infection in the real natural host of the virus. These findings open the avenue to target DCs for therapeutic interventions in viral hemorrhagic diseases.

4.1182 Aging-Induced Dysregulation of Dicer1-Dependent MicroRNA Expression Impairs Angiogenic Capacity of Rat Cerebromicrovascular Endothelial Cells

Ungvari, Z., Tucsek, Z., Sosnowska, D., Toth, P., Gautam, T., Podlutsky, A., Csiszar, A., Losonczy, G., Valcarcel-Ares, M.N., Sonntag, W.E. and Csiszar, A.
J. Gerontol. A Biol. Sci. Med. Sci., ***68(8)****, 877-891 (2013)*

Age-related impairment of angiogenesis is likely to play a central role in cerebromicrovascular rarefaction and development of vascular cognitive impairment, but the underlying mechanisms remain elusive. To test the hypothesis that dysregulation of Dicer1 (ribonuclease III, a key enzyme of the microRNA [miRNA]

machinery) impairs endothelial angiogenic capacity in aging, primary cerebromicrovascular endothelial cells (CMVECs) were isolated from young (3 months old) and aged (24 months old) Fischer 344 × Brown Norway rats. We found an age-related downregulation of Dicer1 expression both in CMVECs and in small cerebral vessels isolated from aged rats. In aged CMVECs, Dicer1 expression was increased by treatment with polyethylene glycol–catalase. Compared with young cells, aged CMVECs exhibited altered miRNA expression profile, which was associated with impaired proliferation, adhesion to vitronectin, collagen and fibronectin, cellular migration (measured by a wound-healing assay using electric cell–substrate impedance sensing technology), and impaired ability to form capillary-like structures. Overexpression of Dicer1 in aged CMVECs partially restored miRNA expression profile and significantly improved angiogenic processes. In young CMVECs, downregulation of Dicer1 (siRNA) resulted in altered miRNA expression profile associated with impaired proliferation, adhesion, migration, and tube formation, mimicking the aging phenotype. Collectively, we found that Dicer1 is essential for normal endothelial angiogenic processes, suggesting that age-related dysregulation of Dicer1-dependent miRNA expression may be a potential mechanism underlying impaired angiogenesis and cerebromicrovascular rarefaction in aging.

4.1183 The neurotrophic properties of progranulin depend on the granulin E domain but do not require sortilin binding

De Muynck, L., Herdewyn, S., Beel, S., Scheveneels, W., Van Den Bosch, L., Robberecht, W. and Van Damme P.

*Neurobiol. Of Aging, **34**, 2541-2547 (2013)*

Progranulin (PGRN) is a growth factor involved in wound healing, inflammation, tumor growth, and neurodegeneration. Mutations in the gene encoding PGRN give rise to shortage of PGRN and cause familial frontotemporal lobar degeneration. PGRN exerts neurotrophic functions and binding of PGRN to the membrane receptor sortilin (SORT1) mediates the endocytosis of PGRN. SORT1-mediated uptake plays an important role in the regulation of extracellular PGRN levels. We studied the role of SORT1 in PGRN-mediated neuroprotection in vitro and in vivo. The survival-enhancing effect of PGRN seemed to be dependent on the granulin E (GRN E) domain. Pharmacologic inhibition of the GRN E–SORT1 interaction or deletion of the SORT1 binding site of GRN E did not abolish its neurotrophic function. In addition, the in vivo phenotype of PGRN knockdown in zebrafish embryos was not phenocopied by SORT1 knockdown. These results suggest that GRN E mediates the neurotrophic properties of PGRN and that binding to SORT1 is not required for this effect.

4.1184 A DISINTEGRIN AND METALLOPROTEINASE 17 REGULATES TNF AND TNFR1 LEVELS IN INFLAMMATION AND LIVER REGENERATION IN MICE

McMahan, R.S., Riehle, K.J., Fausto, N. and Campbell, J.S.

*Am. J: Physiol. Gastrointest. Liver Physiol., **305**, G25-G34 (2013)*

A Disintegrin And Metalloproteinase 17 (ADAM17), or TNF-alpha Converting Enzyme (TACE), is a key metalloproteinase and physiological convertase for a number of putative targets that play critical roles in cytokine and growth factor signaling. These interdependent pathways are essential components of the signaling network that links liver function with the compensatory growth that occurs during liver regeneration following 2/3 partial hepatectomy (PH) or chemically induced hepatotoxicity. Despite identification of many soluble factors needed for efficient liver regeneration, very little is known about how such ligands are regulated in the liver. To directly study the role of ADAM17 in the liver, we employed two cell-specific ADAM17 KO mouse models. Using LPS as a robust stimulus for TNF release, we found attenuated levels of circulating TNF in myeloid-specific ADAM17 KO mice (ADAM17 m-KO) and unexpectedly, in mice with hepatocyte-specific ADAM17 deletion (ADAM17 h-KO), indicating that ADAM17 expression in both cell types plays a role in TNF shedding. After 2/3 PH, induction of TNF, TNFR1, and amphiregulin (AR) was significantly attenuated in ADAM17 h-KO mice, implicating ADAM17 as the primary sheddase for these factors in the liver. Surprisingly, the extent and timing of hepatocyte proliferation were not affected after PH or carbon tetrachloride (CCl_4) injection in ADAM17 h-KO or ADAM17 m-KO mice. We conclude that ADAM17 regulates TNF, TNFR1, and AR in the liver, and its expression in both hepatocytes and myeloid cells is important for TNF regulation after LPS injury or 2/3 PH, but is not required for liver regeneration.

4.1185 Dendritic cell immunotherapy combined with gemcitabine chemotherapy enhances survival in a murine model of pancreatic carcinoma

Ghansah, T., Vohra, N., Kinney, K., Weber, A., Kodumudi, K., Springett, G., Sarnaik, A.A. and Pilon-Thomas, S.

*Cancer Immunol. Immunother.,**62(6)**, 1083-1091 (2013)*

Pancreatic cancer is an extremely aggressive malignancy with a dismal prognosis. Cancer patients and tumor-bearing mice have multiple immunoregulatory subsets including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC) that may limit the effectiveness of anti-tumor immunotherapies for pancreatic cancer. It is possible that modulating these subsets will enhance anti-tumor immunity. The goal of this study was to explore depletion of immunoregulatory cells to enhance dendritic cell (DC)-based cancer immunotherapy in a murine model of pancreatic cancer. Flow cytometry results showed an increase in both Tregs and MDSC in untreated pancreatic cancer–bearing mice compared with control. Elimination of Tregs alone or in combination with DC-based vaccination had no effect on pancreatic tumor growth or survival. Gemcitabine (Gem) is a chemotherapeutic drug routinely used for the treatment for pancreatic cancer patients. Treatment with Gem led to a significant decrease in MDSC percentages in the spleens of tumor-bearing mice, but did not enhance overall survival. However, combination therapy with DC vaccination followed by Gem treatment led to a significant delay in tumor growth and improved survival in pancreatic cancer–bearing mice. Increased MDSC were measured in the peripheral blood of patients with pancreatic cancer. Treatment with Gem also led to a decrease of this population in pancreatic cancer patients, suggesting that combination therapy with DC-based cancer vaccination and Gem may lead to improved treatments for patients with pancreatic cancer.

4.1186 A Comparative Toxicogenomic Investigation of Oil Sand Water and Processed Water in Rainbow Trout Hepatocytes

Gagne, F., Andre, C., Turcotte, P., Gagnon, C., Sherry, J. and Talbot, A.

*Arch. Environ Contam. Toxicol., **65(2)**, 309-323 (2013)*

The purpose of this study was to compare the expression of gene transcripts involved in toxic stress in rainbow trout hepatocytes exposed to oil sand water (OSW), lixiviate (OSLW), and processed water (OSPW). We pose the hypothesis that the changes in gene expression responses in cells exposed to a simulated oil sand extraction procedure (OSPW) differ from the gene expression responses of OSLW and OS. Rainbow trout hepatocytes were exposed to increasing concentrations of OSW, OSLW, and OSPW for 48 h at 15 °C. Cell viability was assessed by measuring membrane permeability, total RNA levels, and gene expression using an array of 16 genes involved in xenobiotic biotransformation (GST, CYP1A1, CYP3A4, MDR), metal homeostasis and oxidative stress (MT, SOD, and CAT), estrogenicity (VTG, ERβ), DNA repair (LIG, APEX, UNG, and OGG), cell growth (GADD45 and PCNA), and glycolysis (GAPDH). The results showed that the toxicogenomic properties of OSPW differed from those of OSLW and OSW. Gene transcripts that were influenced by OSW and OSLW, and strongly expressed in OSPW, were MT, CAT, GST (induction), CYP1A1, VTG, UNG/OGG, and PCNA. These genes are therefore considered not entirely specific to OSPW but to water in contact with OS. We also found gene transcripts that responded only with OSPW: SOD, GST (inhibition), MDR (inhibition), CYP3A4, GAPDH, GADD45, and APEX. Of these gene transcripts, the ones strongly associated with toxicity (loss of cell viability and RNA levels) were CYP3A4, GST, and GAPDH. Genes involved in DNA repair were also strongly related to the loss of cell viability but responded to both OSLW and OSPW. The observed changes in cell toxicity and gene expression therefore support the hypothesis that OSPW has a distinct toxic fingerprint from OSLW and OSW.

4.1187 Expression Pattern of Interferon-Inducible Transcriptional Genes in Neutrophils During Bovine Tuberculosis Infection no access

Wang, J., Zhou, X., Pan, B., Wang, H., Shi, F., Gan, W., Yang, L., Yin, X., Xu, B. and Zhao, D.

*DNA and Cell Biol., **32(8)**, 480-486 (2013)*

Mycobacterium bovis, the classical causative agent of bovine tuberculosis (BTB), infects animals of agricultural importance and other mammals, including humans. Neutrophils are one of the first lines of defense against all microbes and produce a diverse collection of antimicrobial molecules, which play an important role in the early control of tuberculosis progression. An interferon (IFN)-inducible neutrophil-driven blood transcriptional signature that consisted of both IFN-γ and type I IFN-α/β signaling has been identified in human tuberculosis, supporting a role for neutrophils in the pathogenesis of tuberculosis disease. However, it is unknown whether bovine neutrophils play a similar role during *M. bovis* infection.

Thus, we assessed the expression levels of ten IFN-inducible transcriptional genes in neutrophils from healthy cattle stimulated by *M. bovis* and neutrophils isolated from three groups of cattle of different infection status, and in addition, examined the changes in the expression of myeloperoxidase (*MPO*) and pentraxin-related protein pentraxin-inducible protein (*PTX3*) genes during bovine tuberculosis infection. Our results demonstrated a specific expression pattern of IFN-inducible transcriptional genes and *MPO* and *PTX3* genes in neutrophils during bovine tuberculosis infection. The observed expression pattern provides a potential diagnostic tool, which may have implications for vaccine and therapeutic development to combat the bovine tuberculosis epidemic.

4.1188 Truncated Form of TGF-βRII, But Not Its Absence, Induces Memory CD8+ T Cell Expansion and Lymphoproliferative Disorder in Mice
Ishigame, H., Mosaheb, M.M., Sanjabi, S. and Flavell, R.A.
J. Immunol., ***190(12)****, 6340-6350 (2013)*

Inflammatory and anti-inflammatory cytokines play an important role in the generation of effector and memory $CD8^+$ T cells. We used two different models, transgenic expression of truncated (dominant negative) form of TGF-βRII (dnTGFβRII) and Cre-mediated deletion of the floxed TGF-βRII to examine the role of TGF-β signaling in the formation, function, and homeostatic proliferation of memory $CD8^+$ T cells. Blocking TGF-β signaling in effector $CD8^+$ T cells using both of these models demonstrated a role for TGF-β in regulating the number of short-lived effector cells but did not alter memory $CD8^+$ T cell formation and their function upon *Listeria monocytogenes* infection in mice. Interestingly, however, a massive lymphoproliferative disorder and cellular transformation were observed in Ag-experienced and homeostatically generated memory $CD8^+$ T cells only in cells that express the dnTGFβRII and not in cells with a complete deletion of TGF-βRII. Furthermore, the development of transformed memory $CD8^+$ T cells expressing dnTGFβRII was IL-7– and IL-15–independent, and MHC class I was not required for their proliferation. We show that transgenic expression of the dnTGFβRII, rather than the absence of TGF-βRII–mediated signaling, is responsible for dysregulated expansion of memory $CD8^+$ T cells. This study uncovers a previously unrecognized dominant function of the dnTGFβRII in $CD8^+$ T cell proliferation and cellular transformation, which is caused by a mechanism that is different from the absence of TGF-β signaling. These results should be considered during both basic and translational studies where there is a desire to block TGF-β signaling in $CD8^+$ T cells.

4.1189 Macrophage Migration Inhibitory Factor Potentiates Autoimmune-Mediated Neuroinflammation
Cox, G.M., Kithcart, A.P., Pitt, D., Guan, Z., Alexander, J., Williams, J.L., Shawler, T., Dagia, N.M., Popovich, P.G., Satoskar, A.R. and Whittacre, C.C.
J. Immunol., ***191(3)****, 1043-1054 82013)*

Macrophage migration inhibitory factor (MIF) is a multipotent cytokine that is associated with clinical worsening and relapses in multiple sclerosis (MS) patients. The mechanism through which MIF promotes MS progression remains undefined. In this study, we identify a critical role for MIF in regulating CNS effector mechanisms necessary for the development of inflammatory pathology in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Despite the ability to generate pathogenic myelin-specific immune responses peripherally, MIF-deficient mice have reduced EAE severity and exhibit less CNS inflammatory pathology, with a greater percentage of resting microglia and fewer infiltrating inflammatory macrophages. We demonstrate that MIF is essential for promoting microglial activation and production of the innate soluble mediators IL-1β, IL-6, TNF-α, and inducible NO synthase. We propose a novel role for MIF in inducing microglial C/EBP-β, a transcription factor shown to regulate myeloid cell function and play an important role in neuroinflammation. Intraspinal stereotaxic microinjection of MIF resulted in upregulation of inflammatory mediators in microglia, which was sufficient to restore EAE-mediated inflammatory pathology in MIF-deficient mice. To further implicate a role for MIF, we show that MIF is highly expressed in human active MS lesions. Thus, these results illustrate the ability of MIF to influence the CNS cellular and molecular inflammatory milieu during EAE and point to the therapeutic potential of targeting MIF in MS.

4.1190 IL-17A Plays a Critical Role in the Pathogenesis of Liver Fibrosis through Hepatic Stellate Cell Activation
Tan, Z., Xiaofeng, Q., Jiang, R., Liu, Q., Wang, Y., Chen. C.,Wang, X., Ryffel, B. and Sun, B.
J. Immunol., ***191(4)****, 1835-1844 (2013)*

Liver fibrosis is a severe, life-threatening clinical condition resulting from nonresolving hepatitis of

different origins. IL-17A is critical in inflammation, but its relation to liver fibrosis remains elusive. We find increased IL-17A expression in fibrotic livers from HBV-infected patients undergoing partial hepatectomy because of cirrhosis-related early-stage hepatocellular carcinoma in comparison with control nonfibrotic livers from uninfected patients with hepatic hemangioma. In fibrotic livers, IL-17A immunoreactivity localizes to the inflammatory infiltrate. In experimental carbon tetrachloride–induced liver fibrosis of IL-17RA–deficient mice, we observe reduced neutrophil influx, proinflammatory cytokines, hepatocellular necrosis, inflammation, and fibrosis as compared with control C57BL/6 mice. IL-17A is produced by neutrophils and T lymphocytes expressing the Th17 lineage–specific transcription factor Retinoic acid receptor–related orphan receptor γt. Furthermore, hepatic stellate cells (HSCs) isolated from naive C57BL/6 mice respond to IL-17A with increased IL-6, α-smooth muscle actin, collagen, and TGF-β mRNA expression, suggesting an IL-17A–driven fibrotic process. Pharmacologic ERK1/2 or p38 inhibition significantly attenuated IL-17A–induced HSC activation and collagen expression. In conclusion, IL-17A$^+$ Retinoic acid receptor–related orphan receptor γt$^+$ neutrophils and T cells are recruited into the injured liver driving a chronic, fibrotic hepatitis. IL-17A–dependent HSC activation may be critical for liver fibrosis. Thus, blockade of IL-17A could potentially benefit patients with chronic hepatitis and liver fibrosis.

4.1191 P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway
Gandelman, M., Levy, M., Cassina, P., Barbeito, L. and Beckman, J.S.
J. Neurochem., ***126(3)****, 382-388 (2013)*

The P2X7 receptor/channel responds to extracellular ATP and is associated with neuronal death and neuroinflammation in spinal cord injury and amyotrophic lateral sclerosis. Whether activation of P2X7 directly causes motor neuron death is unknown. We found that cultured motor neurons isolated from embryonic rat spinal cord express P2X7 and underwent caspase-dependent apoptosis when exposed to exceptionally low concentrations of the P2X7 agonist 2′(3′)-*O*-(4-Benzoylbenzoyl)-ATP. The P2X7 inhibitors BBG, oATP, and KN-62 prevented 2′(3′)-*O*-(4-Benzoylbenzoyl)-ATP-induced motor neuron death. The endogenous P2X7 agonist ATP induced motor neuron death at low concentrations (1-100 μM). High concentrations of ATP (1 mM) paradoxically became protective due to degradation in the culture media to produce adenosine and activate adenosine receptors. P2X7-induced motor neuron death was dependent on neuronal nitric oxide synthase-mediated production of peroxynitrite, p38 activation, and autocrine FAS signaling. Taken together, our results indicate that motor neurons are highly sensitive to P2X7 activation, which triggers apoptosis by activation of the well-established peroxynitrite/FAS death pathway in motor neurons.

4.1192 Neonatal macrophages express elevated levels of interleukin-27 that oppose immune responses
Kraft, J.D., Horzempa, J., Davis, C., Jung, J-Y., Pena, M.M. and Robinson, C.M.
Immunology, ***139(4)****, 484-493 (2013)*

Microbial infections are a major cause of infant mortality worldwide because of impaired immune defences in this population. The nature of this work was to further understand the mechanistic limitations of the neonatal and infant immune response. Interleukin-27 (IL-27) is a heterodimeric cytokine of the IL-12 family that is produced primarily by antigen-presenting cells and is immunosuppressive toward a variety of immune cell types. We show that IL-27 gene expression is elevated in cord blood-derived macrophages relative to macrophages originating from healthy adults. We also evaluated the duration over which elevated IL-27 gene expression may impact immune responses in mice. Age-dependent analysis of IL-27 gene expression indicated that levels of IL-27 remained significantly elevated throughout infancy and then declined in adult mice. Flow cytometric analysis of intracellular cytokine-stained splenocytes further confirmed these results. Interleukin-27 may be induced during pregnancy to contribute to the immunosuppressive environment at the fetal–maternal interface because we demonstrate dose-responsive gene expression to progesterone in macrophages. Neutralization of IL-27 in neonatal macrophages improved the ability of these cells to limit bacterial replication. Moreover, neutralization of IL-27 during incubation with the *Mycobacterium bovis* bacillus Calmette–Guérin vaccine augmented the level of interferon-ϒ elicited from allogeneic CD4$^+$ T lymphocytes. This suggests that blocking IL-27 during vaccination and infection may improve immune responses in newborn and infant populations. Furthermore, mice will be a suitable model system to further address these possibilities.

4.1193 Venezuelan equine encephalitis virus glycoprotein pseudotyping confers neurotropism to lentiviral vectors

Trabalza, A., Geortgiadis, C., Eleftheriadou, I., Hislop, J.N., Karavassilis, M.E. and Mazarakis, N.D.
Gene Therapy, ***20(7)***, *723-732 (2013)*

We have produced high-titre HIV-1 green fluorescent protein-expressing lentiviral (LV) vectors pseudotyped with strain 3908 Venezuelan equine encephalitis virus glycoprotein (VEEV-G) and used them to study transduction of: (1) rat embryonic motor neuron (MN) and striatal neuron primary cultures, (2) differentiated MN cell line NSC-34 and (3) adult rat striatum. In primary neuronal cultures, transduction with VEEV-G-pseudotyped LV was more efficient and more neuronal than with vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped LV. In NSC-34 cells clear retrograde transport of VEEV-G vector particles was observed. In the striatum at the injection site, transduction with the VEEV-G vectors driven by cytomegalovirus or phosphoglycerate kinase promoters exhibited a distinct neuronal tropism with no microglial and only a minor astroglial component, superior to that obtained with VSV-G-pseudotyped LV, irrespective of the promoter used. Neuronal transduction efficiency increased over time. Distal to the injection site transduction of mitral cells in the olfactory bulb, thalamic neurons and dopaminergic neurons in the substantia nigra pars compacta was detected. This, together with observations of retrograde axonal trafficking *in vitro* indicates that these vectors also possess low level of retrograde neuronal transduction capability *in vivo*. In this study, we demonstrate both strong neurotropism as well as sustainability of expression and minimal host immune response *in vivo*, making the VEEV-G-pseudotyped LV vectors potentially useful for gene therapy of neurodegenerative diseases.

4.1194 Paracrine Wnt signaling both promotes and inhibits human breast tumor growth

Green, J.L., La, J., Yum, K.W., Desai, P., Redewald, L-W., Zhang, X., leblanc, M., Nusse, R., lewis, M.T. and Wahl, G.M.
PNAS, ***110(17)***, *6991-6996 (2013)*

Wnt signaling in mouse mammary development and tumorigenesis has been heavily studied and characterized, but its role in human breast cancer remains elusive. Although Wnt inhibitors are in early clinical development, it is unclear whether they will be of therapeutic benefit to breast cancer patients, and subsequently, to which ones. To address this, we generated a panel of Wnt reporting human breast cancer cell lines and identified a previously unrecognized enrichment for the ability to respond to Wnt in the basal B or claudin-low subtype, which has a poor prognosis and no available targeted therapies. By co-injecting Wnt3A expressing human mammary fibroblasts with human breast cancer cell lines into mouse mammary fat pads, we showed that elevated *paracrine* Wnt signaling was correlated with accelerated tumor growth. Using this heterotypic system and a dual lentiviral reporter system that enables simultaneous real-time measurement of both Wnt-responsive cells and bulk tumor cells, we analyzed the outcome of elevated Wnt signaling in patient-derived xenograft (PDX) models. Interestingly, the PDX models exhibited responses not observed in the cell lines analyzed. Exogenous WNT3A promoted tumor growth in one human epidermal growth factor receptor 2-overexpressing PDX line but inhibited growth in a second PDX line obtained from a patient with triple-negative breast cancer. Tumor suppression was associated with squamous differentiation in the latter. Thus, our work suggests that paracrine Wnt signaling can either fuel or repress the growth of human breast cancers depending on yet to be determined aspects of the molecular pathways they express.

4.1195 MST1 functions as a key modulator of neurodegeneration in a mouse model of ALS

Lee, J.K:, Shin, J.H., Hwang, S.G., Gwag, B.J., McKee, A.C., Lee, J., Kowall, N.W., Ryu, H., Lim, D.S. and Choi, E-J.
PNAS, ***110(29)***, *12066-12071 (2013)*

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by loss of motor neurons. Dominant mutations in the gene for superoxide dismutase 1 (SOD1) give rise to familial ALS by an unknown mechanism. Here we show that genetic deficiency of mammalian sterile 20-like kinase 1 (MST1) delays disease onset and extends survival in mice expressing the ALS-associated G93A mutant of human SOD1. SOD1(G93A) induces dissociation of MST1 from a redox protein thioredoxin-1 and promotes MST1 activation in spinal cord neurons in a reactive oxygen species–dependent manner. Moreover, MST1 was found to mediate SOD1(G93A)-induced activation of p38 mitogen-activated protein kinase and caspases as well as impairment of autophagy in spinal cord motoneurons of SOD1(G93A) mice. Our findings implicate MST1 as a key determinant of neurodegeneration in ALS.

4.1196 The Intracellular Environment of Human Macrophages That Produce Nitric Oxide Promotes Growth of Mycobacteria

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Infect. Immun., ***81(9)***, *3198-3209 (2013)*

Nitric oxide (NO) is a diffusible radical gas produced from the activity of nitric oxide synthase (NOS). NOS activity in murine macrophages has a protective role against mycobacteria through generation of reactive nitrogen intermediates (RNIs). However, the production of NO by human macrophages has remained unclear due to the lack of sensitive reagents to detect NO directly. The purpose of this study was to investigate NO production and the consequence to mycobacteria in primary human macrophages. We found that Mycobacterium bovis BCG or Mycobacterium tuberculosis infection of human macrophages induced expression of NOS2 and NOS3 that resulted in detectable production of NO. Treatment with gamma interferon (IFN-γ), l-arginine, and tetrahydrobiopterin enhanced expression of NOS2 and NOS3 isoforms, as well as NO production. Both of these enzymes were shown to contribute to NO production. The maximal level of NO produced by human macrophages was not bactericidal or bacteriostatic to M. tuberculosis or BCG. The number of viable mycobacteria was increased in macrophages that produced NO, and this requires expression of nitrate reductase. An *narG* mutant of M. tuberculosis persisted but was unable to grow in human macrophages. Taken together, these data (i) enhance our understanding of primary human macrophage potential to produce NO, (ii) demonstrate that the level of RNIs produced in response to IFN-γ *in vitro* is not sufficient to limit intracellular mycobacterial growth, and (iii) suggest that mycobacteria may use RNIs to enhance their survival in human macrophages.

4.1197 In vivo CD8+ T Cell Dynamics in the Liver of Plasmodium yoelii Immunized and Infected Mice

Cabrera, M., Pewe, L.L., Harty, J.T. and Frevert, U.

PloS One, ***8(8)***, *e70842 (2013)*

Plasmodium falciparum malaria remains one of the most serious health problems globally and a protective malaria vaccine is desperately needed. Vaccination with attenuated parasites elicits multiple cellular effector mechanisms that lead to *Plasmodium* liver stage elimination. While granule-mediated cytotoxicity requires contact between CD8+ effector T cells and infected hepatocytes, cytokine secretion should allow parasite killing over longer distances. To better understand the mechanism of parasite elimination *in vivo*, we monitored the dynamics of CD8+ T cells in the livers of naïve, immunized and sporozoite-infected mice by intravital microscopy. We found that immunization of BALB/c mice with attenuated *P. yoelii* 17XNL sporozoites significantly increases the velocity of CD8+ T cells patrolling the hepatic microvasculature from 2.69±0.34 μm/min in naïve mice to 5.74±0.66 μm/min, 9.26±0.92 μm/min, and 7.11±0.73 μm/min in mice immunized with irradiated, early genetically attenuated (Pyuis4-deficient), and late genetically attenuated (Pyfabb/f-deficient) parasites, respectively. Sporozoite infection of immunized mice revealed a 97% and 63% reduction in liver stage density and volume, respectively, compared to naïve controls. To examine cellular mechanisms of immunity in *situ*, naïve mice were passively immunized with hepatic or splenic CD8+ T cells. Unexpectedly, adoptive transfer rendered the motile CD8+ T cells from immunized mice immotile in the liver of *P. yoelii* infected mice. Similarly, when mice were simultaneously inoculated with viable sporozoites and CD8+ T cells, velocities 18 h later were also significantly reduced to 0.68±0.10 μm/min, 1.53±0.22 μm/min, and 1.06±0.26 μm/min for CD8+ T cells from mice immunized with irradiated wild type sporozoites, Pyfabb/f-deficient parasites, and *P. yoelii* $CS_{280–288}$ peptide, respectively. Because immobilized CD8+ T cells are unable to make contact with infected hepatocytes, soluble mediators could potentially play a key role in parasite elimination under these experimental conditions.

4.1198 Sorafenib perpetuates cellular anticancer effector functions by modulating the crosstalk between macrophages and natural killer cells

Sprinzl, M.F., Reisinger, F., Puschnik, A., Ringelhan, M., Ackermann, K., Hartmann, D., Schiemann, M., Weinmann, A., Galle, P.R., Schuchmann, M., Friess, H., Otto, G., Heikenwalder, M. and Protzer, U.

Hepatology, ***57(6)***, *2358-2368 (2013)*

Alternatively polarized macrophages (Mϕ) shape the microenvironment of hepatocellular carcinoma (HCC) and temper anticancer immune responses. We investigated if sorafenib alters the HCC microenvironment by restoring classical macrophage polarization and triggering tumor-directed natural killer (NK) cell responses. *In vivo* experiments were conducted with sorafenib (25 mg/kg)-treated C57BL/6 wildtype as well as hepatitis B virus (HBV) and lymphotoxin transgenic mice with and without

HCC. Monocyte-derived Mϕ or tumor-associated macrophages (TAM) isolated from HCC tissue were treated with sorafenib (0.07-5.0 μg/mL) and cocultured with autologous NK cells. Mϕ and NK cell activation was analyzed by flow cytometry and killing assays, respectively. Cytokine and growth factor release was measured by enzyme-linked immunosorbent assay. Short-term administration of sorafenib triggered activation of hepatic NK cells in wildtype and tumor-bearing mice. *In vitro*, sorafenib sensitized Mϕ to lipopolysaccharide, reverted alternative Mϕ polarization and enhanced IL12 secretion ($P = 0.0133$). NK cells activated by sorafenib-treated Mϕ showed increased degranulation (15.3 ± 0.2% versus 32.0 ± 0.9%, $P < 0.0001$) and interferon-gamma (IFN-ϒ) secretion (2.1 ± 0.2% versus 8.0 ± 0.2%, $P < 0.0001$) upon target cell contact. Sorafenib-triggered NK cell activation was verified by coculture experiments using TAM. Sorafenib-treated Mϕ increased cytolytic NK cell function against K562, Raji, and HepG2 target cells in a dose-dependent manner. Neutralization of interleukin (IL)12 or IL18 as well as inhibition of the nuclear factor kappa B (NF-κB) pathway reversed NK cell activation in Mϕ/NK cocultures. *Conclusion*: Sorafenib triggers proinflammatory activity of TAM and subsequently induces antitumor NK cell responses in a cytokine- and NF-κB-dependent fashion. This observation is relevant for HCC therapy, as sorafenib is a compound in clinical use that reverts alternative polarization of TAM in HCC

4.1199 Maternal obesity programs offspring nonalcoholic fatty liver disease by innate immune dysfunction in mice

Mouralidarane, A., Soeda, J., Visconti-Pugmire, C., Samuelsson, A-M., Pombo, J., maragkoudaki, X., Butt, A., Saraswati, R., Novelli, M., Fusai, G., Poston, L., Taylor, P.D. and oben, J.A.
Hepatology, ***58(1)***, *128-138 (2013)*

The global prevalence of obesity-induced liver disease (nonalcoholic fatty liver disease; NAFLD) is rising. Suggested causes include a role for *in utero* influences of maternal obesity compounded by the availability of energy-dense foods throughout postnatal life. Using a physiologically relevant model, we investigated the role of the innate immune system in liver injury induced by maternal obesity followed by a postnatal obesogenic diet. Female C57BL/6J mice were fed a standard or obesogenic diet before and throughout pregnancy and during lactation. Female offspring were weaned onto a standard or obesogenic diet at 3 weeks postpartum. Biochemical and histological indicators of dysmetabolism, NAFLD and fibrosis, analysis of profibrotic pathways, liver innate immune cells, and reactive oxygen species (ROS) were investigated at 3, 6, and 12 months. Female offspring exposed to a postweaning obesogenic diet (OffCon-OD) demonstrated evidence of liver injury, which was exacerbated by previous exposure to maternal obesity (OffOb-OD), as demonstrated by raised alanine aminotransferase, hepatic triglycerides, and hepatic expression of interleukin (IL)-6, tumor necrosis factor alpha, transforming growth factor beta, alpha smooth muscle actin, and collagen ($P < 0.01$). Histological evidence of hepatosteatosis and a more-robust NAFLD phenotype with hepatic fibrosis was observed at 12 months in OffOb-OD. A role for the innate immune system was indicated by increased Kupffer cell numbers with impaired phagocytic function and raised ROS synthesis ($P < 0.01$), together with reduced natural killer T cells and raised interleukin (IL)-12 and IL-18. *Conclusion:* Maternal obesity in the context of a postnatal hypercalorific obesogenic diet aggressively programs offspring NAFLD associated with innate immune dysfunction, resulting in a comprehensive phenotype that accurately reflects the human disease.

4.1200 Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice

Henning, J.R., Graffeo, C.S., Rehman, A., fallon, N.C., Zambirinis, C.P., Ochi, A., Barilla, R., Jamal, M., Deutsch, M., Greco, S., Ego-Osuala, M., Bin-Saeed, U., Rao, R.S., Badar, S., Quesada, J.P., Acehan, D. and Miller, G.
Hepatology, ***58(2)***, *589-602 (2013)*

Nonalcoholic steatohepatitis (NASH) is the most common etiology of chronic liver dysfunction in the United States and can progress to cirrhosis and liver failure. Inflammatory insult resulting from fatty infiltration of the liver is central to disease pathogenesis. Dendritic cells (DCs) are antigen-presenting cells with an emerging role in hepatic inflammation. We postulated that DCs are important in the progression of NASH. We found that intrahepatic DCs expand and mature in NASH liver and assume an activated immune phenotype. However, rather than mitigating the severity of NASH, DC depletion markedly exacerbated intrahepatic fibroinflammation. Our mechanistic studies support a regulatory role for DCs in NASH by limiting sterile inflammation through their role in the clearance of apoptotic cells and necrotic debris. We found that DCs limit $CD8^+$ T-cell expansion and restrict Toll-like receptor expression and cytokine production in innate immune effector cells in NASH, including Kupffer cells, neutrophils, and inflammatory monocytes. Consistent with their regulatory role in NASH, during the recovery phase of disease, ablation of DC populations results in delayed resolution of intrahepatic inflammation and

fibroplasia. *Conclusion*: Our findings support a role for DCs in modulating NASH. Targeting DC functional properties may hold promise for therapeutic intervention in NASH.

4.1201 Mutational Analyses on X-Linked Adrenoleukodystrophy Reveal a Novel Cryptic Splicing and Three Missense Mutations in the ABCD1 Gene

Hung, K-L., Wang, J-S., Keng, W.T., Chen, H-J., Liang, J-S., Ngu, L.H. and Lu, J-F.
Pediatric Neurol., ***49***, *185-190 (2013)*

Background
X-linked adrenoleukodystrophy is caused by a defective peroxisomal membrane transporter, ABCD1, responsible for transporting very-long-chain fatty acid substrate into peroxisomes for degradation. The main biochemical defect, which is also one of the major diagnostic hallmarks, of X-linked adrenoleukodystrophy is the accumulation of saturated very-long-chain fatty acids in all tissues and body fluids.
Methods
Direct and reverse-transcribed polymerase chain reactions followed by DNA sequencing-based mutational analyses were performed on one Taiwanese and three Malaysian X-linked adrenoleukodystrophy families.
Results
A novel splicing donor site mutation (c.1272+1g>a) was identified in a Taiwanese X-linked adrenoleukodystrophy patient, resulting in a deletion of 121 bp and a premature stop codon (p.Val425fs*92) in messenger-RNA transcript. This deletion is caused by the activation of a cryptic splicing donor site in exon 4 of the *ABCD1* gene, which is consistent with the prediction by several online algorithms. In addition, three previously described missense mutations (c.965T>C, c.1978C>T, and c.2006A>G), leading to aberrant ABCD1 of p.Leu322Pro, p.Arg660Trp, and p.His669Arg, were also identified in Malaysian probands.
Conclusions
This is the first report to unveil unequivocally that cryptic splicing-induced aberrant messenger-RNA carrying an internal frameshift deletion results from an intronic mutation in the *ABCD1* gene. Furthermore, a polymorphism in intron 9 (c.1992-32c/t; refSNP: rs4898368) of the *ABCD1* gene was commonly observed in both Taiwanese and Malaysian populations.

4.1202 Interleukin-33-Dependent Innate Lymphoid Cells Mediate Hepatic Fibrosis

Mchedlidze, T., Waldner, M., Zopf, S., Walker, J., Rankin, A.L., Schuchmann, M., Voehringer, D., McKenzie, A.N.J., Neurarath, M.F., Pflanz, S. and Wirtz, S.
Immunity, ***39***, *357-371 (2013)*

Liver fibrosis is a consequence of chronic liver diseases and thus a major cause of mortality and morbidity. Clinical evidence and animal studies suggest that local tissue homeostasis is disturbed due to immunological responses to chronic hepatocellular stress. Poorly defined stress-associated inflammatory networks are thought to mediate gradual accumulation of extracellular-matrix components, ultimately leading to fibrosis and liver failure. Here we have reported that hepatic expression of interleukin-33 (IL-33) was both required and sufficient for severe hepatic fibrosis in vivo. We have demonstrated that IL-33's profibrotic effects related to activation and expansion of liver resident innate lymphoid cells (ILC2). We identified ILC2-derived IL-13, acting through type-II IL-4 receptor-dependent signaling via the transcription factor STAT6 and hepatic stellate-cell activation, as a critical downstream cytokine of IL-33-dependent pathologic tissue remodeling and fibrosis. Our data reveal key immunological networks implicated in hepatic fibrosis and support the concept of modulation of IL-33 bioactivity for therapeutic purposes.

4.1203 Differentiation of glutamatergic neurons from mouse embryonic stem cells requires raptor S6K signaling

Chuang, J-H., Tung, L-C., Yin, Y. and Lin, Y.
Stem Cell Res., ***11***, *1117-1128 (2013)*

Although the mammalian target of rapamycin complex 1 (mTORC1) functions as an important signaling complex in many cellular processes, the role of mTORC1 in neurons derived from embryonic stem cells (ESCs) has been less explored. Here, using a modified protocol to differentiate mouse ESCs (mESCs) into almost uniform glutamatergic neurons, we explored the importance of raptor/mTORC1 in the differentiation of mESCs. Raptor gene-trap mESCs, and raptor-knockdown mESCs formed smaller-sized

embryonic bodies than the wild type and failed to undergo neuronal differentiation. Treatment with 1 μM rapamycin starting at the point when neuronal precursors began to differentiate from mESCs caused the gradual loss of neurites, shrinkage of soma, and a decreased ratio of neurite length to cell number over 48 to 72 h of treatment. This change was accompanied by activation of caspase-3 and S6 kinase (S6K), but not 4E-binding protein 1 (4EBP1). Knockdown of raptor during neuronal differentiation from mESCs also resulted in gradual loss of neurites and shrinkage of cell bodies. Loss of neurite density resulting from rapamycin treatment could be reversed by overexpression of S6K T389E. Taken together, these data demonstrate that raptor/mTORC1/S6K plays a critical role in the differentiation and survival of neurons derived from mESCs.

4.1204 Cushioned versus noncushioned centrifugation: Sperm recovery rate and integrity
Len, J.A., Beehan, D.P., Lyle, S.K. and Eilts, B.E.
Theriogenology, ***80***, *648-653 (2013)*

It was hypothesized that optimal sperm recovery rate (RR) without damage to the sperm would be obtained after centrifugation without a cushion solution. Semen collected three times from six light breed stallions was extended to 25 × 10^6 sperm/mL and centrifuged at CON (noncentrifuged), 900NC (no-cushion), 900C (cushion), 1800NC, and 1800C × *g* for 10 minutes. Sperm concentration, motility (TM and PM), and intact plasma membranes (PLM) and acrosomes (ACR) pre- and postcentrifugation (D0) and after 24 hours (D1) of cooling were evaluated. The RR in the CON (100 ± 0.0), 900NC (93.7 ± 2.9), and 1800NC (96.7 ± 2.6) groups was significantly higher than the 900C (68.7 ± 4.6) and 1800C (79.6 ± 3.5) groups. The D0 TM and PM were not different between the CON, 900NC, 900C, and 1800C, but were lower for the 1800NC group. The D1 TM and PM of the 900NC (75.2 ± 3.8 and 71.1 ± 4.1) and 900C (76.2 ± 3.7 and 72.4 ± 4.0) groups were significantly higher than the 1800NC (71.7 ± 4.1 and 67.3 ± 4.4) and 1800C (71.6 ± 4.1 and 67.2 ± 4.4) groups, and the CON (66.2 ± 4.5 and 60.0 ± 4.8) group was significantly lower than the other groups. The D1 PLM of the CON, 900NC, 900C, 1800NC, and 1800C groups were not different. The ACR on D1 was significantly lower for the CON (93.0 ± 2.4) group compared with all other groups. Optimal RR preserving sperm integrity was obtained in the 900NC group.

4.1205 Influences of cerebral stent implantation on CD4+CD25+FOXP3+Treg, Th1 and Th17 cells
Wang, S., Ni, B., Chen, K. and Shi, S.
Int. Immunopharmacol., ***17***, *519-525 (2013)*

Stent implantation is primarily used for the treatment of artery stenosis. However, the application and use of stent struts induces local and systemic inflammation, leading to intractable neointimal hyperplasia. $CD4^+$ T cells are involved in artery stenosis diseases, but little is known about the influence of the $CD4^+$ T cells on the inflammation reaction after stent implantation. In this study, we analyzed the frequency of signature transcription factors and proinflammatory cytokine expression from each subtype of $CD4^+$ T cells in 50 patients receiving intracranial or cervical stent implantations from December 2011 to June 2012. The results showed that the frequency of signature transcription factor/cytokine production in Treg cells was reduced in the first week and returned to control levels at 3 months after stent implantation. However, we observed opposite trends for Th17 cells, showing increased signature transcription factor/cytokine production during the acute phase, which returned to control levels after 3 months. No significant difference in the frequency of signature transcription factor/cytokine expression was observed in Th1 cells from patients before and after stent implantation. We speculate that the maintenance of the frequency and function of Tregs controls the inflammatory response, which otherwise induces acute inflammation after stent implantation.

4.1206 Small Molecule Suppressors of Drosophila Kinesin Deficiency Rescue Motor Axon Development in a Zebrafish Model of Spinal Muscular Atrophy
Gassmann, A., Hao, L.T., Bhoite, L., Braadford, C.L., Chien, C-B., Beattie, C.E. and Manfredi, J.P.
PloS One, ***8(9)***, *e74325 (2013)*

Proximal spinal muscular atrophy (SMA) is the most common inherited motor neuropathy and the leading hereditary cause of infant mortality. Currently there is no effective treatment for the disease, reflecting a need for pharmacologic interventions that restore performance of dysfunctional motor neurons or suppress

the consequences of their dysfunction. In a series of assays relevant to motor neuron biology, we explored the activities of a collection of tetrahydroindoles that were reported to alter the metabolism of amyloid precursor protein (APP). In *Drosophila* larvae the compounds suppressed aberrant larval locomotion due to mutations in the *Khc* and *Klc* genes, which respectively encode the heavy and light chains of kinesin-1. A representative compound of this class also suppressed the appearance of axonal swellings (alternatively termed axonal spheroids or neuritic beads) in the segmental nerves of the kinesin-deficient *Drosophila* larvae. Given the importance of kinesin-dependent transport for extension and maintenance of axons and their growth cones, three members of the class were tested for neurotrophic effects on isolated rat spinal motor neurons. Each compound stimulated neurite outgrowth. In addition, consistent with SMA being an axonopathy of motor neurons, the three axonotrophic compounds rescued motor axon development in a zebrafish model of SMA. The results introduce a collection of small molecules as pharmacologic suppressors of SMA-associated phenotypes and nominate specific members of the collection for development as candidate SMA therapeutics. More generally, the results reinforce the perception of SMA as an axonopathy and suggest novel approaches to treating the disease

4.1207 Apoptotic cell administration enhances pancreatic islet engraftment by induction of regulatory T cells and tolerogenic dendritic cells

Wu, C., Zhang, Y., Jiang, Y., Wang, Q., Long, Y., Wang, C., Cao, X. and Chen, G.
Cell. Mol. Immunol., ***10***, *393-402 (2013)*

Apoptotic cell transfer has been found to be able to facilitate engraftment of allograft. However, the underlying mechanisms remain to be fully understood. Here we demonstrate that intravenous administration of donor apoptotic splenocytes can promote pancreatic islet engraftment by inducing generation of tolerogenic dendritic cells (Tol-DCs) and expansion of $CD4^{+}Foxp3^{+}$ regulatory T cells (Tregs). *In vivo* clearance of either dendritic cells (DCs) or Tregs prevented the induction of immune tolerance by apoptotic cell administration. Transient elimination of Tregs using anti-CD25, monoclonal antibody (mAb) abrogated the generation of Tol-DCs after administration of apoptotic splenocytes. Reciprocally, depletion of DCs within CD11c-DTR mice using diphtheria toxin (DT) prevented the generation of Tregs in the recipients with administration of apoptotic splenocytes. Induction of Tregs by Tol-DCs required direct cell contact between the two cell types, and programmed death 1 ligand (PD-L1) played important role in the Tregs expansion. Apoptotic cell administration failed to induce Tol-DCs in IL-10-deficient and Smad3-deficient mice, suggesting that IL-10 and transforming growth factor-β (TGF-β) are needed to maintain DCs in the tolerogenic state. Therefore, we demonstrate that Tol-DCs promote the expansion of Tregs *via* PD-L1 on their surface and reciprocally Tregs facilitate Tol-DCs to maintain transplantation tolerance induced by apoptotic cells *via* secreting IL-10 and TGF-β.

4.1208 TLR2 Mediates Helicobacter pylori–Induced Tolerogenic Immune Response in Mice

Sun, X., Zhang, M., El-Zataari, M., Qwyang, S.Y., Eaton, K.A., Liu, M., Chang, Y-M., Zou, W. and Kao, J.Y.
PloS One, ***8(9)****, e74595 (2013)*

We have shown that *Helicobacter pylori* induces tolerogenic programming of dendritic cells and inhibits the host immune response. Toll-like receptors (TLRs) represent a class of transmembrane pattern recognition receptors essential for microbial recognition and control of the innate immune response. In this study, we examined the role of TLRs in mediating *H. pylori* tolerogenic programming of dendritic cells and their impact on anti–*H. pylori* immunity using C57BL/6 wild-type and TLR2-knockout (TLR2KO) mice. We analyzed the response of TLR2KO bone marrow-derived dendritic cells (BMDCs) to *H. pylori* SS1 stimulation and the outcome of chronic *H. pylori* infection in TLR2KO mice. We showed that *H. pylori*–stimulated BMDCs upregulated the expression of TLR2, but not TLR4, TLR5, or TLR9. *H. pylori*-stimulated BMDCs from TLRKO mice induced lower Treg and Th17 responses, but a higher IFN-γ response compared to *H. pylori*-stimulated BMDCs from wild-type mice. In vivo analyses following an *H. pylori* infection of 2 months duration showed a lower degree of gastric *H. pylori* colonization in TLR2KO mice and more severe gastric immunopathology compared to WT mice. The gastric mucosa of the infected TLR2KO mice showed a lower mRNA expression of Foxp3, IL-10, and IL-17A, but higher expression of IFN-γ compared to the gastric mRNA expression in infected wild-type mice. Moreover, the *H. pylori*–specific Th1 response was higher and the Treg and Th17 responses were lower in the spleens of infected TLR2KO mice compared to infected WT mice. Our data indicate that *H. pylori* mediates immune tolerance through TLR2-derived signals and inhibits Th1 immunity, thus evading host defense. TLR2 may be an important target in the modulation of the host response to *H. pylori*.

4.1209 Dynamics of the Major Histocompatibility Complex Class I Processing and Presentation Pathway in the Course of Malaria Parasite Development in Human Hepatocytes: Implications for Vaccine Development

Ma, J., Trop, S., Baer, S., Rakjmanaliev, E., Arany, Z., Dumoulin, P., Zhang, H., Romano, J., Coppens, I., levitsky, V. and Levitskaya, J.
PloS One, ***8(9)***, *e75321 (2013)*

Control of parasite replication exerted by MHC class I restricted CD8+ T-cells in the liver is critical for vaccination-induced protection against malaria. While many intracellular pathogens subvert the MHC class I presentation machinery, its functionality in the course of malaria replication in hepatocytes has not been characterized. Using experimental systems based on specific identification, isolation and analysis of human hepatocytes infected with *P. berghei* ANKA GFP or *P. falciparum* 3D7 GFP sporozoites we demonstrated that molecular components of the MHC class I pathway exhibit largely unaltered expression in malaria-infected hepatocytes until very late stages of parasite development. Furthermore, infected cells showed no obvious defects in their capacity to upregulate expression of different molecular components of the MHC class I machinery in response to pro-inflammatory lymphokines or trigger direct activation of allo-specific or peptide-specific human CD8+ T-cells. We further demonstrate that ectopic expression of circumsporozoite protein does not alter expression of critical genes of the MHC class I pathway and its response to pro-inflammatory cytokines. In addition, we identified supra-cellular structures, which arose at late stages of parasite replication, possessed the characteristic morphology of merosomes and exhibited nearly complete loss of surface MHC class I expression. These data have multiple implications for our understanding of natural T-cell immunity against malaria and may promote development of novel, efficient anti-malaria vaccines overcoming immune escape of the parasite in the liver.

4.1210 Definition of a third VLR gene in hagfish

Li, J., Das, S., Herrin, B.R., Hirano, M. and Cooper, M.D.
PNAS, ***110(37)***, *15013-15018 (2013)*

Jawless vertebrates (cyclostomes) have an alternative adaptive immune system in which lymphocytes somatically diversify their variable lymphocyte receptors (VLR) through recombinatorial use of leucine-rich repeat cassettes during *VLR* gene assembly. Three types of these anticipatory receptors in lampreys (*VLRA*, *VLRB*, and *VLRC*) are expressed by separate lymphocyte lineages. However, only two *VLR* genes (*VLRA* and *VLRB*) have been found in hagfish. Here we have identified a third hagfish *VLR*, which undergoes somatic assembly to generate sufficient diversity to encode a large repertoire of anticipatory receptors. Sequence analysis, structural comparison, and phylogenetic analysis indicate that the unique hagfish VLR is the counterpart of lamprey VLRA and the previously identified hagfish "VLRA" is the lamprey VLRC counterpart. The demonstration of three orthologous *VLR* genes in both lampreys and hagfish suggests that this anticipatory receptor system evolved in a common ancestor of the two cyclostome lineages around 480 Mya.

4.1211 Histones Activate the NLRP3 Inflammasome in Kupffer Cells during Sterile Inflammatory Liver Injury

Huang, H., Chen, H-W., Evankovich, J., Yan, W., Rosborough, B.R., Nace, G.W., Ding, Q., Loughran, P., Beer-Stolz, D., Billiar, T.R., Esmon, C.T. and Tsung, A.
J. Immunol., ***191***, *2665-2679 (2013)*

Cellular processes that drive sterile inflammatory injury after hepatic ischemia/reperfusion (I/R) injury are not completely understood. Activation of the inflammasome plays a key role in response to invading intracellular pathogens, but mounting evidence suggests that it also plays a role in inflammation driven by endogenous danger-associate molecular pattern molecules released after ischemic injury. The nucleotide-binding domain, leucine-rich repeat containing protein 3 (NLRP3) inflammasome is one such process, and the mechanism by which its activation results in damage and inflammatory responses following liver I/R is unknown. In this article, we report that both NLRP3 and its downstream target caspase-1 are activated during I/R and are essential for hepatic I/R injury, because both NLRP3 and caspase-1 knockout mice are protected from injury. Furthermore, inflammasome-mediated injury is dependent on caspase-1 expression in liver nonparenchymal cells. Although upstream signals that activate the inflammasome during ischemic injury are not well characterized, we show that endogenous extracellular histones activate the NLRP3 inflammasome during liver I/R through TLR9. This occurs through TLR9-dependent generation of reactive oxygen species. This mechanism is operant in resident liver Kupffer cells, which drive innate immune responses after I/R injury by recruiting additional cell types, including neutrophils and inflammatory

monocytes. These novel findings illustrate a new mechanism by which extracellular histones and activation of NLRP3 inflammasome contribute to liver damage and the activation of innate immunity during sterile inflammation.

4.1212 OPEN

4.1213 Ultrahigh-Throughput Mammalian Single-Cell Reverse-Transcriptase Polymerase Chain Reaction in Microfluidic Drops
Eastburn, D.J., Sciambi, A. and Abate, A.R.
*Anal. Chem., **85(16)**, 8016-8021 (2013)*

The behaviors of complex biological systems are often dictated by the properties of their heterogeneous and sometimes rare cellular constituents. Correspondingly, the analysis of individual cells from a heterogeneous population can reveal information not obtainable by ensemble measurements. Reverse-transcriptase polymerase chain reaction (RT-PCR) is a widely used method that enables transcriptional profiling and sequencing analysis on bulk populations of cells. Major barriers to successfully implementing this technique for mammalian single-cell studies are the labor, cost, and low-throughput associated with current approaches. In this report, we describe a novel droplet-based microfluidic system for performing ~50000 single-cell RT-PCR reactions in a single experiment while consuming a minimal amount of reagent. Using cell type-specific staining and TaqMan RT-PCR probes, we demonstrate the identification of specific cells from a mixed human cell population. The throughput, robust detection rate and specificity of this method makes it well-suited for characterizing large, heterogeneous populations of cells at the transcriptional level.

4.1214 Absence of Siglec-H in MCMV Infection Elevates Interferon Alpha Production but Does Not Enhance Viral Clearance
Puttur, F., Arnold-Schrauf, C., Lahl, K., Solmaz, G., Lindenberg, M., Mayer, C.T., Gohmert, M., Swallow, M., van Helt, C., Schmitt, H., Nitschke, L., Lambrecht, B.N., Lang, R., Messerle, M. and Sparwasser, T.
*PloS One, **9(9)**, e1003648 (2013)*

Plasmacytoid dendritic cells (pDCs) express the I-type lectin receptor Siglec-H and produce interferon α (IFNα), a critical anti-viral cytokine during the acute phase of murine cytomegalovirus (MCMV) infection. The ligands and biological functions of Siglec-H still remain incompletely defined *in vivo*. Thus, we generated a novel bacterial artificial chromosome (BAC)-transgenic "pDCre" mouse which expresses Cre recombinase under the control of the Siglec-H promoter. By crossing these mice with a Rosa26 reporter strain, a representative fraction of Siglec-H^+ pDCs is terminally labeled with red fluorescent protein (RFP). Interestingly, systemic MCMV infection of these mice causes the downregulation of Siglec-H surface expression. This decline occurs in a TLR9- and MyD88-dependent manner. To elucidate the functional role of Siglec-H during MCMV infection, we utilized a novel Siglec-H deficient mouse strain. In the absence of Siglec-H, the low infection rate of pDCs with MCMV remained unchanged, and pDC activation was still intact. Strikingly, Siglec-H deficiency induced a significant increase in serum IFNα levels following systemic MCMV infection. Although Siglec-H modulates anti-viral IFNα production, the control of viral replication was unchanged *in vivo*. The novel mouse models will be valuable to shed further light on pDC biology in future studies.

4.1215 Aging affects AO rat splenic conventional dendritic cell subset composition, cytokine synthesis and T-helper polarizing capacity
Stojic-Vukanic, Z., Bufan, B., Arsenovic-Eanin. N., Kosec, D., Pilipovic, I., Nanut, M.P. and Leposavic, G.
*Biogerontology, **14**, 443-459 (2013)*

It is well-established that almost all cellular components of innate and adaptive immunity undergo age-related remodelling. The findings on age-related changes in both human and mouse dendritic cells (DCs) are conflicting, whereas there are no data on the influence of aging on rat DCs. In an attempt to fill this gap, freshly isolated splenic DCs expressing CD103 (α_{OX-62} integrin), a DC specific marker recognized by MRC OX62 monoclonal antibody, from 3- (young) and 26-month-old (aged) Albino Oxford rats were examined for subset composition, expression of activation/differentiation markers (CD80, CD86 and CD40 and MHC II molecules) and endocytic capacity using flow cytometric analysis (FCA). In addition, splenic OX62+ DCs cultured in the presence or absence of LPS were analysed for the activation marker and TNF-α, IL-6, IL-12, IL-23, TGF-β1, IL-10 expression using FCA, RT-PCR and ELISA, respectively. Moreover,

the allostimulatory capacity of OX62+ DCs and IFN-γ, IL-4 and IL-17 production by CD4+ T cells in mixed leukocyte reaction was quantified using FCA and ELISA, respectively. It was found that aging: i) shifts the CD4+:CD4− subset ratio in the OX62+ DCs population towards the CD4− subset and ii) influences DCs maturation (judging by activation marker expression and efficiency of endocytosis) by affecting the expression of intrinsic (TNF-α and IL-10) and extrinsic maturation regulators. Furthermore, in LPS-matured OX62+ DCs from aged rats expression of TNF-α, IL-12, IL-23 and IL-6 was increased, whereas that of IL-10 was diminished compared with the corresponding cells from young rats. Moreover, in MLR, OX62+ DCs from aged rats exhibited enhanced Th1/Th17 driving force and diminished allostimulatory capacity compared with those from young rats.

4.1216 Down-regulation of microglial activity attenuates axotomized nigral dopaminergic neuronal cell loss
Song, D-Y., Yu, H-N., Park, C-R., Lee, J-S., Lee, J-Y., park, B-G., Woo, R-S., Han, J-T., Cho, B-P. and Baik, T-K.
*BMC Neurosci., **14**:112 (2013)*

Background
There is growing evidence that inflammatory processes of activated microglia could play an important role in the progression of nerve cell damage in neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease which harbor features of chronic microglial activation, though the precise mechanism is unknown. In this study, we presented *in vivo* and *ex vivo* experimental evidences indicating that activated microglia could exacerbate the survival of axotomized dopaminergic neurons and that appropriate inactivation of microglia could be neuroprotective.
Results
The transection of medial forebrain bundle (MFB) of a rat induced loss of dopaminergic neurons in a time-dependent manner and accompanied with microglial activation. Along with microglial activation, production of reactive oxygen species (ROS) was upregulated and TH/OX6/hydroethidine triple-immunofluorescence showed that the microglia mainly produced ROS. When the activated microglial cells that were isolated from the substantia nigra of the MFB axotomized animal, were transplanted into the substantia nigra of which MFB had been transected at 7 days ago, the survival rate of axotomized dopaminergic neurons was significantly reduced as compared with sham control. Meanwhile, when the microglial activation was attenuated by administration of tuftsin fragment 1-3 (microglia inhibitory factor) into the lateral ventricle using mini-osmotic pump, the survival rate of axotomized dopaminergic neurons was increased.
Conclusion
The present study suggests that activated microglia could actively produce and secrete unfavorable toxic substances, such as ROS, which could accelerate dopaminergic neuronal cell loss. So, well-controlled blockade of microglial activation might be neuroprotective in some neuropathological conditions.

4.1217 Characterization of functional capacity of adult ventricular myocytes in long-term culture
Liu, S.J.
*Int. J. Cardiol., **168**, 1923-1936 (2013)*

Background
Functional properties of freshly isolated adult ventricular myocytes (AVMs) or those of AVMs during first few weeks in culture were well described. However, the functional capacity of these AVMs such as regenerative potential remains unknown, in part, due to the short lifespan of AVMs in culture. This study modified culture conditions that extended the lifespan of AVMs, isolated from adult rat hearts, longer than 6 months.
Methods
Temporal changes in the morphology of individual AVMs, cell–cell interaction, formation of myofibers, self-repair capacity after injury, expression of senescence biomarkers, and contractile function of AVMs over 5 weeks (defined as long-term culture) were chronologically characterized and quantified with live-cell video and fluorescence microscopy, and immunocytochemistry.
Results
Cell growth in size reached a plateau after 4 weeks in culture concomitantly with continuous increase in structural remodeling in long-term culture. Dynamic remodeling of AVMs promoted self-contact of filopodia and cell–cell contact where these contained abundant myofilaments, connexin 43 proteins, and high density and high integrity of mitochondria. Such high capacity also enabled self-repair of AVMs after injury, cytokinesis, and formation of myofibers. AVMs in long-term culture displayed spontaneous

contraction and importantly were responsive to electrical stimulation. Moreover, AVMs expressed senescence-associated β-galactosidase, p16, and stress-associated atrial natriuretic peptides that resulted likely from cellular modeling.
Conclusions
Prolonged longevity of AVMs in culture with characteristics of high functional capacity of organelle regeneration and contraction makes them invaluable for further longitudinal mechanistic studies in cardiac (patho)physiology (e.g., hypertrophy and aging), single-cell analysis (e.g., function of hetero-phenotypes) and drug discovery.

4.1218 Tolerance develops to the antiallodynic effects of the peripherally acting opioid loperamide hydrochloride in nerve-injured rats

He, S-Q., Yang, F., Perez, F.M., Xu, Q., Shechter, R., Cheong, Y-K., Carteret, A.F., Dong, X., Sweitzer, S.M., Raja, S.N. and Guan, Y.
Pain, ***154***, *2477-2486 (2013)*

Peripherally acting opioids are potentially attractive drugs for the clinical management of certain chronic pain states due to the lack of centrally mediated adverse effects. However, it remains unclear whether tolerance develops to peripheral opioid analgesic effects under neuropathic pain conditions. We subjected rats to L5 spinal nerve ligation (SNL) and examined the analgesic effects of repetitive systemic and local administration of loperamide hydrochloride, a peripherally acting opioid agonist. We found that the inhibition of mechanical hypersensitivity, an important manifestation of neuropathic pain, by systemic loperamide (1.5 mg/kg subcutaneously) decreased after repetitive drug treatment (tolerance-inducing dose: 0.75 to 6.0 mg/kg subcutaneously). Similarly, repeated intraplantar injection of loperamide (150 μg/50 μL intraplantarly) and D-Ala^2-MePhe^4-Glyol^5 enkephalin (300 μg/50 μL), a highly selective mu-opioid receptor (MOR) agonist, also resulted in decreased inhibition of mechanical hypersensitivity. Pretreatment with naltrexone hydrochloride (5 mg/kg intraperitoneally) and MK-801 (0.2 mg/kg intraperitoneally) attenuated systemic loperamide tolerance. Western blot analysis showed that repetitive systemic administration of morphine (3 mg/kg subcutaneously), but not loperamide (3 mg/kg subcutaneously) or saline, significantly increased MOR phosphorylation in the spinal cord of SNL rats. In cultured rat dorsal root ganglion neurons, loperamide dose-dependently inhibited KCl-induced increases in $[Ca^{2+}]_i$. However, this drug effect significantly decreased in cells pretreated with loperamide (3 μM, 72 hours). Intriguingly, in loperamide-tolerant cells, the delta-opioid receptor antagonist naltrindole restored loperamide's inhibition of KCl-elicited $[Ca^{2+}]_i$ increase. Our findings indicate that animals with neuropathic pain may develop acute tolerance to the antiallodynic effects of peripherally acting opioids after repetitive systemic and local drug administration.

4.1219 A Specific and Facile Method for Isolating Murine Liver Sinusoidal Endothelial Cells using a CD32B Monoclonal Antibody

Donofrio, B., Kasumov, T., Dasarathy, S. and McCullough, A.J.
Hepatology, ***58(4)***, *455A-459A (2013)*

Background: Liver sinusoidal endothelial cells (LSEC) comprise 20% of all liver cells and are now recognized to be both a target and mediator of injury in a number of liver diseases. Despite their emerging importance, current methods of LSEC isolation (centrificugal elutriation or percoll adherence) are cumbersome, produce variable yields and have limited utility as a practical laboratory method. The proposed technique is based on the differential staining characteristics between CD31 (a marker of all endothelial cells) and CD32B (a marker specific for LSEC). Aim: To develop an isolation method for LSEC that would reliably obtain high yield with a specificity and reproducibility that would be easily available for wide spread use.
Methods: We used an immunomagnetic method with the monoclonal antibodies: Rat Anti-Mouse F4/80 and Rat Anti-Mouse Fc gamma RIIB/CD32B. Non-Parenchymal cells were obtained by 0.05% Collagenase liver digestion and a 17% OptiPrep (iodixanol) density gradient. Cells were then subjected to Rat Anti-Mouse F4/80-conjugated Dynabeads to remove Kupffer cells and blood Monocytes. The remaining cells were subjected to magnetic bead cell isolation using Rat Anti-Mouse Fc gamma RIIB/CD32B. The attached LSEC were released from the beads with a specific decoupling buffer. Cells were then plated on a fibronectin matrix. Ninety percent of bead isolated cells were recovered with an average yield of 5.5 x10exp6 ±2 LSEC per mouse liver. Both mouse liver tissue and CD32B isolated cells were stained with immune labeled LSEC markers; CD31,CD32B and Stabilin-2

(Stab-2). Isotype controls were run for CD31, CD32B and a blocking peptide control was run for Stab-2. Results: All three markers stained positive in both mouse liver tissue and CD32B isolated cells. A co-stain with CD32B and Stab-2 showed co-localization in isolated LSEC and in liver tissue in a sinusoidal distribution. The CD32B isolated cells also showed typical LSEC fenestrae and sieve plates on scanning electron microscopy. Importantly, 99% of the isolated cells took up formaldehyde-treated serum albumin and oxidized low-density lipoprotein (oxLDL), which are functions and markers specific for LSEC. Conclusion: This straight forward isolation method allows for the culture of LSEC with high purity and reproducibility with a potential for more wide spread use than currently available methods.

4.1220 Manual adult porcine islet isolation technique and optimal condition for adult pig islets

Okitsu, T.

*Xenotransplantation, **20(5)**, 349 (2013)*

In the case of requiring a high islet yield obtained efficiently through a single series of pancreas procurement and islet isolation procedure, adult pigs, especially retired breeders, are considered suitable to the islet donors. Besides the age of the pig, its race and particular feeding protocols could also influence the number and the morphology of the islets in the pancreas. Because islet yield depends on the na€õve number and how well morphology is preserved in the isolated islets, a series of pancreas procurement and islet isolation is recognized to be an important factor to determine islet yield. This presentation will introduce a simple islet isolation procedure that is modified based upon the static digestion method originally described by O'Neil et al [2001 Cell Transplantation]. In the procedure, at slaughterhouse, a whole pancreas from a retired breeder pig is procured within 30 min of warm ischemic time. A cannula is inserted into the main pancreatic duct, which had been connected to the duodenum. Through the cannula, 200–300 ml of M-Kyoto solution is infused into the pancreatic duct. Then the pancreas is preserved in oxygenized perfluorocarbon and transported to the laboratory. At the isolation laboratory, pancreas is distended using 300–400ml of Hank's balanced salt solution containing 0.5 mg/ml of collagenase. The pancreas is cut into seven to nine pieces, put into a one liter Nalgene jar, and left in water bath at 37^a„*f* for approximately 1 h until almost all the pancreas is digested. After the serous membrane of the pancreas is teared off using two pairs of forceps to allow the dissociated tissue to be released into the solution, all the tissue is filtrated through 500 lm mesh in a large filtration chamber and collected in 250 ml conical tubes. For islet purification, four 500 ml plastic containers with flat bottom are used to load discontinuous density gradients. This system allows us to purify up to 120 ml of dissociated pancreas tissue at a time. The discontinuous gradients consist of 100 ml each of M-Kyoto solution containing iodixanol at the density of 1.060, 1.096 and 1.110 g/ml; the bottom gradient of 1.110 g/ml solution is mixed with the dissociated pancreas tissue in advance. This system is centrifuged at 1000 rpm (240 9 g) for 5 min at 4^a„*f*. The purified islets form a layer between the top and the second gradients and they are carefully collected and washed three times. Using this islet isolation method, we obtained a mean of 560,000 islet equivalent after purification with islet purity of greater than 60% (n = 6 isolations) from one adult pig. This method would be useful to isolate porcine islets of quite a few numbers in a stable and efficient manner through a single series of pancreas procurement and islet isolation using adult pigs.

4.1221 Optimization of a porcine islet isolation and purification procedure that utilizes recombinant collagenase

Green, M., Beechler, C., Breite, D., Dwulet, F. and McCarthy,R.

*Xenotransplantation, **20(5)**, 334 (2013)*

Porcine pancreata represent an alternative xenogeneic source of islets for the clinical treatment of type 1 diabetes. However, the isolation and subsequent purification of porcine islets presents some challenging technical hurdles. Donor characteristics, procurement steps, type and level of enzymes used for tissue dissociation, digestion method, and purification protocol all significantly influence the isolation outcome. Using a standardized Ricordi procedure, which had been previously optimized for use with natural collagenases purified from C. histolyticum fermentation broth, we evaluated the performance of recombinant collagenases expressed in E. coli for use in porcine islet isolation. Pancreata from retired Landrace breeders (18–36 months of age) were divided into the splenic and duodenal/connecting lobes such that direct comparisons between test formulations could be made. Equivalent enzymatic target activities per gram of tissue were maintained between test formulations using specific activities determined from in-house substrate-specific assays. Recombinantly-expressed class II collagenase (rC2) targeted at 7.5

Wunsch units/g pancreas caused a 33% reduction ($P < 0.001$) in the mass of undigested tissue, an approximate 5 min quicker ($P < 0.001$) switch time and a 55% increase ($P < 0.01$) in packed tissue volume relative to naturally-derived C2 at the same target levels. Moreover, a further improvement ($P = 0.03$) in digestion was observed when recombinant C1 (rC1) rather than natural C1 was used in combination with rC2. Molecular form [i.e. intact (rC1) vs. truncated protein (rC1c)]of class I collagenase had no effect on any digestion parameter measured when equivalent collagen degrading activity was targeted, however, achieving such targets required approximately 19-fold higher enzyme masses for rC1c. Clostripain, a tryptic-like protease co-expressed in C. histolyticum fermentations, reduced ($P < 0.01$ and $P < 0.001$) the amount of undigested tissue by 21.6 and 35.5% when combined with naturally-derived and recombinant collagenase, respectively. Addition of a serine protease inhibitor (AEBSF; 0.4 mM) to the Ricordi circuit dramatically reduced the diameter of the liberated exocrine tissue pieces, and, consequently, improved islet release. Discontinuous polysucrose density gradients were inadequate in separating porcine islets from the contaminating exocrine tissue when recombinant collagenase was used for tissue dissociation. However, purification via a continuous iodixanol density gradient (1.069–1.108 g/cm3) resulted in high purity (>90%) preparations of porcine islets with high viability (>85%) as determined by SYTO13/propidium iodide staining. In summary, recombinantlyexpressed collagenases possess some inherent advantages over their naturallyderived counterparts that improve some digestion parameters, but also mandate modifications to the porcine islet isolation and purification protocol. The optimal formulation of rC2, rC1 and neutral protease for maximizing porcine islet yield and function is currently bein determined in a 23 factorial design.

4.1222 **Biliary obstruction results in PD-1-dependent liver T cell dysfunction and acute inflammation mediated by Th17 cells and neutrophils**
Licata, L.A., Ngyuen, C.T., Burga, R.A., Falanga, V., Espat, N.J., Ayala, A., Thorn, M., Junghans, R.P. and katz, S.C.
J. Leukoc. Biol., ***94(4)***, *813-823 (2013)*

Biliary obstruction is a common clinical problem that is associated with intrahepatic inflammation and impaired immunity. PD-1 is well known to mediate T cell dysfunction but has been reported to promote and attenuate acute inflammation in various injury models. With the use of a well-established murine model of BDL, we studied the effects of intrahepatic PD-1 expression on LTC function, inflammation, and cholestasis. Following BDL, PD-1 expression increased significantly among LTCs. Increased PD-1 expression following BDL was associated with decreased LTC proliferation and less IFN-γ production. Elimination of PD-1 expression resulted in significantly improved proliferative capacity among LTC following BDL, in addition to a more immunostimulatory cytokine profile. Not only was LTC function rescued in PD-$1^{-/-}$ mice, but also, the degrees of biliary cell injury, cholestasis, and inflammation were diminished significantly compared with WT animals following BDL. PD-1-mediated acute inflammation following BDL was associated with expansions of intrahepatic neutrophil and Th17 cell populations, with the latter dependent on IL-6. PD-1 blockade represents an attractive strategy for reversing intrahepatic immunosuppression while limiting inflammatory liver damage.

4.1223 **Th9 Cells Drive Host Immunity against Gastrointestinal Worm Infection**
Licona-Limon, P., Henao-Mejia, J., Temann, A.U., Gagliani, N., Licona.Limon, I., Ishigame, H., Hao, L., Herbert, D.B.R. and Flavell, A.
Immunity, ***39(4)***, *744-757 (2013)*

Type 2 inflammatory cytokines, including interleukin-4 (IL-4), IL-5, IL-9, and IL-13, drive the characteristic features of immunity against parasitic worms and allergens. Whether IL-9 serves an essential role in the initiation of host-protective responses is controversial, and the importance of IL-9- versus IL-4-producing $CD4^+$ effector T cells in type 2 immunity is incompletely defined. Herein, we generated IL-9-deficient and IL-9-fluorescent reporter mice that demonstrated an essential role for this cytokine in the early type 2 immunity against *Nippostrongylus brasiliensis*. Whereas T helper 9 (Th9) cells and type 2 innate lymphoid cells (ILC2s) were major sources of infection-induced IL-9 production, the adoptive transfer of Th9 cells, but not Th2 cells, caused rapid worm expulsion, marked basophilia, and increased mast cell numbers in *Rag2*-deficient hosts. Taken together, our data show a critical and nonredundant role for Th9 cells and IL-9 in host-protective type 2 immunity against parasitic worm infection.

4.1224 **3,3′-Diindolylmethane ameliorates experimental hepatic fibrosis via inhibiting miR-21 expression**
Zhang, Z., Gao, Z., Hu, W., Yin, S., Wang, C., Zang, Y., Chen, J., Zhang, J. and Dong, L.

Br. J. Pharmacol., ***170(3)****, 649-660 (2013)*

Background and Purpose
Hepatic fibrosis is a type of liver disease characterized by excessive collagen deposition produced by activated hepatic stellate cells (HSCs), and no appropriate drug treatment is available clinically. The microRNA, miR-21 exhibits an important role in the pathogenesis and progression of hepatic fibrosis. 3,3′-Diindolylmethane (DIM) is a natural autolytic product in plants and can down-regulate miR-21 expression. Here we have assessed the therapeutic effects of DIM against hepatic fibrosis and investigated the underlying mechanisms.
Experimental Approach
The effects of DIM on HSC activation were measured by analysing the expression of α-smooth muscle actin and collagen I in both HSC-T6 cell line and primary HSCs. Expression of miR-21 was also measured after DIM treatment and the therapeutic effect of DIM was further studied *in vivo*, using the model of hepatic fibrosis induced by thioacetamide in mice. The antagonist oligonucleotide, antagomir-21, was also used to suppress the effects of miR-21.
Key Results
DIM suppressed the central TGF-β signalling pathway underlying HSC activation by down-regulating the expression of miR-21. The decreased miR-21 expression was achieved by inhibiting the activity of the transcription factor, AP-1. Moreover, DIM blunted the activation phenotype of primary HSCs. Administration of DIM *in vivo* attenuated liver fibrosis induced by thioacetamide, as assessed by collagen deposition and profiles of profibrogenic markers.
Conclusions and Implications
DIM shows potential as a therapeutic agent for the treatment of hepatic fibrosis.

4.1225 Metabolic Assessment Prior to Total Pancreatectomy and Islet Autotransplant: Utility, Limitations and Potential

Lundberg, R., Beilman, G.J., Dunn, T.B., Pruett, T.L., Chinnakotla, S.C., Radosevich, D.M., Robertson, R.P., Ptacek, P., Balamurugan, A.N., Wilhelm, J.J., Hering, B.J., Sutherland, D.E.R., Moran, A. and Bellin, M.D.
Am. J. Transplant., ***13(10)****, 2664-2671 (2013)*

Islet autotransplant (IAT) may ameliorate postsurgical diabetes following total pancreatectomy (TP), but outcomes are dependent upon islet mass, which is unknown prior to pancreatectomy. We evaluated whether preoperative metabolic testing could predict islet isolation outcomes and thus improve assessment of TPIAT candidates. We examined the relationship between measures from frequent sample IV glucose tolerance tests (FSIVGTT) and mixed meal tolerance tests (MMTT) and islet mass in 60 adult patients, with multivariate logistic regression modeling to identify predictors of islet mass ≥2500 IEQ/kg. The acute C-peptide response to glucose (ACRglu) and disposition index from FSIVGTT correlated modestly with the islet equivalents per kilogram body weight (IEQ/kg). Fasting and MMTT glucose levels and HbA1c correlated inversely with IEQ/kg (r values −0.33 to −0.40, $p \leq 0.05$). In multivariate logistic regression modeling, normal fasting glucose (<100 mg/dL) and stimulated C-peptide on MMTT ≥4 ng/mL were associated with greater odds of receiving an islet mass ≥2500 IEQ/kg (OR 0.93 for fasting glucose, CI 0.87–1.0; OR 7.9 for C-peptide, CI 1.75–35.6). In conclusion, parameters obtained from FSIVGTT correlate modestly with islet isolation outcomes. Stimulated C-peptide ≥4 ng/mL on MMTT conveyed eight times the odds of receiving ≥2500 IEQ/kg, a threshold associated with reasonable metabolic control postoperatively.

4.1226 TLR 2 and 4 Responsiveness from Isolated Peripheral Blood Mononuclear Cells from Rats and Humans as Potential Chronic Pain Biomarkers

Kwok, Y.H., Tuke, J., Nicotra, L.L., Grace, P.M., Rolan, R.E. and Hutchinson, M.R.
PloS One, ***8(10)****, e77799 (2013)*

Background
Chronic pain patients have increased peripheral blood mononuclear cell Interkeukin-1β production following TLR2 and TLR4 simulation. Here we have used a human-to-rat and rat-to-human approach to further investigate whether peripheral blood immune responses to TLR agonists might be suitable for development as possible systems biomarkers of chronic pain in humans.
Methods and Results
Study 1: using a graded model of chronic constriction injury in rats, behavioral allodynia was assessed followed by *in vitro* quantification of TLR2 and TLR4 agonist-induced stimulation of IL-1β release by

PBMCs and spinal cord tissues (n = 42; 6 rats per group). Statistical models were subsequently developed using the IL-1β responses, which distinguished the pain/no pain states and predicted the degree of allodynia. Study 2: the rat-derived statistical models were tested to assess their predictive utility in determining the pain status of a published human cohort that consists of a heterogeneous clinical pain population (n = 19) and a pain-free population (n = 11). The predictive ability of one of the rat models was able to distinguish pain patients from controls with a ROC AUC of 0.94. The rat model was used to predict the presence of pain in a new chronic pain cohort and was able to accurately predict the presence of pain in 28 out of the 34 chronic pain participants.

Conclusions

These clinical findings confirm our previous discoveries of the involvement of the peripheral immune system in chronic pain. Given that these findings are reflected in the prospective graded rat data, it suggests that the TLR response from peripheral blood and spinal cord were related to pain and these clinical findings do indeed act as system biomarkers for the chronic pain state. Hence, they provide additional impetus to the neuroimmune interaction to be a drug target for chronic pain.

4.1227 Gephyrin plays a key role in BDNF-dependent regulation of amygdala surface GABAARs

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Neuroscience, ***255***, *33-44 (2013)*

Brain-derived neurotrophic factor (BDNF) is critically involved in synaptic plasticity and neurotransmission. Our lab has previously found that BDNF activation of neurotrophic tyrosine kinase, receptor, type 2 (TrkB) is required for fear memory formation and that $GABA_A$ receptor ($GABA_AR$) subunits and the $GABA_A$ clustering protein gephyrin are dynamically regulated during fear memory consolidation. We hypothesize that TrkB-dependent internalization of $GABA_A$Rs may partially underlie a transient period of amygdala hyperactivation during fear memory consolidation. We have previously reported that BDNF modulates $GABA_AR$ α1 subunit sequestration in cultured hippocampal and amygdala neurons by differential phosphorylation pathways. At present, no studies have investigated the regulation of gephyrin and $GABA_AR$ α1 subunits following BDNF activation in the amygdala. In this study, we confirm the association of $GABA_AR$ α1 and γ2 subunits with gephyrin on mouse amygdala neurons by coimmunoprecipitation and immunocytochemistry. We then demonstrate that rapid BDNF treatment, as well as suppression of gephyrin protein levels on amygdala neurons, induced sequestration of surface α1 subunits. Further, we find that rapid exposure of BDNF to primary amygdala cultures produced decreases in gephyrin levels, whereas longer exposure resulted in an eventual increase. While total α1 subunit levels remained unchanged, gephyrin was downregulated in whole cell homogenates, but enhanced in complexes with $GABA_A$Rs. Our data with anisomycin suggest that BDNF may rapidly induce gephyrin protein degradation, with subsequent gephyrin synthesis occurring. Together, these findings suggest that gephyrin may be a key factor in BDNF-dependent $GABA_AR$ regulation in the amygdala. This work may inform future studies aimed at elucidating the pathways connecting BDNF, $GABA_A$ systems, gephyrin, and their role in underlying amygdala-dependent learning.

4.1228 Ionizing Radiation Promotes the Acquisition of a Senescence-Associated Secretory Phenotype and Impairs Angiogenic Capacity in Cerebromicrovascular Endothelial Cells: Role of Increased DNA Damage and Decreased DNA Repair Capacity in Microvascular Radiosensitivity

Ungvari, Z., Podlutski, A., Sosnowska, D., Tucsek, Z., Toth, P., Deak, F., Gautman, T., Csiszar, A. and Sonntag, W.E.

J. Gerontol. A Biol. Sci. Med. Sci., ***68(12)***, *1443-1457 (2013)*

Cerebromicrovascular rarefaction is believed to play a central role in cognitive impairment in patients receiving whole-brain irradiation therapy. To elucidate the mechanism underlying the deleterious effects of γ-irradiation on the cerebral microcirculation, rat primary cerebromicrovascular endothelial cells (CMVECs) were irradiated in vitro. We found that in CMVECs, γ-irradiation (2–8 Gy) elicited increased DNA damage, which was repaired less efficiently in CMVECs compared with neurons, microglia, and astrocytes. Increased genomic injury in CMVECs associated with increased apoptotic cell death. In the surviving cells, γ-irradiation promotes premature senescence (indicated by SA-β-galactosidase positivity and upregulation of $p16^{INK4a}$), which was associated with impaired angiogenic capacity (decreased proliferation and tube-forming capacity). γ-Irradiated CMVECs acquired a senescence-associated secretory phenotype, characterized by upregulation of proinflammatory cytokines and chemokines (including IL-6, IL-1α, and MCP-1). Collectively, increased vulnerability of γ-irradiated CMVECs and their impaired

angiogenic capacity likely contribute to cerebromicrovascular rarefaction and prevent regeneration of the microvasculature postirradiation. The acquisition of a senescence-associated secretory phenotype in irradiated CMVECs is biologically highly significant as changes in the cytokine microenvironment in the hippocampus may affect diverse biological processes relevant for normal neuronal function (including regulation of neurogenesis and the maintenance of the blood brain barrier).

4.1229 Toll-Like Receptor 4–Dependent Microglial Activation Mediates Spinal Cord Ischemia–Reperfusion Injury

Bell, M.T., Puskas, F., Agoston, V.A., Cleveland Jr., J.C., Freeman, K.A., Gamboni, F., Herson, P.S., Meng, X., Smith, P.D., Weyant, M.J., Fullerton, D.A. and Reece, T.B.
*Circulation, **128**, S152-S156 (2013)*

Background—Paraplegia continues to complicate thoracoabdominal aortic interventions. The elusive mechanism of spinal cord ischemia–reperfusion injury has delayed the development of pharmacological adjuncts. Microglia, the resident macrophages of the central nervous system, can have pathological responses after a variety of insults. This can occur through toll-like receptor 4 (TLR-4) in stroke models. We hypothesize that spinal cord ischemia–reperfusion injury after aortic occlusion results from TLR-4–mediated microglial activation in mice.
Methods and Results—TLR-4 mutant and wild-type mice underwent aortic occlusion for 5 minutes, followed by 60 hours of reperfusion when spinal cords were removed for analysis. Spinal cord cytokine production and microglial activation were assessed at 6 and 36 hours after surgery. Isolated microglia from mutant and wild-type mice were subjected to oxygen and glucose deprivation for 24 hours, after which the expression of TLR-4 and proinflammatory cytokines was analyzed. Mice without functional TLR-4 demonstrated decreased microglial activation and cytokine production and had preserved functional outcomes and neuronal viability after thoracic aortic occlusion. After oxygen and glucose deprivation, wild-type microglia had increased TLR-4 expression and production of proinflammatory cytokines.
Conclusions—The absence of functional TLR-4 attenuated neuronal injury and microglial activation after thoracic aortic occlusion in mice. Furthermore, microglial upregulation of TLR-4 occurred after oxygen and glucose deprivation, and the absence of functional TLR-4 significantly attenuated the production of proinflammatory cytokines. In conclusion, TLR-4–mediated microglia activation in the spinal cord after aortic occlusion is critical in the mechanism of paraplegia after aortic cross-clamping and may provide targets for pharmacological intervention.

4.1230 Purification and Culture of Spinal Motor Neurons from Rat Embryos

Graber, D.J. and Harris, B.T.
*Cold Spring Harb. Protoc., **pdb.top070920**, 310-311 (2013)*

We describe an immunopanning protocol to isolate, enrich, and culture spinal motor neurons from rat embryonic spinal cords. The method takes advantage of several distinct properties of rat lower motor neurons to isolate them from neighboring cells. First, an ideal stage in development after motor neurons are born (embryonic day 14 during rat gestation), but prior to extensive axonal extension or developmental apoptosis, is exploited. Lower motor neurons cannot be viably isolated using this method after birth. After dissociating embryonic spinal cord tissue, which contains lower motor neurons among many other cell types, the uniquely large motor neurons are enriched using density gradient centrifugation. Finally, the collected cell population is further purified based on selective immunopanning for motor neurons, which express the low-affinity nerve growth factor (NGF) receptor often referred to as p75. The near-pure lower motor neuron cultures are plated and seeded in defined conditions optimal for survival and can be maintained for several weeks. The expected yield is approximately 70,000 cells per embryonic spinal cord.

4.1231 Induction of pulmonary hypertensive changes by extracellular vesicles from monocrotaline-treated mice

Aliotta, J.M., Pereira, M., Amaral, A., Sorokina, A., Igbinoba, Z., Hasslinger, A., El-Bizri, R., Rounds, S.I., Quesenberry, P.J. and Klinger, J.R.
*Cardiovasc. Res.,**100**, 354-362 (2013)*

Aims Circulating endothelium-derived extracellular vesicles (EV) levels are altered in pulmonary arterial hypertension (PAH) but whether they are biomarkers of cellular injury or participants in disease pathogenesis is unknown. Previously, we found that lung-derived EVs (LEVs) induce bone marrow-derived progenitor cells to express lung-specific mRNA and protein. In this study, we sought to determine whether LEV or plasma-derived EV (PEV) alter pulmonary vascular endothelial or marrow progenitor cell

phenotype to induce pulmonary vascular remodelling.
Methods and results LEV, PEV isolated from monocrotaline (MCT-EV)- or vehicle-treated mice (vehicle-EV) were injected into healthy mice. Right ventricular (RV) hypertrophy and pulmonary vascular remodelling were assessed by RV-to-body weight (RV/BW) and blood vessel wall thickness-to-diameter (WT/D) ratios. RV/BW, WT/D ratios were elevated in MCT- vs. vehicle-injected mice (1.99 ± 0.09 vs. 1.04 ± 0.09 mg/g; 0.159 ± 0.002 vs. 0.062 ± 0.009%). RV/BW, WT/D ratios were higher in mice injected with MCT-EV vs. mice injected with vehicle-EV (1.63 ± 0.09 vs. 1.08 ± 0.09 mg/g; 0.113 ± 0.02 vs. 0.056 ± 0.01%). Lineage-depleted bone marrow cells incubated with MCT-EV and marrow cells isolated from mice infused with MCT-EV had greater expression of endothelial progenitor cell mRNAs and mRNAs abnormally expressed in PAH than cells incubated with vehicle-EV or isolated from vehicle-EV infused mice. MCT-EV induced an apoptosis-resistant phenotype in murine pulmonary endothelial cells and lineage-depleted bone marrow cells incubated with MCT-EV induced pulmonary hypertension when injected into healthy mice.
Conclusions EV from MCT-injured mice contribute to the development of MCT-induced pulmonary hypertension. This effect may be mediated directly by EV on the pulmonary vasculature or by differentiation of bone marrow cells to endothelial progenitor cells that induce pulmonary vascular remodelling.

4.1232 A method for biomarker measurements in peripheral blood mononuclear cells isolated from anxious and depressed mice: β-arrestin 1 protein levels in depression and treatment
Mendez-David, I., El-Ali, Z., Hen, R., Falissard, B., Corruble, E., gardier, A.M., Kerdine-Römer, S. and David, D.J.
*Frontiers in Pharmacol., **4**:124 (2013)*

A limited number of biomarkers in the central and peripheral systems which are known may be useful for diagnosing major depressive disorders and predicting the effectiveness of antidepressant (AD) treatments. Since 60% of depressed patients do not respond adequately to medication or are resistant to ADs, it is imperative to delineate more accurate biomarkers. Recent clinical studies suggest that β-arrestin 1 levels in human mononuclear leukocytes may be an efficient biomarker. If potential biomarkers such as β-arrestin 1 could be assessed from a source such as peripheral blood cells, then they could be easily monitored and used to predict therapeutic responses. However, no previous studies have measured β-arrestin 1 levels in peripheral blood mononuclear cells (PBMCs) in anxious/depressive rodents. This study aimed to develop a method to detect β-arrestin protein levels through immunoblot analyses of mouse PBMCs isolated from whole blood. In order to validate the approach, β-arrestin levels were then compared in naïve, anxious/depressed mice, and anxious/depressed mice treated with a selective serotonin reuptake inhibitor (fluoxetine, 18 mg/kg/day in the drinking water). The results demonstrated that mouse whole blood collected by submandibular bleeding permitted isolation of enough PBMCs to assess circulating proteins such as β-arrestin 1. β-Arrestin 1 levels were successfully measured in healthy human subject and naïve mouse PBMCs. Interestingly, PBMCs from anxious/depressed mice showed significantly reduced β-arrestin 1 levels. These decreased β-arrestin 1 expression levels were restored to normal levels with chronic fluoxetine treatment. The results suggest that isolation of PBMCs from mice by submandibular bleeding is a useful technique to screen putative biomarkers of the pathophysiology of mood disorders and the response to ADs. In addition, these results confirm that β-arrestin 1 is a potential biomarker for depression.

4.1233 Investigation of the effect of Mycobacterium bovis infection on bovine neutrophils functions
Wang, J., Zhou, X., Pan, B., Yang, L., Yin, X., Xu, B. and Zhao, D.
*Tuberculosis, **93**, 675-687 (2013)*

Bovine tuberculosis is a disease in cattle caused by infection with *Mycobacterium bovis*. The disease has posed significant economic losses and remains a public health hazard worldwide. Interactions between *M. bovis* and bovine macrophages have been extensively characterized in various studies, while similar analyses in neutrophils, which are one of the other types of white blood cells in mammals, were often overlooked. Neutrophils provide defense against all microbes and can present a diverse collection of antimicrobial molecules, which play an important role in the control of tuberculosis progression. Much of the available data about the involvement of neutrophils in the killing *M. bovis* is controversial. In this study, we assessed the effect of *in vitro* infection with *M. bovis* on some parameters of neutrophils functions including phenotypic changes, apoptosis rate and inflammatory cytokines production. Our results demonstrated that phagocytosis of *M. bovis* activated and enhanced bovine neutrophils functions as well as initialed their defense mechanism, but failed to eliminate the mycobacteria. Moreover, autophagy might

get involved in the defense infection process functioning as a protective mechanism, and inducible-autophagy by lipopolysaccharides stimulation and starvation treatment could efficiently reverse the inability of neutrophils for killing *M. bovis*, suggesting a potential target for anti-mycobacterial drug-therapy.

4.1234 Stage specific reprogramming of mouse embryo liver cells to a beta cell-like phenotype
Yang, Y., Akinci, E., Dutton, J.R., banga, A. and Slack, J.M.W.
*Mechanisms of Development, **130**, 602-612 (2013)*

We show that cultures of mouse embryo liver generate insulin-positive cells when transduced with an adenoviral vector encoding the three genes: *Pdx1*, *Ngn3* and *MafA* (*Ad-PNM*). Only a proportion of transduced cells become insulin-positive and the highest yield occurs in the period E14–16, declining at later stages. Insulin-positive cells do not divide further although they can persist for several weeks. RT-PCR analysis of their gene expression shows the upregulation of a whole battery of genes characteristic of beta cells including upregulation of the endogenous counterparts of the input genes. Other features, including a relatively low insulin content, the expression of genes for other pancreatic hormones, and the fact that insulin secretion is not glucose-sensitive, indicate that the insulin-positive cells remain immature. The origin of the insulin-positive cells is established both by co-immunostaining for α-fetoprotein and albumin, and by lineage tracing for *Sox9*, which is expressed in the ductal plate cells giving rise to biliary epithelium. This shows that the majority of insulin-positive cells arise from hepatoblasts with a minority from the ductal plate cells.

4.1235 121 Effects of iodixanol during rat epididymal sperm cryopreservation
Kim, S., Agca, C.a dn Agca, Y.
*Cryobiology, **67(3)**, 432 (2013)*

Sperm cryopreservation is an effective method of maintaining valuable strains for biomedical research. However, successful cryopreservation of rat spermatozoa remains a challenge. The objective of this study was to determine if OptiPrepTM (60% iodixanol in water) acts as a protectant during Sprague Dawley (SD) rat sperm cryopreservation. We first evaluated OptiPrepTM concentration effect on sperm motility 10 min and 3 h after freezing-thawing. Acrosomal integrity and mitochondrial membrane potential (MMP) were also evaluated for frozen-thawed spermatozoa. In addition, the toxic effects of OptiPrepTM were tested via motility after 1 h or 3 h incubation with OptiPrepTM in fresh and chilled rat spermatozoa. The concentrations of OptiPrepTM used for these studies were 0% (control), 1%, 2%, 3%, and 4% (v/v). There were no significant differences on motility among OptiPrepTM treatment groups in fresh and chilled rat spermatozoa. OptiPrepTM did not have toxic effect on SD rat spermatozoa. In frozen-thawed samples, 2% OptiPrepTM improved total motility at 10 min after thawing and progressive motility and average path velocity at 3 h after thawing ($P < 0.05$) and MMP ($P < 0.05$) while OptiPrepTM did not show difference for acrosomal integrity compared to control. Intra uterine insemination (IUI) was performed for OptiPrepTM -contained cryopreserved spermatozoa showing best results on sperm evaluation parameters. While 9 out of 10 (90%) rats become pregnant after intrauterine insemination (IUI), 4 out of 11 (36%) rats become pregnant after IUI using frozen-thaw SD sperm. These data suggest that iodixanol may have cryoprotective effect during rat sperm freezing without any toxic effects.

4.1236 CD4 blockade directly inhibits mouse and human CD4+ T cell functions independent of Foxp3+ Tregs
Mayer, C.T., Huntenberg, J., Nandan, A., Schmitt, E., Czeloth, N. and Sparwasser, T.
*J. Autoimunity, **47**, 73-82 (2013)*

$CD4^+$ helper T cells orchestrate protective immunity against pathogens, yet can also induce undesired pathologies including allergies, transplant rejection and autoimmunity. Non-depleting CD4-specific antibodies such as clone YTS177.9 were found to promote long-lasting T cell tolerance in animal models. Thus, CD4 blockade could represent a promising therapeutic approach for human autoimmune diseases. However, the mechanisms underlying anti-CD4-induced tolerance are incompletely resolved. Particularly, multiple immune cells express CD4 including $Foxp3^+$ regulatory T cells (Tregs) and dendritic cells (DCs), both controlling the activation of $CD4^+Foxp3^-$ helper T cells. Utilizing mixed leukocyte reactions (MLRs) reflecting physiological interactions between T cells and DCs, we report that anti-CD4 treatment inhibits $CD4^+Foxp3^-$ T cell proliferation in an IL-2-independent fashion. Notably, YTS177.9 binding induces a

rapid internalization of CD4 on both $CD4^+Foxp3^-$ T cells and $Foxp3^+$ Tregs. However, no expansion or activation of immunosuppressive $CD4^+Foxp3^+$ Tregs was observed following anti-CD4 treatment. Additionally, cytokine production, maturation and T cell priming capacity of DCs are not affected by anti-CD4 exposure. In line with these data, the selective ablation of $Foxp3^+$ Tregs from MLRs by the use of diphtheria toxin (DT)-treated bacterial artificial chromosome (BAC)-transgenic DEREG mice completely fails to abrogate the suppressive activity of multiple anti-CD4 antibodies. Instead, tolerization is associated with the defective expression of various co-stimulatory receptors including OX40 and CD30, suggesting altered signaling through the TCR complex. Consistent with our findings in mice, anti-CD4 treatment renders human $CD4^+$ T cells tolerant in the absence of Tregs. Thus, our results establish that anti-CD4 antibodies can directly tolerize pathogenic $CD4^+Foxp3^-$ helper T cells. This has important implications for the treatment of human inflammatory diseases.

4.1237 GABA Protects Human Islet Cells Against the Deleterious Effects of Immunosuppressive Drugs and Exerts Immunoinhibitory Effects Alone

Prud'homme, G.J., Glinka, Y., Hasilo, C., Paraskevas, S., Li, X. and Wang, Q.
Transplantation, ***98(7)****, 616-623 (2013)*

Background: We recently found that γ-aminobutyric acid (GABA) protects mouse islet β cells. It prevented autoimmune type 1 diabetes in mice, induced islet β-cell regeneration, and exerted immunoinhibitory effects. However, it is not known whether GABA would be equally active on human islet and immune cells.
Methods: In vitro culture of human islets and immune cells with or without GABA and immunosuppressive drugs. In vitro analysis of apoptosis, proliferation, nuclear factor (NF)-κB activation, calcium signaling, and insulin secretion.
Results: GABA reduced human islet cell apoptosis in culture, such that the yield of live cells was approximately tripled after 1 week, and it stimulated insulin secretion. It protected against the deleterious effects of rapamycin, tacrolimus, and mycophenolate mofetil. In human immune cells, GABA had inhibitory effects similar to mouse cells, such as suppressed anti-CD3–stimulated T-cell proliferation, in a GABA type A receptor–dependent fashion. The immunosuppressive mechanisms have been unclear, but we found that GABA blocked calcium influx, which is a key activation signal. GABA also suppressed NF-κB activation in both human islet cells and immune cells. We found that it could be combined with rapamycin to increase its suppressive effects.
Conclusions: GABA improved human islet cell survival and had suppressive effects on human immune cells. It inhibited canonical NF-κB activation in both islet and immune cells. This is important because activation of this pathway is detrimental to islet cells and likely promotes damaging autoimmunity and alloreactivity against transplanted islets. These findings suggest that GABA might find applications in clinical islet transplantation.

4.1238 Long-Term Functions of Encapsulated Islets Grafted in Nonhuman Primates Without Immunosuppression

Sasikala, M., Rao, G.V., Vijayalakshmi, V., Pradeep, R., Pothani, S., Kumar, P.P., Gaddipati, R., Sirisha, G., Cheemalakonda, R., Tandan, M., Subramanyam, C., Vasudevan, S. and Reddy, D.N.
Transplantation, ***96(7)****, 624-632 (2013)*
Background: Long-term survival and functions of encapsulated islet grafts need to be evaluated in the absence of immunosuppression. The present study aimed to assess the viability and functions of macroencapsulated islets grafted in nonhuman primates without immunosuppression for 1 year.
Methods: Islet transplantations were performed in partially pancreatectomized rhesus monkeys (two autologous and four allogenic) without immunosuppression using immunoisolatory devices. Macroencapsulated islets were implanted subcutaneously (5000–8000 IEQ/device) at two sites (left thigh and interscapular region) and were explanted at 2, 6, and 12 months after implantation. Staining for viability and apoptosis, in vivo and in vitro glucose-stimulated insulin release, expression of insulin and glucagon genes, and histopathologic examination of the device were used to assess engraftment potential, viability, and functions of islets. Animals were regularly monitored for dietary intake, body weight, and fasting blood glucose levels after islet transplantation.
Results: Devices explanted showed vascularization at the end of 2, 6, and 12 months with occasional lymphocytes and minimal fibrosis outside the device. Flow cytometric analysis revealed 97.9%±1.5% and 94.3%±5.71% viable β cells in interscapular site and thigh in autologous recipients and 85.6%±4.01% (interscapular site) and 74.1%±12.05% (thigh) viable β cells in allogenic islet recipients. In vivo glucose challenge test revealed significantly increased glucose-stimulated insulin release (P=0.028) in the left thigh

with implant (17.58±3.13 mU/L) compared with the thigh without implant (9.86±1.063 mU/L). Insulin and glucagon gene expression was evident in islets recovered from explanted device.
Conclusions: These results indicate that subcutaneous implantation of macroencapsulated islets is minimally invasive and has potential for transplantation without immunosuppression.

4.1239 Electrical Stimulation of Embryonic Neurons for 1 Hour Improves Axon Regeneration and the Number of Reinnervated Muscles That Function
Liu, Y., Grumbles, R.M. and Thomas, C.K.
J. Neuropathol. Exp. Neurol., ***72(7)***, *697-707 (2013)*

Motoneuron death after spinal cord injury or disease results in muscle denervation, atrophy, and paralysis. We have previously transplanted embryonic ventral spinal cord cells into the peripheral nerve to reinnervate denervated muscles and to reduce muscle atrophy, but reinnervation was incomplete. Here, our aim was to determine whether brief electrical stimulation of embryonic neurons in the peripheralnerve changes motoneuron survival, axon regeneration, and muscle reinnervation and function because neural depolarization is crucial for embryonic neuron survival and may promote activity-dependent axon growth. At 1 week after denervation by sciatic nerve section, embryonic day 14 to 15 cells were purified for motoneurons, injected into the tibial nerve of adult Fischer rats, and stimulated immediatelyfor up to 1 hour. More myelinated axons were present in tibial nerves 10 weeks after transplantation when transplants had been stimulated acutely at 1 Hz for 1 hour. More muscles were reinnervated if the stimulation treatment lasted for 1 hour. Reinnervation reduced muscle atrophy, with or without the stimulation treatment. These data suggest that brief stimulation of embryonic neurons promotes axon growth, which has a long-term impact on muscle reinnervation and function. Muscle reinnervation is important because it may enable the use of functional electrical stimulation to restore limb movements.

4.1240 The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons
Wang, W., Li, L., Lin, W-L., Dickson, D.W., Petrucelli, L., Zhang, T. and Wang, X.
Human Mol. Genet., ***22(23)***, *4706-4719 (2013)*

Mutations in TDP-43 lead to familial ALS. Expanding evidence suggests that impaired mitochondrial dynamics likely contribute to the selective degeneration of motor neurons in SOD1-associated ALS. In this study, we investigated whether and how TDP-43 mutations might impact mitochondrial dynamics and function. We demonstrated that overexpression of wild-type TDP-43 resulted in reduced mitochondrial length and density in neurites of primary motor neurons, features further exacerbated by ALS-associated TDP-43 mutants Q331K and M337V. In contrast, suppression of TDP-43 resulted in significantly increased mitochondrial length and density in neurites, suggesting a specific role of TDP-43 in regulating mitochondrial dynamics. Surprisingly, both TDP-43 overexpression and suppression impaired mitochondrial movement. We further showed that abnormal localization of TDP-43 in cytoplasm induced substantial and widespread abnormal mitochondrial dynamics. TDP-43 co-localized with mitochondria in motor neurons and their colocalization was enhanced by ALS associated mutant. Importantly, co-expression of mitochondrial fusion protein mitofusin 2 (Mfn2) could abolish TDP-43 induced mitochondrial dynamics abnormalities and mitochondrial dysfunction. Taken together, these data suggest that mutant TDP-43 impairs mitochondrial dynamics through enhanced localization on mitochondria, which causes mitochondrial dysfunction. Therefore, abnormal mitochondrial dynamics is likely a common feature of ALS which could be potential new therapeutic targets to treat ALS.

4.1241 Fortilin reduces apoptosis in macrophages and promotes atherosclerosis
Pinkaew, D., Le, R.J., Chen, Y., Eltorky, M., teng, B-B. and Fujise, K.
Am. J. Physiol. Heart Circ. Physiol., ***305***, *H1519-H1529 (2013)*

Atherosclerosis, a deadly disease insufficiently addressed by cholesterol-lowering drugs, needs new therapeutic strategies. Fortilin, a 172-amino acid multifunctional polypeptide, binds p53 and blocks its transcriptional activation of Bax, thereby exerting potent antiapoptotic activity. Although fortilin-overexpressing mice reportedly exhibit hypertension and accelerated atherosclerosis, it remains unknown if fortilin, not hypertension, facilitates atherosclerosis. Our objective was to test the hypothesis that fortilin in and of itself facilitates atherosclerosis by protecting macrophages against apoptosis. We generated fortilin-deficient (*fortilin*$^{+/-}$) mice and wild-type counterparts (*fortilin*$^{+/+}$) on a LDL receptor (*Ldlr*)$^{-/-}$ apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (*Apobec1*)$^{-/-}$ hypercholesterolemic genetic background, incubated them for 10 mo on a normal chow diet, and assessed the degree and extent

of atherosclerosis. Despite similar blood pressure and lipid profiles, *fortilin*$^{+/-}$ mice exhibited significantly less atherosclerosis in their aortae than their *fortilin*+/+ littermate controls. Quantitative immunostaining and flow cytometry analyses showed that the atherosclerotic lesions of *fortilin*$^{+/-}$ mice contained fewer macrophages than those of *fortilin*$^{+/+}$ mice. In addition, there were more apoptotic cells in the intima of *fortilin*$^{+/-}$ mice than in the intima of *fortilin*$^{+/+}$ mice. Furthermore, peritoneal macrophages from *fortilin*$^{+/-}$ mice expressed more *Bax* and underwent increased apoptosis, both at the baseline level and in response to oxidized LDL. Finally, hypercholesterolemic sera from *Ldlr*$^{-/-}$*Apobec1*$^{-/-}$ mice induced fortilin in peritoneal macrophages more robustly than sera from control mice. In conclusion, fortilin, induced in the proatherosclerotic microenvironment in macrophages, protects macrophages against Bax-induced apoptosis, allows them to propagate, and accelerates atherosclerosis. Anti-fortilin therapy thus may represent a promising next generation antiatherosclerotic therapeutic strategy.

4.1242 Dendritic Cell-Specific Delivery of Flt3L by Coronavirus Vectors Secures Induction of Therapeutic Antitumor Immunity

Perez-Shibayama, C., Gil-Cruz, C., Nussbacher, M., Allgäuer, E., Cervantes-Barragan, L., Züst, R. and Ludewig, B.
*PloS One, **8(11)**, e81442 (2013)*

Efficacy of antitumor vaccination depends to a large extent on antigen targeting to dendritic cells (DCs). Here, we assessed antitumor immunity induced by attenuated coronavirus vectors which exclusively target DCs in vivo and express either lymphocyte- or DC-activating cytokines in combination with a GFP-tagged model antigen. Tracking of *in vivo* transduced DCs revealed that vectors encoding for Fms-like tyrosine kinase 3 ligand (Flt3L) exhibited a higher capacity to induce DC maturation compared to vectors delivering IL-2 or IL-15. Moreover, Flt3L vectors more efficiently induced tumor-specific CD8$^+$ T cells, expanded the epitope repertoire, and provided both prophylactic and therapeutic tumor immunity. In contrast, IL-2- or IL-15-encoding vectors showed a substantially lower efficacy in CD8$^+$ T cell priming and failed to protect the host once tumors had been established. Thus, specific *in vivo* targeting of DCs with coronavirus vectors in conjunction with appropriate conditioning of the microenvironment through Flt3L represents an efficient strategy for the generation of therapeutic antitumor immunity.

4.1243 Ezh1 and Ezh2 differentially regulate PSD-95 gene transcription in developing hippocampal neurons

Henriquez, B., Bustos, F., Aguilar, R., Becerra, A., Simon, F., Montecino, M. and van Zundert, B.
*Mol. Cell. Neurosci., **57**, 130-143 (2013)*

Polycomb Repressive Complex 2 (PRC2) mediates transcriptional silencing by catalyzing histone H3 lysine 27 trimethylation (H3K27me3), but its role in the maturation of postmitotic mammalian neurons remains largely unknown. We report that the PRC2 paralogs Ezh1 and Ezh2 are differentially expressed during hippocampal development. We show that depletion of Ezh2 leads to increased expression of PSD-95, a critical plasticity gene, and that reduced PSD-95 gene transcription is correlated with enrichment of Ezh2 at the PSD-95 gene promoter; however, the H3K27me3 epigenetic mark is not present at the PSD-95 gene promoter, likely due to the antagonizing effects of the H3S28P and H3K27Ac marks and the activity of the H3K27 demethylases JMJD3 and UTX. In contrast, increased PSD-95 gene transcription is accompanied by the presence of Ezh1 and elongation-engaged RNA Polymerase II complexes at the PSD-95 gene promoter, while knock-down of Ezh1 reduces PSD-95 transcription. These results indicate that Ezh1 and Ezh2 have antagonistic roles in regulating PSD-95 transcription.

4.1244 Engineering the type III secretion system in non-replicating bacterial minicells for antigen delivery

Carleton, H.A., Lara-Tejero, M., Liu, X. and Galan, J.E.
*Nature Communications, **4**:1590 (2013)*

Type III protein secretion systems are being considered for vaccine development as virtually any protein antigen can be engineered for delivery by these nanomachines into the class I antigen presentation pathway to stimulate antigen-specific CD8$^+$ T cells. A limitation in the use of this system is that it requires live virulence-attenuated bacteria, which may preclude its use in certain populations such as children and the immunocompromised. Here we report the engineering of the *Salmonella* Typhimurium type III secretion system in achromosomal, non-replicating nanoparticles derived from bacterial minicells. The engineered system is shown to be functional and capable of delivering heterologous antigens to the class I antigen presentation pathway stimulating immune responses both *in vitro* and *in vivo*. This antigen delivery platform offers a novel approach for vaccine development and cellular immunotherapy.

4.1245 **The single-nucleotide polymorphism (GPX4c718t) in the glutathione peroxidase 4 gene influences endothelial cell function: Interaction with selenium and fatty acids**
Crosley, L.K., Bashir, S., Nicol., F., Arthur,J.R., Hesketh, J.E. and Sneddon, A.A.
Mol. Nutr. Food Res., ***57(12)***, *2185-2194 (2013)*

Scope
Selenium (Se) is incorporated into selenoproteins as selenocysteine, which requires structures in the 3′-untranslated region (3′-UTR) of selenoprotein mRNAs. The functional consequences of a single nucleotide polymorphism (SNP) within the 3′-UTR of the selenoprotein *GPX4* gene (GPX4c718t) was assessed in human umbilical vein endothelial cells (HUVECs) and monocytes from human volunteers.
Methods and results
HUVEC and monocytes homozygous for the T- or C-variant of the GPX4c718t SNP were assessed for monocyte–endothelial cell adhesion, expression of VCAM-1 and sensitivity to oxidative challenge. Interaction of the SNP with Se and different PUFA and effects on selenoprotein expression were also investigated. HUVEC and monocytes homozygous for the T-variant showed elevated adhesion levels compared to cells of the C-variant. This effect was modified by Se and PUFA. HUVEC homozygous for the T-variant showed elevated levels of VCAM-1 protein in the presence of arachidonic acid, were more sensitive to oxidative challenge and showed Se-dependant changes in lipid peroxide levels and expression of additional selenoproteins.
Conclusion
These findings demonstrate functional effects of the GPX4c718t SNP in endothelial cells and may suggest that individuals with the TT genotype have impaired endothelial function and are at greater risk of vascular disease compared to individuals with the CC genotype.

4.1246 **Anti-Inflammatory Potency of Nano-Formulated Puerarin and Curcumin in Rats Subjected to the Lipopolysaccharide-Induced Inflammation no access**
Singh, A.K:, Jiang, Y., Gupta, s., Younus, M. and Ramzan, M.
J. Medicinal Food, ***16(10)***, *899-911 (2013)*

Puerarin (PU) and curcumin (CU), used commonly in traditional Chinese medicine and Ayurveda, have been shown to possess potent anti-inflammatory, anti-oxidation, and neuro-protective properties. Despite the experimental success of CU and PU in *in vitro* and animal models, their effectiveness has not yet been demonstrated in clinical trials, possibly because of their poor bioavailability. We hypothesized that gold nanoparticle (AuNP)-formulated PU (PU-AuNP), CU (CU-AuNP), or a combination of PU and CU (PU-CU-AuNP) were a more effective and nontoxic alternative to their bulk (nonformulated) counterparts. To test the hypothesis, bioavailability, therapeutic potency, and toxicity of bulk CU and/or PU were compared with those of their nanotized counterparts in rats subjected to the lipopolysaccharide (LPS)-induced inflammation. This study showed that a 20-mg/kg dose of bulk PU or a mixture of PU and CU did not, while their nanotized counterparts, PU-AuNP, CU-AuNP, or PU-CU-AuNP, effectively suppressed the LPS-induced inflammation and cytotoxicity in rats. In addition, PU-CU-AuNP was more potent than PU-AuNP or CU-AuNP alone. The blank AuNP (bAuNP) at ≤40 mg/kg dose did not cause any adverse effects (blood and brain lactic acid concentrations, kidney function, and neuronal apoptosis were measured) in animals. Therefore, the present observations suggest that a bi-functional AuNP loaded with CU and PU may effectively suppress the LPS-induced inflammation and cytotoxicity provided the following conditions are met: (1) The AuNP dose is at or below the no-effect dose; (2) the nanoparticles release a therapeutic dose of CU and PU *in vivo*; and (3) the active ingredients are released into the intracellular component of the brain.

4.1247 **Upregulation of axon guidance molecules in the adult central nervous system of Nogo-A knockout mice restricts neuronal growth and regeneration**
Kempf, A., Montani, L., Petrinovic, M.M., Schroeter, A., Weinmann, O., Patrigani, A. and Schwab, M.E.
Eur. J. Neurosci., ***38(11)***, *3567-3579 (2013)*

Adult central nervous system axons show restricted growth and regeneration properties after injury. One of the underlying mechanisms is the activation of the Nogo-A/Nogo receptor (NgR1) signaling pathway. Nogo-A knockout (KO) mice show enhanced regenerative growth *in vivo*, even though it is less pronounced than after acute antibody-mediated neutralization of Nogo-A. Residual inhibition may involve a compensatory component. By mRNA expression profiling and immunoblots we show increased

expression of several members of the Ephrin/Eph and Semaphorin/Plexin families of axon guidance molecules, e.g. EphrinA3 and EphA4, in the intact spinal cord of adult Nogo-A KO vs. wild-type (WT) mice. EphrinA3 inhibits neurite outgrowth of EphA4-positive neurons *in vitro*. In addition, EphrinA3 KO myelin extracts are less growth-inhibitory than WT but more than Nogo-A KO myelin extracts. EphA4 KO cortical neurons show decreased growth inhibition on Nogo-A KO myelin as compared with WT neurons, supporting increased EphA4-mediated growth inhibition in Nogo-A KO mice. Consistently, *in vivo*, Nogo-A/EphA4 double KO mice show increased axonal sprouting and regeneration after spinal cord injury as compared with EphA4 KO mice. Our results reveal the upregulation of developmental axon guidance cues following constitutive Nogo-A deletion, e.g. the EphrinA3/EphA4 ligand/receptor pair, and support their role in restricting neurite outgrowth in the absence of Nogo-A.

4.1248 Salvianolic acid B inhibits hepatic stellate cell activation through transforming growth factor beta-1 signal transduction pathway in vivo and in vitro
Tao, Y-Y., Wang, Q-L., Shen, L., Fu, W-W. and Liu, C-H.
Exp.Biol. Med., ***238(11)****, 1284-1296 (2013)*

Salvianolic acid B (Sal B) is a major water soluble component extracted from *Radix Salviae miltiorrhizae*, a traditional Chinese herb widely used for treating cardiovascular and hepatic diseases. Sal B has been reported to inhibit transforming growth factor (TGF)-β1-stimulated hepatic stellate cells (HSCs) activation and collagen type I expression. In this study, we further investigated the mechanisms of Sal B on liver fibrosis relating to TGF-β/Smads signalling pathway, especially to TGF-β1 receptors. Liver fibrosis model was induced by intraperitoneal injection of dimethylnitrosamine (DMN) for four weeks. Rats were randomly divided into three groups: normal, model, and Sal B groups. Rats in Sal B group were treated by oral administration of Sal B for four weeks from the first day of DMN exposure. Hydroxyproline (Hyp) content in liver tissue was assayed using Jamall's method and collagen deposition was visualized using Sirius red staining. HSCs were isolated from normal rats, and were cultured primarily in uncoated plastics. At day 4 after isolation, cells were stimulated with 2.5 ng/mL TGF-β1, and treated with 1 and 10 μmol/L Sal B and 10 μmol/L SB-431542 (TβR-I inhibitor) for 24 h, respectively. Cell proliferation was examined with 5-ethynyl-2'-deoxyuridine assay. The expressions of alpha smooth muscle actin (α-SMA) and Smad3 were assayed by immunofluorescent stain and Western blotting. The expression of TβR-I was analysed by Western blotting and real-time polymerase chain reaction. The activity of TβR-I kinase was measured by ADP-Glo kinase assay. The results showed that Sal B could inhibit collagen deposition and reduce Hyp content significantly, and decrease expressions of TGF-β1 and TβR-I in fibrotic liver *in vivo*. Also, Sal B decreased the expressions of α-SMA and TβR-I, inhibited Smad3 nuclear translocation and down-regulated TβR-I kinase activity *in vitro*. These findings suggested that Sal B could prevent HSCs activation through TGF-β signalling pathway, i.e. inhibiting TGF-β1 expression, activity of TβR-I kinase and Smads phosphorylation.

4.1249 Selective Nanovector Mediated Treatment of Activated Proinflammatory Microglia/Macrophages in Spinal Cord Injury
Papa, S.et al
ACSNano, ***7(11)****, 9881-9895 (2013)*

Much evidence shows that acute and chronic inflammation in spinal cord injury (SCI), characterized by immune cell infiltration and release of inflammatory mediators, is implicated in development of the secondary injury phase that occurs after spinal cord trauma and in the worsening of damage. Activation of microglia/macrophages and the associated inflammatory response appears to be a self-propelling mechanism that leads to progressive neurodegeneration and development of persisting pain state. Recent advances in polymer science have provided a huge amount of innovations leading to increased interest for polymeric nanoparticles (NPs) as drug delivery tools to treat SCI. In this study, we tested and evaluated *in vitro* and *in vivo* a new drug delivery nanocarrier: minocycline loaded in NPs composed by a polymer based on poly-ε-caprolactone and polyethylene glycol. These NPs are able to selectively target and modulate, specifically, the activated proinflammatory microglia/macrophages in subacute progression of the secondary injury in SCI mouse model. After minocycline-NPs treatment, we demonstrate a reduced activation and proliferation of microglia/macrophages around the lesion site and a reduction of cells with round shape phagocytic-like phenotype in favor of a more arborized resting-like phenotype with low CD68 staining. Treatment here proposed limits, up to 15 days tested, the proinflammatory stimulus associated with microglia/macrophage activation. This was demonstrated by reduced expression of proinflammatory cytokine IL-6 and persistent reduced expression of CD68 in traumatized site. The nanocarrier drug delivery tool developed here shows potential advantages over the conventionally administered anti-

inflammatory therapy, maximizing therapeutic efficiency and reducing side effects.

4.1250 The Autoregulatory Feedback Loop of MicroRNA-21/Programmed Cell Death Protein 4/Activation Protein-1 (MiR-21/PDCD4/AP-1) as a Driving Force for Hepatic Fibrosis Development
Zhang, Z., Zha, Y., Hu, W., Huang, Z., Gao, Z., Zang, Y., Chen, J. Dong, L. and Zhang, J.
J. Biol. Chem., ***288(52)***, *37082-37093 (2013)*

Sustained activation of hepatic stellate cells (HSCs) leads to hepatic fibrosis, which is characterized by excessive collagen production, and for which there is no available drug clinically. Despite tremendous progress, the cellular activities underlying HSC activation, especially the driving force in the perpetuation stage, are only partially understood. Recently, microRNA-21 (miR-21) has been found to be prevalently up-regulated during fibrogenesis in different tissues, although its detailed role needs to be further elucidated. In the present study, miR-21 expression was examined in human cirrhotic liver samples and in murine fibrotic livers induced by thioacetamide or carbon tetrachloride. A dramatic miR-21 increase was noted in activated HSCs. We further found that miR-21 maintained itself at constant high levels by using a microRNA-21/programmed cell death protein 4/activation protein-1 (miR-21/PDCD4/AP-1) feedback loop. Disrupting this loop with miR-21 antagomir or AP-1 inhibitors significantly suppressed fibrogenic activities in HSCs and ameliorated liver fibrosis. In contrast, reinforcing this loop with small interfering RNA (siRNA) against PDCD4 promoted fibrogenesis in HSCs. Further analysis indicated that the up-regulated miR-21 promoted the central transforming growth factor-β (TGF-β) signaling pathway underlying HSC activation. In summary, we suggest that the miR-21/PDCD4/AP-1 autoregulatory loop is one of the main driving forces for hepatic fibrosis progression. Targeting this aberrantly activated feedback loop may provide a new therapeutic strategy and facilitate drug discovery against hepatic fibrosis.

4.1251 Activation of the PD-1 Pathway Contributes to Immune Escape in EGFR-Driven Lung Tumors
Akbay, E.A., Koyama, S., Carretero, J. et al
Cancer Discovery, ***3***, *1355-1363 (2013)*

The success in lung cancer therapy with programmed death (PD)-1 blockade suggests that immune escape mechanisms contribute to lung tumor pathogenesis. We identified a correlation between EGF receptor (EGFR) pathway activation and a signature of immunosuppression manifested by upregulation of PD-1, PD-L1, CTL antigen-4 (CTLA-4), and multiple tumor-promoting inflammatory cytokines. We observed decreased CTLs and increased markers of T-cell exhaustion in mouse models of EGFR-driven lung cancer. PD-1 antibody blockade improved the survival of mice with EGFR-driven adenocarcinomas by enhancing effector T-cell function and lowering the levels of tumor-promoting cytokines. Expression of mutant EGFR in bronchial epithelial cells induced PD-L1, and *PD-L1* expression was reduced by EGFR inhibitors in non–small cell lung cancer cell lines with activated EGFR. These data suggest that oncogenic EGFR signaling remodels the tumor microenvironment to trigger immune escape and mechanistically link treatment response to PD-1 inhibition.
Significance: We show that autochthonous EGFR-driven lung tumors inhibit antitumor immunity by activating the PD-1/PD-L1 pathway to suppress T-cell function and increase levels of proinflammatory cytokines. These findings indicate that EGFR functions as an oncogene through non–cell-autonomous mechanisms and raise the possibility that other oncogenes may drive immune escape

4.1252 Adenosine is required for sustained inflammasome activation via the A2A receptor and the HIF-1α pathway
Ouyang, X., Ghani, A., Malik, A., Wilder, T., Colegio, O.R., Flavell, R.A., Cronstein, B.N. and Mehal, W.Z.
Nature Communications, ***4****:2909 (2013)*

Inflammasome pathways are important in chronic diseases; however, it is not known how the signalling is sustained after initiation. Inflammasome activation is dependent on stimuli such as lipopolysaccharide (LPS) and ATP that provide two distinct signals resulting in rapid production of interleukin (IL)-1β, with the lack of response to repeat stimulation. Here we report that adenosine is a key regulator of inflammasome activity, increasing the duration of the inflammatory response via the A_{2A} receptor. Adenosine does not replace signals provided by stimuli such as LPS or ATP but sustains inflammasome activity via a cAMP/PKA/CREB/HIF-1α pathway. In the setting of the lack of IL-1β responses after previous exposure to LPS, adenosine can supersede this tolerogenic state and drive IL-1β production. These data reveal that inflammasome activity is sustained, after initial activation, by A_{2A} receptor-mediated signalling.

4.1253 **Particle Focusing in Curved Microfluidic Channels**
Martel, J.M. and Toner, M.
*Scientific Reports, **3:**3340 (2013)*

The decoupled effects of Reynolds and Dean numbers are examined in inertial focusing flows. In doing so, a complex set of inertial focusing behavioral regimes is discovered within curved microfluidic channels over a range of channel Reynolds numbers, curvature ratios and particle confinement ratios. These regimes are characterized by particle migration either towards or away from the center of curvature as the channel Reynolds number is increased. The transition between these two regimes is shown to be a set of conditions where single-point equilibrium position focusing of particles of different sizes is achieved. A mechanism describing the observed motion of particles in such flows is hypothesized incorporating the redistribution of the main flow velocities caused by Dean flow and its effect on the balance forces on suspended particles.

4.1254 **Quiescent Hepatic Stellate Cells Functionally Contribute to the Hepatic Innate Immune Response via TLR3**
Wilson, C.L., Mann, J., Walsh, M., Perrugoria, M.J., Oakley, F., Wright, M.C., Brignole, C., Di Paolo, D., Perri, P., Ponzoni, M., Karin, M. and Mann, D.A.
*PloS One, **9(1)**, e83391 (2014)*

Toll-like Receptor 3 (TLR3) is a pathogen pattern recognition receptor that plays a key role in innate immunity. TLR3 signalling has numerous functions in liver, both in health and disease. Here we report that TLR3 is expressed by quiescent hepatic stellate cells (HSC) where it functions to induce transcription and secretion of functional interferons as well as a number of other cytokines and chemokines. Upon transdifferentiation into myofibroblasts, HSCs rapidly loose the ability to produce interferon gamma (IFNγ). Mechanistically, this gene silencing may be due to Polycomb complex mediated repression via methylation of histone H3 lysine 27. In contrast to wild type, quiescent HSC isolated from *tlr3* knockout mice do not produce IFNγ in response to Poly(I:C) treatment. Therefore, quiescent HSC may contribute to induction of the hepatic innate immune system in response to injury or infection.

4.1255 **Overcoming barriers in clinical islet transplantation: Current limitations and future prospects**
Chhabra, P. et al
*Current Problems in Surgery, **51**, 49-86 (2014)*

Type 1 diabetes (T1D) has been determined to be an autoimmune disease involving the selective destruction of insulin-producing β-cells in the pancreatic islets of Langerhans that results in the progressive loss of insulin secretion and ultimately in glycometabolic dysregulation.[1] As many as 3 million Americans may have T1D, and according to the most recent population-based estimates of diabetes incidence and prevalence, the number of youth with T1D will increase by more than 20% over the next 40 years.[2] Complications associated with long-standing T1D include retinopathy, neuropathy, and nephropathy, as well as cerebrovascular and cardiovascular disease.[1] Discovering strategies that could prevent or arrest the onset and progression of autoimmune T1D, reverse β-cell destruction, and permanently restore glycometabolic control preferably without long-term immunosuppression is the ultimate goal of clinical intervention.
The current treatment for T1D is primarily focused on the combination of intensive diet management along with lifelong insulin administration, either by multiple daily injections or more recently through pump delivery.[3 and 4] Tight glucose control through insulin therapy has succeeded in preventing and even reversing long-term complications of T1D in some patients,[5] but the treatment often renders patients susceptible to severe episodes of hypoglycemia and hypoglycemic unawareness.[6 and 7] Recent innovations in insulin therapy have succeeded in achieving more physiological glycometabolic control. For instance, genetically modified recombinant human insulin analogues, aspart and lispro insulin, are rapid acting, with faster onset and offset than regular insulin given subcutaneously, which allows both prandial and corrective boluses.[8] BIOD-123 and BIOD-125 are ultrarapid-acting recombinant human insulin analogues with acceptable injection site toleration that have an even faster absorption rate and onset of action compared with insulin lispro.[9] Degludec, an ultralong-acting basal insulin analogue, was shown to improve glycemic control and lower the risk of nocturnal hypoglycemia better than long-acting insulin analogue, glargine, in basal-bolus treatment with mealtime insulin aspart in patients with T1D.[10] Similarly, development of the “artificial pancreas,” an integrated closed-loop control system that combines continuous

glucose monitoring with subcutaneous insulin infusion, has been shown to significantly improve glycemic control, although refinement for routine ambulatory use is still in evolution.[11] Despite advances such as these, insulin therapy alone has not been successful in reducing the frequency of hypoglycemic episodes and unawareness, highlighting the necessity for developing alternative strategies to permanently restore glycometabolic homeostasis.

To date, pancreas or islet transplantation has been the most reliable clinical approach to cure T1D.[12, 13 and 14] The success rate of whole-pancreas transplantation alone has continued to improve, with graft survival rates of up to 86% at 1 year and 69% at 3 years with tacrolimus-based maintenance therapy.[15 and 16] Moreover, successful β-cell replacement through transplantation is often accompanied by a reduction in secondary complications of T1D, such as autonomic neuropathy and retinopathy.[17] However, major drawbacks of the pancreas transplant procedure persist, including the risks of major surgery with associated morbidity or mortality and complications, such as graft thrombosis, graft pancreatitis, fistulae, sepsis, and peritonitis.[18 and 19] Importantly, the side effects associated with long-term immunosuppressive therapy (infection and malignancy) are also of concern.[13, 20 and 21] Pancreas transplantation is often restricted to patients with T1D receiving simultaneous kidney-pancreas transplants or those that have undergone successful kidney transplantation who are already on immunosuppressive therapy,[19, 20, 21, 22 and 23] and often patient selection excludes high-risk older patients or those with coronary artery disease.[19, 24 and 25]
In contrast to whole-pancreas transplantation, isolated islet transplantation represents a safe, effective, and definitive treatment option, offering substantial benefits in terms of lowering daily insulin requirements, improving levels of glycated hemoglobin (HbA1c), reducing incidences of debilitating hypoglycemic episodes and unawareness, and affording potential insulin independence.[19, 26, 27, 28 and 29] It is also less invasive than whole-pancreas transplantation and considered hypothetically superior from a metabolic, immunologic, and patient-ease perspective. Islet transplantation restores insulin sensitivity and glucose disposal, as well as improves free fatty acid clearance dynamics.[30 and 31] According to the most recent Collaborative Islet Transplant Registry (CITR) report, the periprocedural complications of islet transplantation have an estimated 20-fold lower morbidity risk when compared with pancreatic transplantation.[19] An additional advantage of islet transplantation includes the utilization of islets isolated from deceased donor pancreas that are considered unsuitable for whole-pancreas transplantation, a decided plus given the shortage of donor organs.[32] Ex vivo manipulation of isolated islet tissue also allows a window of opportunity, before transplantation, for attempting various therapeutic manipulations aimed at achieving superior transplant outcomes as well as bypassing the need for long-term immunosuppression.[33] An overview of the procedure is provided in Figure 1.[29, 34, 35 and 36] However, despite impressive advances in the field in the last decade, widespread application of the procedure is hindered by an inadequate supply of donor pancreata, innate and alloimmune graft rejection, recurrence of autoimmunity,[37] diabetogenic and nephrotoxic side effects related to long-term immunosuppression,[38] and financial considerations related to the fact that adequate funding streams for the procedure have not advanced through Medicare and private, commercial sources.[38 and 39] This article reviews the strategies that are being employed to overcome some of these hurdles to help transition islet cell transplantation into a widespread, accepted, and fundable clinical option as a permanent cure for T1D (Fig 2).

4.1256 Dual-energy precursor and nuclear erythroid–related factor 2 activator treatment additively improve redox glutathione levels and neuron survival in aging and Alzheimer mouse neurons upstream of reactive oxygen species
Ghosh, D., LeVault, K.R. and Brewer, G.J.
*Neurobiology of Aging, **35**, 179-190 (2014)*

To determine whether glutathione (GSH) loss or increased reactive oxygen species (ROS) are more important to neuron loss, aging, and Alzheimer's disease (AD), we stressed or boosted GSH levels in neurons isolated from aging 3xTg-AD neurons compared with those from age-matched nontransgenic (non-Tg) neurons. Here, using titrating with buthionine sulfoximine, an inhibitor of γ-glutamyl cysteine synthetase (GCL), we observed that GSH depletion increased neuronal death of 3xTg-AD cultured neurons at increasing rates across the age span, whereas non-Tg neurons were resistant to GSH depletion until old age. Remarkably, the rate of neuron loss with ROS did not increase in old age and was the same for both genotypes, which indicates that cognitive deficits in the AD model were not caused by ROS. Therefore, we targeted for neuroprotection activation of the redox sensitive transcription factor, nuclear erythroid–related factor 2 (Nrf2) by 18 alpha glycyrrhetinic acid to stimulate GSH synthesis through GCL. This balanced stimulation of a number of redox enzymes restored the lower levels of Nrf2 and GCL seen in 3xTg-AD neurons compared with those of non-Tg neurons and promoted translocation of Nrf2 to the nucleus. By

combining the Nrf2 activator together with the NADH precursor, nicotinamide, we increased neuron survival against amyloid beta stress in an additive manner. These stress tests and neuroprotective treatments suggest that the redox environment is more important for neuron survival than ROS. The dual neuroprotective treatment with nicotinamide and an Nrf2 inducer indicates that these age-related and AD-related changes are reversible.

4.1257 Differential effects of cadmium administration on peripheral blood granulocytes in rats
Djokic, J., Ninkov, M., Mirkov, I., Popov, A., Aleksandrov, A.P., Zolotarevski, L., Kataranovski, D. and Kataranovski, M.
*Environ. Toxicol. Pharmacol., **37**, 210-219 (2014)*

Infiltration of circulatory inflammatory cells is a common histopathological finding in target organs following cadmium administration, but there is paucity of data concerning their activity. In this study, the effects of sublethal (1 mg/kg) cadmium on peripheral blood polymorphonuclear (PMN) cells were examined 48 h following administration in rats, when tissue (liver and lung) infiltration of these cells was observed. Cadmium administration resulted in systemic inflammatory cytokine and acute phase response with an increase in circulatory neutrophil numbers and cells that express CD11b molecules. Rise in basic aspects of oxidative activity including intracellular myeloperoxidase (MPO), reactive oxygen (nitroblue tetrazolium/NBT cytochemical assay) and nitrogen (Griess assay) species production was observed in PMNs from cadmium-administered rats. A decrease in levels of mRNA for IL-1β, TNF-α and IL-6 was noted, but production of these cytokines was affected differentially. Described effects of cadmium on PMNs add further to the understanding of inflammatory potential of this environmental contaminant.

4.1258 In situ measurement of superoxide and hydroxyl radicals by frequency mixing detection technique
Hong, H., Krause, H.J., Sohn, S.W., Baik, T., Park, J.H., Shin, S., Park, C. and Song, D.
*Anal. Biochem., **447**, 141-145 (2014)*

Frequency mixing magnetic detection (FMMD) was used to detect superoxide from hypoxanthine and xanthine reaction and to detect hydroxyl radical from the Fenton reaction. FMMD was also applied to measure the reactive oxygen species (ROS) level released from microglial cells. We could assess the formation and extinction of the free radicals without a spin trap reagent. The FMMD signal amplitude scaled with the concentration of the radicals. It was verified that no signals are obtained from the substrates and reagents. Based on the observations and on previous research, we suggest that the FMMD signals originate from superoxide and hydroxyl radicals, indicating that FMMD can be used to detect O-centered radicals. Subsequent analysis of free radicals generated from living microglial cells showed that there were significant differences between the activated microglial cells and resting ones. The results of this research are promising regarding the applications of FMMD for in situ measurement of free radicals from various sources, including the cell.

4.1259 Expression of antimicrobial peptides in coelomocytes and embryos of the green sea urchin (Strongylocentrotus droebachiensis)
Li, C., Blencke, H-M., Haug, T., Jørgensen, Ø. And Stensvågt, K.
*Developmental and Comparative Immunol., **43**, 106-113 (2014)*

Antimicrobial peptides (AMPs) play a crucial role in innate immunity. We have previously reported the isolation and characterization of the AMPs, strongylocins 1 and 2, and centrocin 1, from coelomocyte extracts of *Strongylocentrotus droebachiensis*. Here we show that these AMPs were expressed in phagocytes. In addition, transcripts of strongylocin 1 were detected in vibratile cells and/or colorless spherule cells, while transcripts of strongylocin 2 were found in red spherule cells. Results from immunoblotting and immunocytochemistry studies showed that centrocin 1 was produced by phagocytes and stored in granular vesicles. Co-localization of centrocin 1 and phagocytosed bacteria suggests that the granular vesicles containing centrocin 1 may be involved in the formation of phagolysosomes. We also analyzed the temporal and spatial expression of AMPs throughout larval development. Strongylocins were expressed in the early pluteus stage, while centrocin 1 was expressed in the mid pluteus stage. The spatial expression pattern showed that centrocin 1 was mainly located in blastocoelar cells (BCs) around the stomach and the esophagus. In addition, a few patrolling BCs were detected in some larval arms. Together, these results suggest that AMPs are expressed in different types of coelomocytes and that centrocin 1 is involved in response against bacteria. Furthermore, the expression of AMPs in larval pluteus stage, especially in BCs, indicates that AMPs and BCs are engaged in the larval immune system.

4.1260 **Polymeric nanoparticle system to target activated microglia/macrophages in spinal cord injury**
Papa, S. et al
J. Controlled Release, ***174****, 15-26 (2014)*

The possibility to control the fate of the cells responsible for secondary mechanisms following spinal cord injury (SCI) is one of the most relevant challenges to reduce the post traumatic degeneration of the spinal cord. In particular, microglia/macrophages associated inflammation appears to be a self-propelling mechanism which leads to progressive neurodegeneration and development of persisting pain state. In this study we analyzed the interactions between poly(methyl methacrylate) nanoparticles (PMMA-NPs) and microglia/macrophages *in vitro* and *in vivo*, characterizing the features that influence their internalization and ability to deliver drugs. The uptake mechanisms of PMMA-NPs were in-depth investigated, together with their possible toxic effects on microglia/macrophages. In addition, the possibility to deliver a mimetic drug within microglia/macrophages was characterized *in vitro* and *in vivo*. Drug-loaded polymeric NPs resulted to be a promising tool for the selective administration of pharmacological compounds in activated microglia/macrophages and thus potentially able to counteract relevant secondary inflammatory events in SCI.

4.1261 **Curcumin up-regulates phosphatase and tensin homologue deleted on chromosome 10 through microRNA-mediated control of DNA methylation – a novel mechanism suppressing liver fibrosis**
Zheng, J., Wu, C., Lin, Z., Guo, Y., Shi, L., Dong, P., Lu, Z., Gao, S., Liao, Y., Chen, B. and Yu, F.
FEBS J., ***281****, 88-103 (2014)*

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) has been reported to play a role in the suppression of activated hepatic stellate cells (HSCs). Moreover, it has been demonstrated that hypermethylation of the PTEN promoter is responsible for the loss of PTEN expression during HSC activation. Methylation is now established as a fundamental regulator of gene transcription. MicroRNAs (miRNAs), which can control gene expression by binding to their target genes for degradation and/or translational repression, were found to be involved in liver fibrosis. However, the mechanism responsible for miRNA-mediated epigenetic regulation in liver fibrosis still remained unclear. In the present study, curcumin treatment significantly resulted in the inhibition of cell proliferation and an increase in the apoptosis rate through the up-regulation of PTEN associated with a decreased DNA methylation level. Only DNA methyltransferase 3b (DNMT3b) was reduced *in vivo* and *in vitro* after curcumin treatment. Further studies were performed aiming to confirm that the knockdown of DNMT3b enhanced the loss of PTEN methylation by curcumin. In addition, miR-29b was involved in the hypomethylation of PTEN by curcumin. MiR-29b not only was increased by curcumin in activated HSCs, but also was confirmed to target DNMT3b by luciferase activity assays. Curcumin-mediated PTEN up-regulation, DNMT3b down-regulation and PTEN hypomethylation were all attenuated by miR-29b inhibitor. Collectively, it is demonstrated that curcumin can up-regulate miR-29b expression, resulting in DNMT3b down-regulation in HSCs and epigenetically-regulated PTEN involved in the suppression of activated HSCs. These results indicate that miRNA-mediated epigenetic regulation may be a novel mechanism suppressing liver fibrosis.

4.1262 **Mycobacterium tuberculosis infection of human dendritic cells decreases integrin expression, adhesion and migration to chemokines**
Roberts, L.L. and Robinson, M.
Immunology, ***141****, 39-51 (2014)*

Tuberculosis (TB) remains a major global health problem accounting for millions of deaths annually. Approximately one-third of the world's population is infected with the causative agent *Mycobacterium tuberculosis*. The onset of an adaptive immune response to *M. tuberculosis* is delayed compared with other microbial infections. This delay permits bacterial growth and dissemination. The precise mechanism(s) responsible for this delay have remained obscure. T-cell activation is preceded by dendritic cell (DC) migration from infected lungs to local lymph nodes and synapsis with T cells. We hypothesized that *M. tuberculosis* may impede the ability of DCs to reach lymph nodes and initiate an adaptive immune response. We used primary human DCs to determine the effect of *M. tuberculosis* on expression of heterodimeric integrins involved in cellular adhesion and migration. We also evaluated the ability of infected DCs to adhere to and migrate through lung endothelial cells, which is necessary to reach lymph nodes. We show by flow cytometry and confocal microscopy that *M. tuberculosis*-infected DCs exhibit a significant reduction in surface expression of the β_2 (CD18) integrin. Distribution of integrin β_2 is also

markedly altered in *M. tuberculosis*-infected DCs. A corresponding reduction in the αL (CD11a) and αM (CD11b) subunits that associate with integrin β_2 was also observed. Consistent with reduced integrin surface expression, we show a significant reduction in adherence to lung endothelial cell monolayers and migration towards lymphatic chemokines when DCs are infected with *M. tuberculosis*. These findings suggest that *M. tuberculosis* modulates DC adhesion and migration to increase the time required to initiate an adaptive immune response.

4.1263 Impact of Surfactant Protein D, Interleukin-5, and Eosinophilia on Cryptococcosis
Holmer, S.M., Evans, K.S., Asfaw, Y.G., Saini, D., Schell, W.A., Ledford, J.G., Frothingham, R., Wright, J.R., Sempowski, G.D.and Perfect, J.R.
Infect. Immun., ***82(2)***, *683-693 (2014)*

Cryptococcus neoformans is an opportunistic fungal pathogen that initiates infection following inhalation. As a result, the pulmonary immune response provides a first line of defense against C. neoformans. Surfactant protein D (SP-D) is an important regulator of pulmonary immune responses and is typically host protective against bacterial and viral respiratory infections. However, SP-D is not protective against C. neoformans. This is evidenced by previous work from our laboratory demonstrating that SP-D-deficient mice infected with C. neoformans have a lower fungal burden and live longer than wild-type (WT) control animals. We hypothesized that SP-D alters susceptibility to C. neoformans by dysregulating the innate pulmonary immune response following infection. Thus, inflammatory cells and cytokines were compared in the bronchoalveolar lavage fluid from WT and SP-$D^{-/-}$ mice after C. neoformans infection. Postinfection, mice lacking SP-D have reduced eosinophil infiltration and interleukin-5 (IL-5) in lung lavage fluid. To further explore the interplay of SP-D, eosinophils, and IL-5, mice expressing altered levels of eosinophils and/or IL-5 were infected with C. neoformans to assess the role of these innate immune mediators. IL-5-overexpressing mice have increased pulmonary eosinophilia and are more susceptible to C. neoformans infection than WT mice. Furthermore, susceptibility of SP-$D^{-/-}$ mice to C. neoformans infection could be restored to the level of WT mice by increasing IL-5 and eosinophils by crossing the IL-5-overexpressing mice with SP-$D^{-/-}$ mice. Together, these studies support the conclusion that SP-D increases susceptibility to C. neoformans infection by promoting C. neoformans-driven pulmonary IL-5 and eosinophil infiltration.

4.1264 Paracrine activation of hepatic stellate cells in platelet-derived growth factor C transgenic mice: Evidence for stromal induction of hepatocellular carcinoma
Wright, J.H., Johnson, M.M., Shimizu-Albergine, M., Bauer, R.L., Hayes, B.J., Surapisitchat, J., Hudkins, K.L., Riehle, K.J., Johnson, S.C., Yeh, M.M., Bammler, T.K., Beyer, R.P., Gilbertson, D.G., Alpers, C.E., Fausto, N. and Campell, J.S.
Int. J. Cancer, ***134(4)***, *778-788 (2014)*

Cirrhosis is the primary risk factor for the development of hepatocellular carcinoma (HCC), yet the mechanisms by which cirrhosis predisposes to carcinogenesis are poorly understood. Using a mouse model that recapitulates many aspects of the pathophysiology of human liver disease, we explored the mechanisms by which changes in the liver microenvironment induce dysplasia and HCC. Hepatic expression of platelet-derived growth factor C (PDGF-C*)* induces progressive fibrosis, chronic inflammation, neoangiogenesis and sinusoidal congestion, as well as global changes in gene expression. Using reporter mice, immunofluorescence, immunohistochemistry and liver cell isolation, we demonstrate that receptors for PDGF-CC are localized on hepatic stellate cells (HSCs), which proliferate, and transform into myofibroblast-like cells that deposit extracellular matrix and lead to production of growth factors and cytokines. We demonstrate induction of cytokine genes at 2 months, and stromal cell-derived hepatocyte growth factors that coincide with the onset of dysplasia at 4 months. Our results support a paracrine signaling model wherein hepatocyte-derived PDGF-C stimulates widespread HSC activation throughout the liver leading to chronic inflammation, liver injury and architectural changes. These complex changes to the liver microenvironment precede the development of HCC. Further, increased PDGF-CC levels were observed in livers of patients with nonalcoholic fatty steatohepatitis and correlate with the stage of disease, suggesting a role for this growth factor in chronic liver disease in humans. PDGF-C transgenic mice provide a unique model for the *in vivo* study of tumor–stromal interactions in the liver.

4.1265 Study of Adenovirus and CAR Axonal Transport in Primary Neurons
Zussy, C. and Salinas, S.
Methods in Mol. Biol., ***1089***, *71-78 (2014)*

Vectors derived from the canine adenovirus serotype 2 (CAV-2) possess a high neurotropism and efficient retrograde transport that lead to widespread neuronal transduction in the central nervous system (CNS) of various animals. These abilities are due to the engagement of virions to the coxsackievirus and adenovirus receptor at the surface of neurons, which is linked to the endocytic and axonal transport machineries. The trafficking of CAV-2 and the coxsackievirus and adenovirus receptor (CAR) can be visualized ex vivo by incubating primary neurons (e.g., motoneurons and hippocampal neurons) with fluorescently labeled virions or recombinant viral proteins. Using this approach, we could recapitulate the mechanisms responsible for long-range transport of adenovirus in neurons.

4.1266 Neural progenitor cells from human induced pluripotent stem cells generated less autogenous immune response

Huang, K., Liu, P., Li, X., Chen, S., Wang, L., Qin, L., Su, Z., Huang, W., Liu, J., Jia, B., Liu, J., Cai, J., Pei, D. and Pan, G.
*Science China Life Sciences, **57(2)**, 162-170 (2014)*

The breakthrough development of induced pluripotent stem cells (iPSCs) raises the prospect of patient-specific treatment for many diseases through the replacement of affected cells. However, whether iPSC-derived functional cell lineages generate a deleterious immune response upon auto-transplantation remains unclear. In this study, we differentiated five human iPSC lines from skin fibroblasts and urine cells into neural progenitor cells (NPCs) and analyzed their immunogenicity. Through co-culture with autogenous peripheral blood mononuclear cells (PBMCs), we showed that both somatic cells and iPSC-derived NPCs do not stimulate significant autogenous PBMC proliferation. However, a significant immune reaction was detected when these cells were co-cultured with allogenous PBMCs. Furthermore, no significant expression of perforin or granzyme B was detected following stimulation of autogenous immune effector cells ($CD3^+CD8^-$ T cells, $CD3^+CD8^+$ T cells or $CD3^-CD56^+$ NK cells) by NPCs in both PBMC and T cell co-culture systems. These results suggest that human iPSC-derived NPCs may not initiate an immune response in autogenous transplants, and thus set a base for further preclinical evaluation of human iPSCs.

4.1267 Vitamin D confers protection to motoneurons and is a prognostic factor of amyotrophic lateral sclerosis

Camu, W., Tremblier, B., Plassot, C., Alphandery, S., Salsac, C., Pageot, N., Juntas-Morales, R., Scamps, F., Daures, J-P. and Raoul, C.
*Neurobiology of Aging, **35**, 1198-1205 (2014)*

Amyotrophic lateral sclerosis (ALS) is an incurable paralytic disorder primarily typified by the selective and progressive degeneration of motoneurons in the brain and spinal cord. ALS causes muscle wasting and atrophy, resulting eventually in respiratory failure and death within 3–5 years of diagnosis. Vitamin D is a potent secosteroid hormone with diverse biological functions that include protection against neuronal damage. The detrimental consequences of vitamin D dietary deficiency have been documented in other neurodegenerative diseases. However, the protective effect of vitamin D on motoneuron and the influence of its levels on disease course remains elusive. Here we found that the biologically active form of vitamin D significantly potentiated the effect of neurotrophic factors and prevented motoneurons from a Fas-induced death, while electrophysiological properties of motoneurons were not affected. In ALS patients, we report that a severe vitamin D deficiency accelerates by 4 times the rate of decline and were associated with a marked shorter life expectancy. Our findings support a neuroprotective function of vitamin D on motoneurons and propose vitamin D as a reliable prognostic factor of ALS.

4.1268 No Major Role for Insulin-Degrading Enzyme in Antigen Presentation by MHC Molecules

Culina, S., Mauvais, F-X., Hsu, H-T., Burgevin, A., Guenette, S., Moser, A. and van Endert, P.
*PloS One, **9(2)**, e88365 (2014)*

Antigen presentation by MHC class I molecules requires degradation of epitope source proteins in the cytosol. Although the preeminent role of the proteasome is clearly established, evidence suggesting a significant role for proteasome-independent generation of class I ligands has been reported repeatedly. However, an enzyme responsible for such a role has not been identified. Recently insulin-degrading enzyme (IDE) was shown to produce an antigenic peptide derived from the tumor antigen MAGE-A3 in an entirely proteasome-independent manner, raising the question of the global impact of IDE in MHC class I antigen processing. Here we report that IDE knockdown in human cell lines, or knockout in two different mouse strains, has no effect on cell surface expression of various MHC class I molecules, including allomorphs such as HLA-A3 and HLA-B27 suggested to be loaded in an at least a partly proteasome-

independent manner. Moreover, reduced or absent IDE expression does not affect presentation of five epitopes including epitopes derived from beta amyloid and proinsulin, two preferred IDE substrates. Thus, IDE does not play a major role in MHC class I antigen processing, confirming the dominant and almost exclusive role of the proteasome in cytosolic production of MHC class I ligands.

4.1269 OX40 ligand regulates splenic CD8− dendritic cell-induced Th2 responses in vivo
Kamachi, F., Harada, N., Usui, Y., Sakanishi, T., Ishii, N., Okumura, K., Miyake, S. and Akiba, H.
Biochem. Biophys. Res. Comm., ***444***, *235-240 (2014)*

In mice, splenic conventional dendritic cells (cDCs) can be separated, based on their expression of CD8α into $CD8^-$ and $CD8^+$ cDCs. Although previous experiments demonstrated that injection of antigen (Ag)-pulsed $CD8^-$ cDCs into mice induced CD4 T cell differentiation toward Th2 cells, the mechanism involved is unclear. In the current study, we investigated whether OX40 ligand (OX40L) on $CD8^-$ cDCs contributes to the induction of Th2 responses by Ag-pulsed $CD8^-$ cDCs *in vivo*, because OX40–OX40L interactions may play a preferential role in Th2 cell development. When unseparated Ag-pulsed OX40L-deficient cDCs were injected into syngeneic BALB/c mice, Th2 cytokine (IL-4, IL-5, and IL-10) production in lymph node cells was significantly reduced. Splenic cDCs were separated to $CD8^-$ and $CD8^+$ cDCs. OX40L expression was not observed on freshly isolated $CD8^-$ cDCs, but was induced by anti-CD40 mAb stimulation for 24 h. Administration of neutralizing anti-OX40L mAb significantly inhibited IL-4, IL-5, and IL-10 production induced by Ag-pulsed $CD8^-$ cDC injection. Moreover, administration of anti-OX40L mAb with Ag-pulsed $CD8^-$ cDCs during a secondary response also significantly inhibited Th2 cytokine production. Thus, OX40L on $CD8^-$ cDCs physiologically contributes to the development of Th2 cells and secondary Th2 responses induced by Ag-pulsed $CD8^-$ cDCs *in vivo*.

4.1270 Abstract 192: Bone Marrow Mononuclear Cells May Enhance Recovery After Stroke By Modulating the Microglial Response
Yang, B., Parsha, K., Migliati, E. and Savitz, S.
Stroke, ***45***, *A192 (2014)*

Background: Autologous bone marrow mononuclear cells (MNCs) have been shown in multiple labs to improve stroke recovery in animal models but the mechanisms remain unclear. We assessed whether MNCs modulate macrophage-microglia responses in acute stroke.
Methods: C57/BL mice were subjected to middle cerebral artery occlusion (MCAo) for 60 minutes or sham surgery. 24 hours later, they were randomized to receive saline infusion IV or 1x 10^6 autologous MNCs IV. At various time points after stroke, 1ml peripheral blood was collected and brains were harvested up to day14. Peripheral blood was labeled with anti-mouse CD45, CD115, F4/80 or Gr-1 antibodies and then assessed by flow cytometry. Microglia were isolated from brains by Optiprep gradient and stained with anti-mouse CD11b, CD45, and CD86 antibodies for evaluation by flow cytometry. For gene expression analyses by RT-PCR, microglia-macrophages were further sorted by magnetic activated-cells sorting using CD11b antibody.
Results: 1) At day 2 after stroke, the percentage of $CD115^+$ monocytes and $CD115^+$/Gr-$1^{\mathbf{low}}$cells were significantly decreased in whole peripheral blood but $F4/80^+$ macrophages were increased. MNCs increased the abundance of $CD115^+$/Gr-$1^{\mathbf{low}}$ cells and decreased the abundance of $F4/80^+$ macrophages in peripheral blood compared to saline control at day 2 after stroke (n=5, $p<0.05$); 2)Compared to saline, MNCs significantly reduced the total number of $CD11b^+$ microglia-macrophages in the stroke-affected hemisphere at day 2 to day 6. MNCs significantly decreased the proportion of $CD11b^+$/$CD45^{\mathbf{high}}$ among $CD11b^+$ cells at day 2 and day 4 and $CD86^+$ cells among $CD11b^+$ cells at day 2. Among $CD11b^+$ microglia-macrophages isolated from the ipsilateral hemisphere of MNC treated mice, compared to saline treated controls, iNOS and IL-1β genes expression were down-regulated at day 2. At day 6, iNOS remained down-regulated while now IL-10, Arganase-1 and CD206 genes expression (markers for the "healing" macrophage phenotype) were up-regulated (n=3 to 5, $p<0.05$).
Conclusions: MNCs may enhance recovery after stroke by altering monocyte trafficking and changing microglial polarization into a healing phenotype.

4.1271 Dendritic Cells Coordinate Innate Immunity via MyD88 Signaling to Control Listeria monocytogenes Infection

Arnold-Schrauf, C., Dudek, M., Dielmann, A., Pace, L., Swallow, M., Kruse, F., Kühl, A.A., Holzmann, B., Berod, L. and Sparwasser, T.
Cell Reports, ***6(4)***, *698-708 (2014)*

Listeria monocytogenes (LM), a facultative intracellular Gram-positive pathogen, can cause life-threatening infections in humans. In mice, the signaling cascade downstream of the myeloid differentiation factor 88 (MyD88) is essential for proper innate immune activation against LM, as MyD88-deficient mice succumb early to infection. Here, we show that MyD88 signaling in dendritic cells (DCs) is sufficient to mediate the protective innate response, including the production of proinflammatory cytokines, neutrophil infiltration, bacterial clearance, and full protection from lethal infection. We also demonstrate that MyD88 signaling by DCs controls the infection rates of $CD8\alpha^+$ cDCs and thus limits the spread of LM to the T cell areas. Furthermore, in mice expressing MyD88 in DCs, inflammatory monocytes, which are required for bacterial clearance, are activated independently of intrinsic MyD88 signaling. In conclusion, $CD11c^+$ conventional DCs critically integrate pathogen-derived signals via MyD88 signaling during early infection with LM in vivo.

4.1272 Schistosoma japonicum soluble egg antigens induce apoptosis and inhibit activation of hepatic stellate cells: a possible molecular mechanism

Duan, Y., Gu, X., Zhu, D., Sun, W., Chen, J., Feng, J., Song, K., Xu, F., He, X. and He, X.
Int. J. Parasitol., ***44***, *217-224 (2014)*

Hepatic stellate cells play a key role in the development of hepatic fibrosis. Activated hepatic stellate cells can be reversed to a quiescent-like state or apoptosis can be induced to reverse fibrosis. Some studies have recently shown that Schistosoma mansoni eggs could suppress the activation of hepatic stellate cells and that soluble egg antigens from schistosome eggs could promote immunocyte apoptosis. Hence, in this study, we attempt to assess the direct effects of Schistosoma japonicum soluble egg antigens on hepatic stellate cell apoptosis, and to explore the mechanism by which the apoptosis of activated hepatic stellate cells can be induced by soluble egg antigens, as well as the mechanism by which hepatic stellate cell activation is inhibited by soluble egg antigens. Here, it was shown that S. japonicum-infected mouse livers had increased apoptosis phenomena and a variability of peroxisome proliferator-activated receptor γ expression. Soluble egg antigens induce morphological changes in the hepatic stellate cell LX-2 cell line, inhibit cell proliferation and induce cell-cycle arrest at the G_1 phase. Soluble egg antigens also induce apoptosis in hepatic stellate cells through the TNF-related apoptosis-inducing ligand/death receptor 5 and caspase-dependent pathways. Additionally, soluble egg antigens could inhibit the activation of hepatic stellate cells through peroxisome proliferator-activated receptor γ and the transforming growth factor β signalling pathways. Therefore, our study provides new insights into the anti-fibrotic effects of S. japonicum soluble egg antigens on hepatic stellate cell apoptosis and the underlying mechanism by which the liver fibrosis could be attenuated by soluble egg antigens.

4.1273 GAP, an aequorin-based fluorescent indicator for imaging Ca2+ in organelles

Rodriguez-Garcia, A., Rojo-Ruiz, J., Navas-Navarro, P., Aulestia, F.J., Gallego-Sendin, S., Garcia-Sancho, J. and Alonso, M.T.
PNAS, ***111(7)***, *2584-2589 (2014)*

Genetically encoded calcium indicators allow monitoring subcellular Ca^{2+} signals inside organelles. Most genetically encoded calcium indicators are fusions of endogenous calcium-binding proteins whose functionality in vivo may be perturbed by competition with cellular partners. We describe here a novel family of fluorescent Ca^{2+} sensors based on the fusion of two *Aequorea victoria* proteins, GFP and apo-aequorin (GAP). GAP exhibited a unique combination of features: dual-excitation ratiometric imaging, high dynamic range, good signal-to-noise ratio, insensitivity to pH and Mg^{2+}, tunable Ca^{2+} affinity, uncomplicated calibration, and targetability to five distinct organelles. Moreover, transgenic mice for endoplasmic reticulum-targeted GAP exhibited a robust long-term expression that correlated well with its reproducible performance in various neural tissues. This biosensor fills a gap in the actual repertoire of

Ca^{2+} indicators for organelles and becomes a valuable tool for in vivo Ca^{2+} imaging applications.

4.1274 Isolation and culture of hepatic stellate cells from mouse liver
Chang, W., Yang, M., Song, L., Shen, K., Wang, H., Gao, X., Li, M., Niu, W. and Qin, X.
Acta Biochim. Biophys. Sin., ***46(4)***, *291-298 (2014)*

Hepatic stellate cells (HSCs) are the primary extracellular matrix-producing cells within the liver and have numerous vital functions. A robust protocol for the isolation and culture of HSCs is important for further investigations of cell functions and related mechanisms in liver disease. The volume of the mouse liver is much smaller than that of the rat liver, which makes it much more difficult to isolate mouse HSCs (mHSCs) than rat HSCs. At present, isolating mHSCs is still a challenge because there is no efficient, robust method to isolate and culture these cells. In the present study, C57BL/6J mice were intravenously injected with liposome-encapsulated dichloromethylene diphosphate (CL2MDP) to selectively eliminate Kupffer cells from the liver. The mouse livers were then perfused in situ, and the mHSCs were isolated with an optimized density gradient centrifugation technique. In the phosphate buffer solution (PBS)-liposome group, the yield of mHSCs was $(1.37 \pm 0.23) \times 10^6$/g liver, the cell purity was (90.18 ± 1.61)%, and the cell survival rate was (94.51 ± 1.61)%. While in the CL2MDP-liposome group, the yield of mHSCs was $(1.62 \pm 0.34) \times 10^6$/g liver, the cell purity was (94.44 ± 1.89)%, and the cell survival rate was (94.41 ± 1.50)%. Based on the yield and purity of mHSCs, the CL2MDP-liposome treatment was superior to the PBS-liposome treatment ($P < 0.05$, $P < 0.01$). This study established successfully a robust and efficient protocol for the separation and purification of mHSCs, and both a high purity and an adequate yield of mHSCs were obtained.

4.1275 Adoptive cytotoxic T lymphocyte therapy triggers a counter-regulatory immunosuppressive mechanism via recruitment of myeloid-derived suppressor cells
Hosoi, A., Matsushita, H., Shimizu, K., Fujii, S-i.,. Ueha, S., Abe, J., Kurach, M., Maekawa, R., Matsushima, K. and Kakimi, K.
Int. J. Cancer, ***134(8)***, *1810-1822 (2014)*

Complex interactions among multiple cell types contribute to the immunosuppressive milieu of the tumor microenvironment. Using a murine model of adoptive T-cell immunotherapy (ACT) for B16 melanoma, we investigated the impact of tumor infiltrating cells on this complex regulatory network in the tumor. Transgenic pmel-1-specific cytotoxic T lymphocytes (CTLs) were injected intravenously into tumor-bearing mice and could be detected in the tumor as early as on day 1, peaking on day 3. They produced IFN-γ, exerted anti-tumor activity and inhibited tumor growth. However, CTL infiltration into the tumor was accompanied by the accumulation of large numbers of cells, the majority of which were $CD11b^+Gr1^+$ myeloid-derived suppressor cells (MDSCs). Notably, $CD11b^+Gr1^{int}Ly6G^-Ly6C^+$ monocytic MDSCs outnumbered the CTLs by day 5. They produced nitric oxide, arginase I and reactive oxygen species, and inhibited the proliferation of antigen-specific $CD8^+$ T cells. The anti-tumor activity of the adoptively-transferred CTLs and the accumulation of MDSCs both depended on IFN-γ production on recognition of tumor antigens by the former. In $CCR2^{-/-}$ mice, monocytic MDSCs did not accumulate in the tumor, and inhibition of tumor growth by ACT was improved. Thus, ACT triggered counter-regulatory immunosuppressive mechanism *via* recruitment of MDSCs. Our results suggest that strategies to regulate the treatment-induced recruitment of these MDSCs would improve the efficacy of immunotherapy.

4.1276 Evidence for Instant Blood-Mediated Inflammatory Reaction in Clinical Autologous Islet Transplantation
Naziruddin, B., Iwahashi, S., Kanak, M.A., Takita, M., Itoh, T. and Levy, M.F.
Am. J. Transplant., ***14(2)***, *428-437 (2014)*

A nonspecific inflammatory and thrombotic reaction termed instant blood-mediated inflammatory reaction (IBMIR) has been reported when allogenic or xenogenic islets come into contact with blood. This reaction is known to cause significant loss of transplanted islets. We hypothesized that IBMIR occurs in patients undergoing total pancreatectomy followed by autologous islet transplantation (TP-AIT) and tested this hypothesis in 24 patients and in an *in vitro* model. Blood samples drawn during the peritransplant period showed a significant and rapid increase of thrombin–anti-thrombin III complex (TAT) and C-peptide during islet infusion, which persisted for up to 3 h, along with a decreased platelet count. A concomitant increase in levels of inflammatory proteins IL-6, IL-8 and interferon-inducible protein-10 was observed. An *in vitro* model composed of pure islets plus autologous blood also demonstrated significantly increased levels of TAT ($p < 0.05$), C-peptide ($p < 0.05$), tumor necrosis factor-alpha ($p < 0.05$) and MCP-1 ($p < 0.05$),

as well as strong tissue factor expression in islets. Islet viability decreased significantly but was rescued by the presence of low-molecular-weight dextran sulfate. In conclusion, AIT-induced elevation of TAT and destruction of islets suggests that IBMIR might occur during AIT. Modulating this process may help improve islet engraftment and the insulin independence rate in TP-AIT patients.

4.1277 Concentration, Activity and Biochemical Characterization of Myeloperoxidase in Fresh and Post-Thaw Equine Semen and their Implication on Freezability

Ponthier, J., Franck, T., Parrilla-Hernandez, S., Niesten, A., de la Rebiere, G., Serteyn, D. and Deleuze, S.
Reproduction in Domestic Animals, ***49(2)***, *285-291 (2014)*

Myeloperoxidase (MPO) is a pro-oxidant enzyme associated with decreased motility in thawed equine semen. This study aimed to describe MPO concentration, activity and subunits in raw and thawed semen and to correlate these data with motilities in raw and thawed semen. Semen samples from five stallions were collected four times. Motilities were assessed in raw and thawed semen. MPO assays were performed in raw seminal plasma, raw sperm-rich pellet and thawed semen. Total and active MPO concentrations were, respectively, assayed by enzyme-linked immunosorbent assay and specific immunological extraction followed by enzymatic detection. MPO subunits present in semen were characterized by Western blot. Purified active MPO was added in saline solution and freezing extender to control its activity during freezing procedure. Differences between medians were determined using *Kruskal–Wallis* test, and correlations were determined using *Spearman's test* for nonparametric data. Active MPO concentration was low in seminal plasma and thawed semen, but high in pellet ($p = 0.0058$), as the opposite relation was observed for total MPO concentration ($p < 0.0001$). In seminal plasma and post-thaw semen, inactive 86-kDa MPO precursor was mainly observed. Purified MPO activity was decreased in the extender ($p = 0.0286$). MPO activity in pellet was highly correlated with thawed progressive motility ($r = -0.5576$, $p = 0.0086$). Inactive MPO precursor and unknown low molecular weight inactive MPO precursor subunits explain low MPO activity in semen. Major MPO activity was observed in pellet, and post-thaw loss of activity is partially explained by MPO inactivation in extender. Thawed semen motility was negatively correlated with MPO activity in pellet, becoming a potential freezability predictor.

4.1278 Cerebrospinal fluid-targeted delivery of neutralizing anti-IFNγ antibody delays motor decline in an ALS mouse model

Otsmane, B., Aebischer, J., Moumen, A. and Raoul, C.
Neuroreport, ***25(1)***, *49-54 (2014)*

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the selective and gradual loss of motoneurons in the brain and spinal cord. A persistent inflammation, typified by the activation of astrocytes and microglia, accompanies the progressive degeneration of motoneurons. Interferon gamma (IFNγ), a potent proinflammatory cytokine that is aberrantly present in the spinal cord of ALS mice and patients, has been proposed to contribute to motoneuron death by eliciting the activation of the lymphotoxin-β receptor (LT-βR) through its ligand LIGHT. However, the implication of IFNγ in the pathogenic process remains elusive. Here, we show that an antagonistic anti-IFNγ antibody efficiently rescues motoneurons from IFNγ-induced death. When transiently delivered in the cerebrospinal fluid through a subcutaneously implanted osmotic minipump, the neutralizing anti-IFNγ antibody significantly retarded motor function decline in a mouse model of ALS. However, this transient infusion of anti-IFNγ antibody did not increase the life expectancy of ALS mice. Our results suggest that IFNγ contributes to ALS pathogenesis and represents a potential therapeutic target for ALS.

4.1279 Chronic Pancreatitis and Primary Sclerosing Cholangitis—First Report of Intrahepatic Autologous Islet Transplantation

Wang, L-j., Young, S., Misawa, r., Azzam, R., Wang, X., Golab, K., Cochet, O., Savari, O., Tibudan, M., Millis, J.M., Matthews, J.B. and Witkowski, P.
J. Gastrointest. Surg., ***18(4)***, *845-850 (2014)*

Background

We are reporting first successful intrahepatic autologous islet transplantation after total pancreatectomy in a patient with chronic pancreatitis and primary sclerosing cholangitis.

Methods

Total pancreatectomy and subsequent islet autotransplantation were performed in a 16-year-old boy with intractable pain due to chronic pancreatitis in the setting of ulcerative colitis and primary sclerosing cholangitis (PSC). Liver biopsy revealed PSC with focal bridging fibrosis. The pancreas was surgically

removed and digested, and islets were isolated, highly purified, and infused intraportally.
Results
Over 18-month follow-up, the patient did not show progression of chronic liver disease or signs of portal hypertension. Magnetic resonance cholangiopancreatography revealed no new changes, and liver biopsy did not show progression of the periportal fibrosis. Pain medication was weaned over 12 months at which time glycemic control was excellent without exogenous insulin supplementation. HbgA1c was 5.9. Fifteen months after the procedure, stimulation with a mixed meal led to a fourfold increase of serum C-peptide and an eightfold increase of insulin level.
Conclusion
Pancreatic autologous islets can be successfully transplanted into a liver affected by PSC without compromising hepatic or graft function. Durability of the procedure may be limited in the future by the natural course of the liver injury caused by PSC.

4.1280 Microfluidic encapsulation of cells in alginate particles via an improved internal gelation approach
Akbari, S. and Pirbodaghi, T.
Microfluid. Nanofluid., ***16(3)****, 773-777 (2014)*

An improved internal gelation approach is developed to encapsulate single mammalian cells in monodisperse alginate microbeads as small as 26 μm in diameter and at rates of up to 1 kHz with high cell viability. The cell damage resulting from contact with calcium carbonate nanoparticles as gelation reagents is eliminated by employing a co-flow microfluidic device, and the cell exposure to low pH is minimized by a chemically balanced off-chip gelation step. These modifications significantly improve the viability of cells encapsulated in gelled alginate particles. Two different mammalian cell types are encapsulated with viability of over 84 %. The cells are functional and continue to grow inside the microparticles.

4.1281 Resveratrol treatment rescues neurovascular coupling in aged mice: role of improved cerebromicrovascular endothelial function and downregulation of NADPH oxidase
Toth, P., Tarantini, S., Tucsek, Z., Ashpole, N.M., Sosnowska, D., Gautam, T., Ballabh, P., Koller, A., Sonntag, W., Csiszar, a. and Ungvari, Z.
Am. J. Physiol. Heart Circ. Physiol., ***30683)****, H299-H308 (2014)*

Moment-to-moment adjustment of cerebral blood flow (CBF) to neuronal activity via neurovascular coupling is essential for the maintenance of normal neuronal function. Increased oxidative stress that occurs with aging was shown to impair neurovascular coupling, which likely contributes to a significant age-related decline in higher cortical function, increasing the risk for vascular cognitive impairment. Resveratrol is a polyphenolic compound that exerts significant antiaging protective effects in large vessels, but its effects on the cerebromicrovasculature remain poorly defined. The present study was undertaken to investigate the capacity of resveratrol to improve neurovascular coupling in aging. In aged (24-mo-old) C57BL/6 mice N^{ω}-nitro-l-arginine methyl ester-sensitive, nitric oxide-mediated CBF responses to whisker stimulation and to the endothelium-dependent dilator acethylcholine (ACh) were impaired compared with those in young (3-mo-old) mice. Treatment of aged mice with resveratrol rescued neurovascular coupling and ACh-induced responses, which was associated with downregulation of cortical expression of NADPH oxidase and decreased levels of biomarkers of oxidative/nitrative stress (3-nitrotyrosine, 8-isoprostanes). Resveratrol also attenuated age-related increases in reactive oxygen species (ROS) production in cultured cerebromicrovascular endothelial cells (DCF fluorescence, flow cytometry). In conclusion, treatment with resveratrol rescues cortical neurovascular coupling responses to increased neuronal activity in aged mice, likely by restoring cerebromicrovascular endothelial function via downregulation of NADPH oxidase-derived ROS production. Beneficial cerebromicrovascular effects of resveratrol may contribute to its protective effects on cognitive function in aging.

4.1282 Involvement of IGF-II receptors in the antioxidant and neuroprotective effects of IGF-II on adult cortical neuronal cultures
Martin-Montanez, E., pavia, J., Santin, L.J., Boraldi, F., Estivill-Torrus, G., Aguirre, J.A. and Garcia-Fernandez, M.
Biochim. Biophys. Acta, ***1842****, 1041-1051 (2014)*

Insulin-like growth factor-II (IGF-II) is a naturally occurring peptide that exerts known pleiotropic effects ranging from metabolic modulation to cellular development, growth and survival. IGF-II triggers its actions by binding to and activating IGF (IGF-I and IGF-II) receptors. In this study, we assessed the neuroprotective effect of IGF-II on corticosterone-induced oxidative damage in adult cortical neuronal

cultures and the role of IGF-II receptors in this effect. We provide evidence that treatment with IGF-II alleviates the glucocorticoid-induced toxicity to neuronal cultures, and this neuroprotective effect occurred due to a decrease in reactive oxygen species (ROS) production and a return of the antioxidant status to normal levels. IGF-II acts via not only the regulation of synthesis and/or activity of antioxidant enzymes, especially manganese superoxide dismutase, but also the restoration of mitochondrial cytochrome *c* oxidase activity and mitochondrial membrane potential. Although the antioxidant effect of IGF-I receptor activation has been widely reported, the involvement of the IGF-II receptor in these processes has not been clearly defined. The present report is the first evidence describing the involvement of IGF-II receptors in redox homeostasis. IGF-II may therefore contribute to the mechanisms of neuroprotection by acting as an antioxidant, reducing the neurodegeneration induced by oxidative insults. These results open the field to new pharmacological approaches to the treatment of diseases involving imbalanced redox homeostasis. In this study, we demonstrated that the antioxidant effect of IGF-II is at least partially mediated by IGF-II receptors.

4.1283 IL-12 and IL-27 regulate the phagolysosomal pathway in mycobacteria-infected human macrophages

Jung, J-Y. and Robinson, C.M.
*Cell Communication and Signaling, **12**:16 (2014)*

Background
The cytokine environment at the site of infection is important to the control of mycobacteria by host macrophages. During chronic infection immunosuppressive cytokines are likely to favor mycobacterial growth, persistence, and an avoidance of proper antigen processing and presentation. The activity of interleukin (IL)-27 toward macrophages is anti-inflammatory and this compromises control of mycobacteria. Modulation of the cytokine environment may enhance both protective and vaccine-induced responses.
Results
In this study we showed that supplying IL-12 and neutralizing IL-27 enhanced acidification and fusion of mycobacterial-containing phagosomes with lysosomes. This was achieved by phagosomal acquisition of vacuolar ATPase (V-ATPase) and CD63. Both V-ATPase and CD63 protein levels were increased by the addition of IL-12 and neutralization of IL-27. In addition, cathepsin D associated with the bacteria and matured to the active form when IL-12 was supplied and IL-27 was neutralized. Lysosomal acidification and cathepsin D activity were associated with control of mycobacteria. The acidification of lysosomes, association with mycobacteria, and maturation of cathepsin D required macrophage production of IFN-γ and signaling through signal transducer and activator of transcription (STAT)-1. In contrast, STAT-3 signaling opposed these events.
Conclusions
Our results have identified novel influences of IL-12, IL-27, and STAT-3 on lysosomal activity and further demonstrate that modulating the cytokine environment promotes enhanced trafficking of mycobacteria to lysosomes in human macrophages. This has important implications in approaches to control infection and improve vaccination. Overcoming bacterial resistance to lysosomal fusion may expand the repertoire of antigens presented to the adaptive arm of the immune response.

4.1284 Long-chain Acyl-CoA Dehydrogenase Deficiency as a Cause of Pulmonary Surfactant Dysfunction

Goetzman, E.S. et al
*J. Biol. Chem., **289(15)**, 10668-10679 (2014)*

Long-chain acyl-CoA dehydrogenase (LCAD) is a mitochondrial fatty acid oxidation enzyme whose expression in humans is low or absent in organs known to utilize fatty acids for energy such as heart, muscle, and liver. This study demonstrates localization of LCAD to human alveolar type II pneumocytes, which synthesize and secrete pulmonary surfactant. The physiological role of LCAD and the fatty acid oxidation pathway in lung was subsequently studied using LCAD knock-out mice. Lung fatty acid oxidation was reduced in $LCAD^{-/-}$ mice. $LCAD^{-/-}$ mice demonstrated reduced pulmonary compliance, but histological examination of lung tissue revealed no obvious signs of inflammation or pathology. The changes in lung mechanics were found to be due to pulmonary surfactant dysfunction. Large aggregate surfactant isolated from $LCAD^{-/-}$ mouse lavage fluid had significantly reduced phospholipid content as well as alterations in the acyl chain composition of phosphatidylcholine and phosphatidylglycerol. $LCAD^{-/-}$ surfactant demonstrated functional abnormalities when subjected to dynamic compression-expansion cycling on a constrained drop surfactometer. Serum albumin, which has been shown to degrade and inactivate pulmonary surfactant, was significantly increased in $LCAD^{-/-}$ lavage fluid, suggesting

increased epithelial permeability. Finally, we identified two cases of sudden unexplained infant death where no lung LCAD antigen was detectable. Both infants were homozygous for an amino acid changing polymorphism (K333Q). These findings for the first time identify the fatty acid oxidation pathway and LCAD in particular as factors contributing to the pathophysiology of pulmonary disease.

4.1285 **A comparison of the main structures of N-glycans of porcine islets with those from humans**
Miyagawa, S., Maeda, A., Kawamura, T., Ueno, T., Usui, N., Kondo, S., Matsumoto, S., Okitsu, T., Goto, M. and Nagashima, H.
*Glycobiology, **24(2)**, 125-138 (2014)*

After producing α1-3-galactosyltransferase knockout (GKO) pigs, most of the organs of these pigs showed less antigenicity to the human body. However, wild-type adult pig islets (API) that originally contained negligible levels of α-galactosidase now showed a clear antigenicity to human serum. In this study, *N*-glycans were isolated from both APIs and human islets. Their structures were then analyzed by a mapping technique based on their high-performance liquid chromatography elution positions and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometric data. Both preparations contained substantial amounts of high-mannose structures. The *N*-glycans from human islets were separated into 17 neutral, 8 mono-sialyl and 4 di-sialyl glycans, and the API glycans were comprised of 11 neutral, 8 mono-sialyl, 3 di-sialyl, 2 mono-sulfated, 3 mono-sialyl-mono-sulfated and 1 di-sulfated glycans. Among them, the API preparation contained one neutral, five mono-sialyl glycans and six sulfated glycans that were not detected in human islets. The structures of 9 of these 12 could be clearly determined. In addition, a study of the sulfate-depleted API suggests that sulfate residues could be antigenic to humans. The data herein will be helpful for future studies of the antigenicity associated with API.

4.1286 **Upregulation of cannabinoid receptor-1 and fibrotic activation of mouse hepatic stellate cells during Schistosoma J. infection: Role of NADPH oxidase**
Wang, M., Abais, J.M., Meng, N., Zhang, Y., Ritter, J.K., Li, P-L. and Tang, W-X.
*Free Radical Biology abd Medicine, **71**, 109-120 (2014)*

The endocannabinoid system (CS) has been implicated in the development of hepatic fibrosis such as schistosomiasis-associated liver fibrosis (SSLF). However, the mechanisms mediating the action of the CS in hepatic fibrosis are unclear. The present study hypothesized that *Schistosoma J.* infection upregulates cannabinoid receptor 1 (CB1) due to activation of NADPH oxidase leading to a fibrotic phenotype in hepatic stellate cells (HSCs). The SSLF model was developed by infecting mice with *Schistosoma J.* cercariae in the skin, and HSCs from control and infected mice were then isolated, cultured, and confirmed by analysis of HSC markers α-SMA and desmin. CB1 significantly increased in HSCs isolated from mice with SSLF, which was accompanied by a greater expression of fibrotic markers α-SMA, collagen I, and TIMP-1. CB1 upregulation and enhanced fibrotic changes were also observed in normal HSCs treated with soluble egg antigen (SEA) from *Schistosoma J.* Electron spin resonance (ESR) analysis further demonstrated that superoxide ($O_2^{\bullet -}$) production was increased in infected HSCs or normal HSCs stimulated with SEA. Both Nox4 and Nox1 siRNA prevented SEA-induced upregulation of CB1, α-SMA, collagen I, and TIMP-1 by inhibition of $O_2^{\bullet -}$ production, while CB1 siRNA blocked SEA-induced fibrotic changes without effect on $O_2^{\bullet -}$ production in these HSCs. Taken together, these data suggest that the fibrotic activation of HSCs on *Schistosoma J.* infection or SEA stimulation is associated with NADPH oxidase-mediated redox regulation of CB1 expression, which may be a triggering mechanism for SSLF.

4.1287 **Nanoparticle Incorporation of Melittin Reduces Sperm and Vaginal Epithelium Cytotoxicity**
Jallouk, A.P., Moley, K.H., Omurtag, K., Hu, G., Lanza, G.M., Wickline, S.A. and Hood, J.L.
*PloS One, **9(4)**, e95411 (2014)*

Melittin is a cytolytic peptide component of bee venom which rapidly integrates into lipid bilayers and forms pores resulting in osmotic lysis. While the therapeutic utility of free melittin is limited by its cytotoxicity, incorporation of melittin into the lipid shell of a perfluorocarbon nanoparticle has been shown to reduce its toxicity *in vivo*. Our group has previously demonstrated that perfluorocarbon nanoparticles containing melittin at concentrations <10 μM inhibit HIV infectivity *in vitro*. In the current study, we

assessed the impact of blank and melittin-containing perfluorocarbon nanoparticles on sperm motility and the viability of both sperm and vaginal epithelial cells. We found that free melittin was toxic to sperm and vaginal epithelium at concentrations greater than 2 μM ($p<0.001$). However, melittin nanoparticles were not cytotoxic to sperm ($p = 0.42$) or vaginal epithelium ($p = 0.48$) at an equivalent melittin concentration of 10 μM. Thus, nanoparticle formulation of melittin reduced melittin cytotoxicity fivefold and prevented melittin toxicity at concentrations previously shown to inhibit HIV infectivity. Melittin nanoparticles were toxic to vaginal epithelium at equivalent melittin concentrations ≥20 μM ($p<0.001$) and were toxic to sperm at equivalent melittin concentrations ≥40 μM ($p<0.001$). Sperm cytotoxicity was enhanced by targeting of the nanoparticles to the sperm surface antigen sperm adhesion molecule 1. While further testing is needed to determine the extent of cytotoxicity in a more physiologically relevant model system, these results suggest that melittin-containing nanoparticles could form the basis of a virucide that is not toxic to sperm and vaginal epithelium. This virucide would be beneficial for HIV serodiscordant couples seeking to achieve natural pregnancy.

4.1288 Endoplasmic reticulum stress is accompanied by activation of NF-κB in amyotrophic lateral sclerosis

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J. Neuroimmunol., ***270***, *29-36 (2014)*

Background
Recent studies have indicated that endoplasmic reticulum (ER) stress is involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). ER stress occurs when the ER–mitochondria calcium cycle is disturbed and misfolded proteins accumulate in the ER. To cope with ER stress, cells activate the unfolded protein response (UPR). Accumulating evidence from non-neuronal cell models suggests that there is extensive cross-talk between the UPR and the NF-κB pathway.
Methods
Here we investigated the expression of NF-κB and the main UPR markers X-box binding protein 1 (XBP1), basic leucine-zipper transcription factor 6 (ATF6) and phosphorylated eukaryotic initiation factor-2α (p-eIF2) in mutated $SOD1^{G93A}$ cell models of ALS, as well as their modulation by lipopolysaccharide and ER-stressing (tunicamycin) stimuli.
Results
Expression of NF-κB was enhanced in the presence of $SOD1^{G93A}$. Lipopolysaccharide did not induce the UPR in NSC34 cells and motor neurons in a mixed motor neuron–glia coculture system. The induction of the UPR by tunicamycin was accompanied by activation of NF-κB in NSC34 cells and motor neurons.
Conclusion
Our data linked two important pathogenic mechanisms of ALS, ER stress and NF-κB signalling, in motor neurons.

4.1289 Optimization of murine small intestine leukocyte isolation for global immune phenotype analysis

Goodyear, A.W., Kumar, A., Dow, S. and Ryan, E.P.
J. Immunol. Methods, ***405***, *97-108 (2014)*

New efforts to understand complex interactions between diet, gut microbiota, and intestinal immunity emphasize the need for a standardized murine protocol that has been optimized for the isolation of lamina propria immune cells. In this study multiple mouse strains including BALB/c, 129S6/Sv/EvTac and ICR mice were utilized to develop an optimal protocol for global analysis of lamina propria leukocytes. Incubation temperature was found to significantly improve epithelial cell removal, while changes in media formulation had minor effects. Tissue weight was an effective method for normalization of solution volumes and incubation times. Collagenase digestion in combination with thermolysin was identified as the optimal method for release of leukocytes from tissues and global immunophenotyping, based on the criteria of minimizing marker cleavage, improving cell viability, and reagent cost. The effects of collagenase in combination with dispase or thermolysin on individual cell surface markers revealed diverse marker specific effects. Aggressive formulations cleaved CD8α, CD138, and B220 from the cell surface, and resulted in relatively higher expression levels of CD3, γδ TCR, CD5, DX5, Ly6C, CD11b, CD11c, MHC-II and CD45. Improved collagenase digestion significantly improved viability and reduced debris

formation, eliminating the need for density gradient purification. Finally, we demonstrate that two different digestion protocols yield significant differences in detection of $CD4^+$ and $CD8^+$ T cells, NK cells, monocytes and interdigitating DC (iDC) populations, highlighting the importance and impact of cell collection protocols on assay outputs. The optimized protocol described herein will help assure the reproducibility and robustness of global assessment of lamina propria immune responses. Moreover, this technique may be applied to isolation of leukocytes from the entire gastrointestinal tract.

4.1290 The contribution of the glycine cleavage system to the pathogenesis of Francisella tularensis
Brown, M.J., Russo, B.C., O'Dee, D.M., Schmitt, D.M. and Nau, G.J.
*Microbes and Infection, **16**, 300-309 (2014)*

Biosynthesis and acquisition of nutrients during infection are integral to pathogenesis. Members of a metabolic pathway, the glycine cleavage system, have been identified in virulence screens of the intracellular bacterium *Francisella tularensis* but their role in pathogenesis remains unknown. This system generates 5,10-methylenetetrahydrofolate, a precursor of amino acid and DNA synthesis, from glycine degradation. To characterize this pathway, deletion of the *gcvT* homolog, an essential member of this system, was performed in attenuated and virulent *F. tularensis* strains. Deletion mutants were auxotrophic for serine but behaved similar to wild-type strains with respect to host cell invasion, intracellular replication, and stimulation of TNF-α. Unexpectedly, the glycine cleavage system was required for the pathogenesis of virulent *F. tularensis* in a murine model. Deletion of the *gcvT* homolog delayed mortality and lowered bacterial burden, particularly in the liver and bloodstream. To reconcile differences between the cell culture model and animal model, minimal tissue culture media was employed to mimic the nutritionally limiting environment of the host. This reevaluation demonstrated that the glycine cleavage system contributes to the intracellular replication of virulent *F. tularensis* in serine limiting environments. Thus, the glycine cleavage system is the serine biosynthetic pathway of *F. tularensis* and contributes to pathogenesis in vivo.

4.1291 Genotoxic potential of several naphthenic acids and a synthetic oil sands process-affected water in rainbow trout (Oncorhynchus mykiss)
Lacaze, E., Devaux, A., Bruneau, A., Bony, S., Sheery, J. and gagne, F.
*Aquatic Toxicol., **152**, 291-299 (2014)*

The exploitation of oil sands has raised major environmental concerns, particularly regarding the presence of high concentration in contaminants such as polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (NAs) in oil sands process-affected water (OSPW). The purpose of this study was, first to evaluate the genotoxic impact of OSPW-related compounds such as NAs and PAHs in a salmonid species and secondly to assess if OSPW exposure leads to genotoxicity. For this purpose, rainbow trout hepatocytes were exposed *in vitro* to environmentally relevant concentrations of synthetic NAs, naphtalene, benzo(a)pyrene, and extracts of synthetic OSPW (generated by a laboratory bitumen extraction) and of oil sands leaching water (OSLW, mimicking leaching of oil sands in river water). Primary DNA damage was assessed by the formamidopyrimidine-DNA glycolyase (Fpg)-modified comet assay. Genotoxicity was observed in hepatocytes exposed to several NAs, mixture of them, OSPW and OSLW extracts. The chemical structure of NAs influences the genotoxicity potential: among the NAs tested, the most cyclic NA was the most genotoxic. It also appears that genotoxicity was more marked for OSPW than for OSLW. Because exposure to OSPW led to oxidative DNA damage, while after exposure to several NAs, these types of DNA damage were limited, the NAs tested in this study could not be qualified as the only major contaminants responsible for OSPW genotoxicity. Notwithstanding, it should be noteworthy that exposure to NAs resulted in genotoxic impact at concentrations lower than those documented by literature for fresh OSPW. Further research is needed to explore the relationships between the chemical structure of NAs and their genotoxicity in the light of the distribution of NAs in fresh OSPW samples as well as in surface waters.

4.1292 Mass and Density Measurements of Live and Dead Gram-Negative and Gram-Positive Bacterial Populations
Lewis, C.L., Craig, C.C. and Senecal, A.G.
*Appl. Environ. Microbiol., **80(12)**, 3622-3631 (2014)*

Monitoring cell growth and measuring physical features of food-borne pathogenic bacteria are important for better understanding the conditions under which these organisms survive and proliferate. To address this challenge, buoyant masses of live and dead Escherichia coli O157:H7 and Listeria innocua were

measured using Archimedes, a commercially available suspended microchannel resonator (SMR). Cell growth was monitored with Archimedes by observing increased cell concentration and buoyant mass values of live growing bacteria. These growth data were compared to optical density measurements obtained with a Bioscreen system. We observed buoyant mass measurements with Archimedes at cell concentrations between 10^5 and 10^8 cells/ml, while growth was not observed with optical density measurements until the concentration was 10^7 cells/ml. Buoyant mass measurements of live and dead cells with and without exposure to hydrogen peroxide stress were also compared; live cells generally had a larger buoyant mass than dead cells. Additionally, buoyant mass measurements were used to determine cell density and total mass for both live and dead cells. Dead E. coli cells were found to have a larger density and smaller total mass than live E. coli cells. In contrast, density was the same for both live and dead L. innocua cells, while the total mass was greater for live than for dead cells. These results contribute to the ongoing challenge to further develop existing technologies used to observe cell populations at low concentrations and to measure unique physical features of cells that may be useful for developing future diagnostics.

4.1293 Saxitoxins induce cytotoxicity, genotoxicity and oxidative stress in teleost neurons in vitro
Da Silva, C.A., de Morais, E.C.P., Costa, M.D.M., Ribas, J.L.C., Guiloski, I.C., Ramsdorf, W.A., Zanata, S.M., Cestari, M.M., Ribeiro, C.A.O., magalhaes, V.F., Trudeau, V.L. and de Assis, H.C.S.
*Toxicon, **86**, 8-15 (2014)*

The aim of this study was establish a protocol for isolation and primary culture of neurons from tropical freshwater fish species *Hoplias malabaricus* for assessment of the effects of neurotoxic substances as saxitoxins (STXs). Cells from brain of *H. malabaricus* were treated with different concentrations of trypsin, dispase and papain for tissue dissociation. Cells type was separated by cellular gradient and basic fibroblast growth factor (bFGF) supplement nutrition media were added. The dissociated cells were plated with medium and different STXs concentrations and the toxic cellular effects such as oxidative stress, neurotoxicity, and genotoxicity and apoptosis process were evaluated. Cultures treated with bFGF showed the greatest adherence, survival and cellular development. STXs increased specific activity of glutathione peroxidase and lipoperoxidation levels, were cytotoxic and genotoxic indicated by the comet assay. Although the STXs effects due the blockage of sodium channels is reported to be reversible, the time exposure and concentration of STXs suggested cellular injuries which can lead to neuropathology. The establishment of primary neuronal culture protocol enables new applications for neurotoxicological assessments.

4.1294 Macrophagic and microglial responses after focal traumatic brain injury in the female rat
Turtzo, L.C., Lescher, J., Janes, L., Dean, D.D., Budde, M.D. and Frank, J.A.
*J. Neuroinflammation, **11**:82 (2014)*

Background
After central nervous system injury, inflammatory macrophages (M1) predominate over anti-inflammatory macrophages (M2). The temporal profile of M1/M2 phenotypes in macrophages and microglia after traumatic brain injury (TBI) in rats is unknown. We subjected female rats to severe controlled cortical impact (CCI) and examined the postinjury M1/M2 time course in their brains.
Methods
The motor cortex (2.5 mm left laterally and 1.0 mm anteriorly from the bregma) of anesthetized female Wistar rats (ages 8 to 10 weeks; $N = 72$) underwent histologically moderate to severe CCI with a 5-mm impactor tip. Separate cohorts of rats had their brains dissociated into cells for flow cytometry, perfusion-fixed for immunohistochemistry (IHC) and *ex vivo* magnetic resonance imaging or flash-frozen for RNA and protein analysis. For each analytical method used, separate postinjury times were included for 24 hours; 3 or 5 days; or 1, 2, 4 or 8 weeks.
Results
By IHC, we found that the macrophagic and microglial responses peaked at 5 to 7 days post-TBI with characteristics of mixed populations of M1 and M2 phenotypes. Upon flow cytometry examination of immunological cells isolated from brain tissue, we observed that peak M2-associated staining occurred at 5 days post-TBI. Chemokine analysis by multiplex assay showed statistically significant increases in macrophage inflammatory protein 1α and keratinocyte chemoattractant/growth-related oncogene on the ipsilateral side within the first 24 hours after injury relative to controls and to the contralateral side. Quantitative RT-PCR analysis demonstrated expression of both M1- and M2-associated markers, which peaked at 5 days post-TBI.
Conclusions

The responses of macrophagic and microglial cells to histologically severe CCI in the female rat are maximal between days 3 and 7 postinjury. The response to injury is a mixture of M1 and M2 phenotypes.

4.1295 Differential expression of CD45 isoforms in canine leukocytes
Goto-Koshino, Y., Tomiyasu, H., Suzuki, H., Tamamoto, T., Mitzutani, N., Fujino, Y., Ohno, K. and Tsujimoto, H.
Veterinary Immunol. Immunopathol., ***160****, 118-122 (2014)*

CD45 is one of the most abundant molecules expressed on the white blood cell surface in various mammals. In this study, we investigated the differential expression of CD45 isoforms in normal canine white blood cells. It has been shown that all canine nucleated blood cells express CD45. We characterized two major isoforms of canine CD45 derived from alternative splicing: a higher molecular weight isoform, CD45RA, and a lower molecular weight isoform, CD45RO. The nucleotide sequences of the two isoforms were identical, except for the region corresponding to a part in the extracellular domain. Flow cytometry analysis using an antibody that recognizes CD45RA, but not CD45RO, revealed that granulocytes did not express CD45RA, and monocytes express low levels of CD45RA. We further analyzed the expression levels of CD45RA in each lymphocyte subpopulation and found that the expression of CD45RA on CD21+ B cells was uniform. On the other hand, expression of CD45RA on CD3+ T cells was variable. Upon stimulation of lymphocytes with Con A, the CD45RA+ fraction increased, indicating that not only the phenotypes but also the activation status influences the isoform expression pattern of CD45. Our finding provides a basic knowledge of the expression of canine CD45, which could be a tool to study lymphocytes with various phenotypes, developmental stages, and activation status.

4.1296 Rabies Virus Envelope Glycoprotein Targets Lentiviral Vectors to the Axonal Retrograde Pathway in Motor Neurons
Hislop, J.N., Islam, T.A., eleftheriadou, I., Carpentier, D.C.J., Trabalza, A., Parkinson, M., Schhiavo, G. and Mazarakis, N.D.
J. Biol. Chem., ***289(23)****, 16148-16163 (2014)*

Rabies pseudotyped lentiviral vectors have great potential in gene therapy, not least because of their ability to transduce neurons following their distal axonal application. However, very little is known about the molecular processes that underlie their retrograde transport and cell transduction. Using multiple labeling techniques and confocal microscopy, we demonstrated that pseudotyping with rabies virus envelope glycoprotein (RV-G) enabled the axonal retrograde transport of two distinct subtypes of lentiviral vector in motor neuron cultures. Analysis of this process revealed that these vectors trafficked through Rab5-positive endosomes and accumulated within a non-acidic Rab7 compartment. RV-G pseudotyped vectors were co-transported with both the tetanus neurotoxin-binding fragment and the membrane proteins thought to mediate rabies virus endocytosis (neural cell adhesion molecule, nicotinic acetylcholine receptor, and p75 neurotrophin receptor), thus demonstrating that pseudotyping with RV-G targets lentiviral vectors for transport along the same pathway exploited by several toxins and viruses. Using motor neurons cultured in compartmentalized chambers, we demonstrated that axonal retrograde transport of these vectors was rapid and efficient; however, it was not able to transduce the targeted neurons efficiently, suggesting that impairment in processes occurring after arrival of the viral vector in the soma is responsible for the low transduction efficiency seen *in vivo*, which suggests a novel area for improvement of gene therapy vectors.

4.1297 Efficient Transient Transfection of Human Multiple Myeloma Cells by Electroporation – An Appraisal
Steinbrunn, T., Chatterjee, M., Bargou, R.C. and Stühmer, T.
PloS One, ***9(6)****, e97443 (2014)*

Cell lines represent the everyday workhorses for in vitro research on multiple myeloma (MM) and are regularly employed in all aspects of molecular and pharmacological investigations. Although loss-of-function studies using RNA interference in MM cell lines depend on successful knockdown, no well-established and widely applied protocol for efficient transient transfection has so far emerged. Here, we provide an appraisal of electroporation as a means to introduce either short-hairpin RNA expression vectors or synthesised siRNAs into MM cells. We found that electroporation using siRNAs was much more efficient than previously anticipated on the basis of transfection efficiencies deduced from EGFP-expression off protein expression vectors. Such knowledge can even confidently be exploited in "hard-to-transfect" MM cell lines to generate large numbers of transient knockdown phenotype MM cells. In addition, special attention was given to developing a protocol that provides easy implementation, good

reproducibility and manageable experimental costs.

4.1298 Human Tonsil-derived Dendritic Cells are Poor Inducers of T cell Immunity to Mucosally Encountered Pathogens

Hallisey, C.M., Heydeman, R.S. and Williams, N.A.
*J. Infectious Disease, **209**, 1847-1856 (2014)*

The mucosal immune system must initiate and regulate protective immunity, while balancing this immunity with tolerance to harmless antigens and bacterial commensals. We have explored the hypothesis that mucosal dendritic cells (DC) control the balance between regulation and immunity, by studying the responses of human tonsil-derived DC to *Neisseria meningitidis* as a model organism. We show that tonsil DC are able to sample their antigenic environment, internalizing Nm and expressing high levels of HLA-DR and CD86. However, in comparison to monocyte-derived DC (moDC), they respond to pathogen encounter with only low level cytokine production, largely dominated by TGFβ. Functionally, tonsil DC also only stimulated low levels of antigen-specific T cell proliferation and cytokine production when compared to moDC. We therefore propose that the default role for DC in the nasopharynx is to maintain tolerance/ignorance of the large volume of harmless antigens and bacterial commensals encountered at the nasopharyngeal mucosa.

4.1299 Dietary Zinc Supplementation to the Donor Improves Insulin Secretion After Islet Transplantation in Chemically Induced Diabetic Rats

Mishima, T., Kuroki, T., Tajima, Y., Adachi, T., Hirabaru, M., Tanaka, T., Kitasato, A., Takatsuki, M. and Eguchi, S.
*Pancreas, **43(2)**, 236-239 (2014)*

Objectives
Zinc (Zn) is related to insulin synthesis, storage, and secretion. This study demonstrates the effects of Zn supplementation in donor rats on the outcomes of islet transplantation.

Methods
Donor rats received 3 different regimens of dietary Zn supplementation for 2 weeks before undergoing pancreas donation: a standard diet containing Zn at 50 ppm (control), 1 ppm (low-Zn group) or 1000 ppm (high-Zn group), respectively. Diabetic recipient rats underwent islet transplantation, and the blood glucose levels and insulin secretion were monitored for 7 days after transplantation.

Results
The serum and pancreatic Zn levels at the time of donation were significantly lower in the low-Zn group (48.8 ± 25.5 μg/dL and 11.3 ± 1.9 μg/g) and higher in the high-Zn group (147.3 ± 17.6 μg/dL and 18.7 ± 2.2 μg/g) when compared with those observed in the controls (118.7 ± 7.9 μg/dL and 14.6 ± 2.0 μg/g) ($P < 0.05$). The blood glucose levels became re-elevated 2 days after transplantation in rats receiving islet grafts from the controls and the low-Zn groups. In contrast, in the rats that received islets from the high-Zn groups, these were maintained within a reference range ($P < 0.01$).

Conclusions
These data indicate that a Zn-rich diet for donor rats improves the function of islet grafts in chemically induced diabetic rats.

4.1300 Long-Term Function of Islets Encapsulated in a Redesigned Alginate Microcapsule Construct in Omentum Pouches of Immune-Competent Diabetic Rats

Pareta, R., McQuilling, J.P., Sittadjody, S., Jenkins, R., Bowden, S., Orlando, G., Farney, A.C., Brey, E.M. and Opara, E.C.
*Pancreas, **43(4),** 605-613 (2014)*

Objective
Our study aim was to determine encapsulated islet graft viability in an omentum pouch and the effect of fibroblast growth factor 1 (FGF-1) released from our redesigned alginate microcapsules on the function of the graft.
Methods
Isolated rat islets were encapsulated in an inner core made with 1.5% low-viscosity–high-mannuronic-acid alginate followed by an external layer made with 1.25% low-viscosity high-guluronic acid alginate with or

without FGF-1, in microcapsules measuring 300 to 400 μm in diameter. The 2 alginate layers were separated by a perm-selective membrane made with 0.1% poly-l-ornithine, and the inner low-viscosity–high-mannuronic-acid core was partially chelated using 55 mM sodium citrate for 2 minutes.
Results
A marginal mass of encapsulated islet allografts (~2000 islets/kg) in streptozotocin-diabetic Lewis rats caused significant reduction in blood glucose levels similar to the effect observed with encapsulated islet isografts. Transplantation of alloislets coencapsulated with FGF-1 did not result in better glycemic control, but induced greater body weight maintenance in transplant recipients compared with those that received only alloislets. Histological examination of the retrieved tissue demonstrated morphologically and functionally intact islets in the microcapsules, with no signs of fibrosis.
Conclusions
We conclude that the omentum is a viable site for encapsulated islet transplantation.

4.1301 Lactate Reduces Liver and Pancreatic Injury in Toll-Like Receptor– and Inflammasome-Mediated Inflammation via GPR81-Mediated Suppression of Innate Immunity
Hoque, R., Farooq, A., Ghani, A., Gorelick, F. and Mehal, W.Z.
*Gastroenterology, **146**, 1763-1774 (2014)*

Background & Aims

The NACHT, LRR, and pyrin domain–containing protein 3 (NLRP3) inflammasome induces inflammation in response to organ injury, but little is known about its regulation. Toll-like receptors (TLRs) provide the first signal required for activation of the inflammasome and stimulate aerobic glycolysis to generate lactate. We examined whether lactate and the lactate receptor, G_i-protein–coupled receptor 81 (GPR81), regulate TLR induction of signal 1 and limit inflammasome activation and organ injury.
Methods
Primary mouse macrophages and human monocytes were incubated with TLR4 agonists and lactate and assayed for levels of pro-interleukin (IL)1β, NLRP3, and caspase-1 (CASP1); release of IL1β; and activation of nuclear factor-κB (NF-κB) and caspase-1. Small interfering RNAs were used to reduce levels of GPR81 and arrestin β-2 (ARRB2), and an NF-κB luciferase reporter transgene was transfected in RAW 264.7 cells. Cell lysates were analyzed by immunoprecipitation with an antibody against GPR81. Acute hepatitis was induced in C56BL/6N mice by administration of lipopolysaccharide and D-galactosamine. Acute pancreatitis was induced by administration of lipopolysaccharide and cerulein. Some mice were given intraperitoneal injections of sodium lactate or small interfering RNA against Gpr81. Activation of NF-κB in tissue macrophages was assessed in mice that expressed a reporter transgene.
Results
In macrophages and monocytes, increasing concentrations of lactate reduced TLR4-mediated induction of *Il1B, Nlrp3,* and *Casp1*; activation of NF-κB; release of IL1β; and cleavage of CASP1. GPR81 and ARRB2 physically interacted and were required for these effects. The administration of lactate reduced inflammation and organ injury in mice with immune hepatitis; this reduction required Gpr81 dependence in vivo. Lactate also prevented activation of NF-κB in macrophages of mice, and, when given after injury, reduced the severity of acute pancreatitis and acute liver injury.
Conclusions
Lactate negatively regulates TLR induction of the NLRP3 inflammasome and production of IL1β, via ARRB2 and GPR81. Lactate could be a promising immunomodulatory therapy for patients with acute organ injury.

4.1302 Despite Differences in Cytosolic Calcium Regulation, Lidocaine Toxicity Is Similar in Adult and Neonatal Rat Dorsal Root Ganglia In Vitro
Doan, L.V., Eydlin, O., Piskoun, B., Kline, R., Recio-Pinto, E., Rosenberg, A.D., Blanck, T.J.J. and Xu, F.
*Anesthesiology,**120(1)**, 50-61 (2014)*

Background: Neuraxial local anesthetics may have neurological complications thought to be due to neurotoxicity. A primary site of action of local anesthetics is the dorsal root ganglia (DRG) neuron. Physiologic differences have been noted between young and adult DRG neurons; hence, the authors examined whether there were any differences in lidocaine-induced changes in calcium and lidocaine toxicity in neonatal and adult rat DRG neurons. Methods: DRG neurons were cultured from postnatal day

7 (P7) and adult rats. Lidocaine-induced changes in cytosolic calcium were examined with the calcium indicator Fluo-4. Cells were incubated with varying concentrations of lidocaine and examined for viability using calcein AM and ethidium homodimer-1 staining. Live imaging of caspase-3/7 activation was performed after incubation with lidocaine.
Results: The mean KCl-induced calcium transient was greater in P7 neurons ($P < 0.05$), and lidocaine significantly inhibited KCl-induced calcium responses in both ages ($P < 0.05$). Frequency distribution histograms of KCl-evoked calcium increases were more heterogeneous in P7 than in adult neurons. With lidocaine, KCl-induced calcium transients in both ages became more homogeneous but remained different between the groups. Interestingly, cell viability was decreased by lidocaine in a dose-dependent manner similarly in both ages. Lidocaine treatment also activated caspase-3/7 in a dose- and time-dependent manner similarly in both ages. Conclusions: Despite physiological differences in P7 and adult DRG neurons, lidocaine cytotoxicity is similar in P7 and adult DRG neurons in vitro. Differences in lidocaine- and KCl-evoked calcium responses suggest the similarity in lidocaine cytotoxicity involves other actions in addition to lidocaine-evoked effects on cytosolic calcium responses.

4.1303 NKT Cells Determine Titer and Subtype Profile of Virus-Specific IgG Antibodies during Herpes Simplex Virus Infection
Rafter, M.J., Wolter, E., Fillatreau, S., Meisel, H., Kaufmann, H.E. and Schönrich, G.
J. Immunol., ***192(9)***, *4294-4302 (2014)*

Invariant NKT cells (iNKT cells) are innate lymphocytes that recognize lipid-derived Ags presented by the MHC class I–related protein CD1d. In this study, we analyzed the role of iNKT cells in the generation of Abs against HSV type 1 (HSV-1). In sera from healthy hman donors, we found a correlation between HSV-1–specific IgG titers and proportions of $CD4^+$ iNKT cells. In HSV-1–infected iNKT cell–deficient mice, the amount of specific IgM and IgG Abs were significantly reduced compared with wild-type mice. Moreover, iNKT cell–deficient mice were unable to upregulate CD1d on B cells and failed to establish an IFN-γ–driven subtype profile of HSV-1–specific IgG Abs. In spleens of HSV-1–infected wild-type mice, the percentage of iNKT cells expressing CCR6, a marker for inflammatory iNKT cells secreting IFN-γ, was significantly decreased at 6 mo postinfection, suggesting that these cells were released from the spleen to other tissues. Finally, in vitro experiments showed that in the absence of CD1d-restricted cells, HSV-1 induced markedly lower IFN-γ production in splenocytes from naive mice. Taken together, our results indicate that iNKT cells shape the Ab response to HSV-1 infection and provide a basis for rational development of antiviral vaccines.

4.1304 Adult Porcine Islet Isolation Using a Ductal Preservation Method and Purification With a Density Gradient Composed of Histidine-Tryptophan-Ketoglutarate Solution and Iodixanol
Jin, S-M.,Lee, H-S., Oh, S-H., Park, H.J., Park, J.B., Kim, J.H. and Kim, S.J
Transplantation Proceedings, ***46***, *1628-1632 (2014)*

Background
Given the fragility of adult porcine islets, reduction of shearing stress in islet purification using histidine-tryptophan-ketoglutarate (HTK) solution and iodixanol could be an effective strategy. We examined the effect of ductal preservation with HTK solution and an islet purification protocol that utilizes HTK solution and iodixanol in adult porcine islet isolation.
Methods
Islets were isolated with a modified Ricordi method using adult Prestige World Genetics (PWG) and Yucatan pigs. The discontinuous density gradient was composed of either HTK solution/iodixanol ($n = 23$, iodixanol group) or Hank's balanced salt solution (HBSS)/Ficoll ($n = 17$, Ficoll group). In the iodixanol group, ductal injection of HTK solution was performed before purification.
Results
In PWG pigs, significantly higher islet yield after purification (3480 ± 214.2 islet equivalent [IEQ]/g, $P = .003$) and higher recovery rate (85.45% ± 3.49%, $P = .0043$) were obtained from the HTK/iodixanol group as compared to the HBSS/Ficoll group (1905 ± 323.2 IEQ/g, and 67.22% ± 4.77%, respectively). Similar results were obtained in Yucatan pigs with greater body weight.
Conclusion
Ductal preservation and iodixanol-based islet purification using HTK solution improved the yield of adult porcine islet isolation compared to the conventional method using HBSS and Ficoll. The results of this study support the feasibility of an adult porcine islet isolation protocol using HTK solution and iodixanol, which have the favorable physical properties.

4.1305 Endoplasmic reticulum stress in spinal and bulbar muscular atrophy: a potential target for therapy
Montague, K., Malik, B., Gray, A.L., La Spada, A.R., Hanna, M.G., Szabadkai, G. and Linda Greensmith
*Brain, **137**, 1894-1906 (2014)*

Spinal and bulbar muscular atrophy is an X-linked degenerative motor neuron disease caused by an abnormal expansion in the polyglutamine encoding CAG repeat of the androgen receptor gene. There is evidence implicating endoplasmic reticulum stress in the development and progression of neurodegenerative disease, including polyglutamine disorders such as Huntington's disease and in motor neuron disease, where cellular stress disrupts functioning of the endoplasmic reticulum, leading to induction of the unfolded protein response. We examined whether endoplasmic reticulum stress is also involved in the pathogenesis of spinal and bulbar muscular atrophy. Spinal and bulbar muscular atrophy mice that carry 100 pathogenic polyglutamine repeats in the androgen receptor, and develop a late-onset neuromuscular phenotype with motor neuron degeneration, were studied. We observed a disturbance in endoplasmic reticulum-associated calcium homeostasis in cultured embryonic motor neurons from spinal and bulbar muscular atrophy mice, which was accompanied by increased endoplasmic reticulum stress. Furthermore, pharmacological inhibition of endoplasmic reticulum stress reduced the endoplasmic reticulum-associated cell death pathway. Examination of spinal cord motor neurons of pathogenic mice at different disease stages revealed elevated expression of markers for endoplasmic reticulum stress, confirming an increase in this stress response *in vivo*. Importantly, the most significant increase was detected presymptomatically, suggesting that endoplasmic reticulum stress may play an early and possibly causal role in disease pathogenesis. Our results therefore indicate that the endoplasmic reticulum stress pathway could potentially be a therapeutic target for spinal and bulbar muscular atrophy and related polyglutamine diseases.

4.1306 Microglial VPAC1R mediates a novel mechanism of neuroimmune-modulation of hippocampal precursor cells via IL-4 release
Nunan, R., Sivasathiaseelan, H., Khan, D., Zaben, M. and Gray, W.
*Glia, **62**, 1313-1327 (2014)*

Neurogenesis, the production of new neurons from neural stem/progenitor cells (NSPCs), occurs throughout adulthood in the dentate gyrus of the hippocampus, where it supports learning and memory. The innate and adaptive immune systems are increasingly recognized as important modulators of hippocampal neurogenesis under both physiological and pathological conditions. However, the mechanisms by which the immune system regulates hippocampal neurogenesis are incompletely understood. In particular, the role of microglia, the brains resident immune cell is complex, as they have been reported to both positively and negatively regulate neurogenesis. Interestingly, neuronal activity can also regulate the function of the immune system. Here, we show that depleting microglia from hippocampal cultures reduces NSPC survival and proliferation. Furthermore, addition of purified hippocampal microglia, or their conditioned media, is trophic and proliferative to NSPCs. VIP, a neuropeptide released by dentate gyrus interneurons, enhances the proliferative and pro-neurogenic effect of microglia via the VPAC1 receptor. This VIP-induced enhancement is mediated by IL-4 release, which directly targets NSPCs. This demonstrates a potential neuro-immuno-neurogenic pathway, disruption of which may have significant implications in conditions where combined cognitive impairments, interneuron loss, and immune system activation occurs, such as temporal lobe epilepsy and Alzheimer's disease.

4.1307 Control of Insulin Secretion by Cytochrome c and Calcium Signaling in Islets with Impaired Metabolism
Rountree, A.M., Neal, A.S., Lisowsky, M., Rizzo, M., Radtke, J., White, S., Luciani, D.S., Kim, F., Hampe, C.S. and Sweet, I.R.
*J. Biol. Chem., **289(27)**, 19110-19119 (2014)*

The aim of the study was to assess the relative control of insulin secretion rate (ISR) by calcium influx and signaling from cytochrome *c* in islets where, as in diabetes, the metabolic pathways are impaired. This was achieved either by culturing isolated islets at low (3 mm) glucose or by fasting rats prior to the isolation of the islets. Culture in low glucose greatly reduced the glucose response of cytochrome *c* reduction and translocation and ISR, but did not affect the response to the mitochondrial fuel α-ketoisocaproate. Unexpectedly, glucose-stimulated calcium influx was only slightly reduced in low glucose-cultured islets and was not responsible for the impairment in glucose-stimulated ISR. A glucokinase activator acutely restored cytochrome *c* reduction and translocation and ISR, independent of effects on calcium influx. Islets

from fasted rats had reduced ISR and cytochrome *c* reduction in response to both glucose and α-ketoisocaproate despite normal responses of calcium. Our data are consistent with the scenario where cytochrome *c* reduction and translocation are essential signals in the stimulation of ISR, the loss of which can result in impaired ISR even when calcium response is normal.

4.1308 Islet cell transplantation
McCall, M. and Shapiro, A.M.J.
*Seminars in Pediatric Surgery, **23**, 83-90 (2014)*

Islet transplantation has become a promising treatment for selected patients with type 1 diabetes. Here we provide an overview of the procedure including its history, the process of donor selection, and the techniques and procedures involved in a successful transplant. A brief overview of the current immunosuppressive regimens, the long-term follow-up and the reported outcomes will also be discussed. While islet transplantation is currently generally reserved for adults with type 1 diabetes with severe hypoglycemia or glycemic lability, we herein consider the possibility of its application to the pediatric population.

4.1309 Isolation and characterization of resident endogenous c-Kit+ cardiac stem cells from the adult mouse and rat heart
Smith, A.J., lewis, F.C., Aquila, I., Waring, C.D., Nocera, A., Agosti, V., Nadal-Ginard, b., Torella, D. and Ellison, G.M.
*Nature Protocols, **9(7)**, 1662-1681 (2014)*

This protocol describes the isolation of endogenous c-Kit (also known as CD117)-positive (c-Kit$^+$), CD45-negative (CD45$^-$) cardiac stem cells (eCSCs) from whole adult mouse and rat hearts. The heart is enzymatically digested via retrograde perfusion of the coronary circulation, resulting in rapid and extensive breakdown of the whole heart. Next, the tissue is mechanically dissociated further and cell fractions are separated by centrifugation. The c-Kit$^+$CD45$^-$ eCSC population is isolated by magnetic-activated cell sorting technology and purity and cell numbers are assessed by flow cytometry. This process takes ~4 h for mouse eCSCs or 4.5 h for rat eCSCs. We also describe how to characterize c-Kit$^+$CD45$^-$ eCSCs. The c-Kit$^+$CD45$^-$ eCSCs exhibit the defining characteristics of stem cells: they are self-renewing, clonogenic and multipotent. This protocol also describes how to differentiate eCSCs into three main cardiac lineages: functional, beating cardiomyocytes, smooth muscle, and endothelial cells. These processes take 17–20 d.

4.1310 Specific Retrograde Transduction of Spinal Motor Neurons Using Lentiviral Vectors Targeted to Presynaptic NMJ Receptors
Eleftheriadou, I., trabalza, A., Ellison, S.M., Gharun, K. and Mazarakis, N.D.
*Molecular Therapy, **22(7)**, 1285-1298 (2014)*

To understand how receptors are involved in neuronal trafficking and to be able to utilize them for specific targeting via the peripheral route would be of great benefit. Here, we describe the generation of novel lentiviral vectors with tropism to motor neurons that were made by coexpressing onto the lentiviral surface a fusogenic glycoprotein (mutated sindbis G) and an antibody against a cell-surface receptor (Thy1.1, p75^{NTR}, or coxsackievirus and adenovirus receptor) on the presynaptic terminal of the neuromuscular junction. These vectors exhibit binding specificity and efficient transduction of receptor positive cell lines and primary motor neurons *in vitro*. Targeting of each of these receptors conferred to these vectors the capability of being transported retrogradely from the axonal tip, leading to transduction of motor neurons *in vitro* in compartmented microfluidic cultures. *In vivo* delivery of coxsackievirus and adenovirus receptor-targeted vectors in leg muscles of mice resulted in predicted patterns of motor neuron labeling in lumbar spinal cord. This opens up the clinical potential of these vectors for minimally invasive administration of central nervous system-targeted therapeutics in motor neuron diseases.

4.1311 Micropatterned Cell–Cell Interactions Enable Functional Encapsulation of Primary Hepatocytes in Hydrogel Microtissues No Access
Li, C.Y., Stevens, K:R., Schwartz, R.E., Alejandro, B.S., Huang, J.H. and Bhatia, S.N:
*Tissue Eingineering Part A, **20(15)**, 2200-2212 (2014)*

Drug-induced liver injury is a major cause of drug development failures and postmarket withdrawals. In vitro models that incorporate primary hepatocytes have been shown to be more predictive than model

systems which rely on liver microsomes or hepatocellular carcinoma cell lines. Methods to phenotypically stabilize primary hepatocytes ex vivo often rely on mimicry of hepatic microenvironmental cues such as cell–cell interactions and cell–matrix interactions. In this work, we sought to incorporate phenotypically stable hepatocytes into three-dimensional (3D) microtissues, which, in turn, could be deployed in drug-screening platforms such as multiwell plates and diverse organ-on-a-chip devices. We first utilize micropatterning on collagen I to specify cell–cell interactions in two-dimensions, followed by collagenase digestion to produce well-controlled aggregates for 3D encapsulation in polyethylene glycol (PEG) diacrylate. Using this approach, we examined the influence of homotypic hepatocyte interactions and composition of the encapsulating hydrogel, and achieved the maintenance of liver-specific function for over 50 days. Optimally preaggregated structures were subsequently encapsulated using a microfluidic droplet-generator to produce 3D microtissues. Interactions of engineered hepatic microtissues with drugs was characterized by flow cytometry, and yielded both induction of P450 enzymes in response to prototypic small molecules and drug–drug interactions that give rise to hepatotoxicity. Collectively, this study establishes a pipeline for the manufacturing of 3D hepatic microtissues that exhibit stabilized liver-specific functions and can be incorporated into a wide array of emerging drug development platforms.

4.1312 Cutting Edge: Antigen-Specific Thymocyte Feedback Regulates Homeostatic Thymic Conventional Dendritic Cell Maturation

Spidale, N.A., Wang, B. and Tisch, R.
J. Immunol., ***193(1)***, *21-25 (2014)*

Thymic dendritic cells (DC) mediate self-tolerance by presenting self-peptides to and depleting autoreactive thymocytes. Despite a significant role in negative selection, the events regulating thymic DC maturation and function under steady-state conditions are poorly understood. We report that cross-talk with thymocytes regulates thymic conventional DC (cDC) numbers, phenotype, and function. In mice lacking TCR-expressing thymocytes, thymic cDC were reduced and exhibited a less mature phenotype. Furthermore, thymic cDC in TCR-transgenic mice lacking cognate Ag expression in the thymus were also immature; notably, however, thymic cDC maturation was re-established by an Ag-specific cognate interaction with $CD4^+$ or $CD8^+$ single-positive thymocytes (SP). Blockade of CD40L during Ag-specific interactions with CD4 SP, but not CD8 SP, limited the effect on cDC maturation. Together, these novel findings demonstrate that homeostatic maturation and function of thymic cDC are regulated by feedback delivered by CD4 SP and CD8 SP via distinct mechanisms during a cognate Ag–specific interaction.

4.1313 GM-CSF–Licensed CD11b+ Lung Dendritic Cells Orchestrate Th2 Immunity to Blomia tropicalis

Zhou, Q., Ho, A.W.S., Schlitzer, A., Tang, Y., Wwong, K.H.S., Wong, F.H.S., Chua, Y.L, Angeli, V., Mortellaro, A., Ginhoux, F. and Kemeny, D.M.
J. Immunol., ***193(2)***, *496-509 (2014)*

The *Blomia tropicalis* dust mite is prevalent in tropical and subtropical regions of the world. Although it is a leading cause of asthma, little is known how it induces allergy. Using a novel murine asthma model induced by intranasal exposure to *B. tropicalis*, we observed that a single intranasal sensitization to *B. tropicalis* extract induces strong Th2 priming in the lung draining lymph node. Resident $CD11b^+$ dendritic cells (DCs) preferentially transport Ag from the lung to the draining lymph node and are crucial for the initiation of Th2 $CD4^+$ T cell responses. As a consequence, mice selectively deficient in $CD11b^+$ DCs exhibited attenuated Th2 responses and more importantly did not develop any allergic inflammation. Conversely, mice deficient in $CD103^+$ DCs and CCR2-dependent monocyte-derived DCs exhibited similar allergic inflammation compared with their wild-type counterparts. We also show that $CD11b^+$ DCs constitutively express higher levels of GM-CSF receptor compared with $CD103^+$ DCs and are thus selectively licensed by lung epithelial-derived GM-CSF to induce Th2 immunity. Taken together, our study identifies GM-CSF–licensed $CD11b^+$ lung DCs as a key component for induction of Th2 responses and represents a potential target for therapeutic intervention in allergy.

4.1314 Multiple pathogenic proteins implicated in neuronopathic Gaucher disease mice

Xu, Y-h., Xu, K., Sun, Y., Liou, B., Quinn, B., Li, R-h., Xue, L., Zhang, W., Setchell, K.D.R., Witte, D. and Grabowski, G.A.
Hum. Mol. Genet., ***23(15)***, *3943-3957 (2014)*

Gaucher disease, a prevalent lysosomal storage disease (LSD), is caused by insufficient activity of acid β-glucosidase (GCase) and the resultant glucosylceramide (GC)/glucosylsphingosine (GS) accumulation in visceral organs (Type 1) and the central nervous system (Types 2 and 3). Recent clinical and genetic studies implicate a pathogenic link between Gaucher and neurodegenerative diseases. The aggregation and

inclusion bodies of α-synuclein with ubiquitin are present in the brains of Gaucher disease patients and mouse models. Indirect evidence of β-amyloid pathology promoting α-synuclein fibrillation supports these pathogenic proteins as a common feature in neurodegenerative diseases. Here, multiple proteins are implicated in the pathogenesis of chronic neuronopathic Gaucher disease (nGD). Immunohistochemical and biochemical analyses showed significant amounts of β-amyloid and amyloid precursor protein (APP) aggregates in the cortex, hippocampus, stratum and substantia nigra of the nGD mice. APP aggregates were in neuronal cells and colocalized with α-synuclein signals. A majority of APP co-localized with the mitochondrial markers TOM40 and Cox IV; a small portion co-localized with the autophagy proteins, P62/LC3, and the lysosomal marker, LAMP1. In cultured wild-type brain cortical neural cells, the GCase-irreversible inhibitor, conduritol B epoxide (CBE), reproduced the APP/α-synuclein aggregation and the accumulation of GC/GS. Ultrastructural studies showed numerous larger-sized and electron-dense mitochondria in nGD cerebral cortical neural cells. Significant reductions of mitochondrial adenosine triphosphate production and oxygen consumption (28–40%) were detected in nGD brains and in CBE-treated neural cells. These studies implicate defective GCase function and GC/GS accumulation as risk factors for mitochondrial dysfunction and the multi-proteinopathies (α-synuclein-, APP- and Aβ-aggregates) in nGD.

4.1315 Connective tissue growth factor (CCN2) and microRNA-21 are components of a positive feedback loop in pancreatic stellate cells (PSC) during chronic pancreatitis and are exported in PSC-derived Exosomes

Charrier, A., Chen, R., Chen, L., Kemper, S., Hattori, T., Takigawa, M. and Brigstock, D.R.
J. Cell Commun. Signal., ***8***, *147-156 (2014)*

Pancreatitis is an inflammatory condition of the pancreas which, in its chronic form, involves tissue destruction, exocrine and endocrine insufficiency, increased risk of pancreatic cancer, and an extensive fibrotic pathology which is due to unrelenting collagen deposition by pancreatic stellate cells (PSC). In response to noxious agents such as alcohol—excessive consumption of which is a major cause of pancreatitis in the West—normally quiescent PSC undergo a phenotypic and functional transition to activated myofibroblasts which produce and deposit collagen at high levels. This process is regulated by connective tissue growth factor (CCN2), expression of which is highly up-regulated in activated PSC. We show that CCN2 production by activated PSC is associated with enhanced expression of microRNA-21 (miR-21) which was detected at high levels in activated PSC in a murine model of alcoholic chronic pancreatitis. A positive feedback loop between CCN2 and miR-21 was identified that resulted in enhancement of their respective expression as well as that of collagen α1(I). Both miR-21 and CCN2 mRNA were present in PSC-derived exosomes, which were characterized as 50–150 nm CD9-positive nano-vesicles. Exosomes from CCN2-GFP- or miR-21-GFP-transfected PSC were taken up by other PSC cultures, as shown by direct fluorescence or qRT-PCR for GFP. Collectively these studies establish miR-21 and CCN2 as participants in a positive feedback loop during PSC activation and as components of the molecular payload in PSC-derived exosomes that can be delivered to other PSC. Thus interactions between cellular or exosomal miR-21 and CCN2 represent novel aspects of fibrogenic regulation in PSC. *Summary* Chronic injury in the pancreas is associated with fibrotic pathology which is driven in large part by CCN2-dependent collagen production in pancreatic stellate cells. This study shows that CCN2 up-regulation in PSC is associated with increased expression of miR-21 which, in turn, is able to stimulate CCN2 expression further via a positive feedback loop. Additionally miR-21 and CCN2 were identified in PSC-derived exosomes which effected their delivery to other PSC. The cellular and exosomal miR-21-CCN2 axis is a novel component in PSC fibrogenic signaling.

4.1316 Mesodermal mesenchymal cells give rise to myofibroblasts, but not epithelial cells, in mouse liver injury

Lua, I., James, D., Wang, J., Wang, K.S. and Asahina, K.
Hepatology, ***60(1)***, *311-322 (2014)*

Hepatic stellate cells (HSCs) and portal fibroblasts (PFs) are believed to be the major source of myofibroblasts that participate in fibrogenesis by way of synthesis of proinflammatory cytokines and extracellular matrices. Previous lineage tracing studies using MesP1^{Cre} and Rosa26lacZ^{flox} mice demonstrated that MesP1+ mesoderm gives rise to mesothelial cells (MCs), which differentiate into HSCs and PFs during liver development. In contrast, several *in vivo* and *in vitro* studies reported that HSCs can differentiate into other cell types, including hepatocytes, cholangiocytes, and progenitor cell types known as oval cells, thereby acting as stem cells in the liver. To test whether HSCs give rise to epithelial cells in adult liver, we determined the hepatic lineages of HSCs and PFs using MesP1^{Cre} and Rosa26mTmG^{flox}

mice. Genetic cell lineage tracing revealed that the MesP1+ mesoderm gives rise to MCs, HSCs, and PFs, but not to hepatocytes or cholangiocytes, in the adult liver. Upon carbon tetrachloride injection or bile duct ligation surgery-mediated liver injury, mesodermal mesenchymal cells, including HSCs and PFs, differentiate into myofibroblasts but not into hepatocytes or cholangiocytes. Furthermore, differentiation of the mesodermal mesenchymal cells into oval cells was not observed. These results indicate that HSCs are not sufficiently multipotent to produce hepatocytes, cholangiocytes, or oval cells by way of mesenchymal-epithelial transition *in vivo*. *Conclusion*: Cell lineage tracing demonstrated that mesodermal mesenchymal cells including HSCs are the major source of myofibroblasts but do not differentiate into epithelial cell types such as hepatocytes, cholangiocytes, and oval cells

4.1317 Lung fibroblasts accelerate wound closure in human alveolar epithelial cells through hepatocyte growth factor/c-Met signaling

Ito, Y., Correll, K., Schiel, J.A., Finigan, J.H., Prekeris, R. and Mason, R.J.
Am. J. Physiol. Lung Cell Mol. Physiol., ***307(1)***, *L94-L105 (2014)*

There are 190,600 cases of acute lung injury/acute respiratory distress syndrome (ALI/ARDS) each year in the United States, and the incidence and mortality of ALI/ARDS increase dramatically with age. Patients with ALI/ARDS have alveolar epithelial injury, which may be worsened by high-pressure mechanical ventilation. Alveolar type II (ATII) cells are the progenitor cells for the alveolar epithelium and are required to reestablish the alveolar epithelium during the recovery process from ALI/ARDS. Lung fibroblasts (FBs) migrate and proliferate early after lung injury and likely are an important source of growth factors for epithelial repair. However, how lung FBs affect epithelial wound healing in the human adult lung has not been investigated in detail. Hepatocyte growth factor (HGF) is known to be released mainly from FBs and to stimulate both migration and proliferation of primary rat ATII cells. HGF is also increased in lung tissue, bronchoalveolar lavage fluid, and serum in patients with ALI/ARDS. Therefore, we hypothesized that HGF secreted by FBs would enhance wound closure in alveolar epithelial cells (AECs). Wound closure was measured using a scratch wound-healing assay in primary human AEC monolayers and in a coculture system with FBs. We found that wound closure was accelerated by FBs mainly through HGF/c-Met signaling. HGF also restored impaired wound healing in AECs from the elderly subjects and after exposure to cyclic stretch. We conclude that HGF is the critical factor released from FBs to close wounds in human AEC monolayers and suggest that HGF is a potential strategy for hastening alveolar repair in patients with ALI/ARDS.

4.1318 The Ca2+/calmodulin-dependent kinase kinase β-AMP-activated protein kinase-α1 pathway regulates phosphorylation of cytoskeletal targets in thrombin-stimulated human platelets

Onselaer, M.B., Oury, C., Hunter, R.W., eeckhoudt, S., Barile, N., Lecut, C., Morel, N., Viollet, B., Jacquet, L.M., Bertrand, L., Sakamoto, K., Vanoverschelde, J.L., Beauloye, C. and Horman, S.
J. Thrombosis and Haemostatis, ***12***, *973-986 (2014)*

Background
Platelet activation requires sweeping morphologic changes, supported by contraction and remodeling of the platelet actin cytoskeleton. In various other cell types, AMP-activated protein kinase (AMPK) controls the phosphorylation state of cytoskeletal targets.
Objective
To determine whether AMPK is activated during platelet aggregation and contributes to the control of cytoskeletal targets.
Results
We found that AMPK-α1 was mainly activated by thrombin, and not by other platelet agonists, in purified human platelets. Thrombin activated AMPK-α1 *ex vivo* via a Ca^{2+}/calmodulin-dependent kinase kinase β (CaMKKβ)-dependent pathway. Pharmacologic inhibition of CaMKKβ blocked thrombin-induced platelet aggregation and counteracted thrombin-induced phosphorylation of several cytoskeletal proteins, namely, regulatory myosin light chains (MLCs), cofilin, and vasodilator-stimulated phosphoprotein (VASP), three key elements involved in actin cytoskeletal contraction and polymerization. Platelets isolated from mice lacking AMPK-α1 showed reduced aggregation in response to thrombin, and this was associated with defects in MLC, cofilin and VASP phosphorylation and actin polymerization. More importantly, we show, for the first time, that the AMPK pathway is activated in platelets of patients undergoing major cardiac surgery, in a heparin-sensitive manner.
Conclusion
AMPK-α1 is activated by thrombin in human platelets. It controls the phosphorylation of key cytoskeletal targets and actin cytoskeletal remodeling during platelet aggregation.

4.1319 Targeted delivery of antisense oligonucleotides to hepatocytes using triantennary N-acetyl galactosamine improves potency 10-fold in mice
Prakash, T. et al
Nucleic Acids Res., ***42(13)***, *8796-8807 (2014)*

Triantennary *N*-acetyl galactosamine (GalNAc, **GN3**), a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR), enhances the potency of second-generation gapmer antisense oligonucleotides (ASOs) 6–10-fold in mouse liver. When combined with next-generation ASO designs comprised of short *S*-cEt (*S*-2′-*O*-Et-2′,4′-bridged nucleic acid) gapmer ASOs, ~60-fold enhancement in potency relative to the parent MOE (2′-*O*-methoxyethyl RNA) ASO was observed. **GN3**-conjugated ASOs showed high affinity for mouse ASGPR, which results in enhanced ASO delivery to hepatocytes versus non-parenchymal cells. After internalization into cells, the **GN3**-ASO conjugate is metabolized to liberate the parent ASO in the liver. No metabolism of the **GN3**-ASO conjugate was detected in plasma suggesting that **GN3** acts as a hepatocyte targeting prodrug that is detached from the ASO by metabolism after internalization into the liver. GalNAc conjugation also enhanced potency and duration of the effect of two ASOs targeting human apolipoprotein C-III and human transthyretin (TTR) in transgenic mice. The unconjugated ASOs are currently in late stage clinical trials for the treatment of familial chylomicronemia and TTR-mediated polyneuropathy. The ability to translate these observations in humans offers the potential to improve therapeutic index, reduce cost of therapy and support a monthly dosing schedule for therapeutic suppression of gene expression in the liver using ASOs.

4.1320 Limited Type I Interferons and Plasmacytoid Dendritic Cells during Neonatal Respiratory Syncytial Virus Infection Permit Immunopathogenesis upon Reinfection
Cormier, S.A., Shrestha, B., Saravia, J., Lee, G.I., Shen, L., DeVincenzo, J.P., Kim, Y-i. and You, D.
J. Virol., ***88(16)***, *9350-9360 (2014)*

Respiratory syncytial virus (RSV) infection is the number one cause of bronchiolitis in infants, yet no vaccines are available because of a lack of knowledge of the infant immune system. Using a neonatal mouse model, we previously revealed that mice initially infected with RSV as neonates develop Th2-biased immunopathophysiologies during reinfection, and we demonstrated a role for enhanced interleukin-4 receptor α (IL-4Rα) expression on T helper cells in these responses. Here we show that RSV infection in neonates induced limited type I interferon (IFN) and plasmacytoid dendritic cell (pDC) responses. IFN alpha (IFN-α) treatment or adoptive transfer of adult pDCs capable of inducing IFN-α prior to neonatal RSV infection decreased Th2-biased immunopathogenesis during reinfection. A reduced viral load and downregulation of IL-4Rα on Th2 cells were observed in IFN-α-treated neonatal mice, suggesting dual mechanisms of action.

4.1321 Intrinsic Innate Immunity Fails To Control Herpes Simplex Virus and Vesicular Stomatitis Virus Replication in Sensory Neurons and Fibroblasts
Rosato, P.C. and Leib, D.A.
J. Virol., ***88(17)***, *9991-10001 (2014)*

Herpes simplex virus 1 (HSV-1) establishes lifelong latent infections in the sensory neurons of the trigeminal ganglia (TG), wherein it retains the capacity to reactivate. The interferon (IFN)-driven antiviral response is critical for the control of HSV-1 acute replication. We therefore sought to further investigate this response in TG neurons cultured from adult mice deficient in a variety of IFN signaling components. Parallel experiments were also performed in fibroblasts isolated concurrently. We showed that HSV-1 replication was comparable in wild-type (WT) and IFN signaling-deficient neurons and fibroblasts. Unexpectedly, a similar pattern was observed for the IFN-sensitive vesicular stomatitis virus (VSV). Despite these findings, TG neurons responded to IFN-β pretreatment with STAT1 nuclear localization and restricted replication of both VSV and an HSV-1 strain deficient in γ34.5, while wild-type HSV-1 replication was unaffected. This was in contrast to fibroblasts in which all viruses were restricted by the addition of IFN-β. Taken together, these data show that adult TG neurons can mount an effective antiviral response only if provided with an exogenous source of IFN-β, and HSV-1 combats this response through γ34.5. These results further our understanding of the antiviral response of neurons and highlight the importance of paracrine IFN-β signaling in establishing an antiviral state.

4.1322 A genetic fiber modification to achieve matrix-metalloprotease-activated infectivity of oncolytic adenovirus

Jose, A., Rovira-Rigau, M., Luna, J., Gimenez-Alejandre, M., Vaquero, E., de la Torre, B.G., Andreu, D., Alemany, R. and Fillat, C.
*J. Controlled Release, **192**, 148-156 (2014)*

Selective tumor targeting of oncolytic adenovirus at the level of cell entry remains a major challenge to improve efficacy and safety. Matrix metalloproteases (MMPs) are overexpressed in a variety of tumors and in particular in pancreatic cancer. In the current work, we have exploited the expression of MMPs together with the penetration capabilities of a TAT-like peptide to engineer tumor selective adenoviruses. We have generated adenoviruses containing CAR-binding ablated fibers further modified with a C-terminus TAT-like peptide linked to a blocking domain by an MMP-cleavable sequence. This linker resulted in a MMP-dependent cell transduction of the reporter MMP-activatable virus AdTATMMP and in efficient transduction of neoplastic cells and cancer-associated fibroblasts. Intravenous and intraductal administration of AdTATMMP into mice showed very low AdTATMMP activity in the normal pancreas, whereas increased transduction was observed in pancreatic tumors of transgenic Ela-myc mice. Intraductal administration of AdTATMMP into mice bearing orthotopic tumors led to a 25-fold increase in tumor targeting compared to the wild type fiber control. A replication competent adenovirus, Ad^{RC}MMP, with the MMP-activatable fiber showed oncolytic efficacy and increased antitumor activity compared to Adwt in a pancreatic orthotopic model. Reduced local and distant metastases were observed in Ad^{RC}MMP treated-mice. Moreover, no signs of pancreatic toxicity were detected. We conclude that MMP-activatable adenovirus may be beneficial for pancreatic cancer treatment.

4.1323 Activation of AMP-activated Protein Kinase Regulates Hippocampal Neuronal pH by Recruiting Na+/H+ Exchanger NHE5 to the Cell Surface

Jinadasa, T.S., Szabo, E.Z., Numata, M. and Orlowski, J.
*J. Biol. Chem.,**289(30)**, 20879-20897 (2014)*

Strict regulation of intra- and extracellular pH is an important determinant of nervous system function as many voltage-, ligand-, and H^+-gated cationic channels are exquisitely sensitive to transient fluctuations in pH elicited by neural activity and pathophysiologic events such as hypoxia-ischemia and seizures. Multiple Na^+/H^+ exchangers (NHEs) are implicated in maintenance of neural pH homeostasis. However, aside from the ubiquitous NHE1 isoform, their relative contributions are poorly understood. NHE5 is of particular interest as it is preferentially expressed in brain relative to other tissues. In hippocampal neurons, NHE5 regulates steady-state cytoplasmic pH, but intriguingly the bulk of the transporter is stored in intracellular vesicles. Here, we show that NHE5 is a direct target for phosphorylation by the AMP-activated protein kinase (AMPK), a key sensor and regulator of cellular energy homeostasis in response to metabolic stresses. In NHE5-transfected non-neuronal cells, activation of AMPK by the AMP mimetic AICAR or by antimycin A, which blocks aerobic respiration and causes acidification, increased cell surface accumulation and activity of NHE5, and elevated intracellular pH. These effects were effectively blocked by the AMPK antagonist compound C, the NHE inhibitor HOE694, and mutation of a predicted AMPK recognition motif in the NHE5 C terminus. This regulatory pathway was also functional in primary hippocampal neurons, where AMPK activation of NHE5 protected the cells from sustained antimycin A-induced acidification. These data reveal a unique role for AMPK and NHE5 in regulating the pH homeostasis of hippocampal neurons during metabolic stress.

4.1324 Activation of Nuclear Factor Kappa B in the Hepatic Stellate Cells of Mice with Schistosomiasis Japonica

He, X., Pu, G., Tang, R., Zhang, D. and Pan, W.
*PloS One, **9(8)**, e104323 (2014)*

Schistosomiasis japonica is a serious tropical parasitic disease in humans, which causes inflammation and fibrosis of the liver. Hepatic stellate cells (HSCs) are known to play an important role in schistosome-induced fibrosis, but their role in schistosome-induced inflammation is still largely unknown. Here, we use a murine model of schistosomiasis japonica to investigate the role that nuclear factor kappa B (NF-κB), a critical mediator of inflammatory responses, plays in schistosome-induced inflammation. We revealed that NF-κB was significantly activated in HSCs at the early stage of infection, but not at later stages. We also show that the expression levels of several chemokines regulated by NF-κB signaling (Ccl2, Ccl3 and Ccl5) were similarly elevated at early infection. TLR4 signaling, one of the strongest known inducers of NF-κB

activation, seemed not activated in HSCs post-infection. Importantly, we found that levels of miR-146 (a known negative regulator of NF-κB signaling) in HSCs opposed those of NF-κB signaling, elevating at later stage of infection. These results indicate that HSCs might play an important role in the progression of hepatic schistosomiasis japonica by linking liver inflammation to fibrosis via NF-κB signaling. Moreover, our work suggests that miR-146 appeared to regulate this process. These findings are significant and imply that manipulating the function of HSCs by targeting either NF-κB signaling or miR-146 expression may provide a novel method of treating hepatic schistosomiasis japonica.

4.1325 Pretreatment of Donor Pigs With a Diet Rich in Soybean Oil Increases the Yield of Isolated Islets
Loganathan, G., Graham, M.L., Spizzo, T., Tiwari, M., Lockridge, A.D., Soltani, S., Wilhelm, J.J., Balamurugan, A.N. and Hering, B.J.
Transplant. Proceedings, ***46***, *1945-1949 (2014)*

Introduction
The pig is considered the donor species of choice for islet xenotransplantation. However, isolation of porcine islets is difficult, particularly from young pigs. Early life exposure to a high-fat diet (HFD) reportedly encourages islet β-cell expansion in neonatal rodents and improves islet viability in culture from pretreated weanling pigs. In this study, we examined the influence of young donor pretreatment with a soybean oil–enriched HFD on porcine islet mass and yield after islet isolation.
Materials and Methods
Postweaning and between days 70 and 250, pigs were fed either a standard diet (control group; n = 5) or an HFD (experimental group; n = 6). Biochemical blood parameters and acute C-peptide response to intravenous glucose were monitored before pancreas procurement. The study was blinded to objectively evaluate the influence of treated diet. After procurement, pancreas biopsy samples were taken from control and pretreated donor pigs to assess islet number by using a dithizone scoring method and histologic islet area fraction determination. Control and HFD donor pig islets were isolated by using our standard isolation protocol to determine islet yield. Islet isolation characteristics and islet quality were assessed in both groups, and the results were compared.
Results
There were no significant differences in the donor characteristics (age, body weight, glucose disposal rate, acute C-peptide response to intravenous glucose, cholesterol, and aspartate aminotransferase) except fasting blood glucose level between the control and treatment groups (84 ± 6 vs 99 ± 12 mg/dL; $P = .0317$). The stimulated insulin and C-peptide levels between groups were similar. However, the dithizone score was slightly higher in the treatment group compared with the control group (95.4 ± 38.5 vs 62.6 ± 23.9; $P = .1208$). Digestion time, digested pancreas weight, pellet volume, and the fragility index were similar in both groups. However, the average islet count (islet equivalent number/g pancreas) at the digest level was significantly higher in the HFD group than in the control group (1578 ± 994 vs 738 ± 202; $P = .0344$). The functional viability of 2- and 7 day-cultured islets, as assessed by using oxygen consumption rate corrected for DNA, was similar in both groups.
Conclusions
Pretreatment of pigs with HFD enriched with soybean oil could potentially be used to improve the islet mass in donor pigs. Further studies are needed to confirm and optimize the use of HFD for the purpose of increasing islet yield from young donor pigs.

4.1326 TGF-β-dependent induction of CD4+CD25+Foxp3+ Tregs by liver sinusoidal endothelial cells
Carambia, A., Freund, B., Schwinge, D., Heine, M., Laschtowitz, A., Huber, S., Wraith, D.C., Korn, T., Schramm, C., Lohse, A.W., Heeren, J. and Herkel, J.
J. Hepatol., ***61***, *594-599 (2014)*

Background & Aims
$CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells (Tregs) have a profound ability to control immune responses. We have previously shown that the liver is a major source of peripherally induced Tregs. Here, we investigate the liver cell types and molecular mechanisms responsible for hepatic Treg induction.
Methods
To assess the Treg-inducing potential of liver resident antigen-presenting cell types, we studied the conversion of $Foxp3^-$ non-Tregs into $Foxp3^+$ Tregs induced by liver dendritic cells (DCs), liver sinusoidal endothelial cells (LSECs), or Kupffer cells (KCs). The dependency of Treg induction on TGF-β was tested

in Treg conversion assays using T cells with reduced TGF-β sensitivity. The suppressive potential of liver cell-induced Tregs was assessed by an *in vitro* suppression assay and *in vivo*, in the model of experimental autoimmune encephalomyelitis (EAE).
Results
All tested liver cell types were capable of inducing $Foxp3^+$ Tregs; however, LSECs were most efficient in inducing Tregs. Treg-induction was antigen-specific and depended on TGF-β. LSECs featured membrane-bound LAP/TGF-β and the anchor molecule GARP, which is required for tethering LAP/TGF-β to the cell membrane. LSEC-induced Tregs suppressed proliferation and cytokine secretion of effector T cells *in vitro*. LSEC-induced Tregs were also functional suppressors *in vivo*, as neuroantigen-specific Tregs induced by LSECs were able to suppress EAE.
Conclusions
We demonstrate that LSECs are the major liver cell type responsible for TGF-β dependent hepatic Treg induction. The extraordinary capacity of LSECs to induce Tregs was associated with their unique ability to tether TGF-β to their membrane.

4.1327 Evaluation of the Contribution of Multiple DAMPs and DAMP Receptors in Cell Death-Induced Sterile Inflammatory Responses
Kataoka, H., Kono, H., patel, Z. and Rock, K.L.
*PloS One, **9(8)**, e104741 (2014)*

When cells die by necrosis *in vivo* they stimulate an inflammatory response. It is thought that this response is triggered when the injured cells expose proinflammatory molecules, collectively referred to as damage associated molecular patterns (DAMPs), which are recognized by cells or soluble molecules of the innate or adaptive immune system. Several putative DAMPs and/or their receptors have been identified, but whether and how much they participate in responses *in vivo* is incompletely understood, and they have not previously been compared side-by-side in the same models. This study focuses on evaluating the contribution of multiple mechanisms that have been proposed to or potentially could participate in cell death-induced inflammation: The third component of complement (C3), ATP (and its receptor P2X7), antibodies, the C-type lectin receptor Mincle (Clec4e), and protease-activated receptor 2 (PAR2). We investigate the role of these factors in cell death-induced inflammation to dead cells in the peritoneum and acetaminophen-induced liver damage. We find that mice deficient in antibody, C3 or PAR2 have impaired inflammatory responses to dying cells. In contrast there was no reduction in inflammation to cell death in the peritoneum or liver of mice that genetically lack Mincle, the P2X7 receptor or that were treated with apyrase to deplete ATP. These results indicate that antibody, complement and PAR2 contribute to cell death-induced inflammation but that Mincle and ATP- P2X7 receptor are not required for this response in at least 2 different *in vivo* models.

4.1328 131. Centrifugation of Semen: Cushion Technique
Bradecamp, E.A.
Equine Reproductive Procedures, 429-432 (2014)

Centrifugation of semen can be performed with or without the aid of a cushion medium. The purpose of the cushion is to allow the semen to be centrifuged at a higher g force to maximize the percentage of sperm harvested post-centrifugation without having a detrimental effect on the viability of the sperm. This chapter tabulates iodixanol-based cushions for the centrifugation of equine semen. It summarizes step by step procedure for centrifugation of semen, and the equipment and supplies needed.

4.1329 Reduction of ARNT in myeloid cells causes immune suppression and delayed wound healing
Scott, C., Bonner, J., Min, D., Boughton, P., Stokes, R., Cha, K.M., Walters, S.N., Maskowski, K., Sierro, F., Grey, S.T., Twigg, S., McLennan, S. and Gunton, J.E.
*Am. J. Physiol. Cell Physiol., **307**, C349-C357 (2014)*

Aryl hydrocarbon receptor nuclear translocator (ARNT) is a transcription factor that binds to partners to mediate responses to environmental signals. To investigate its role in the innate immune system, floxed ARNT mice were bred with lysozyme M-Cre recombinase animals to generate lysozyme M-ARNT (LAR) mice with reduced ARNT expression. Myeloid cells of LAR mice had altered mRNA expression and delayed wound healing. Interestingly, when the animals were rendered diabetic, the difference in wound healing between the LAR mice and their littermate controls was no longer present, suggesting that

decreased myeloid cell ARNT function may be an important factor in impaired wound healing in diabetes. Deferoxamine (DFO) improves wound healing by increasing hypoxia-inducible factors, which require ARNT for function. DFO was not effective in wounds of LAR mice, again suggesting that myeloid cells are important for normal wound healing and for the full benefit of DFO. These findings suggest that myeloid ARNT is important for immune function and wound healing. Increasing ARNT and, more specifically, myeloid ARNT may be a therapeutic strategy to improve wound healing

4.1330 Caloric restriction confers persistent anti-oxidative, pro-angiogenic, and anti-inflammatory effects and promotes anti-aging miRNA expression profile in cerebromicrovascular endothelial cells of aged rats

Csiszar, A., Gautam, T., Sosnowska, D., Tarantini, S., Banki, E., Tucsek, Z., Toth, P., Losonczy, G., Koller, A., Reglodi, D., Giles, C.B., Wren, J.D., Sonntag, W.E. and Ungvari, Z.
*Am. J. Physiol. Heart Circ. Physiol., **307**, H292-H306 (2014)*

In rodents, moderate caloric restriction (CR) without malnutrition exerts significant cerebrovascular protective effects, improving cortical microvascular density and endothelium-dependent vasodilation, but the underlying cellular mechanisms remain elusive. To elucidate the persisting effects of CR on cerebromicrovascular endothelial cells (CMVECs), primary CMVECs were isolated from young (3 mo old) and aged (24 mo old) ad libitum-fed and aged CR F344xBN rats. We found an age-related increase in cellular and mitochondrial oxidative stress, which is prevented by CR. Expression and transcriptional activity of Nrf2 are both significantly reduced in aged CMVECs, whereas CR prevents age-related Nrf2 dysfunction. Expression of miR-144 was upregulated in aged CMVECs, and overexpression of miR-144 significantly decreased expression of Nrf2 in cells derived from both young animals and aged CR rats. Overexpression of a miR-144 antagomir in aged CMVECs significantly decreases expression of miR-144 and upregulates Nrf2. We found that CR prevents age-related impairment of angiogenic processes, including cell proliferation, adhesion to collagen, and formation of capillary-like structures and inhibits apoptosis in CMVECs. CR also exerts significant anti-inflammatory effects, preventing age-related increases in the transcriptional activity of NF-κB and age-associated pro-inflammatory shift in the endothelial secretome. Characterization of CR-induced changes in miRNA expression suggests that they likely affect several critical functions in endothelial cell homeostasis. The predicted regulatory effects of CR-related differentially expressed miRNAs in aged CMVECs are consistent with the anti-aging endothelial effects of CR observed in vivo. Collectively, we find that CR confers persisting anti-oxidative, pro-angiogenic, and anti-inflammatory cellular effects, preserving a youthful phenotype in rat cerebromicrovascular endothelial cells, suggesting that through these effects CR may improve cerebrovascular function and prevent vascular cognitive impairment.

4.1331 Soluble Adenylyl Cyclase Is Necessary and Sufficient to Overcome the Block of Axonal Growth by Myelin-Associated Factors

Martinez, J., Stressin, A., Campana, A., Hou, J., Nikulina, E., Buck, J., Levin, L.R. and Filbin, M.T.
*J. Neurosci., **34(28)**, 9281-9289 (2014)*

Neurons in the CNS do not regenerate following injury; regeneration is blocked by inhibitory proteins in myelin, such as myelin-associated glycoprotein (MAG). Elevating neuronal levels of the second messenger cAMP overcomes this blocked axonal outgrowth. One way to elevate cAMP is pretreating neurons with neurotrophins, such as brain-derived neurotrophic factor (BDNF). However, pleiotropic effects and poor bioavailability make exogenous administration of neurotrophins *in vivo* problematic; therefore, alternative targets must be considered. In neurons, two families of adenylyl cyclases synthesize cAMP, transmembrane adenylyl cyclases (tmACs), and soluble adenylyl cyclase (sAC). Here, we demonstrate that sAC is the essential source of cAMP for BDNF to overcome MAG-dependent inhibition of neurite outgrowth. Elevating sAC in rat and mouse neurons is sufficient to induce neurite outgrowth on myelin *in vitro* and promotes regeneration *in vivo*. These results suggest that stimulators of sAC might represent a novel therapeutic strategy to promote axonal growth and regeneration.

4.1332 Electrical stimulation of transplanted motoneurons improves motor unit formation

Liu, Y., Grumbles, R.M. and Thomas, C.K.
*J. Neurophysiol., **112**, 660-670 (2014)*

Motoneurons die following spinal cord trauma and with neurological disease. Intact axons reinnervate nearby muscle fibers to compensate for the death of motoneurons, but when an entire motoneuron pool dies, there is complete denervation. To reduce denervation atrophy, we have reinnervated muscles in

Fisher rats from local transplants of embryonic motoneurons in peripheral nerve. Since growth of axons from embryonic neurons is activity dependent, our aim was to test whether brief electrical stimulation of the neurons immediately after transplantation altered motor unit numbers and muscle properties 10 wk later. All surgical procedures and recordings were done in anesthetized animals. The muscle consequences of motoneuron death were mimicked by unilateral sciatic nerve section. One week later, 200,000 embryonic *day 14* and *15* ventral spinal cord cells, purified for motoneurons, were injected into the tibial nerve 10–15 mm from the gastrocnemii muscles as the only neuron source for muscle reinnervation. The cells were stimulated immediately after transplantation for up to 1 h using protocols designed to examine differential effects due to pulse number, stimulation frequency, pattern, and duration. Electrical stimulation that included short rests and lasted for 1 h resulted in higher motor unit counts. Muscles with higher motor unit counts had more reinnervated fibers and were stronger. Denervated muscles had to be stimulated directly to evoke contractions. These results show that brief electrical stimulation of embryonic neurons, in vivo, has long-term effects on motor unit formation and muscle force. This muscle reinnervation provides the opportunity to use patterned electrical stimulation to produce functional movements.

4.1333 TLR2 and TLR4 mediate the TNFα response to Vibrio vulnificus biotype 1
Stamm, L.V. and Drapp, R.L.
*Pathogens and Disease, **71(3)**, 357-361 (2014)*

Vibrio vulnificus (*Vv*) is a pathogenic bacterium that can cause life-threatening infections in humans. Most fatal cases are due to septic shock that results from dysregulation of cytokines, particularly TNFα, which plays a critical role in the outcome of *Vv* infection. The goal of this study was to investigate the Toll-like receptor (TLR)-mediated TNFα response to four *Vv* biotype 1 strains using mice deficient for TLR2, TLR4, and TLR2/TLR4. *Ex vivo* assays were performed with blood, splenocytes, and Kupffer cells (KC) from wild-type (WT) and TLR-knockout (KO) mice using formalin-inactivated *Vv* (f-*Vv*) as stimulant. All f-*Vv* biotype 1 strains elicited strong TNFα production by WT mouse blood and cells, which was TLR2 and TLR4 dependent. OxPAPC, an inhibitor of TLR2 and TLR4 signaling, effectively blunted the TLR-mediated TNFα response to f-*Vv*. Furthermore, TLR2 KO and TLR2/TLR4 KO mice were more resistant to lethal infection with *Vv* ATCC 27562 than WT mice, perhaps due to attenuation of the TNFα response. These data suggest that it may be possible to devise strategies to specifically target the harmful TLR-mediated TNFα response as an adjunct to antibiotic treatment of severe *Vv* infection.

4.1334 Identification of an Atg8-Atg3 Protein–Protein Interaction Inhibitor from the Medicines for Malaria Venture Malaria Box Active in Blood and Liver Stage Plasmodium falciparum Parasites
Hain, A.U.P., bartee, D., Sanders, N.G., Miller, A.S., Sullivan, D.J., Levitskaya, J., Meyers, C.F. and Bosch, J.
*J. Med. Chem., **57(11)**, 4521-4531 (2014)*

Atg8 is a ubiquitin-like autophagy protein in eukaryotes that is covalently attached (lipidated) to the elongating autophagosomal membrane. Autophagy is increasingly appreciated as a target in diverse diseases from cancer to eukaryotic parasitic infections. Some of the autophagy machinery is conserved in the malaria parasite, *Plasmodium*. Although Atg8's function in the parasite is not well understood, it is essential for *Plasmodium* growth and survival and partially localizes to the apicoplast, an indispensable organelle in apicomplexans. Here, we describe the identification of inhibitors from the Malaria Medicine Venture Malaria Box against the interaction of *Pf*Atg8 with its E2-conjugating enzyme, *Pf*Atg3, by surface plasmon resonance. Inhibition of this protein–protein interaction prevents *Pf*Atg8 lipidation with phosphatidylethanolamine. These small molecule inhibitors share a common scaffold and have activity against both blood and liver stages of infection by *Plasmodium falciparum*. We have derivatized this scaffold into a functional platform for further optimization.

4.1335 Nrf2 Activation in Astrocytes Contributes to Spinal Cord Ischemic Tolerance Induced by Hyperbaric Oxygen Preconditioning No Access
Xu, J., Huang, G., Zhang, K., Sun, J., Xu, T., Li, R., Tao, H. and Xu, W.
*J. Neurotrauma, **31(15)**, 1343-1353 (2014)*

In this study, we investigated whether nuclear factor erythroid 2-related factor 2 (Nrf2) activation in astrocytes contributes to the neuroprotection induced by a single hyperbaric oxygen preconditioning (HBO-PC) against spinal cord ischemia/reperfusion (SCIR) injury. *In vivo*: At 24 h after a single HBO-PC at 2.5 atmospheres absolute for 90 min, the male ICR mice underwent SCIR injury by aortic cross-clamping surgery and observed for 48 h. HBO-PC significantly improved hindlimb motor function,

reduced secondary spinal cord edema, ameliorated the reactivity of spinal motor-evoked potentials, and slowed down the process of apoptosis to exert neuroprotective effects against SCIR injury. At 12 h or 24 h after HBO-PC without aortic cross-clamping surgery, Western blot, enzyme-linked immunosorbent assay, realtime-polymerase chain reaction and double-immunofluorescence staining were used to detect the Nrf2 activity of spinal cord tissue, such as mRNA level, protein content, DNA binding activity, and the expression of downstream gene, such as glutamate-cysteine ligase, γ-glutamyltransferase, multidrug resistance protein 1, which are key proteins for intracellular glutathione synthesis and transit. The Nrf2 activity and downstream genes expression were all enhanced in normal spinal cord with HBO-PC. Glutathione content of spinal cord tissue with HBO-PC significantly increased at all time points after SCIR injury. Moreover, Nrf2 overexpression mainly occurs in astrocytes. *In vitro:* At 24 h after HBO-PC, the primary spinal astrocyte-neuron co-cultures from ICR mouse pups were subjected to oxygen-glucose deprivation (OGD) for 90 min to simulate the ischemia-reperfusion injury. HBO-PC significantly increased the survival rate of neurons and the glutathione content in culture medium, which was mainly released from asctrocytes. Moreover, the Nrf2 activity and downstream genes expression induced by HBO-PC were mainly enhanced in astrocytes, but not in neurons. In conclusion, our findings demonstrated that spinal cord ischemic tolerance induced by HBO-PC may be mainly related to Nrf2 activation in astrocytes.

4.1336 Subchronic olanzapine treatment decreases the expression of pancreatic glucose transporter 2 in rat pancreatic β cells

Shu, S., Liu, H., Wang, M., Su, D., Yao, L. and Wang, G.
J. Endocrinol. Invest., ***37***, *667-673 (2014)*

Background
Olanzapine is a second generation antipsychotic. A common side effect in humans is weight gain, but the mechanisms are mostly unknown.
Aim
To study the effects of subchronic olanzapine treatment on body weight, fasting plasma glucose (FPG), fasting insulin (FINS), C-peptide, insulin sensitivity index (ISI), and expression of glucose transporter 2 (GLUT2) in rat pancreatic β cells.
Materials and methods
Female Sprague-Dawley rats were randomly divided into two groups: the olanzapine-treated group and the control group (each $n = 8$). Rats in the olanzapine-treated group intragastrically received olanzapine 5 mg/kg/day for 28 days; the rats in the control group received the same volume of vehicle. FPG and body weight were measured on the 1st, 7th, 14th and 28th day. FINS and C-peptide were measured using immunoradiometric assays at baseline and on the 28th day. GLUT2 mRNA and protein expressions in pancreatic β cells were analyzed by RT-PCR and western blot.
Results
Olanzapine-treated rats had higher body weight (227.4 ± 8.9 vs. 211.0 ± 9.9 g), FPG (5.86 ± 0.42 vs. 4.24 ± 0.29 mmol/L), FINS (17.34 ± 3.64 vs. 10.20 ± 1.50 μIU/mL), and C-peptide (0.154 ± 0.027 vs. 0.096 ± 0.009 ng/mL) than those in controls (all $P < 0.05$) at the 28th day. Pancreatic β cells of the olanzapine-treated group showed lower ISI (−4.60 ± 0.23 vs. −3.76 ± 0.20) and GLUT2 levels (mRNA: 1.12 ± 0.02 vs. 2.00 ± 0.03; protein: 0.884 ± 0.134 vs. 1.118 ± 0.221) than those in controls (all $P < 0.05$).
Conclusions
Subchronic olanzapine treatment inhibited expression of GLUT2 in rat pancreatic β cells. Therefore, it may disturb glucose metabolism via the insulin resistance of β cells, but confirmation in humans is needed.

4.1337 Blood-Derived Mesenchymal Stem Cells Heal Calvarial Defects and Promote Wound Healing

Hu, M., Huang, K-J., Li, S., Wu, J-C., Lo, D.D., Hyun, J.S., Chung, M.T., Hu, M., Longaker, M. and Lorenz, H.P.
J. American College of Surgeons, ***219(S3)***, *S85-S86 (2014)*

Introduction
Mesenchymal stem cells (MSCs) are promising for their potential in cell-based regenerative therapeutics. However, typically obtained from bone marrow, MSC harvesting techniques are invasive and costly. Here we demonstrate the capacity of peripheral blood-derived MSCs (BD-MSCs) for cell-based calvarial defect and wound healing applications.
Methods
BD-MSCs were obtained from the peripheral blood of 8-10 week-old CD1 wild type or FVB-Tg(CAG-luc,-GFP)L2G85Chco/J mice, which express firefly luciferase and cytoplasmic eGFP constitutively in all cells, by a novel method utilizing OptiPrep density gradient isolation and hepatocyte stimulation. Cells

were characterized by fluorescence-activated cell sorting (FACS). For calvarial defect healing, cells (5.0x105) were seeded onto hydroxyapatite-poly(lactic-co-glycolic acid) (HA-PLGA) scaffolds and placed onto critical-sized (4 mm) calvarial defects on CD1 athymic nude mice. For wound healing, BD-MSCs were seeded onto pullulan-collagen composite dermal hydrogels, and transplanted (2.5×105 cells per wound) onto 6 mm splinted full thickness excisional wounds on the dorsum of FVB/NJ mice.
Results
Quantification of calvarial defect healing demonstrated increased bone formation in defects treated with HA-PLGA scaffold seeded with BD-MSCs vs HA-PLGA scaffold alone (*p<0.01). BD-MSC-seeded hydrogels demonstrated improved wound healing as compared to un-seeded hydrogel controls on days 8-12 (*p<0.05). The average time for complete wound healing was 12.7 days in the BD-MSC group vs 14 days in the control group (*p<0.05).
Conclusions
We demonstrate the ability of peripheral blood-derived MSCs to heal calvarial defects and accelerate wound healing in vivo. This novel, less invasive, approach to obtaining MSCs may provide a promising alternative to available techniques for cell-based regenerative applications.

4.1338 Anti-Inflammatory Activity of Bone Morphogenetic Protein Signaling Pathways in Stomachs of Mice

Takabayashi, H., Shinohara, M., Mao, M., Phaosawasdi, P., El-Zaatari, M., Zhang, M., Ji, T., Eaton, K.A., Dang, D., Kao, J. and Todisco, A.
*Gastroenterology, **147**, 396-406 (2014)*

Background & Aims
Bone morphogenetic protein (BMP)4 is a mesenchymal peptide that regulates cells of the gastric epithelium. We investigated whether BMP signaling pathways affect gastric inflammation after bacterial infection of mice.
Methods
We studied transgenic mice that express either the BMP inhibitor noggin or the β- galactosidase gene under the control of a BMP-responsive element and BMP4$^{\beta gal/+}$ mice. Gastric inflammation was induced by infection of mice with either *Helicobacter pylori* or *Helicobacter felis*. Eight to 12 weeks after inoculation, gastric tissue samples were collected and immunohistochemical, quantitative, reverse-transcription polymerase chain reaction and immunoblot analyses were performed. We used enzyme-linked immunosorbent assays to measure cytokine levels in supernatants from cultures of mouse splenocytes and dendritic cells, as well as from human gastric epithelial cells (AGS cell line). We also measured the effects of BMP-2, BMP-4, BMP-7, and the BMP inhibitor LDN-193189 on the expression of interleukin (IL)8 messenger RNA by AGS cells and primary cultures of canine parietal and mucus cells. The effect of BMP-4 on NFkB activation in parietal and AGS cells was examined by immunoblot and luciferase assays.
Results
Transgenic expression of noggin in mice increased *H pylori*–or *H felis*–induced inflammation and epithelial cell proliferation, accelerated the development of dysplasia, and increased expression of the signal transducer and activator of transcription 3 and activation-induced cytidine deaminase. BMP-4 was expressed in mesenchymal cells that expressed α-smooth muscle actin and activated BMP signaling pathways in the gastric epithelium. Neither BMP-4 expression nor BMP signaling were detected in immune cells of C57BL/6, BRE–β-galactosidase, or BMP-4$^{\beta gal/+}$ mice. Incubation of dendritic cells or splenocytes with BMP-4 did not affect lipopolysaccharide-stimulated production of cytokines. BMP-4, BMP-2, and BMP-7 inhibited basal and tumor necrosis factor α–stimulated expression of IL8 in canine gastric epithelial cells. LDN-193189 prevented BMP4-mediated inhibition of basal and tumor necrosis factor α–stimulated expression of IL8 in AGS cells. BMP-4 had no effect on TNFα-stimulated phosphorylation and degradation of IκBα, or on TNFα induction of a NFκβ reporter gene.
Conclusions
BMP signaling reduces inflammation and inhibits dysplastic changes in the gastric mucosa after infection of mice with *H pylori* or *H felis*.

4.1339 Interleukin 17–Producing γδT Cells Promote Hepatic Regeneration in Mice

Rao, R., Graffeo, C.S., Gulati, R., Jamal, M., Narayan, S., Zambirinis, C.R., Barilla, R., Deutsch, M., Greco, S.H., Ochi, A., Tomkötter, L., Blobstein, R., Avanzi, A., Tippens, D.M., Gelstein, Y., Van Heerden,

E. and Miller, G.
*Gastroenterology, **147**, 473-484 (2014)*

Background & Aims

Subsets of leukocytes synergize with regenerative growth factors to promote hepatic regeneration. γδT cells are early responders to inflammation-induced injury in a number of contexts. We investigated the role of γδT cells in hepatic regeneration using mice with disruptions in *Tcrd* (encodes the T-cell receptor δ chain) and *Clec7a* (encodes C-type lectin domain family 7 member a, also known as *DECTIN1*).
Methods

We performed partial hepatectomies on wild-type C57BL/6, CD45.1, *Tcrd*$^{-/-}$, or *Clec7a*$^{-/-}$ mice. Cells were isolated from livers of patients and mice via mechanical and enzymatic digestion. γδT cells were purified by fluorescence-activated cell sorting.
Results

In mice, partial hepatectomy up-regulated expression of CCL20 and ligands of Dectin-1, which was associated with recruitment and activation of γδT cells and their increased production of interleukin (IL)-17 family cytokines. Recruited γδT cells induced production of IL-6 by antigen-presenting cells and suppressed expression of interferon gamma by natural killer T cells, promoting hepatocyte proliferation. Absence of IL-17–producing γδT cells or deletion of Dectin-1 prevented development of regenerative phenotypes in subsets of innate immune cells. This slowed liver regeneration and was associated with reduced expression of regenerative growth factors and cell cycle regulators. Conversely, exogenous administration of IL-17 family cytokines or Dectin-1 ligands promoted regeneration. More broadly, we found that γδT cells are required for inflammatory responses mediated by IL-17 and Dectin-1.
Conclusions

γδT cells regulate hepatic regeneration by producing IL-22 and IL-17, which have direct mitogenic effects on hepatocytes and promote a regenerative phenotype in hepatic leukocytes, respectively. Dectin-1 ligation is required for γδT cells to promote hepatic regeneration.

4.1340 **Small-molecule screening identifies inhibition of salt-inducible kinases as a therapeutic strategy to enhance immunoregulatory functions of dendritic cells**
Sundberg, T.B. et al
*PNAS, **111(34)**, 12468-12473 (2014)*

Genetic alterations that reduce the function of the immunoregulatory cytokine IL-10 contribute to colitis in mouse and man. Myeloid cells such as macrophages (MΦs) and dendritic cells (DCs) play an essential role in determining the relative abundance of IL-10 versus inflammatory cytokines in the gut. As such, using small molecules to boost IL-10 production by DCs–MΦs represents a promising approach to increase levels of this cytokine specifically in gut tissues. Toward this end, we screened a library of well-annotated kinase inhibitors for compounds that enhance production of IL-10 by murine bone-marrow–derived DCs stimulated with the yeast cell wall preparation zymosan. This approach identified a number of kinase inhibitors that robustly up-regulate IL-10 production including the Food and Drug Administration (FDA)-approved drugs dasatinib, bosutinib, and saracatinib that target ABL, SRC-family, and numerous other kinases. Correlating the kinase selectivity profiles of the active compounds with their effect on IL-10 production suggests that inhibition of salt-inducible kinases (SIKs) mediates the observed IL-10 increase. This was confirmed using the SIK-targeting inhibitor HG-9-91-01 and a series of structural analogs. The stimulatory effect of SIK inhibition on IL-10 is also associated with decreased production of the proinflammatory cytokines IL-1β, IL-6, IL-12, and TNF-α, and these coordinated effects are observed in human DCs–MΦs and anti-inflammatory $CD11c^{+}$ CX_3CR1^{hi} cells isolated from murine gut tissue. Collectively, these studies demonstrate that SIK inhibition promotes an anti-inflammatory phenotype in activated myeloid cells marked by robust IL-10 production and establish these effects as a previously unidentified activity associated with several FDA-approved multikinase inhibitors.

4.1341 **Long-Acting Atypical Antipsychotics: Characterization of the Local Tissue Response**
Paquette, S.M., Dawit, H., Hickey, M.B., Merisko-Liversidge, E., Almarsson, Ö and Deaver, D.R.
*Pharm. Res., **31**, 2065-2077 (2014)*

Purpose
Long-acting injectables (LAIs) are increasingly recognized as an effective therapeutic approach for treating chronic conditions. Many LAIs are formulated to create a poorly soluble depot from which the active agent is delivered over time. This long residing depot can cause localized chronic-active inflammation in the tissue, which has not been well defined in the literature. The purpose of this work is to establish an experimental baseline for describing these responses.
Methods
Non-human primates and rodents were used to examine the response to LAI formulations of two clinically relevant atypical antipsychotics, aripiprazole monohydrate and olanzapine pamoate monohydrate.
Results
A foreign body response develops with elevations of key cytokines such as IL-1α, IL-1β, TNFα, and IL6 at the site of injection. However, the tissue response for the two atypical antipsychotics compounds diverge as evidenced by quantitative differences observed in cytokine levels at various time points after dosing.
Conclusions
Our studies show that, while the drugs are in the same therapeutic class, the response to each of these compounds can be distinguished qualitatively and quantitatively, supporting the idea that the injection site reaction involves a multiplicity of factors including the properties of the compound and cellular dynamics at the site of injection.

4.1342 Distinct Dictation of Japanese Encephalitis Virus-Induced Neuroinflammation and Lethality via Triggering TLR3 and TLR4 Signal Pathways
Han, Y.W., Choi, J.Y., Uyangaa, E., Kim, S.B., Kim, J.H., Kim, B.S., Kim, K. and Eo, S.K.
PloS Pathogens, ***10(9)***, *e1004319 (2014)*

Japanese encephalitis (JE) is major emerging neurologic disease caused by JE virus. To date, the impact of TLR molecules on JE progression has not been addressed. Here, we determined whether each TLR modulates JE, using several TLR-deficient mouse strains (TLR2, TLR3, TLR4, TLR7, TLR9). Surprisingly, among the tested TLR-deficient mice there were contrasting results in $TLR3^{-/-}$ and $TLR4^{-/-}$ mice, *i.e*. $TLR3^{-/-}$ mice were highly susceptible to JE, whereas $TLR4^{-/-}$ mice showed enhanced resistance to JE. TLR3 ablation induced severe CNS inflammation characterized by early infiltration of inflammatory $CD11b^{+}Ly\text{-}6C^{high}$ monocytes along with profoundly increased viral burden, proinflammatory cytokine/chemokine expression as well as BBB permeability. In contrast, $TLR4^{-/-}$ mice showed mild CNS inflammation manifested by reduced viral burden, leukocyte infiltration and proinflammatory cytokine expression. Interestingly, TLR4 ablation provided potent *in vivo* systemic type I IFN innate response, as well as *ex vivo* type I IFN production associated with strong induction of antiviral PRRs (RIG-I, MDA5), transcription factors (IRF-3, IRF-7), and IFN-dependent (PKR, Oas1, Mx) and independent ISGs (ISG49, ISG54, ISG56) by alternative activation of IRF3 and NF-κB in myeloid-derived DCs and macrophages, as compared to $TLR3^{-/-}$ myeloid-derived cells which were more permissive to viral replication through impaired type I IFN innate response. TLR4 ablation also appeared to mount an enhanced type I IFN innate and humoral, $CD4^{+}$ and $CD8^{+}$ T cell responses, which were mediated by altered immune cell populations (increased number of plasmacytoid DCs and NK cells, reduced $CD11b^{+}Ly\text{-}6C^{high}$ monocytes) and $CD4^{+}Foxp3^{+}$ Treg number in lymphoid tissue. Thus, potent type I IFN innate and adaptive immune responses in the absence of TLR4 were closely coupled with reduced JE lethality. Collectively, these results suggest that a balanced triggering of TLR signal array by viral components during JE progression could be responsible for determining disease outcome through regulating negative and positive factors.

4.1343 Identification and genetic analysis of cancer cells with PCR-activated cell sorting
Eastburn, D.J., Sciambi, A. and Abate, A.R.
Nucleic Acids Res., ***42(16)***, *e128 (2014)*

Cell sorting is a central tool in life science research for analyzing cellular heterogeneity or enriching rare cells out of large populations. Although methods like FACS and FISH-FC can characterize and isolate cells from heterogeneous populations, they are limited by their reliance on antibodies, or the requirement to chemically fix cells. We introduce a new cell sorting technology that robustly sorts based on sequence-specific analysis of cellular nucleic acids. Our approach, PCR-activated cell sorting (PACS), uses TaqMan PCR to detect nucleic acids within single cells and trigger their sorting. With this method, we identified and sorted prostate cancer cells from a heterogeneous population by performing >132 000 simultaneous single-cell TaqMan RT-PCR reactions targeting vimentin mRNA. Following vimentin-positive droplet sorting and downstream analysis of recovered nucleic acids, we found that cancer-specific genomes and transcripts were significantly enriched. Additionally, we demonstrate that PACS can be used to sort and

enrich cells via TaqMan PCR reactions targeting single-copy genomic DNA. PACS provides a general new technical capability that expands the application space of cell sorting by enabling sorting based on cellular information not amenable to existing approaches.

4.1344 In vitro antiparasitic activity of new thiosemicarbazones in strains of Trypanosoma cruzi
Moreno-Rodriguez, A., Salazar-Schettino, P.M., Bautista, J.K., hernandez-Luis, F., Torrens, H., Guevara-Gomez, Y., Pina-canseco, S., Torres, M.B., Cabrera-bravo, M., Martinez, C.M. and Perez-campos, E.
Eur. J. Medicinal Chem., ***87****, 23-29 (2014)*

In this study thiosemicarbazones derivatives of 5-[(trifluoromethyl)phenylthio]-2-furaldehyde were synthesized and evaluated in terms of their efficiency in challenging the growth of epimastigote forms of *Trypanosoma cruzi*, the etiological agent of Chagas' disease. A number of compounds were synthesized from 5-bromo-2-furfuraldehyde using nucleophilic aromatic substitution, with a series of trifluoromethyl thiolates, followed by condensation reactions with thiosemicarbazide. Their molecular structures were determined by ^{1}H, ^{13}C and ^{19}F NMR, MS and IR spectroscopy. When tested with *T.cruzi*, they showed a stronger reaction, similar to nifurtimox and benznidazole, with the 5-[nitro-4-(trifluoromethyl)phenyltio]-2-furaldehyde thiosemicarbazone (compound **4**) showing the highest antiparasitic activity. This improved activity may be explained due to the nitro group present in the molecule, which potentiates its activity. The thiosemicarbazone derivatives in this study showed no apoptosis in platelets or monocytes, nor did they induce platelet activation. The trypanocidal activity of these substances represents a good starting point for a medicinal chemistry program aimed at therapy for Chagas' disease.

4.1345 Endothelin-1 induced desensitization in primary afferent neurons
Smith, T.P., Smith, S.N. and Sweitzer, S.M.
Neuroscience Letters, ***582****, 59-64 (2014)*

Endothelin-1 (ET-1) is a known algogen that causes acute pain and sensitization in humans and spontaneous nociceptive behaviors when injected into the periphery in rats, and is elevated during vaso-occlusive episodes (VOEs) in sickle cell disease (SCD) patients. Previously, our lab has shown that a priming dose of ET-1 produces sensitization to capsaicin-induce secondary hyperalgesia. The goal of this study was to determine if the sensitization induced by ET-1 priming is occurring at the level of the primary afferent neuron. Calcium imaging in cultured dorsal root ganglion (DRG) neurons was utilized to examine the effects of ET-1 on primary afferent neurons. ET-1 induces $[Ca^{2+}]_i$ transients in unprimed cells. ET-1 induced $[Ca^{2+}]_i$ transients are attenuated by priming with ET-1. This priming effect occurs whether the priming dose is given 0–4 days prior to the challenge dose. Similarly, ET-1 priming decreases capsaicin-induced $[Ca^{2+}]_i$ transients. At the level of the primary afferent neuron, ET-1 priming has a desensitizing effect on challenge exposures to ET-1 and capsaicin.

4.1346 Identification of the clpB and bipA genes and an evaluation of their expression as related to intracellular survival for the bacterial pathogen Piscirickettsia salmonis
Isla, A., Haussmann, D., Vera, T., Kausel, G. and Figueroa, J.
Vet. Microbiol., ***173****, 390-394 (2014)*

Piscirickettsia salmonis is the pathogen responsible for salmonid rickettsial septicemia (SRS), a disease that affects a wide variety of marine cultivated fish species and causes economic losses for the aquaculture industry worldwide. Many *in vitro* studies have reported on the capacity of this microorganism to replicate in the interior of cytoplasmic vesicles from varied fish cell lines. However, the mechanisms used by this bacteria to survive, replicate, and propagate in cell lines, especially in macrophages and monocytes, are unknown. A number of studies have described the diverse proteins in pathogens such as *Legionella pneumophila*, *Coxiella burnetii*, and *Francisella tularensis* which allow these to evade the cellular immune response and replicate in the interior of macrophages in different hosts. Some of these proteins are the virulence factor BipA/TypA and the heat shock protein ClpB, both of which have been widely characterized. The results of the current study present the complete coding sequence of the genes *clp*B and *bip*A from the *P. salmonis* genome. Moreover, the experimental results suggest that during the infectious process of the SHK-1 cellular line in *P. salmonis*, the pathogen significantly increases the expression of proteins ClpB and BipA. This would permit the pathogen to adapt to the hostile conditions produced by the macrophage and thus evade mechanisms of cellular degradation while facilitating replication in the interior of this salmon cell line.

4.1347 Obesity in Aging Exacerbates Blood–Brain Barrier Disruption, Neuroinflammation, and Oxidative

Stress in the Mouse Hippocampus: Effects on Expression of Genes Involved in Beta-Amyloid Generation and Alzheimer's Disease
Tucsek, Z., Toth, P., Sosnowska, D., Gautam, T., Mitschelen, M., Koller, A., Szalai, G., Sonntag, W.E., Ungvari, Z. and Csiszar, A.
J. Gerontol. A Biol. Sci. Med. Sci., ***69(10)***, *1212-1226 (2014)*

There is growing evidence that obesity has deleterious effects on the brain and cognitive function in the elderly population. However, the specific mechanisms through which aging and obesity interact to promote cognitive decline remain unclear. To test the hypothesis that aging exacerbates obesity-induced cerebromicrovascular damage and neuroinflammation, we compared young (7 months) and aged (24 months) high fat diet–fed obese C57BL/6 mice. Aging exacerbated obesity-induced systemic inflammation and blood–brain barrier disruption, as indicated by the increased circulating levels of proinflammatory cytokines and increased presence of extravasated immunoglobulin G in the hippocampus, respectively. Obesity-induced blood–brain barrier damage was associated with microglia activation, upregulation of activating Fc-gamma receptors and proinflammatory cytokines, and increased oxidative stress. Treatment of cultured primary microglia with sera derived from aged obese mice resulted in significantly more pronounced microglia activation and oxidative stress, as compared with treatment with young sera. Serum-induced activation and oxidative stress were also exacerbated in primary microglia derived from aged animals. Hippocampal expression of genes involved in regulation of the cellular amyloid precursor protein–dependent signaling pathways, beta-amyloid generation, and the pathogenesis of tauopathy were largely unaffected by obesity in aged mice. Collectively, obesity in aging is associated with a heightened state of systemic inflammation, which exacerbates blood–brain barrier disruption. The resulting neuroinflammation and oxidative stress in the mouse hippocampus likely contribute to the significant cognitive decline observed in aged obese animals.

4.1348 Activation of N-methyl-d-aspartate receptor downregulates inflammasome activity and liver inflammation via a β-arrestin-2 pathway
Farooq, A., Hoque, R., Ouyang, X., Farooq, A., Ghani, A., Ahsan, K., Guerra, M. and Mehal, W.Z.
Am. J. Physiol. Gastrointest. Liver Physiol., ***307***, *G732-G740 (2014)*

Activation of the cytosolic inflammasome machinery is responsible for acute and chronic liver inflammation, but little is known about its regulation. The *N*-methyl-d-aspartate (NMDA) receptor families are heterotetrameric ligand-gated ion channels that are activated by a range of metabolites, including aspartate, glutamate, and polyunsaturated fatty acids. In the brain NMDA receptors are present on neuronal and nonneuronal cells and regulate a diverse range of functions. We tested the role of the NMDA receptor and aspartate in inflammasome regulation in vitro and in models of acute hepatitis and pancreatitis. We demonstrate that the NMDA receptor is present on Kupffer cells, and their activation on primary mouse and human cells limits inflammasome activation by downregulating NOD-like receptor family, pyrin domain containing 3 and procaspase-1. The NMDA receptor pathway is active in vivo, limits injury in acute hepatitis, and can be therapeutically further activated by aspartate providing protection in acute inflammatory liver injury. Downregulation of inflammasome activation by NMDA occurs via a β-arrestin-2 NF-kβ and JNK pathway and not via Ca^{2+} mobilization. We have identified the NMDA receptor as a regulator of inflammasome activity in vitro and in vivo. This has identified a new area of immune regulation associated by metabolites that may be relevant in a diverse range of conditions, including nonalcoholic steatohepatitis and total parenteral nutrition-induced immune suppression.

4.1349 Regulator of G-Protein Signaling-5 Is a Marker of Hepatic Stellate Cells and Expression Mediates Response to Liver Injury
Bahrami, A.J., Gunaje, J.J., Hayes, B.J., Riehle, K.J., Kenerson, H.L., Yeung, R.S., Stempien-Otero, A.S., Campbell, J.S. and Mahoney Jr, W.M.
PloS One, ***9(10)***, *e108505 (2014)*

Liver fibrosis is mediated by hepatic stellate cells (HSCs), which respond to a variety of cytokine and growth factors to moderate the response to injury and create extracellular matrix at the site of injury. G-protein coupled receptor (GPCR)-mediated signaling, via endothelin-1 (ET-1) and angiotensin II (AngII), increases HSC contraction, migration and fibrogenesis. Regulator of G-protein signaling-5 (RGS5), an inhibitor of vasoactive GPCR agonists, functions to control GPCR-mediated contraction and hypertrophy in pericytes and smooth muscle cells (SMCs). Therefore we hypothesized that RGS5 controls GPCR signaling in activated HSCs in the context of liver injury. In this study, we localize RGS5 to the HSCs and

demonstrate that *Rgs5* expression is regulated during carbon tetrachloride (CCl_4)-induced acute and chronic liver injury in $Rgs5^{LacZ/LacZ}$ reporter mice. Furthermore, CCl_4 treated RGS5-null mice develop increased hepatocyte damage and fibrosis in response to CCl_4 and have increased expression of markers of HSC activation. Knockdown of *Rgs5* enhances ET-1-mediated signaling in HSCs *in vitro*. Taken together, we demonstrate that RGS5 is a critical regulator of GPCR signaling in HSCs and regulates HSC activation and fibrogenesis in liver injury.

4.1350 Three Distinct Subsets of Thymic Epithelial Cells in Rats and Mice Defined by Novel Antibodies
Sawanobori, Y., Ueta, H., Dijkstra, C.D., park, C.G., Satou, M., Kitazawa, Y. and matsuno, K:
*PloS One, **9(10)**, e109995 (2014)*

Aim
Thymic epithelial cells (TECs) are thought to play an essential role in T cell development and have been detected mainly in mice using lectin binding and antibodies to keratins. Our aim in the present study was to create a precise map of rat TECs using antibodies to putative markers and novel monoclonal antibodies (i.e., ED 18/19/21 and anti-CD205 antibodies) and compare it with a map from mouse counterparts and that of rat thymic dendritic cells.
Results
Rat TECs were subdivided on the basis of phenotype into three subsets; $ED18^{+}ED19^{+/-}$keratin 5 $(K5)^{+}K8^{+}CD205^{+}$ class II MHC $(MHCII)^{+}$ cortical TECs (cTECs), $ED18^{+}ED21^{-}K5^{-}K8^{+}$*Ulex europaeus* lectin 1 $(UEA\text{-}1)^{+}CD205^{-}$ medullary TECs (mTEC1s), and $ED18^{+}ED21^{+}K5^{+}K8^{dull}UEA\text{-}1^{-}CD205^{-}$ medullary TECs (mTEC2s). Thymic nurse cells were defined in cytosmears as an $ED18^{+}ED19^{+/-}K5^{+}K8^{+}$ subset of cTECs. mTEC1s preferentially expressed MHCII, claudin-3, claudin-4, and autoimmune regulator (AIRE). Use of ED18 and ED21 antibodies revealed three subsets of TECs in mice as well. We also detected two distinct TEC-free areas in the subcapsular cortex and in the medulla. Rat dendritic cells in the cortex were $MHCII^{+}CD103^{+}$ but negative for TEC markers, including CD205. Those in the medulla were $MHCII^{+}CD103^{+}$ and $CD205^{+}$ cells were found only in the TEC-free area.
Conclusion
Both rats and mice have three TEC subsets with similar phenotypes that can be identified using known markers and new monoclonal antibodies. These findings will facilitate further analysis of TEC subsets and DCs and help to define their roles in thymic selection and in pathological states such as autoimmune disorders.

4.1351 Morphology and Intrinsic Excitability of Regenerating Sensory and Motor Neurons Grown on a Line Micropattern
Benzina, O., Cloitre, T., martin, M., Raoul, C., gergely, C. and Scamps, F.
*PloS One, **9(10)**, e110687 (2014)*

Axonal regeneration is one of the greatest challenges in severe injuries of peripheral nerve. To provide the bridge needed for regeneration, biological or synthetic tubular nerve constructs with aligned architecture have been developed. A key point for improving axonal regeneration is assessing the effects of substrate geometry on neuronal behavior. In the present study, we used an extracellular matrix-micropatterned substrate comprising 3 µm wide lines aimed to physically mimic the *in vivo* longitudinal axonal growth of mice peripheral sensory and motor neurons. Adult sensory neurons or embryonic motoneurons were seeded and processed for morphological and electrical activity analyses after two days *in vitro*. We show that micropattern-guided sensory neurons grow one or two axons without secondary branching. Motoneurons polarity was kept on micropattern with a long axon and small dendrites. The micro-patterned substrate maintains the growth promoting effects of conditioning injury and demonstrates, for the first time, that neurite initiation and extension could be differentially regulated by conditioning injury among DRG sensory neuron subpopulations. The micro-patterned substrate impacts the excitability of sensory neurons and promotes the apparition of firing action potentials characteristic for a subclass of mechanosensitive neurons. The line pattern is quite relevant for assessing the regenerative and developmental growth of sensory and motoneurons and offers a unique model for the analysis of the impact of geometry on the expression and the activity of mechanosensitive channels in DRG sensory neurons.

4.1352 Transcriptome analysis of peripheral blood mononuclear cells in human subjects following a 36 h fast provides evidence of effects on genes regulating inflammation, apoptosis and energy metabolism
Elliott, R.M., de Roos, B., Duthie, S.J., Bouwman, F.G., Rubio-Aliaga, I., Crosley, L.K., Mayer, C., Polley, A.C., Heim, C., Coort, S.L., Evelo, C.T., Mulholland, F., Daniel, H., mariman, E.C. and Jonhson, L.T.
*Genes Nutr., **9**:432 (2014)*

There is growing interest in the potential health benefits of diets that involve regular periods of fasting. While animal studies have provided compelling evidence that feeding patterns such as alternate-day fasting can increase longevity and reduce incidence of many chronic diseases, the evidence from human studies is much more limited and equivocal. Additionally, although several candidate processes have been proposed to contribute to the health benefits observed in animals, the precise molecular mechanisms responsible remain to be elucidated. The study described here examined the effects of an extended fast on gene transcript profiles in peripheral blood mononuclear cells from ten apparently healthy subjects, comparing transcript profiles after an overnight fast, sampled on four occasions at weekly intervals, with those observed on a single occasion after a further 24 h of fasting. Analysis of the overnight fasted data revealed marked inter-individual differences, some of which were associated with parameters such as gender and subject body mass. For example, a striking positive association between body mass index and the expression of genes regulated by type 1 interferon was observed. Relatively subtle changes were observed following the extended fast. Nonetheless, the pattern of changes was consistent with stimulation of fatty acid oxidation, alterations in cell cycling and apoptosis and decreased expression of key pro-inflammatory genes. Stimulation of fatty acid oxidation is an expected response, most likely in all tissues, to fasting. The other processes highlighted provide indications of potential mechanisms that could contribute to the putative beneficial effects of intermittent fasting in humans.

4.1353 GABA Promotes Human β-Cell Proliferation and Modulates Glucose Homeostasis
Purwana, I. et al
*Diabetes, **63**, 4197-4205 (2014)*

γ-Aminobutyric acid (GABA) exerts protective and regenerative effects on mouse islet β-cells. However, in humans it is unknown whether it can increase β-cell mass and improve glucose homeostasis. To address this question, we transplanted a suboptimal mass of human islets into immunodeficient NOD-scid-γ mice with streptozotocin-induced diabetes. GABA treatment increased grafted β-cell proliferation, while decreasing apoptosis, leading to enhanced β-cell mass. This was associated with increased circulating human insulin and reduced glucagon levels. Importantly, GABA administration lowered blood glucose levels and improved glucose excursion rates. We investigated GABA receptor expression and signaling mechanisms. In human islets, GABA activated a calcium-dependent signaling pathway through both GABA A receptor and GABA B receptor. This activated the phosphatidylinositol 3-kinase–Akt and CREB–IRS-2 signaling pathways that convey GABA signals responsible for β-cell proliferation and survival. Our findings suggest that GABA regulates human β-cell mass and may be beneficial for the treatment of diabetes or improvement of islet transplantation.

4.1354 Virus-Specific Immune Memory at Peripheral Sites of Herpes Simplex Virus Type 2 (HSV-2) Infection in Guinea Pigs
Xia, J., Veselenak, R.L., Gorder, S.R., Bourne, N. and Milligan, G.N.
*PloS One, **9(12)**, e114652 (2014)*

Despite its importance in modulating HSV-2 pathogenesis, the nature of tissue-resident immune memory to HSV-2 is not completely understood. We used genital HSV-2 infection of guinea pigs to assess the type and location of HSV-specific memory cells at peripheral sites of HSV-2 infection. HSV-specific antibody-secreting cells were readily detected in the spleen, bone marrow, vagina/cervix, lumbosacral sensory ganglia, and spinal cord of previously-infected animals. Memory B cells were detected primarily in the spleen and to a lesser extent in bone marrow but not in the genital tract or neural tissues suggesting that the HSV-specific antibody-secreting cells present at peripheral sites of HSV-2 infection represented persisting populations of plasma cells. The antibody produced by these cells isolated from neural tissues of infected animals was functionally relevant and included antibodies specific for HSV-2 glycoproteins and HSV-2 neutralizing antibodies. A vigorous IFN-γ-secreting T cell response developed in the spleen as well as the sites of HSV-2 infection in the genital tract, lumbosacral ganglia and spinal cord following acute HSV-2 infection. Additionally, populations of HSV-specific tissue-resident memory T cells were maintained at these sites and were readily detected up to 150 days post HSV-2 infection. Unlike the persisting plasma cells, HSV-specific memory T cells were also detected in uterine tissue and cervicothoracic region of the spinal cord and at low levels in the cervicothoracic ganglia. Both HSV-specific $CD4^+$ and $CD8^+$ resident memory cell subsets were maintained long-term in the genital tract and sensory ganglia/spinal cord following HSV-2 infection. Together these data demonstrate the long-term maintenance of both humoral and cellular arms of the adaptive immune response at the sites of HSV-2 latency and virus shedding and highlight the utility of the guinea pig infection model to investigate tissue-resident memory in the setting

of HSV-2 latency and spontaneous reactivation.

4.1355 Fuzheng Huayu recipe alleviates hepatic fibrosis via inhibiting TNF-α induced hepatocyte apoptosis
Tao, Y-y., Yan, X-c., Zhou, T., Shen, L., Liu, Z-l. and Liu, C-h.
*BMC Complementary and Alternative Medicine, **14**:449 (2014)*

Background
What was the relationship of Fuzheng Huayu recipe (FZHY) inhibiting hepatocyte apoptosis and HSC activation at different stage of liver fibrosis? In order to answer this question, the study was carried out to dynamically observe FZHY's effect on hepatocyte apoptosis and HSC activation and further explored underling mechanism of FZHY against hepatocyte apoptosis.
Methods
Mice were randomly divided into four groups: normal, model, FZHY, and N-acetylcystein (NAC) groups. Acute hepatic injury and liver fibrosis in mice were induced by CCl_4. Three days before the first CCl_4 injection, treatment with FZHY powder or NAC respectively was started. *In vitro*, primary hepatocytes were pretreated with FZHY medicated serum or Z-VAD-FMK and then incubated with ActD and TNF-α. Primary HSCs were treated with DNA from apoptotic hepatocytes incubated by Act D/TNF-α or FZHY medicated. Liver sections were analyzed for HE staining and immunohistochemical evaluation of apoptosis. Serum ALT and AST, Alb content and TNF-α expression in liver tissue were detected. Hyp content was assayed and collagen deposition was visualized. Expressions of α-SMA and type I collagen were analyzed by immunofluorescence and immunoblotting. Flow cytometry, immunofluorescence, and DNA ladder for hepatocyte apoptosis and immunoblotting for TNF-R1, Bcl-2 and Bax were also analyzed.
Results
Mice showed characteristic features of massive hepatocytes apoptosis in early stage of liver injury and developed severe hepatic fibrosis in later phase. FZHY treatment significantly alleviated acute liver injury and hepatocyte apoptosis, and inhibited liver fibrosis by decreasing α-SMA expression and hepatic Hyp content. *In vitro*, primary hepatocytes were induced by TNF-α and Act D. The anti-apoptotic effect of FZHY was generated by reducing TNFR1 expression and balancing the expressions of Bcl-2 and Bax. Meanwhile, the nuclear DNA from apoptotic hepatocytes stimulated HSC activation in a dose dependent manner, and the DNA from apoptotic hepatocytes treated with FZHY or Z-VAD-FMK reduced HSC activation and type I collagen expression.
Conclusion
These findings suggested that FZHY suppressed hepatocyte apoptosis through regulating mediators in death receptor and mitochondrial pathways, and the effect of FZHY on hepatocyte apoptosis might play an important role in inhibiting liver fibrosis.

4.1356 Aag-initiated base excision repair promotes ischemia reperfusion injury in liver, brain, and kidney
Ebrahimkhani, M.R., Daneshmand, A., Mazumder, A., Allocca, M., Calvo, J.A., Abolhassani, N., Jhun, I., Muthupalani, S., Ayata, C. and Samson, L.D.
*PNAS, **111(43)**, E4878-E4886 (2014)*

Inflammation is accompanied by the release of highly reactive oxygen and nitrogen species (RONS) that damage DNA, among other cellular molecules. Base excision repair (BER) is initiated by DNA glycosylases and is crucial in repairing RONS-induced DNA damage; the alkyladenine DNA glycosylase (Aag/Mpg) excises several DNA base lesions induced by the inflammation-associated RONS release that accompanies ischemia reperfusion (I/R). Using mouse I/R models we demonstrate that $Aag^{-/-}$ mice are significantly protected against, rather than sensitized to, I/R injury, and that such protection is observed across three different organs. Following I/R in liver, kidney, and brain, $Aag^{-/-}$ mice display decreased hepatocyte death, cerebral infarction, and renal injury relative to wild-type. We infer that in wild-type mice, Aag excises damaged DNA bases to generate potentially toxic abasic sites that in turn generate highly toxic DNA strand breaks that trigger poly(ADP-ribose) polymerase (Parp) hyperactivation, cellular bioenergetics failure, and necrosis; indeed, steady-state levels of abasic sites and nuclear PAR polymers were significantly more elevated in wild-type vs. $Aag^{-/-}$ liver after I/R. This increase in PAR polymers was accompanied by depletion of intracellular NAD and ATP levels plus the translocation and extracellular release of the high-mobility group box 1 (Hmgb1) nuclear protein, activating the sterile inflammatory response. We thus demonstrate the detrimental effects of Aag-initiated BER during I/R and sterile inflammation, and present a novel target for controlling I/R-induced injury.

4.1357 Pancreatic Ductal Perfusion at Organ Procurement Enhances Islet Yield in Human Islet Isolation
Takita, M., Itoh, T., Shimoda, M., Kanak, M.A., Shahbazov, R., Kunnathodi, F., Lawrence, M.C.,

Naziruddin, B. and Levy, M.F.
*Pancreas, **43(8)**, 1249-1255 (2014)*

Objective
Pancreas preservation is a major factor influencing the results of islet cell transplantation. This study evaluated the effects of 2 different solutions for pancreatic ductal perfusion (PDP) at organ procurement.
Methods
Eighteen human pancreases were assigned to 3 groups: non-PDP (control), PDP with ET-Kyoto solution, and PDP with cold storage/purification stock solution. Pancreatic islets were isolated according to the modified Ricordi method.
Results
No significant differences in donor characteristics, including cold ischemia time, were observed between the 3 groups. All islet isolations in the PDP groups had more than 400,000 islet equivalence in total islet yield after purification, a significant increase when compared with the control ($P = 0.04$ and $P < 0.01$). The islet quality assessments, including an in vivo diabetic nude mice assay and the response of high-mobility group box protein 1 to cytokine stimulation, also showed no significant differences. The proportion of terminal deoxynucleotidyl transferase dUTP nick-end labeling–positive cells showing apoptosis in islets in the PDP groups was significantly lower than in the control group ($P < 0.05$).
Conclusions
Both ET-Kyoto solution and cold storage/purification stock solution are suitable for PDP and consistently resulted in isolation success. Further studies with a larger number of pancreas donors should be done to compare the effects of the PDP solutions.

4.1358 Neuronal Transgene Expression in Dominant-Negative SNARE Mice
Fujita, T., Chen, M.J., Li, B., Smith, N.A., Peng, W., Sun, W., Toner, M.J., Kress, B.T., Wang, L., Benraiss, A., Takano, T., Wang, S. and Nedergaard, M.
*J. Neurosci., **34(50)**, 16594-16604 (2014)*

Experimental advances in the study of neuroglia signaling have been greatly accelerated by the generation of transgenic mouse models. In particular, an elegant manipulation that interferes with astrocyte vesicular release of gliotransmitters via overexpression of a dominant-negative domain of vesicular SNARE (dnSNARE) has led to documented astrocytic involvement in processes that were traditionally considered strictly neuronal, including the sleep–wake cycle, LTP, cognition, cortical slow waves, depression, and pain. A key premise leading to these conclusions was that expression of the dnSNARE was specific to astrocytes. Inconsistent with this premise, we report here widespread expression of the *dnSNARE* transgene in cortical neurons. We further demonstrate that the activity of cortical neurons is reversibly suppressed in dnSNARE mice. These findings highlight the need for independent validation of astrocytic functions identified in dnSNARE mice and thus question critical evidence that astrocytes contribute to neurotransmission through SNARE-dependent vesicular release of gliotransmitters.

4.1359 Micropatterned Coumarin Polyester Thin Films Direct Neurite Orientation
Mccormick, A.M., Maddipatla, M.V.S.N., Shi, S., Chamsaz, E.A., Yokoyama, H., Joy, A. and Leipzig, N.D.
*ACS Appl. Mater. Interfaces, **6(22)**, 19655-19667 (2014)*

Guidance and migration of cells in the nervous system is imperative for proper development, maturation, and regeneration. In the peripheral nervous system (PNS), it is challenging for axons to bridge critical-sized injury defects to achieve repair and the central nervous system (CNS) has a very limited ability to regenerate after injury because of its innate injury response. The photoreactivity of the coumarin polyester used in this study enables efficient micropatterning using a custom digital micromirror device (DMD) and has been previously shown to be biodegradable, making these thin films ideal for cell guidance substrates with potential for future in vivo applications. With DMD, we fabricated coumarin polyester thin films into 10 × 20 μm and 15 × 50 μm micropatterns with depths ranging from 15 to 20 nm to enhance nervous system cell alignment. Adult primary neurons, oligodendrocytes, and astrocytes were isolated from rat brain tissue and seeded onto the polymer surfaces. After 24 h, cell type and neurite alignment were analyzed using phase contrast and fluorescence imaging. There was a significant difference ($p < 0.0001$) in cell process distribution for both emergence angle (from the body of the cell) and orientation angle (at the tip of the growth cone) confirming alignment on patterned surfaces compared to control substrates (unpatterned polymer and glass surfaces). The expected frequency distribution for parallel alignment (≤15°) is 14% and the two micropatterned groups ranged from 42 to 49% alignment for emergence and

orientation angle measurements, where the control groups range from 12 to 22% for parallel alignment. Despite depths being 15 to 20 nm, cell processes could sense these topographical changes and preferred to align to certain features of the micropatterns like the plateau/channel interface. As a result this initial study in utilizing these new DMD micropatterned coumarin polyester thin films has proven beneficial as an axon guidance platform for future nervous system regenerative strategies.

Molecular aspects, genomic arrangement and immune responsive mRNA expression profiles of two CXC chemokine receptor homologs (CXCR1 and CXCR2) from rock bream, Oplegnathus fasciatus
Umasuthan, N., Wan, Q., Revathy, K.S., Whang, I., Noh, J.K., Kim, S., park, M-A. and Lee, J.
Fish & Shellfish Immunology, ***40(1)****, 304-318 (2014)*

The CXCR1 and CXCR2 are the prototypical receptors and are the only known receptors for mammalian ELR+ (Glu-Leu-Arg) CXC chemokines, including CXCL8 (interleukin 8). These receptors transduce the ELR+ chemokine signals and operate the downstream signaling pathways in inflammation and innate immunity. In this study, we report the identification and characterization of *CXCR1* and *CXCR2* genes from rock bream fish (*OfCXCR1* and *OfCXCR2*) at the molecular level. The cDNA and genomic DNA sequences of the *OfCXCR1* and *OfCXCR2* were identified from a transcriptome library and a custom-constructed BAC library, respectively. Both *OfCXCR* genes consisted of two exons, separated by an intron. The 5′-flanking regions of *OfCXCR* genes possessed multiple putative transcription factor binding sites related to immune response. The coding sequences of *OfCXCR1* and *OfCXCR2* encoded putative peptides of 355 and 360 amino acids (aa), respectively. The deduced aa sequences of OfCXCR1 and OfCXCR2 comprised of a G-protein coupled receptors (GPCR) family 1 profile with a GPCR signature and a DRY motif. In addition, seven conserved transmembrane regions were predicted in both OfCXCRs. While our multiple alignment study revealed the functionally significant conserved elements of the OfCXCR1 and OfCXCR2, phylogeny analyses further confirmed their position in teleost sub clade, in which they manifested an evolutionary relatedness with other fish counterparts. Based on comparative analyses, teleost CXC chemokine receptors appear to be distinct from their non-fish orthologs in terms of evolution (both *CXCR1* and*CXCR2*) and genomic organization (*CXCR2*). Quantitative real-time PCR (qPCR) detected the transcripts of *OfCXCR1* and *OfCXCR2* in eleven examined tissues, with higher levels in head kidney, kidney and spleen highlighting their crucial importance in immunity. *In vitro* stimulation of peripheral blood leukocytes (PBLs) with concanavalin A (Con A) resulted in modulation of *OfCXCR2* transcription, but not that of*OfCXCR1*. In addition, the magnitude of the *OfCXCR1* and *OfCXCR2* transcripts in head kidney and spleen was differentially increased after the *in vivo* administration of immune stimulants, LPS and poly I:C and in the infection models injected with rock bream irido virus, *Edwardsiella tarda* and *Streptococcus iniae*. These lines of evidence suggest that these receptors may play an important role(s) in immune responsive signaling during pathogenesis of rock bream.

Isolation and characterization of platelet-derived extracellular vesicles
Aatonen, M.T., Ôhman, T., Nyman, T.A., laitinen, S., Grönholm, M. and Siljander, P.R.-M.
J. Extracellulaar Vesicles, ***3****:24692 (2014)*

Background: Platelet-derived extracellular vesicles (EVs) participate, for example, in haemostasis, immunity and development. Most studies of platelet EVs have targeted microparticles, whereas exosomes and EV characterization under various conditions have been less analyzed. Studies have been hampered by the difficulty in obtaining EVs free from contaminating cells and platelet remnants. Therefore, we optimized an EV isolation protocol and compared the quantity and protein content of EVs induced by different agonists.
Methods: Platelets isolated with iodixanol gradient were activated by thrombin and collagen, lipopolysaccharide (LPS) or Ca^{2+} ionophore. Microparticles and exosomes were isolated by differential centrifugations. EVs were quantitated by nanoparticle tracking analysis (NTA) and total protein. Size distributions were determined by NTA and electron microscopy. Proteomics was used to characterize the differentially induced EVs.
Results: The main EV populations were 100–250 nm and over 90% were <500 nm irrespective of the activation. However, activation pathways differentially regulated the quantity and the quality of EVs, which also formed constitutively. Thrombogenic activation was the most potent physiological EV-generator. LPS was a weak inducer of EVs, which had a selective protein content from the thrombogenic EVs. Ca^{2+} ionophore generated a large population of protein-poor and unselectively packed EVs. By proteomic analysis, EVs were highly heterogeneous after the different activations and between the vesicle subpopulations.

Conclusions: Although platelets constitutively release EVs, vesiculation can be increased, and the activation pathway determines the number and the cargo of the formed EVs. These activation-dependent variations render the use of protein content in sample normalization invalid. Since most platelet EVs are 100–250 nm, only a fraction has been analyzed by previously used methods, for example, flow cytometry. As the EV subpopulations could not be distinguished and large vesicle populations may be lost by differential centrifugation, novel methods are required for the isolation and the differentiation of all EVs.

4.1360 S100A4 promotes liver fibrosis via activation of hepatic stellate cells
Chen, L. et al
J. Hepatol., ***62***, *156-164 (2015)*

Background & Aims
S100A4 has been linked to the fibrosis of several organs due to its role as a fibroblast-specific marker. However, the role of S100A4 itself in the development of fibrosis has not been much investigated. Here, we determined whether S100A4 regulates liver fibrogenesis and examined its mechanism by focusing on the activation of hepatic stellate cells (HSCs).
Methods
S100A4 deficient mice were used to determine the role of S100A4 in liver fibrogenesis. The effect of S100A4 on HSC activation was estimated by using primary mouse HSCs and the human HSC cell line LX-2. Serum levels of S100A4 in cirrhotic patients were determined by ELISA.
Results
S100A4 was found to be secreted by a subpopulation of macrophages and to promote the development of liver fibrosis. It accumulated in the liver during the progression of liver fibrosis and activated HSCs in mice. *In vitro* studies demonstrated that S100A4 induced the overexpression of alpha-smooth muscle actin through c-Myb in HSCs. Both, the selective depletion of S100A4-expressing cells and knockdown of *S100A4* in the liver by RNA interference, resulted in a reduction of liver fibrosis following injury. Importantly, increased S100A4 levels in both the liver tissue and serum correlated positively with liver fibrosis in humans.
Conclusions
S100A4 promotes liver fibrosis by activating HSCs, which may represent a potential target for anti-fibrotic therapies.

4.1361 The xanthine oxidase inhibitor Febuxostat reduces tissue uric acid content and inhibits injury-induced inflammation in the liver and lung
Kataoka, H., yang, K. and Rock, K.L.
Eur. J. Pharmacol., ***746***, *174-179 (2015)*

Necrotic cell death in vivo induces a robust neutrophilic inflammatory response and the resulting inflammation can cause further tissue damage and disease. Dying cells induce this inflammation by releasing pro-inflammatory intracellular components, one of which is uric acid. Cells contain high levels of intracellular uric acid, which is produced when purines are oxidized by the enzyme xanthine oxidase. Here we test whether a non-nucleoside xanthine oxidase inhibitor, Febuxostat (FBX), can reduce intracellular uric acid levels and inhibit cell death-induced inflammation in two different murine tissue injury models; acid-induced acute lung injury and acetaminophen liver injury. Infiltration of inflammatory cells induced by acid injection into lungs or peritoneal administration of acetaminophen was evaluated by quantification with flow cytometry and tissue myeloperoxidase activity in the presence or absence of FBX treatment. Uric acid levels in serum and tissue were measured before giving the stimuli and during inflammation. The impact of FBX treatment on the peritoneal inflammation caused by the microbial stimulus, zymosan, was also analyzed to see whether FBX had a broad anti-inflammatory effect. We found that FBX reduced uric acid levels in acid-injured lung tissue and inhibited acute pulmonary inflammation triggered by lung injury. Similarly, FBX reduced uric acid levels in the liver and inhibited inflammation in response to acetaminophen-induced hepatic injury. In contrast, FBX did not reduce inflammation to zymosan, and therefore is not acting as a general anti-inflammatory agent. These results point to the potential of using agents like FBX to treat cell death-induced inflammation.

4.1362 Identification of quinoline-chalcone hybrids as potential antiulcer agents
Sashidhara, K.V., Avula, S.R., Mishra, V., Palnati, G.R., Singh, L.R., Singh, N., Chhonker, Y.S., Swami, P., Bhata, R.S. and palit, G.
Eur. J. Medicinal Chem., ***89***, *638-653 (2015)*

Antiulcer activity of novel quinoline-chalcone hybrids (**13**–**37**) was investigated. Among them, eight compounds (**14**, **16**, **17**, **23**, **29**, **31**, **32** and **35**) were found to be active in various ulcer models in Sprague–Dawley (SD) rats. To understand the mechanism of action of these hybrids, the effects of the compounds on antisecretory and cytoprotective activities were studied. All these active hybrids improved the depleted levels of mucin and consequently inhibited the formation of erosions in a pyloric ligated ulcer model. In addition, they also significantly increased the gastric PGE_2 content in an aspirin induced ulcer model. The additional experiments including the *in vitro* metabolic stability and *in vivo* pharmacokinetics led to the identification of compound **17** as an orally active and safe candidate that is worthy of further investigation to be developed as an antiulcer agent.

4.1363 Spinal neuroimmune activation is independent of T-cell infiltration and attenuated by A3 adenosine receptor agonists in a model of oxaliplatin-induced peripheral neuropathy
Janes, K., Wahlman, C., Little, J.W., Doyle, T., Tosh, D.K., Jacobson, K.A. and Salvemini, D.
Brain, Behavior, and Immunity, ***44***, *91-99 (2015)*

Many commonly used chemotherapeutics including oxaliplatin are associated with the development of a painful chemotherapy-induced peripheral neuropathy (CIPN). This dose-limiting complication can appear long after the completion of therapy causing a significant reduction in quality-of-life and impeding cancer treatment. We recently reported that activation of the G_i/G_q-coupled A_3 adenosine receptor (A_3AR) with selective A_3AR agonists (i.e., IB-MECA) blocked the development of chemotherapy induced-neuropathic pain in models evoked by distinct agents including oxaliplatin without interfering with their anticancer activities. The mechanism(s) of action underlying these beneficial effects has yet to be explored. Our results herein demonstrate that the development of oxaliplatin-induced mechano-hypersensitivity (allodynia and hyperalgesia) in rats is associated with the hyperactivation of astrocytes, but not microglial cells, increased production of pro-inflammatory and neuroexcitatory cytokines (TNF, IL-1β), and reductions in the levels of anti-inflammatory/neuroprotective cytokines (IL-10, IL-4) in the dorsal horn of the spinal cord. These events did not require lymphocytic mobilization since oxaliplatin did not induce $CD45^+/CD3^+$ T-cell infiltration into the spinal cord. A_3AR agonists blocked the development of neuropathic pain with beneficial effects strongly associated with the modulation of spinal neuroinflammatory processes: attenuation of astrocytic hyperactivation, inhibition of TNF and IL-1β production, and an increase in IL-10 and IL-4. These results suggest that inhibition of an astrocyte-associated neuroinflammatory response contributes to the protective actions of A_3AR signaling and continues to support the pharmacological basis for selective A_3AR agonists as adjuncts to chemotherapeutic agents for the management of chronic pain.

4.1364 Alcohol directly stimulates epigenetic modifications in hepatic stellate cells
Page, A., Paoli, P.P., Hill, S.J., Howarth, R., Wu, R., Kweon, S-M., French, J., White, S., Tsukamoto, H., Mann, D.E. and Mann, J.
J. Hepatol., ***62***, *388-397 (2015)*

Background & Aims
Alcohol is a primary cause of liver disease and an important co-morbidity factor in other causes of liver disease. A common feature of progressive liver disease is fibrosis, which results from the net deposition of fibril-forming extracellular matrix (ECM). The hepatic stellate cell (HSC) is widely considered to be the major cellular source of fibrotic ECM. We determined if HSCs are responsive to direct stimulation by alcohol.
Methods
HSCs undergoing transdifferentiation were incubated with ethanol and expression of fibrogenic genes and epigenetic regulators was measured. Mechanisms responsible for recorded changes were investigated using ChIP-Seq and bioinformatics analysis. Ethanol induced changes were confirmed using HSCs isolated from a mouse alcohol model and from ALD patient's liver and through precision cut liver slices.
Results
HSCs responded to ethanol exposure by increasing profibrogenic and ECM gene expression including elastin. Ethanol induced an altered expression of multiple epigenetic regulators, indicative of a potential to modulate chromatin structure during HSC transdifferentiation. MLL1, a histone 3 lysine 4 (H3K4) methyltransferase, was induced by ethanol and recruited to the elastin gene promoter where it was associated with enriched H3K4me3, a mark of active chromatin. Chromatin immunoprecipitation sequencing (ChIPseq) revealed that ethanol has broad effects on the HSC epigenome and identified 41 gene loci at which both MML1 and its H3K4me3 mark were enriched in response to ethanol.
Conclusions

Ethanol directly influences HSC transdifferentiation by stimulating global changes in chromatin structure, resulting in the increased expression of ECM proteins. The ability of alcohol to remodel the epigenome during HSC transdifferentiation provides mechanisms for it to act as a co-morbidity factor in liver disease.

4.1365 Decabrominated diphenyl ether and methylmercury impair fetal nervous system development in mice at documented human exposure levels
Mariani, A., Fanelli, R., Re Depaolini, A. and De Paola, M.
Develop. Neurobiol., ***75***, *23-38 (2015)*

The central nervous system (CNS) is extremely vulnerable to the toxic effects of environmental pollutants during development. Polybrominated diphenyl ethers (PBDEs) are persistent contaminants, increasingly present in the environment and in human tissues. Recent investigations identified a correlation between maternal exposure to PBDEs and impairment in fetal neurobehavioral development, suggesting that these contaminants pose a potential risk for children. We investigated on the potential effects of environmental decabrominated diphenyl ether (decaBDE, the fully brominated congener) on key neurodevelopmental molecules (e.g., synaptic proteins and immature neuron markers) in fetal mouse neurons. Methylmercury was used as reference neurotoxic contaminant and to evaluate its possible synergism with decaBDE. The neurotoxic effects of decaBDE and methylmercury were determined in developing cultured neurons from mouse fetal hippocampus and cerebellum. Neuron death, dendritic branching, synaptic protein expression, markers of immature neurons, and microglia activation were evaluated by immunocytochemistry. Brain samples from prenatally treated embryos were also examined for neurotoxicity signs by immunoblotting and histochemistry. DecaBDE significantly affected (down to 0.4 n*M*) the number of dendritic branches, and the levels of synaptic proteins and doublecortin in cultured neurons. Prenatal exposure to decaBDE decreased the synaptic proteins and increased the expression of the immature neuron and microglial markers in mouse fetuses. In conclusion, prenatal exposure to realistic (relevant for human exposure) concentrations of decaBDE induces impairment of fetal CNS development in mice, suggesting a potential risk of fetotoxicity in humans.

4.1366 Myeloid-specific disruption of recombination signal binding protein Jκ ameliorates hepatic fibrosis by attenuating inflammation through cylindromatosis in mice
He, F., Guo, F-C., Li, Z., Yu, H-C., Ma, P-F., Zhao, J-L., Feng, L., Li, W-N., Liu, X-W., Qin, H-Y., Dou, K-F. and Han, H.
Hepatology, ***61***, *303-314 (2015)*

Macrophages play multidimensional roles in hepatic fibrosis, but their control has not been fully understood. The Notch pathway mediated by recombination signal binding protein Jκ (RBP-J), the transcription factor transactivated by signals from four mammalian Notch receptors, is implicated in macrophage activation and plasticity. In this study, by using mouse hepatic fibrosis models, we show that myeloid-specific disruption of RBP-J resulted in attenuated fibrosis. The activation of hepatic stellate cells and production of profibrotic factors including platelet-derived growth factor (PDGF)-B and transforming growth factor beta1 (TGF-β1) reduced significantly in myeloid-specific RBP-J deficient mice. The infiltration of inflammatory cells and production of proinflammatory factors were reduced in liver of myeloid-specific RBP-J-deficient mice during fibrosis. In RBP-J-deficient macrophages, the nuclear factor kappa B (NF-κB) activation was remarkably attenuated as compared with the control. This could be attributed to the up-regulation of cylindromatosis (CYLD), a negative regulator of NF-κB, in Notch signal-compromised macrophages, because the knockdown of CYLD in RBP-J-deficient macrophages or overexpression of p65 in RBP-J knockdown cells both restored NF-κB activation and the production of proinflammatory and/or profibrotic factors by macrophages. In human hepatic fibrosis biopsies, stronger Notch activation is correlated with more severe fibrosis, which is accompanied by a lower level of CYLD but irrespective of etiological reasons. *Conclusion*: RBP-J-mediated Notch signaling is required for macrophages to promote hepatic fibrosis by up-regulation of NF-κB activation through CYLD

4.1367 Inhibition of H3K27me3-Specific Histone Demethylases JMJD3 and UTX Blocks Reactivation of Herpes Simplex Virus 1 in Trigeminal Ganglion Neurons
Messer, H.G.P., Jacobs, D., Dhummakupt, A. and Bloom, D.C.
J. Virol., ***89(6)***, *3417-3420 (2015)*

Herpes simplex virus 1 (HSV-1) genomes are associated with the repressive heterochromatic marks H3K9me2/me3 and H3K27me3 during latency. Previous studies have demonstrated that inhibitors of H3K9me2/me3 histone demethylases reduce the ability of HSV-1 to reactivate from latency. Here we

demonstrate that GSK-J4, a specific inhibitor of the H3K27me3 histone demethylases UTX and JMJD3, inhibits HSV-1 reactivation from sensory neurons *in vitro*. These results indicate that removal of the H3K27me3 mark plays a key role in HSV-1 reactivation.

4.1368 Efficient Uptake and Dissemination of Scrapie Prion Protein by Astrocytes and Fibroblasts from Adult Hamster Brain
Hollister, J.R., Lee, K.S., Dorward, D.W. and baron, G.S.
*PloS One, **10(1)**, e0115351 (2015)*

Prion infections target neurons and lead to neuronal loss. However, the role of non-neuronal cells in the initiation and spread of infection throughout the brain remains unclear despite the fact these cells can also propagate prion infectivity. To evaluate how different brain cells process scrapie prion protein (PrPres) during acute infection, we exposed neuron-enriched and non-neuronal cell cultures from adult hamster brain to fluorescently-labeled purified PrPres and followed the cultures by live cell confocal imaging over time. Non-neuronal cells present in both types of cultures, specifically astrocytes and fibroblasts, internalized PrPres more efficiently than neurons. PrPres was trafficked to late endosomal/lysosomal compartments and rapidly transported throughout the cell bodies and processes of all cell types, including contacts between astrocytes and neurons. These observations suggest that astrocytes and meningeal fibroblasts play an as yet unappreciated role in prion infections via efficient uptake and dissemination of PrPres.

4.1369 Kinetics of sickle cell biorheology and implications for painful vasoocclusive crisis
Du, E., Diez-Silva, M., kato, G.J., Dao, M. and Suresh, S.
*PNAS, **112(5)**, 1422-1427 (2015)*

We developed a microfluidics-based model to quantify cell-level processes modulating the pathophysiology of sickle cell disease (SCD). This in vitro model enabled quantitative investigations of the kinetics of cell sickling, unsickling, and cell rheology. We created short-term and long-term hypoxic conditions to simulate normal and retarded transit scenarios in microvasculature. Using blood samples from 25 SCD patients with sickle hemoglobin (HbS) levels varying from 64 to 90.1%, we investigated how cell biophysical alterations during blood flow correlated with hematological parameters, HbS level, and hydroxyurea (HU) therapy. From these measurements, we identified two severe cases of SCD that were also independently validated as severe from a genotype-based disease severity classification. These results point to the potential of this method as a diagnostic indicator of disease severity. In addition, we investigated the role of cell density in the kinetics of cell sickling. We observed an effect of HU therapy mainly in relatively dense cell populations, and that the sickled fraction increased with cell density. These results lend support to the possibility that the microfluidic platform developed here offers a unique and quantitative approach to assess the kinetic, rheological, and hematological factors involved in vasoocclusive events associated with SCD and to develop alternative diagnostic tools for disease severity to supplement other methods. Such insights may also lead to a better understanding of the pathogenic basis and mechanism of drug response in SCD.

4.1370 Regeneration of whole plants from protoplasts of Gracilaria gracilis (Gracilariales, Rhodophyta)
Huddy, S.M., Meyers, A.E. and Coyne, V.E.
*J. Appl. Phycol., **27(1)**, 427-435 (2015)*

This paper reports the first successful regeneration of whole plants from protoplasts of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham. Protoplasts were isolated and purified using a previously optimized protocol. Protoplasts with regenerated cell walls divided to produce callus-like cell masses which showed the presence of uniseriate, filamentous outgrowths. Bud outgrowth from callus masses was associated with a distinct change in colour intensity at the point of outgrowth. Ultimately, whole plants were regenerated from the callus-like cell masses with overall yields of approximately two to three whole plants per 10^4 protoplasts seeded. Two distinctive patterns of regeneration were observed. In the first case, protoplasts regenerated slowly to produce plants which resembled the parent plants, exhibiting slender, branched thalli. Growth rates of regenerated seaweed were similar to that of wild-type *G. gracilis* cultured under the same conditions with 115 g of seaweed cultured from 15 individually regenerated plants over a year. In the second case, protoplasts regenerated rapidly to produce plants which remained small with thalli that were thick and unbranched and had a limited life span. These results provide an important foundation for the development of a successful tissue culture system for *G. gracilis*.

4.1371 Upregulation of miR21 and Repression of Grhl3 by Leptin Mediates Sinusoidal Endothelial Injury in Experimental Nonalcoholic Steatohepatitis
Pourhoseini, S., Seth, R.K., Das, S., Dattatoy, D., Kadilska, B., Xie, G., Michelotti, G.A., nagarkatti, M., Diehl, A.M. and Chatterjee, S.
*PloS One, **10(2)**, e0116780 (2015)*

Sinusoidal endothelial dysfunction (SED) has been found to be an early event in nonalcoholic steatohepatitis (NASH) progression but the molecular mechanisms underlying its causation remains elusive. We hypothesized that adipokine leptin worsens sinusoidal injury by decreasing functionally active nitric oxide synthase 3 (NOS)3 via miR21. Using rodent models of NASH, and transgenic mice lacking leptin and leptin receptor, results showed that hyperleptinemia caused a 4–5 fold upregulation of hepatic miR21 as assessed by qRTPCR. The upregulation of miR21 led to a time-dependent repression of its target protein Grhl3 levels as shown by western blot analyses. NOS3-p/NOS3 ratio which is controlled by Grhl3 was significantly decreased in NASH models. SED markers ICAM-1, VEGFR-2, and E-selectin as assessed by immunofluorescence microscopy were significantly up regulated in the progressive phases of NASH. Lack of leptin or its receptor in vivo, reversed the upregulation of miR21 and restored the levels of Grhl3 and NOS3-p/NOS3 ratio coupled with decreased SED dysfunction markers. Interestingly, leptin supplementation in mice lacking leptin, significantly enhanced miR21 levels, decreased Grhl3 repression and NOS3 phosphorylation. Leptin supplementation in isolated primary endothelial cells, Kupffer cells and stellate cells showed increased mir21 expression in stellate cells while sinusoidal injury was significantly higher in all cell types. Finally miR21 KO mice showed increased NOS3-p/NOS3 ratio and reversed SED markers in the rodent models of NASH. The experimental results described here show a close association of leptin-induced miR21 in aiding sinusoidal injury in NASH.

4.1372 Cytosolic Access of Mycobacterium tuberculosis: Critical Impact of Phagosomal Acidification Control and Demonstration of Occurrence In Vivo
Simeone, R., Sayes, F., Song, O., Gröschel, M.I., Brodin, P., Brosch, R. and Majlessi, L.
*PloS Pathogens, **10(2)**, e1004650 (2015)*

Mycobacterium tuberculosis (*Mtb*) uses efficient strategies to evade the eradication by professional phagocytes, involving—as recently confirmed—escape from phagosomal confinement. While *Mtb* determinants, such as the ESX-1 type VII secretion system, that contribute to this phenomenon are known, the host cell factors governing this important biological process are yet unexplored. Using a newly developed flow-cytometric approach for *Mtb*, we show that macrophages expressing the phagosomal bivalent cation transporter Nramp-1, are much less susceptible to phagosomal rupture. Together with results from the use of the phagosome acidification inhibitor bafilomycin, we demonstrate that restriction of phagosomal acidification is a prerequisite for mycobacterial phagosomal rupture and cytosolic contact. Using different *in vivo* approaches including an enrichment and screen for tracking rare infected phagocytes carrying the CD45.1 hematopoietic allelic marker, we here provide first and unique evidence of *M. tuberculosis*-mediated phagosomal rupture in mouse spleen and lungs and in numerous phagocyte types. Our results, linking the ability of restriction of phagosome acidification to cytosolic access, provide an important conceptual advance for our knowledge on host processes targeted by *Mtb* evasion strategies.

4.1373 CD40 Is Required for Protective Immunity against Liver Stage Plasmodium Infection
Murray, S.A., Mohar, I., Miller, J.L., Brempelis, K.J., Vaughan, A.M., Kappe, S.I. and Crispe, I.N.
*J. Immunol., **194(5)**, 2268-2279 (2015)*

The costimulatory molecule CD40 enhances immunity through several distinct roles in T cell activation and T cell interaction with other immune cells. In a mouse model of immunity to liver stage *Plasmodium* infection, CD40 was critical for the full maturation of liver dendritic cells, accumulation of $CD8^+$ T cells in the liver, and protective immunity induced by immunization with the *Plasmodium yoelii fabb/f*$^-$ genetically attenuated parasite. Using mixed adoptive transfers of polyclonal wild-type and CD40-deficient $CD8^+$ T cells into wild-type and CD40-deficient hosts, we evaluated the contributions to $CD8^+$ T cell immunity of CD40 expressed on host tissues including APC, compared with CD40 expressed on the $CD8^+$ T cells themselves. Most of the effects of CD40 could be accounted for by expression in the T cells' environment, including the accumulation of large numbers of $CD8^+$ T cells in the livers of immunized mice. Thus, protective immunity generated during immunization with *fabb/f*$^-$ was largely dependent on effective APC licensing via CD40 signaling.

4.1374 Resveratrol Encapsulated in Novel Fusogenic Liposomes Activates Nrf2 and Attenuates Oxidative

Stress in Cerebromicrovascular Endothelial Cells From Aged Rats
Csiszar, A., Csiszar, A., Pinto, J.T., Gautaqm, T., Kleusch, C., Hoffmann, B., Tucsek, Z., Toth, P., Sonntag, W. and Ungvari, Z.
J. Gerontol. A Biol. Sci. Med. Sci., ***70(3)****, 303-314 (2015)*

Resveratrol (3,4′,5-trihydroxystilbene) is a plant-derived polyphenolic trans-stilbenoid, which exerts multifaceted antiaging effects. Here, we propose a novel delivery system for resveratrol, which significantly increases its cellular uptake into aged cells. Combination of resveratrol with a positively charged lipid component to "conventional" liposomes converts these lipid vesicles to a robust fusogenic system. To study their cellular uptake and cellular effects, we treated primary cerebromicrovascular endothelial cells isolated from aged F344xBN rats with resveratrol encapsulated in fusogenic liposomes (FL-RSV). To demonstrate effective cellular uptake of FL-RSV, accumulation of the lipophilic tracer dye, DiR, and resveratrol in cerebromicrovascular endothelial cells was confirmed using flow cytometry and confocal microscopy and high-performance liquid chromatography electrochemical detection. Treatment of aged cerebromicrovascular endothelial cells with FL-RSV activated Nrf2 (assessed with a reporter gene assay), significantly decreased cellular production of reactive oxygen species (assessed by a flow cytometry-based H_2DCFDA fluorescence method), and inhibited apoptosis. Taken together, encapsulation of resveratrol into novel fusogenic liposomes significantly enhances the delivery of resveratrol into aged cells, which subsequently results in rapid activation of cellular Nrf2-driven antioxidant defense mechanisms. Our studies provide proof-of-concept for the development of a novel, translationally relevant interventional strategy for prevention and/or control of oxidative stress-related pathophysiological conditions in aging.

4.1375 Comparison of surface modification chemistries in mouse, porcine, and human islets
SoRelle, J.A., kanak, M.A., Itoh, T., Horton, J.M., Naziruddin, B. and Kane, R.R.
J. Biomed. Mater. Res. part A, ***103A(3)****, 869-877 (2015)*

Beta cell replacement therapy, the transplantation of isolated pancreatic islets by intraportal infusion, offers patients with brittle type 1 diabetes blood glucose regulation with a minimally invasive technique. Chemical modification of islets prior to transplantation, providing a nanothin barrier that potentially includes active protective compounds, has been proposed as a strategy to minimize the inflammatory and immune reactions that often significantly limit graft function and duration. Chemical modification also has the potential to allow the use of alternative sources of islets, such as porcine islets, for transplantation. This investigation compared three orthogonal covalent islet modification techniques across three species (human, porcine, and murine), using multiple measures to determine biocompatibility and effectiveness. All three conjugation chemistries were well tolerated, and the overall efficiency, gross uniformity, and stability of the surface modifications were dependent upon the conjugation chemistry as well as the islet source (human, porcine, or murine). Notably, the reductive modification of surface disulfides was shown to afford intense and long-lasting modification of human islets. This study demonstrates that murine, human, and porcine islets tolerate a variety of covalent modifications, that these modifications are relatively stable, and that the murine islet model may not be predictive for some chemical contexts

4.1376 Use of Density Centrifugation for Delayed Cryopreservation of Stallion Sperm: Perform Sperm Selection Directly after Collection or after Storage?
Heutelbeck, A., Oldenhof, H., Rohn, K., Martinsson, G., Morrell, J.M. and Sieme, H.
Reprod. Dom. Anim., ***50(1)****, 76-83 (2015)*

Equipment for cryopreservation of stallion sperm is not always available. In such cases, diluted semen can be shipped to a facility for later cryopreservation. The aim of this study was to evaluate if selection of sperm via density centrifugation yields higher survival rates when cryopreservation is to be delayed (i.e. carried out after 1 day of storage at 5°C). Two-layer iodixanol as well as single-layer Androcoll density centrifugation were tested and compared with samples prepared with standard centrifugation. Special emphasis was placed on comparing centrifugation on the day of semen collection with centrifugation after 1-day refrigerated storage. Sperm morphology and motility as well as membrane and chromatin integrity were evaluated before and after centrifugation. Sperm motility and membrane integrity were also assessed after cryopreservation. It was found that both two- and single-layer density centrifugation processing resulted in higher percentages of morphologically normal and motile sperm with higher membrane and chromatin integrity, as compared to standard centrifugation or diluted samples. Differences were only in the order of magnitude of 5%. Recovery rates after density centrifugation were only approximately 30–

40%. When cryopreservation was carried out after 1-day refrigerated storage, centrifugation processing of sperm directly after semen collection resulted in higher percentages of plasma membrane intact sperm post-thaw as compared to performing centrifugation processing of stored sperm just prior to cryopreservation. No significant differences in progressively motile sperm post-thaw were seen. Taken together, for delayed cryopreservation, it is best to perform density centrifugation directly after collection rather than immediately prior to cryopreservation.

4.1377 Dual characterization of biological cells by optofluidic microscope and resistive pulse sensor
Guo, J., Chen, L., Huang, X., Li, C.M., Ai, Y. and kang, Y.
*Electrophoresis, **36(3)**, 420-423 (2015)*

Label-free detection technique has emerged as a powerful platform for biomedical applications since it can avoid laborious multi-step sample preparation. In this paper, we demonstrate a dual analysis of biological cells using a single microfluidic system combining optofluidic microscopy and resistive pulse sensing. Both red blood cells (RBCs) and circulating tumor cells (CTCs) have been used to validate the concept of dual analysis and also to test the performance of the microfluidic device. The cell characterization by resistive pulse sensing is in good agreement with the analysis by optofluidic microscopy, further verified by the commercial Beckman-Coulter® FC500 flow cytometry. The present system has attractive merits such as simple fabrication, easy integration, high portability, and low cost. This study has great potentials for the development of innovative on-chip flow cytometry with concurrent imaging sensing and resistive sensing.

4.1378 Generation, cryopreservation, function and in vivo persistence of ex vivo expanded cynomolgus monkey regulatory T cells
Guo, H., Zhang, H., Lu, L., Ezzelarab, M.B. and Thomson, A.W.
*Cell. Immunol., **295**, 19-28 (2015)*

We expanded flow-sorted Foxp3$^+$ cynomolgus monkey regulatory T cells (Treg) >1000-fold after three rounds of stimulation with anti-CD3 mAb-loaded artificial antigen-presenting cells, rapamycin (first round only) and IL-2. The expanded Treg maintained their expression of Treg signature markers, CD25, CD27, CD39, Foxp3, Helios, and CTLA-4, as well as CXCR3, which plays an important role in T cell migration to sites of inflammation. In contrast to expanded effector T cells (Teff), expanded Treg produced minimal IFN-γ and IL-17 and no IL-2 and potently suppressed Teff proliferation. Following cryopreservation, thawed Treg were less viable than their freshly-expanded counterparts, although no significant changes in phenotype or suppressive ability were observed. Additional rounds of stimulation/expansion restored maximal viability. Furthermore, adoptively-transferred autologous Treg expanded from cryopreserved second round stocks and labeled with CFSE or VPD450 were detected in blood and secondary lymphoid tissues of normal or immunosuppressed recipients at least two months after their systemic infusion.

4.1379 Identifying the primary site of pathogenesis in amyotrophic lateral sclerosis – vulnerability of lower motor neurons to proximal excitotoxicity
Blizzard, C.A., Southam, K.A., Dawkins, E., Lewis, K.E., King, A.E., Clark, J.A. and Dickson, ST.C.
*Diseases Modles & Mechanisms, **8**, 215-224 (2015)*

There is a desperate need for targeted therapeutic interventions that slow the progression of amyotrophic lateral sclerosis (ALS). ALS is a disorder with heterogeneous onset, which then leads to common final pathways involving multiple neuronal compartments that span both the central and peripheral nervous system. It is believed that excitotoxic mechanisms might play an important role in motor neuron death in ALS. However, little is known about the mechanisms by which excitotoxicity might lead to the neuromuscular junction degeneration that characterizes ALS, or about the site at which this excitotoxic cascade is initiated. Using a novel compartmentalised model of site-specific excitotoxin exposure in lower motor neurons in vitro, we found that spinal motor neurons are vulnerable to somatodendritic, but not axonal, excitotoxin exposure. Thus, we developed a model of somatodendritic excitotoxicity in vivo using osmotic mini pumps in Thy-1-YFP mice. We demonstrated that in vivo cell body excitotoxin exposure leads to significant motor neuron death and neuromuscular junction (NMJ) retraction. Using confocal real-time live imaging of the gastrocnemius muscle, we found that NMJ remodelling preceded excitotoxin-induced NMJ degeneration. These findings suggest that excitotoxicity in the spinal cord of individuals with ALS might result in a die-forward mechanism of motor neuron death from the cell body outward, leading to initial distal plasticity, followed by subsequent pathology and degeneration.

4.1380 **Physiological Electrical Signals Promote Chain Migration of Neuroblasts by Up-Regulating P2Y1 Purinergic Receptors and Enhancing Cell Adhesion**
Cao, L., Pu, J., Scott, R.H., CHing, J. and McCaig, C.D.
Stem Cell Rev. and Rep., ***11****, 75-86 (2015)*

Neuroblasts migrate as directed chains of cells during development and following brain damage. A fuller understanding of the mechanisms driving this will help define its developmental significance and in the refinement of strategies for brain repair using transplanted stem cells. Recently, we reported that in adult mouse there are ionic gradients within the extracellular spaces that create an electrical field (EF) within the rostral migratory stream (RMS), and that this acts as a guidance cue for neuroblast migration. Here, we demonstrate an endogenous EF in brain slices and show that mimicking this by applying an EF of physiological strength, switches on chain migration in mouse neurospheres and in the SH-SY5Y neuroblastoma cell line. Firstly, we detected a substantial endogenous EF of 31.8 ± 4.5 mV/mm using microelectrode recordings from explants of the subventricular zone (SVZ). Pharmacological inhibition of this EF, effectively blocked chain migration in 3D cultures of SVZ explants. To mimic this EF, we applied a physiological EF and found that this increased the expression of N-cadherin and β-catenin, both of which promote cell-cell adhesion. Intriguingly, we found that the EF up-regulated P2Y purinoceptor 1 (P2Y1) to contribute to chain migration of neuroblasts through regulating the expression of N-cadherin, β-catenin and the activation of PKC. Our results indicate that the naturally occurring EF in brain serves as a novel stimulant and directional guidance cue for neuronal chain migration, via up-regulation of P2Y1.

4.1381 **Blood cell transcriptomic-based early biomarkers of adverse programming effects of gestational calorie restriction and their reversibility by leptin supplementation**
Konieczna, J., Sanchez, J., Palou, M., Pico, C. and Palou, A.
Scientific Reports, ***5****:9088 (2015)*

The challenge of preventing major chronic diseases requires reliable, early biomarkers. Gestational mild undernutrition in rats is enough to program the offspring to develop later pathologies; the intake of leptin, a breastmilk component, during lactation may reverse these programming effects. We used these models to identify, in peripheral blood mononuclear cells (PBMCs), transcriptomic-based early biomarkers of programmed susceptibility to later disorders, and explored their response to neonatal leptin intake. Microarray analysis was performed in PBMCs from the offspring of control and 20% gestational calorie-restricted dams (CR), and CR-rats supplemented with physiological doses of leptin throughout lactation. Notably, leptin supplementation normalised 218 of the 224 mRNA-levels identified in PBMCs associated to undernutrition during pregnancy. These markers may be useful for early identification and subsequent monitoring of individuals who are at risk of later diseases and would specifically benefit from the intake of appropriate amounts of leptin during lactation.

4.1382 **Lipolysis of Visceral Adipocyte Triglyceride by Pancreatic Lipases Converts Mild Acute Pancreatitis to Severe Pancreatitis Independent of Necrosis and Inflammation**
Patel, K., Trivedi, R.N., Durgampudi, C., Noel, P:, Cline, R.A., DeLany, J.P., navina, S. and Singh, V.P:
Am. J. Pathol., ***185(3)****, 808-819 (2015)*

Visceral fat necrosis has been associated with severe acute pancreatitis (SAP) for over 100 years; however, its pathogenesis and role in SAP outcomes are poorly understood. Based on recent work suggesting that pancreatic fat lipolysis plays an important role in SAP, we evaluated the role of pancreatic lipases in SAP-associated visceral fat necrosis, the inflammatory response, local injury, and outcomes of acute pancreatitis (AP). For this, cerulein pancreatitis was induced in lean and obese mice, alone or with the lipase inhibitor orlistat and parameters of AP induction (serum amylase and lipase), fat necrosis, pancreatic necrosis, and multisystem organ failure, and inflammatory response were assessed. Pancreatic lipases were measured in fat necrosis and were overexpressed in 3T3-L1 cells. We noted obesity to convert mild cerulein AP to SAP with greater cytokines, unsaturated fatty acids (UFAs), and multisystem organ failure, and 100% mortality without affecting AP induction or pancreatic necrosis. Increased pancreatic lipase amounts and activity were noted in the extensive visceral fat necrosis of dying obese mice. Lipase inhibition reduced fat necrosis, UFAs, organ failure, and mortality but not the parameters of AP induction. Pancreatic lipase expression increased lipolysis in 3T3-L1 cells. We conclude that UFAs generated via lipolysis of visceral fat by pancreatic lipases convert mild AP to SAP independent of pancreatic necrosis and the inflammatory response.
Supported by grants from the Clinical Translational Science Institute (RO1DK092460 to V.P.S.) and the NIH (UL1RR024153 and UL1TR000005; V.P.S., S.N.). This project used the UPCI Cancer Biomarkers

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4.1383 Complement Protein C1q Modulates Neurite Outgrowth In Vitro and Spinal Cord Axon Regeneration In Vivo

Peterson, S.L., Nguyen, H.X., Mendez, O.A. and Anderson, A.J:
J. Neurosci., ***35(10)***, *4332-4349 (2015)*

Traumatic injury to CNS fiber tracts is accompanied by failure of severed axons to regenerate and results in lifelong functional deficits. The inflammatory response to CNS trauma is mediated by a diverse set of cells and proteins with varied, overlapping, and opposing effects on histological and behavioral recovery. Importantly, the contribution of individual inflammatory complement proteins to spinal cord injury (SCI) pathology is not well understood. Although the presence of complement components increases after SCI in association with axons and myelin, it is unknown whether complement proteins affect axon growth or regeneration. We report a novel role for complement C1q in neurite outgrowth *in vitro* and axon regrowth after SCI. In culture, C1q increased neurite length on myelin. Protein and molecular assays revealed that C1q interacts directly with myelin associated glycoprotein (MAG) in myelin, resulting in reduced activation of growth inhibitory signaling in neurons. In agreement with a C1q-outgrowth-enhancing mechanism in which C1q binding to MAG reduces MAG signaling to neurons, complement C1q blocked both the growth inhibitory and repulsive turning effects of MAG *in vitro*. Furthermore, C1q KO mice demonstrated increased sensory axon turning within the spinal cord lesion after SCI with peripheral conditioning injury, consistent with C1q-mediated neutralization of MAG. Finally, we present data that extend the role for C1q in axon growth and guidance to include the sprouting patterns of descending corticospinal tract axons into spinal gray matter after dorsal column transection SCI.

4.1384 Protein kinase CK2 enables regulatory T cells to suppress excessive TH2 responses in vivo

Ulges, A. et al
Nature Immunol., ***16(3)***, *267-275 (2015)*

The quality of the adaptive immune response depends on the differentiation of distinct $CD4^+$ helper T cell subsets, and the magnitude of an immune response is controlled by $CD4^+Foxp3^+$ regulatory T cells (T_{reg} cells). However, how a tissue- and cell type–specific suppressor program of T_{reg} cells is mechanistically orchestrated has remained largely unexplored. Through the use of T_{reg} cell–specific gene targeting, we found that the suppression of allergic immune responses in the lungs mediated by T helper type 2 (T_H2) cells was dependent on the activity of the protein kinase CK2. Genetic ablation of the β-subunit of CK2 specifically in T_{reg} cells resulted in the proliferation of a hitherto-unexplored $ILT3^+$ T_{reg} cell subpopulation that was unable to control the maturation of $IRF4^+PD\text{-}L2^+$ dendritic cells required for the development of T_H2 responses *in vivo*.

4.1385 Abnormal erythroid maturation leads to microcytic anemia in the TSAP6/Steap3 null mouse model

Blanc, L., Papoin, J., Debnath, G., Vidal, M., Amson, R., Telerman, A., An, X. and Mohandas, N.
Am. J. Hematol., ***90(3)***, *235-241 (2015)*

Genetic ablation of the ferrireductase STEAP3, also known as TSAP6, leads to severe microcytic and hypochromic red cells with moderate anemia in the mouse. However, the mechanism leading to anemia is poorly understood. Previous results indicate that TSAP6/*Steap3* is a regulator of exosome secretion. Using TSAP6/*Steap3* knockout mice, we first undertook a comprehensive hematologic characterization of the red cell compartment, and confirmed a dramatic decrease in the volume and hemoglobin content of these erythrocytes. We observed marked anisocytosis as well as the presence of fragmenting erythrocytes. Consistent with these observations, we found by ektacytometry decreased membrane mechanical stability of knockout red cells. However, we were unable to document significant changes in the expression levels of the major skeletal and transmembrane proteins to account for this decrease in the membrane stability. Furthermore, there were no differences in red cell survival between wild type and knockout animals. However, when we monitored erythropoiesis, we found a decreased number of proerythroblasts in the bone marrow of TSAP6/*Steap3*$^{-/-}$ animals. In addition, progression from the proerythroblastic to the orthochromatic stage was affected, with accumulation of cells at the polychromatic stage. Altogether, our findings demonstrate that abnormal erythroid maturation is the main cause of anemia in these mice

4.1386 The presence of interleukin-27 during monocyte-derived dendritic cell differentiation promotes

improved antigen processing and stimulation of T cells
Jung, J-Y., Roberts, L.L. and Robinson, C.M.
Immunology, ***144(4)***, *649-660 (2015)*

Dendritic cells (DCs) are potent antigen-presenting cells necessary to establish effective adaptive immune responses. The cytokine environment that exists at the time of DC differentiation may be an important but often ignored determinant in the phenotypic and functional properties of DCs. Interleukin-27 (IL-27) is a unique cytokine that has both inflammatory and immune suppressive activities. Although it can both promote and oppose activity of different T-cell subsets, mostly anti-inflammatory activity has been described toward macrophages and DCs. However, the specific effect of IL-27 during DC differentiation and how that may change the nature of the antigen-presenting cell has not been investigated. In this report, we show that IL-27 treatment during monocyte-derived DC differentiation enhanced the ability to process antigens and stimulate T-cell activity. DCs differentiated in the presence of IL-27 showed enhanced acidification of latex bead-containing phagosomes that was consistent with elevated expression of vacuolar-ATPases. This resulted in inhibition of intracellular growth of *Staphylococcus aureus*. In addition, the levels of MHC class II surface expression were higher in DCs differentiated in the presence of IL-27. Production of IL-12 was also significantly increased during *S. aureus* infection of IL-27-differentiated DCs. The net effect of these activities was enhanced $CD4^+$ T-cell proliferation and T helper type 1 cytokine production. These findings are important to a wide number of immunological contexts and should be considered in the development of future vaccines.

4.1387 Testosterone Suppresses Hepatic Inflammation by the Downregulation of IL-17, CXCL-9, and CXCL-10 in a Mouse Model of Experimental Acute Cholangitis
Schwinge, D., Carambia, A., Quaas, A., Krech, T., Wegscheid, CC., Tiegs, G., Prinz, I., Lohse, A.W., Herkel, J. and Schramm, C.
J. Immunol., ***194(6)***, *2522-2530 (2015)*

Autoimmune liver diseases predominantly affect women. In this study, we aimed to elucidate how sex affects autoimmune hepatic inflammation. Acute experimental cholangitis was induced by adoptive transfer of OVA-specific $CD8^+$ T cells into mice, which express the cognate Ag on cholangiocytes. In contrast to previous mouse models of cholangitis, this model displayed a strong sexual dimorphism: female mice developed marked cholangitis, whereas male mice were resistant to cholangitis induction. The recruitment of endogenous $CD4^+$ T cells, but not transferred $CD8^+$ T cells into female livers was strongly increased. These cells expressed higher amounts of the proinflammatory cytokine IL-17, which was at least in part responsible for the liver inflammation observed. The recruitment of endogenous $CD4^+$ T cells was associated with increased expression of the chemokines CXCL-9 and CXCL-10 in female livers. The sex-specific factor responsible for the observed differences was found to be testosterone: male mice could be rendered susceptible to liver inflammation by castration, and testosterone treatment was sufficient to completely suppress liver inflammation in female mice. Accordingly, testosterone treatment of female mice significantly reduced the expression of IL-17A, CXCL-9, and CXCL-10 within the liver. Serum testosterone levels of untreated mice negatively correlated with the IL-17, CXCL-9, and CXCL-10 expression in the liver, further supporting a role for testosterone in hepatic immune homeostasis. In conclusion, testosterone was found to be the major determinant of the observed sexual dimorphism. Further study into the role of testosterone for liver inflammation could lead to novel treatment targets in human autoimmune liver diseases.

4.1388 Disrupting Protein Expression with Peptide Nucleic Acids Reduces Infection by Obligate Intracellular Rickettsia
Pelc, R., McClure, J.C., kaur, S.J., Sears, K., Rahman, M.S. and Ceraul, S.M.
PloS One, ***10(3)***, *e119283 (2015)*

Peptide Nucleic Acids (PNAs) are single-stranded synthetic nucleic acids with a pseudopeptide backbone in lieu of the phosphodiester linked sugar and phosphate found in traditional oligos. PNA designed complementary to the bacterial Shine-Dalgarno or start codon regions of mRNA disrupts translation resulting in the transient reduction in protein expression. This study examines the use of PNA technology to interrupt protein expression in obligate intracellular *Rickettsia* sp. Their historically intractable genetic system limits characterization of protein function. We designed PNA targeting mRNA for *rOmpB* from *Rickettsia typhi* and *rickA* from *Rickettsia montanensis*, ubiquitous factors important for infection. Using an *in vitro* translation system and competitive binding assays, we determined that our PNAs bind target regions. Electroporation of *R. typhi* and *R. montanensis* with PNA specific to *rOmpB* and *rickA*,

respectively, reduced the bacteria's ability to infect host cells. These studies open the possibility of using PNA to suppress protein synthesis in obligate intracellular bacteria.

4.1389 High Expression Level of Tra2-β1 Is Responsible for Increased SMN2 Exon 7 Inclusion in the Testis of SMA Mice
Chen, Y-C., Chang, J-G., Jong, Y-J., Liu, T-Y. and You, C-Y.
*PloS One, **10(3)**, e120721 (2015)*

Spinal muscular atrophy (SMA) is an inherited neuromuscular disease caused by deletion or mutation of *SMN1* gene. All SMA patients carry a nearly identical *SMN2* gene, which produces low level of SMN protein due to mRNA exon 7 exclusion. Previously, we found that the testis of SMA mice (*smn*$^{-/-}$ *SMN2*) expresses high level of *SMN2* full-length mRNA, indicating a testis-specific mechanism for *SMN2* exon 7 inclusion. To elucidate the underlying mechanism, we established primary cultures of testis cells from SMA mice and analyzed them for *SMN2* exon 7 splicing. We found that primary testis cells after a 2-hour culture still expressed high level of *SMN2* full-length mRNA, but the level decreased after longer cultures. We then compared the protein levels of relevant splicing factors, and found that the level of Tra2-β1 also decreased during testis cell culture, correlated with *SMN2* full-length mRNA downregulation. In addition, the testis of SMA mice expressed the highest level of Tra2-β1 among the many tissues examined. Furthermore, overexpression of Tra2-β1, but not ASF/SF2, increased *SMN2* minigene exon 7 inclusion in primary testis cells and spinal cord neurons, whereas knockdown of Tra2-β1 decreased *SMN2* exon 7 inclusion in primary testis cells of SMA mice. Therefore, our results indicate that high expression level of Tra2-β1 is responsible for increased *SMN2* exon 7 inclusion in the testis of SMA mice. This study also suggests that the expression level of Tra2-β1 may be a modifying factor of SMA disease and a potential target for SMA treatment.

4.1390 Monocyte mediated brain targeting delivery of macromolecular drug for the therapy of depression
Qin, J., Yang, X., Zhang, R-X., Luo, Y-X., Li, J-L., Hou, J., Zhang, C., Li, Y-J., Shi, J., Lu, L., Wang, J-X. and Xhu, W.L.
*Nanomedicine: Nanothechnology, Biology, and Medicine, **11**, 391-400 (2015)*

Leukocytes can cross intact blood-brain barrier under healthy conditions and in many neurological diseases, including psychiatric diseases. In present study, a cyclic RGD (cRGD) peptide with high affinity for integrin receptors of leukocytes was used to modify liposomes. The cRGD-modified liposomes (cRGDL) showed high affinity for monocytes *in vitro* and *in vivo* and co-migrated across *in vitro* BBB model with THP-1. The trefoil factor 3 (TFF3), a macromolecular drug, was rapidly and persistently delivered to brain for at least 12 h when loaded into cRGDL while 2.8-fold increase in drug concentration in basolateral amygdala regions related to depression was observed. A systemic administration of cRGDL-TFF3 mimicked antidepressant-like effect of direct intra-basolateral amygdala administration of TFF3 solution in rats subjected to chronic mild stress. The effective dual-brain targeting delivery resulting from the combination and co-migration of cRGDL with leukocyte cross BBB may be a promising strategy for targeted brain delivery.

4.1391 Transcriptome changes upon in vitro challenge with Mycobacterium bovis in monocyte-derived macrophages from bovine tuberculosis-infected and healthy cows
Lin, J., Zhao, D., Wang, J., Wang, Y., Li, H., Yin, X., Yang, L. and Zhou, X.
*Veterinary Immunology and Immunopathology, **163**, 146-156 (2015)*

As innate immune cells, macrophages are expected to respond to mycobacterial infection equally in both *Mycobacterium bovis*-infected cows and healthy cows. We previously found that monocyte-derived macrophages (MDMs) from *M. bovis*-infected cows respond differently than MDMs from healthy cows when exposed to *in vitro M. bovis* challenge. We have now used the Agilent™ Bovine Gene Expression Microarray to examine transcriptional differences between these MDMs. At a high multiplicity of infection (10), *in vitro* challenge led to changes in several thousands of genes, with dysregulation at multiple orders of magnitude. For example, significant changes were seen for colony stimulating factor 3 (granulocyte) (*CSF3*), colony stimulating factor 2 (granulocyte-macrophage) (*CSF2*), and chemokine (C-C motif) ligand 20 (*CCL20*). Classical macrophage activation was also observed, although to a lesser degree in interleukin 12 (*IL12*) expression. For macrophages, kallikrein-related peptidase 12 (*KLK12*) and protease, serine, 2 (trypsin 2) (*PRSS2*), as well as a secreted protein, acidic, cysteine-rich (osteonectin) (*SPARC*)-centered matricellular gene network, were differentially expressed in infected animals. Finally, global transcriptome

fold-changes caused by *in vitro* challenge were higher in healthy cows than in tuberculosis-positive cows, suggesting that healthy macrophages responded marginally better to *in vitro* infection. Macrophages from healthy and already infected animals can both be fully activated during *M. bovis* infection, yet there are differences between these macrophages: distinct expression pattern in matricellular proteins, and their different responses to *in vitro* infection.

4.1392 5-HT4 Receptor Subtype, β-Arrestin Level, and Rapid-Onset Effects of Antidepressant Drugs
Mendez-David, I., David, ; D.J., Guilloux, J-P., Hen, R. and Gardier, A.M.
*Neuromethods, **95**, 101-121 (2015)*

Understanding the pathophysiology of affective disorders and their treatment relies on the availability of experimental models that accurately mimic aspects of the disease. The use of exogenously administered corticosterone (CORT model) can mimic the effects of a chronic stress and has been validated as an animal model to study disease states displaying some hallmark characteristics of anxiety and depression observed in patients.
Recently, we have adapted the CORT model protocol to screen for rapid-onset drugs to treat anxiety/depression disorders. In spite of the fact that selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of depression and several anxiety disorders, the onset of action of SSRIs is often delayed by 3–6 weeks. The existence of this delayed action combined with the fact that one-third of patients do not respond to treatment emphasizes the need for faster acting and more effective antidepressants. This chapter gives laboratory protocols including step-by-step recommendations to explain how the CORT model in mice can be used to screen for candidate drugs. For this purpose we examined the behavioral and cellular effects of a 5-HT_4 receptor ligand, RS67333, and compared it with the SSRI, fluoxetine. Likewise, we emphasize that mononuclear cells (PBMCs) isolated from whole blood in corticosterone-treated mice could serve as a marker of treatment response(s) and fast onset of drug action in the mouse CORT model.

4.1393 Andrographolide inhibits intracellular Chlamydia trachomatis multiplication and reduces secretion of proinflammatory mediators produced by human epithelial cells
Hua, Z., Frolich, K.M., Zhang, Y., Feng, X., Zhang, J. and Shen, L.
Pathog. Dis., 73, 1-11 (2015)

Chlamydia trachomatis is the most common sexually transmitted bacterial disease worldwide. Untreated C. trachomatis infections may cause inflammation and ultimately damage tissues. Here, we evaluated the ability of Andrographolide (Andro), a natural diterpenoid lactone component of Andrographis paniculata, to inhibit C. trachomatis infection in cultured human cervical epithelial cells. We found that Andro exposure inhibited C. trachomatis growth in a dose- and time-dependent manner. The greatest inhibitory effect was observed when exponentially growing C. trachomatis was exposed to Andro. Electron micrographs demonstrated the accumulation of unusual, structurally deficient chlamydial organisms, correlated with a decrease in levels of OmcB expressed at the late stage of infection. Additionally, Andro significantly reduced the secretion of interleukin6, CXCL8 and interferon-γ-induced protein10 produced by host cells infected with C. trachomatis. These results indicate the efficacy of Andro to perturb C. trachomatis transition from the metabolically active reticulate body to the infectious elementary body and concurrently reduce the production of a proinflammatory mediator by epithelial cells in vitro. Further dissection of Andro's anti-Chlamydia action may provide identification of novel therapeutic targets.

4.1394 Alpha-2 agonist attenuates ischemic injury in spinal cord neurons
Freeman, K.A., Puskas, F., Bell, M.T., Mares, J.M., Foley, L.S., Weyant, M.J., Cleveland, J.C., Fullerton, D.A., Meng, X., Herson, P.S. and Reece, T.B:
*J. Surg. Res., **195**, 21-28 (2015)*

Background
Paraplegia secondary to spinal cord ischemia–reperfusion injury remains a devastating complication of thoracoabdominal aortic intervention. The complex interactions between injured neurons and activated leukocytes have limited the understanding of neuron-specific injury. We hypothesize that spinal cord neuron cell cultures subjected to oxygen-glucose deprivation (OGD) would simulate ischemia–reperfusion injury, which could be attenuated by specific alpha-2a agonism in an Akt-dependent fashion.
Materials and methods
Spinal cords from perinatal mice were harvested, and neurons cultured *in vitro* for 7–10 d. Cells were pretreated with 1 μM dexmedetomidine (Dex) and subjected to OGD in an anoxic chamber. Viability was

determined by MTT assay. Deoxyuridine-triphosphate nick-end labeling staining and lactate dehydrogenase (LDH) assay were used for apoptosis and necrosis identification, respectively. Western blot was used for protein analysis.
Results
Vehicle control cells were only 59% viable after 1 h of OGD. Pretreatment with Dex significantly preserves neuronal viability with 88% viable ($P < 0.05$). Dex significantly decreased apoptotic cells compared with that of vehicle control cells by 50% ($P < 0.05$). Necrosis was not significantly different between treatment groups. Mechanistically, Dex treatment significantly increased phosphorylated Akt ($P < 0.05$), but protective effects of Dex were eliminated by an alpha-2a antagonist or Akt inhibitor ($P < 0.05$).
Conclusions
Using a novel spinal cord neuron cell culture, OGD mimics neuronal metabolic derangement responsible for paraplegia after aortic surgery. Dex preserves neuronal viability and decreases apoptosis in an Akt-dependent fashion. Dex demonstrates clinical promise for reducing the risk of paraplegia after high-risk aortic surgery.

4.1395 Plasma bioavailability and changes in PBMC gene expression after treatment of ovariectomized rats with a commercial soy supplement
Islam, M.A., Hooiveld, G.J.E.J., van der berg, J.H.J., Boekschoten, M.V., van der Velpen, V., Murk, A.J., Rietjens, I.M.C.M. and van Leewen, F.X.R.
*Toxicology Reports, **2**, 308-321 82015)*

The health effects of soy supplementation in (post)menopausal women are still a controversial issue. The aim of the present study was to establish the effect of the soy isoflavones (SIF) present in a commercially available supplement on ovariectomized rats and to investigate whether these rats would provide an adequate model to predict effects of SIF in (post)menopausal women. Two dose levels (i.e. 2 and 20 mg/kg b.w.) were used to characterize plasma bioavailability, urinary and fecal concentrations of SIF and changes in gene expression in peripheral blood mononuclear cells (PBMC). Animals were dosed at 0 and 48 h and sacrificed 4 h after the last dose. A clear dose dependent increase of SIF concentrations in plasma, urine and feces was observed, together with a strong correlation in changes in gene expression between the two dose groups. All estrogen responsive genes and related biological pathways (BPs) that were affected by the SIF treatment were regulated in both dose groups in the same direction and indicate beneficial effects. However, in general no correlation was found between the changes in gene expression in rat PBMC with those in PBMC of (post)menopausal women exposed to a comparable dose of the same supplement. The outcome of this short-term study in rats indicates that the rat might not be a suitable model to predict effects of SIF in humans. Although the relative exposure period in this rat study is comparable with that of the human study, longer repetitive administration of rats to SIF may be required to draw a final conclusion on the suitability of the rat a model to predict effects of SIF in humans.

4.1396 Retinoic Acid Can Exacerbate T Cell Intrinsic TLR2 Activation to Promote Tolerance
Nguyen, V., Pearson, K., Kim, J-H., Kamdar, K. and DePaolo, W.
*PloS One, **10(3)**, e0118875 (2015)*

The contribution of vitamin A to immune health has been well established. However, recent evidence indicates that its active metabolite, retinoic acid (RA), has the ability to promote both tolerogenic and inflammatory responses. While the outcome of RA-mediated immunity is dependent upon the immunological status of the tissue, the contribution of specific innate signals influencing this response have yet to be delineated. Here, we found that treatment with RA can dampen inflammation during intestinal injury. Importantly, we report a novel and unexpected requirement for TLR2 in RA-mediated suppression. Our data demonstrate that RA treatment enhances TLR2-dependent IL-10 production from T cells and this, in turn, potentiates T regulatory cell (T_{REG}) generation without the need for activation of antigen presenting cells. These data also suggest that combinatorial therapy using RA and TLR2 ligands may be advantageous in the design of therapies to treat autoimmune or inflammatory disease.

4.1397 Sprouty2 in the Dorsal Hippocampus Regulates Neurogenesis and Stress Responsiveness in Rats
Dow, A.L., Lin, T.V., Chartoff, E.H., Potter, D., McPhie, D.L., Van't Veer, A.V., Knoll, A.T., Lee, K.N., Neve, R.L., Patel, T.B., Ongur, D., Cohen, B.M. and Carlezon Jr., W.A.
*PloS One, **10(3)**, e0120693 (2015)*

Both the development and relief of stress-related psychiatric conditions such as major depression (MD)

and post-traumatic stress disorder (PTSD) have been linked to neuroplastic changes in the brain. One such change involves the birth of new neurons (neurogenesis), which occurs throughout adulthood within discrete areas of the mammalian brain, including the dorsal hippocampus (HIP). Stress can trigger MD and PTSD in humans, and there is considerable evidence that it can decrease HIP neurogenesis in laboratory animals. In contrast, antidepressant treatments increase HIP neurogenesis, and their efficacy is eliminated by ablation of this process. These findings have led to the working hypothesis that HIP neurogenesis serves as a biomarker of neuroplasticity and stress resistance. Here we report that local alterations in the expression of Sprouty2 (SPRY2), an intracellular inhibitor of growth factor function, produces profound effects on both HIP neurogenesis and behaviors that reflect sensitivity to stressors. Viral vector-mediated disruption of endogenous Sprouty2 function (via a dominant negative construct) within the dorsal HIP of adult rats stimulates neurogenesis and produces signs of stress resilience including enhanced extinction of conditioned fear. Conversely, viral vector-mediated elevation of SPRY2 expression intensifies the behavioral consequences of stress. Studies of these manipulations in HIP primary cultures indicate that SPRY2 negatively regulates fibroblast growth factor-2 (FGF2), which has been previously shown to produce antidepressant- and anxiolytic-like effects via actions in the HIP. Our findings strengthen the relationship between HIP plasticity and stress responsiveness, and identify a specific intracellular pathway that could be targeted to study and treat stress-related disorders.

4.1398 Recommendations for the use of the non-obese diabetic/severe combined immunodeficiency mouse model in autoimmune and drug-induced thrombocytopenia: communication from the SSC of the ISTH

Backhoul, T., Fuhrmann, J., Chong, B.H., Boougie, D. and Aster, R.

*J. Thrombosis and Haemostasis, **13**, 1-4 (2015)*

Human platelet survival studies have been hampered by the lack of a suitable animal model. Transfusion of human platelets into immunocompetent animals leads to the rapid destruction of these platelets by naturally occurring xenoantibodies. The non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mouse lacks T and B cells, and therefore lack natural antibodies that could destroy infused human platelets [1]. Because of this property, human platelets given to the mouse intravenously circulate for several days, permitting the model to be used for testing the ability of human antibodies to cause platelet destruction *in vivo* [2-7]. Preliminary studies have demonstrated the usefulness of the NOD/SCID mouse model for monitoring the survival and immune destruction of human platelets. However, differences exist between the research groups regarding the method of platelet injection, the amount and route of antibody injection, and the preparation of blood samples collected from the animal, making the results poorly comparable.

Basically, in all laboratories, resting human platelets are injected intravenously into the mouse circulation, where they can, in the absence of platelet-reactive antibodies, circulate for up to 48 h [8]. After estimatation of a baseline value (100%) of human platelets, platelet-reactive antibodies (with or without drug administration) can be infused. The impact of these antibodies on the survival of human platelets can then be analyzed by taking blood samples from the mouse over time [8].

Methodological details that require attention in this model include: platelet preparation and resuspension in plasma or ‘synthetic plasma’; the concentration and volume of applied analytes (platelet, antibody, or drug); the route of platelet injection (retro-orbital injection or tail vein injection); and the route of antibody injection (intravenous or intraperitoneal). The method of data capture, including time points of blood sampling and subsequent sample preparation for analysis, the percentage of circulating human platelets, and software details, should also be reported in detail. Additional steps required to answer the scientific questions, e.g. platelet preincubation with a drug of interest or an antibody in pooled plasma or ‘synthetic plasma’, should also be reported [2, 9]. Surprisingly, application procedures and the amount of injected platelets and antibodies have been only loosely defined, and standardization is necessary in order to improve the reproducibility of the procedures and to enable reliable comparison of the results.

This report is not didactic in relation to how to measure the survival of human platelets with the NOD/SCID mouse model. Its purpose is to suggest standardized procedures and to define variables that should be considered when presenting methodology in published reports. The presented procedures were introduced and discussed during the meetings of the Subcommittee of Platelet Immunology of the Scientific and Standardization Committee (SSC) in Liverpool 2012 and Milwaukee 2014. Suggestions were introduced to the SSC members and the presented recommendations had unanimous agreement. Adopting these recommendations will be of advantage for investigators and laboratories to reduce imprecision and harmonize results, and will allow other laboratories to readily reproduce reported methods and findings and interpret results appropriately.

4.1399 Bmp6 Expression in Murine Liver Non Parenchymal Cells: A Mechanism to Control their High Iron Exporter Activity and Protect Hepatocytes from Iron Overload?

Rausa, M., Pagani, A., Nai, A., campanella, A., Gilberti, M.E., Apostoli, P., Camaschella, C. and Silvestri, L.

*PloS One, **10(4)**, e0122696 (2015)*

Bmp6 is the main activator of hepcidin, the liver hormone that negatively regulates plasma iron influx by degrading the sole iron exporter ferroportin in enterocytes and macrophages. *Bmp6* expression is modulated by iron but the molecular mechanisms are unknown. Although hepcidin is expressed almost exclusively by hepatocytes (HCs), *Bmp6* is produced also by non-parenchymal cells (NPCs), mainly sinusoidal endothelial cells (LSECs). To investigate the regulation of *Bmp6* in HCs and NPCs, liver cells were isolated from adult wild type mice whose diet was modified in iron content in acute or chronic manner and in disease models of iron deficiency (*Tmprss6* KO mouse) and overload (*Hjv* KO mouse). With manipulation of dietary iron in wild-type mice, *Bmp6* and *Tfr1* expression in both HCs and NPCs was inversely related, as expected. When hepcidin expression is abnormal in murine models of iron overload (*Hjv* KO mice) and deficiency (*Tmprss6* KO mice), *Bmp6* expression in NPCs was not related to *Tfr1*. Despite the low *Bmp6* in NPCs from *Tmprss6* KO mice, *Tfr1* mRNA was also low. Conversely, despite body iron overload and high expression of *Bmp6* in NPCs from *Hjv* KO mice, *Tfr1* mRNA and protein were increased. However, in the same cells ferritin L was only slightly increased, but the iron content was not, suggesting that *Bmp6* in these cells reflects the high intracellular iron import and export. We propose that NPCs, sensing the iron flux, not only increase hepcidin through *Bmp6* with a paracrine mechanism to control systemic iron homeostasis but, controlling hepcidin, they regulate their own ferroportin, inducing iron retention or release and further modulating *Bmp6* production in an autocrine manner. This mechanism, that contributes to protect HC from iron loading or deficiency, is lost in disease models of hepcidin production.

4.1400 Adipose triglyceride lipase is involved in the mobilization of triglyceride and retinoid stores of hepatic stellate cells

Taschler, U., Schreiber, R., Chitraju, C., Grabner, G.F., Romauch, M., Wolinski, H., Haemmerle, G., Breinbauer, R., Zechner, R., Lass, A. and Zimmermann, R.

*Biochim. Biophys. Acta, **1851**, 937-945 (2015)*

Hepatic stellate cells (HSCs) store triglycerides (TGs) and retinyl ester (RE) in cytosolic lipid droplets. RE stores are degraded following retinoid starvation or in response to pathogenic stimuli resulting in HSC activation. At present, the major enzymes catalyzing lipid degradation in HSCs are unknown. In this study, we investigated whether adipose triglyceride lipase (ATGL) is involved in RE catabolism of HSCs. Additionally, we compared the effects of ATGL deficiency and hormone-sensitive lipase (HSL) deficiency, a known RE hydrolase (REH), on RE stores in liver and adipose tissue. We show that ATGL degrades RE even in the presence of TGs, implicating that these substrates compete for ATGL binding. REH activity was stimulated and inhibited by comparative gene identification-58 and G0/G1 switch gene-2, respectively, the physiological regulators of ATGL activity. In cultured primary murine HSCs, pharmacological inhibition of ATGL, but not HSL, increased RE accumulation. In mice globally lacking ATGL or HSL, RE contents in white adipose tissue were decreased or increased, respectively, while plasma retinol and liver RE levels remained unchanged. In conclusion, our study shows that ATGL acts as REH in HSCs promoting the degradation of RE stores in addition to its established function as TG lipase. HSL is the predominant REH in adipocytes but does not affect lipid mobilization in HSCs.

4.1401 Spinal cord protection via alpha-2 agonist-mediated increase in glial cell-line–derived neurotrophic factor

Freeman, K., Fullerton, D.A., Foley, L.S., Bell, M.T., Cleveland Jr., J.C., Weyant, M.J., Mares, J., Meng, X., Puskas, F. and Reece, T.B.

*J. Thorac. Cardiovasc. Surg., **149(2)**, 578-586 (2015)*

Objectives

Delayed paraplegia secondary to ischemia–reperfusion injury is a devastating complication of thoracoabdominal aortic surgery. Alpha-2 agonists have been shown to attenuate ischemia–reperfusion injury, but the mechanism for protection has yet to be elucidated. A growing body of evidence suggests that astrocytes play a critical role in neuroprotection by release of neurotrophins. We hypothesize that

alpha-2 agonism with dexmedetomidine increases glial cell-line–derived neurotrophic factor in spinal cord astrocytes to provide spinal cord protection.
Methods
Spinal cords were isolated en bloc from C57BL/6 mice, and primary spinal cord astrocytes and neurons were selected for and grown separately in culture. Astrocytes were treated with dexmedetomidine, and glial cell-line–derived neurotrophic factor was tested for by enzyme-linked immunosorbent assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to assess neuronal viability.
Results
Spinal cord primary astrocytes treated with dexmedetomidine at 1 μmol/L and 10 μmol/L had significantly increased glial cell-line–derived neurotrophic factor production compared with control ($P < .05$). Neurons subjected to oxygen glucose deprivation had significant preservation ($P < .05$) of viability with use of dexmedetomidine-treated astrocyte media. Glial cell-line–derived neurotrophic factor neutralizing antibody eliminated the protective effects of the dexmedetomidine-treated astrocyte media ($P < .05$).
Conclusions
Astrocytes have been shown to preserve neuronal viability via release of neurotrophic factors. Dexmedetomidine increases glial cell–derived neurotrophic factor from spinal cord astrocytes via the alpha-2 receptor. Treatment with alpha-2 agonist dexmedetomidine may be a clinical tool for use in spinal cord protection in aortic surgery.

4.1402 Vaccine molecules targeting Xcr1 on cross-presenting DCs induce protective CD8+ T-cell responses against influenza virus

Fossum, E., Grødeland, G., Terhorst, D., Tveita, A.A., Vikse, E., Mjaaland, S., Henri, S., Malissen, B. and Bogen, B.
Eur. J. Immunol., ***45(2)***, *624-635 (2015)*

Targeting antigens to cross-presenting dendritic cells (DCs) is a promising method for enhancing $CD8^+$ T-cell responses. However, expression patterns of surface receptors often vary between species, making it difficult to relate observations in mice to other animals. Recent studies have indicated that the chemokine receptor Xcr1 is selectively expressed on cross-presenting murine $CD8\alpha^+$ DCs, and that the expression is conserved on homologous DC subsets in humans ($CD141^+$ DCs), sheep ($CD26^+$ DCs), and macaques ($CADM1^+$ DCs). We therefore tested if targeting antigens to Xcr1 on cross-presenting DCs using antigen fused to Xcl1, the only known ligand for Xcr1, could enhance immune responses. Bivalent Xcl1 fused to model antigens specifically bound $CD8\alpha^+$ DCs and increased proliferation of antigen-specific T cells. DNA vaccines encoding dimeric Xcl1-hemagglutinin (HA) fusion proteins induced cytotoxic $CD8^+$ T-cell responses, and mediated full protection against a lethal challenge with influenza A virus. In addition to enhanced $CD8^+$ T-cell responses, targeting of antigen to Xcr1 induced $CD4^+$ Th1 responses and highly selective production of IgG2a antibodies. In conclusion, targeting of dimeric fusion vaccine molecules to $CD8\alpha^+$ DCs using Xcl1 represents a novel and promising method for induction of protective $CD8^+$ T-cell responses.

4.1403 Vitamin D counteracts fibrogenic TGF-β signalling in human hepatic stellate cells both receptor-dependently and independently

Beilfuss, A., Sowa, J-P., Sydor, S., Beste, M., Bechmann, L.P., Schlattjan, M., Syn, W-K., Wedemeyer, I., Mathe, Z., Jochum, C., Gerken, G., Gieseler, R.K. and Canbay, A.
Gut, ***64(5)***, *791-799 (2015)*

Objective Non-alcoholic fatty liver disease (NAFLD) is closely linked to obesity and constitutes part of the metabolic syndrome, which have been associated with low serum vitamin D (VD). Due to known crosstalk between VD and transforming growth factor (TGF)-β signalling, VD has been proposed as an antifibrotic treatment.
Design We evaluated the association between VD, the vitamin D receptor (VDR) and liver fibrosis in primary human hepatic stellate cells (phHSC) and 106 morbidly obese patients with NAFLD.
Results Treating phHSC with VD ameliorated TGF-β-induced fibrogenesis via both VDR-dependent and VDR-independent mechanisms. Reduction of fibrogenic response was abolished in cells homozygous for GG at the A1012G single nucleotide polymorphisms within the VDR gene. Compared with healthy livers, NAFLD livers expressed higher levels of VDR mRNA and VDR fragments. VDR mRNA was lower in patients homozygous for GG at A1012G and expression of pro-fibrogenic genes was higher in patients carrying the G allele.
Conclusions VD may be an antifibrotic treatment option early in the onset of fibrosis in specific genotypes for VDR. Known polymorphisms of the VDR may influence the response to VD treatment.

4.1404 Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration
Bernard-Marissal, N., Medard, J-J., Azzedine, H. and Chrast, R.
*Brain, **138**, 875-890 (2015)*

Mutations in Sigma 1 receptor (*SIGMAR1*) have been previously identified in patients with amyotrophic lateral sclerosis and disruption of *Sigmar1* in mouse leads to locomotor deficits. However, cellular mechanisms underlying motor phenotypes in human and mouse with disturbed SIGMAR1 function have not been described so far. Here we used a combination of *in vivo* and *in vitro* approaches to investigate the role of SIGMAR1 in motor neuron biology. Characterization of *Sigmar1*$^{-/-}$ mice revealed that affected animals display locomotor deficits associated with muscle weakness, axonal degeneration and motor neuron loss. Using primary motor neuron cultures, we observed that pharmacological or genetic inactivation of SIGMAR1 led to motor neuron axonal degeneration followed by cell death. Disruption of SIGMAR1 function in motor neurons disturbed endoplasmic reticulum–mitochondria contacts, affected intracellular calcium signalling and was accompanied by activation of endoplasmic reticulum stress and defects in mitochondrial dynamics and transport. These defects were not observed in cultured sensory neurons, highlighting the exacerbated sensitivity of motor neurons to SIGMAR1 function. Interestingly, the inhibition of mitochondrial fission was sufficient to induce mitochondria axonal transport defects as well as axonal degeneration similar to the changes observed after SIGMAR1 inactivation or loss. Intracellular calcium scavenging and endoplasmic reticulum stress inhibition were able to restore mitochondrial function and consequently prevent motor neuron degeneration. These results uncover the cellular mechanisms underlying motor neuron degeneration mediated by loss of SIGMAR1 function and provide therapeutically relevant insight into motor neuronal diseases.

4.1405 Peripheral Blood-Derived Mesenchymal Stem Cells: Candidate Cells Responsible for Healing Critical-Sized Calvarial Bone Defects
Li, S., Huang, K-L., Wu, J-C., Hu, M.S., Sanyal, M., Hu, M., Longaker, M.T. and Lorenz, H.P.
*Stem Cells Trans. Med., **4**, 359-368 (2015)*

Postnatal tissue-specific stem/progenitor cells hold great promise to enhance repair of damaged tissues. Many of these cells are retrieved from bone marrow or adipose tissue via invasive procedures. Peripheral blood is an ideal alternative source for the stem/progenitor cells because of its ease of retrieval. We present a coculture system that routinely produces a group of cells from adult peripheral blood. Treatment with these cells enhanced healing of critical-size bone defects in the mouse calvarium, a proof of principle that peripheral blood-derived cells can be used to heal bone defects. From these cells, we isolated a subset of $CD45^-$ cells with a fibroblastic morphology. The $CD45^-$ cells were responsible for most of the differentiation-induced calcification activity and were most likely responsible for the enhanced healing process. These $CD45^-$ fibroblastic cells are plastic-adherent and exhibit a surface marker profile negative for CD34, CD19, CD11b, lineage, and c-kit and positive for stem cell antigen 1, CD73, CD44, CD90.1, CD29, CD105, CD106, and CD140α. Furthermore, these cells exhibited osteogenesis, chondrogenesis, and adipogenesis capabilities. The $CD45^-$ fibroblastic cells are the first peripheral blood-derived cells that fulfill the criteria of mesenchymal stem cells as defined by the International Society for Cellular Therapy. We have named these cells "blood-derived mesenchymal stem cells."

4.1406 Olfactory ensheathing cell–neurite alignment enhances neurite outgrowth in scar-like cultures
Khhankan, R.R., Wanner, I.B. and Phelps, P.E.
*Exp. Neurol., **269**, 93-101 (2015)*

The regenerative capacity of adult CNS neurons after injury is strongly inhibited by the spinal cord lesion site environment that is composed primarily of the reactive astroglial scar and invading meningeal fibroblasts. Olfactory ensheathing cell (OEC) transplantation facilitates neuronal survival and functional recovery after a complete spinal cord transection, yet the mechanisms by which this recovery occurs remain unclear. We used a unique multicellular scar-like culture model to test if OECs promote neurite outgrowth in growth-inhibitory areas. Astrocytes were mechanically injured and challenged by meningeal fibroblasts to produce key inhibitory elements of a spinal cord lesion. Neurite outgrowth of postnatal cerebral cortical neurons was assessed on three substrates: quiescent astrocyte control cultures, reactive astrocyte scar-like cultures, and scar-like cultures with OECs. Initial results showed that OECs enhanced total neurite outgrowth of cortical neurons in a scar-like environment by 60%. We then asked if the neurite

growth-promoting properties of OECs depended on direct alignment between neuronal and OEC processes. Neurites that aligned with OECs were nearly three times longer when they grew on inhibitory meningeal fibroblast areas and twice as long on reactive astrocyte zones compared to neurites not associated with OECs. Our results show that OECs can independently enhance neurite elongation and that direct OEC–neurite cell contact can provide a permissive substrate that overcomes the inhibitory nature of the reactive astrocyte scar border and the fibroblast-rich spinal cord lesion core.

4.1407 A Novel In Vitro Primary Culture Model of the Lower Motor Neuron–Neuromuscular Junction Circuit

Southam, K.A., King, A.E., Blizzard, C.A., McCormack, G.H. and Dickson, T.C.
*Neuromethods, **103**, 181-193 (2015)*

Modelling the complex process of neuromuscular signalling is key to understanding not only normal circuit function but also importantly the mechanisms underpinning a range of degenerative diseases. Here, we describe a compartmented in vitro model of the lower motor neuron–neuromuscular junction circuit, incorporating primary spinal motor neurons, supporting glia and skeletal muscle. This culture model is designed to spatially mimic the unique anatomical and cellular interactions of this circuit in compartmented microfluidic devices, such that the glial cells are located with motor neuron cell bodies in the cell body chamber and motor neuron axons extend to a distal chamber containing skeletal muscle cells whilst simultaneously allowing targeted intervention.

4.1408 Sperm Cleanup and Centrifugation Processing for Cryopreservation

Sieme, H. and Oldenhof, H.
*Methods in Mol. Biol., **1257**, 343-352 (2015)*

Fertility rates with artificial insemination are highest with good-quality sperm samples. Therefore, nonviable sperm, cellular debris, and seminal plasma are preferably removed from semen samples prior to use or for preservation. Such compounds are sources where reactive oxygen species are generated during storage or upon cryopreservation, impairing sperm function. In this chapter we describe methods to remove seminal plasma and cellular debris from sperm samples, and for selecting morphologically normal motile sperm. The methods that are described here include: ordinary centrifugation, sperm swim-up, glass wool and Sephadex filtration/adherence, and single-layer as well as discontinuous two-layer iodixanol density gradient centrifugation.

4.1409 FcRn Rescues Recombinant Factor VIII Fc Fusion Protein from a VWF Independent FVIII Clearance Pathway in Mouse Hepatocytes

Van der Flier, A., Liu, Z., Tan, S., Chen, K., Grager, D., Liu, T., Patarroyo-White, S., Jiang, H. and Light, D.R.
*Plos One, **10(4)**, e0124930 (2015)*

We recently developed a longer lasting recombinant factor VIII-Fc fusion protein, rFVIIIFc, to extend the half-life of replacement FVIII for the treatment of people with hemophilia A. In order to elucidate the biological mechanism for the elongated half-life of rFVIIIFc at a cellular level we delineated the roles of VWF and the tissue-specific expression of the neonatal Fc receptor (FcRn) in the biodistribution, clearance and cycling of rFVIIIFc. We find the tissue biodistribution is similar for rFVIIIFc and rFVIII and that liver is the major clearance organ for both molecules. VWF reduces the clearance and the initial liver uptake of rFVIIIFc. Pharmacokinetic studies in FcRn chimeric mice show that FcRn expressed in somatic cells (hepatocytes or liver sinusoidal endothelial cells) mediates the decreased clearance of rFVIIIFc, but FcRn in hematopoietic cells (Kupffer cells) does not affect clearance. Immunohistochemical studies show that when rFVIII or rFVIIIFc is in dynamic equilibrium binding with VWF, they mostly co localize with VWF in Kupffer cells and macrophages, confirming a major role for liver macrophages in the internalization and clearance of the VWF-FVIII complex. In the absence of VWF a clear difference in cellular localization of VWF-free rFVIII and rFVIIIFc is observed and neither molecule is detected in Kupffer cells. Instead, rFVIII is observed in hepatocytes, indicating that free rFVIII is cleared by hepatocytes, while rFVIIIFc is observed as a diffuse liver sinusoidal staining, suggesting recycling of free-rFVIIIFc out of hepatocytes. These studies reveal two parallel linked clearance pathways, with a dominant pathway in which both rFVIIIFc and rFVIII complexed with VWF are cleared mainly by Kupffer cells without FcRn cycling. In contrast, the free fraction of rFVIII or rFVIIIFc unbound by VWF enters hepatocytes, where FcRn reduces the degradation and clearance of rFVIIIFc relative to rFVIII by cycling rFVIIIFc back to the liver sinusoid and into circulation, enabling the elongated half-life of rFVIIIFc.

4.1410 Lack of GDAP1 Induces Neuronal Calcium and Mitochondrial Defects in a Knockout Mouse Model of Charcot-Marie-Tooth Neuropathy

Barneo-Munoz, M., Juarez, P., Civera-Tregon, A., Yndriago, L., Pla-Martin, D., Zenker, J., Cuevas-Martin, C., Estela, A., Sanchez-Arago, M., Forteza-Villa, J., Cuezva, J.M., Chrast, R. and Palau, F.
*PloS Genetics, **11(4)**, e1005115 (2015)*

Mutations in *GDAP1*, which encodes protein located in the mitochondrial outer membrane, cause axonal recessive (AR-CMT2), axonal dominant (CMT2K) and demyelinating recessive (CMT4A) forms of Charcot-Marie-Tooth (CMT) neuropathy. Loss of function recessive mutations in *GDAP1* are associated with decreased mitochondrial fission activity, while dominant mutations result in impairment of mitochondrial fusion with increased production of reactive oxygen species and susceptibility to apoptotic stimuli. *GDAP1* silencing *in vitro* reduces Ca^{2+} inflow through store-operated Ca^{2+} entry (SOCE) upon mobilization of endoplasmic reticulum (ER) Ca^{2+}, likely in association with an abnormal distribution of the mitochondrial network. To investigate the functional consequences of lack of GDAP1 *in vivo*, we generated a *Gdap1* knockout mouse. The affected animals presented abnormal motor behavior starting at the age of 3 months. Electrophysiological and biochemical studies confirmed the axonal nature of the neuropathy whereas histopathological studies over time showed progressive loss of motor neurons (MNs) in the anterior horn of the spinal cord and defects in neuromuscular junctions. Analyses of cultured embryonic MNs and adult dorsal root ganglia neurons from affected animals demonstrated large and defective mitochondria, changes in the ER cisternae, reduced acetylation of cytoskeletal α-tubulin and increased autophagy vesicles. Importantly, MNs showed reduced cytosolic calcium and SOCE response. The development and characterization of the *GDAP1* neuropathy mice model thus revealed that some of the pathophysiological changes present in axonal recessive form of the *GDAP1*-related CMT might be the consequence of changes in the mitochondrial network biology and mitochondria–endoplasmic reticulum interaction leading to abnormalities in calcium homeostasis.

4.1411 A CXC chemokine gene, CXCL12, from rock bream, Oplegnathus fasciatus: Molecular characterization and transcriptional profile

Thulasitha, W.S., Umasuthan, N., Whang, I., Lim, B-S., Jung, H-B., Noh, J.K. and Lee, J.
*Fish & Shellfish Immunology, **45**, 560-566 (2015)*

Chemokines are small, structurally related chemotactic cytokines characterized by the presence of conserved cysteine residues. In the present study, we identified the cDNA of a CXC chemokine from*Oplegnathus fasciatus*, designated as *OfCXCL12*. An open reading frame of 297 bp encoded a 98 amino acid peptide with a putative signal peptide of 23 amino acids. The CXC family-specific small cytokine domain (SCY), which is highly conserved among vertebrates, was located between residues 29 and 87. The characteristic conserved cysteine residues in the CXC motif of OfCXCL12 were separated by tyrosine (Y). Similar to other vertebrate CXCL12 proteins, OfCXCL12 also lacked the ELR motif and hence belongs to ELR^- subfamily. Phylogenetic analysis revealed two distinct clades, consisting of fish and tetrapod CXCL12 homologs. Constitutive expression with significantly higher levels of *OfCXCL12* mRNA transcription was detected in immune-related organs, including the head kidney, spleen, and kidney. Infection with bacterial and viral agents led to significant upregulation of mRNA expression in both the head kidney and spleen, in a stimulant-specific manner. Stimulation of peripheral blood leukocytes by the mitogen concanavalin-A significantly induced *OfCXCL12* transcription. Results from the present study suggest an important role for *OfCXCL12* in immune defense against bacterial and viral infection in rock bream.

4.1412 Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells

Klein, A.M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Peshkin, L., Weitz, D.A. and Kirschner, M.W.
*Cell, **161**, 1187-1201 (2015)*

It has long been the dream of biologists to map gene expression at the single-cell level. With such data one might track heterogeneous cell sub-populations, and infer regulatory relationships between genes and pathways. Recently, RNA sequencing has achieved single-cell resolution. What is limiting is an effective way to routinely isolate and process large numbers of individual cells for quantitative in-depth sequencing. We have developed a high-throughput droplet-microfluidic approach for barcoding the RNA from thousands of individual cells for subsequent analysis by next-generation sequencing. The method shows a

surprisingly low noise profile and is readily adaptable to other sequencing-based assays. We analyzed mouse embryonic stem cells, revealing in detail the population structure and the heterogeneous onset of differentiation after leukemia inhibitory factor (LIF) withdrawal. The reproducibility of these high-throughput single-cell data allowed us to deconstruct cell populations and infer gene expression relationships.

4.1413 Divergent effects of RIP1 or RIP3 blockade in murine models of acute liver injury
Deutsch, M., Graffeo, C.S., Rokosh, R., pansari, M., Ochi, A., Levie, E.M., Van Heerden, E., Tippens, D.M., Greco, S., Barilla, R., Tomkötter, L., Zambrinis, C.P., Avanzi, N., Gulati, R., pachter, H.L., Torres-Hernandez, A., Eisenthal, A., Daley, D. and Miller, G.
*Cell Death and Disease, **6**, e1759 (2015)*

Necroptosis is a recently described Caspase 8-independent method of cell death that denotes organized cellular necrosis. The roles of RIP1 and RIP3 in mediating hepatocyte death from acute liver injury are incompletely defined. Effects of necroptosis blockade were studied by separately targeting RIP1 and RIP3 in diverse murine models of acute liver injury. Blockade of necroptosis had disparate effects on disease outcome depending on the precise etiology of liver injury and component of the necrosome targeted. In ConA-induced autoimmune hepatitis, RIP3 deletion was protective, whereas RIP1 inhibition exacerbated disease, accelerated animal death, and was associated with increased hepatocyte apoptosis. Conversely, in acetaminophen-mediated liver injury, blockade of either RIP1 or RIP3 was protective and was associated with lower NLRP3 inflammasome activation. Our work highlights the fact that diverse modes of acute liver injury have differing requirements for RIP1 and RIP3; moreover, within a single injury model, RIP1 and RIP3 blockade can have diametrically opposite effects on tissue damage, suggesting that interference with distinct components of the necrosome must be considered separately.

4.1414 Endotoxic shock-expanded murine CD11clowCD45RB+ regulatory dendritic cells modulate inflammatory T cell responses through multiple mechanisms
Wang, X., Wang, Q., Zhang, X., Li, Y., Wang, J., Hou, C., Chen, J., Shen, B., Shi, Y. and Zhang, J.
*Scientific Reports, **5**:10653 (2015)*

Changes in the number and function of dendritic cells (DCs) have been reported to play an important role in endotoxin tolerance. It has been reported that expansion of splenic $CD11c^{low}CD45RB^{+}$ DCs occurs in mice injected with sublethal doses of lipopolysaccharide (LPS). However, the function of endotoxic shock-expanded $CD11c^{low}CD45RB^{+}$ DCs has not been examined. In this work, we show that endotoxic shock promotes the expansion of $CD11c^{low}CD45RB^{+}$ cells with dendritic morphology and the production of low levels of inflammatory cytokines and costimulatory molecules. The expanded cells induce the generation of regulatory T cells (Tregs), show incapability to stimulate T cells, and induce apoptosis of $CD4^{+}$ T cells *in vitro*. As compared to $CD11c^{hi}CD45RB^{-}$ conventional DCs, the expanded cells exert better protection against colitis induction by $CD4^{+}$ $CD25^{-}$ T cells, even though both subpopulations show similar ability to induce Tregs *in vivo.* The better control of proinflammatory cytokine responses *in vivo* by the expanded cells is associated with more apoptosis in the Payer's patches and in colonic tissue-infiltrating cells. Thus, the expanded cells can modulate inflammatory T cell responses through multiple mechanisms. Our study facilitates a better understanding how innate immune responses may shape adaptive immunity and immune suppression following LPS-induced acute inflammation.

4.1415 Influenza induces IL-8 and GM-CSF secretion by human alveolar epithelial cells through HGF/c-Met and TGF-α/EGFR signaling
Ito, Y., Correll, K., Zemans, R.I., Leslie, C.C., Murphy, R.C. and mason, R.J:
*Am. J. Physiol. Lung Cell Mol. Physiol., **308**, L1178-L1188 (2015)*

The most severe complication of influenza is viral pneumonia, which can lead to the acute respiratory distress syndrome. Alveolar epithelial cells (AECs) are the first cells that influenza virus encounters upon entering the alveolus. Infected epithelial cells produce cytokines that attract and activate neutrophils and macrophages, which in turn induce damage to the epithelial-endothelial barrier. Hepatocyte growth factor (HGF)/c-Met and transforming growth factor-α (TGF-α)/epidermal growth factor receptor (EGFR) are well known to regulate repair of damaged alveolar epithelium by stimulating cell migration and proliferation. Recently, TGF-α/EGFR signaling has also been shown to regulate innate immune responses in bronchial epithelial cells. However, little is known about whether HGF/c-Met signaling alters the innate immune responses and whether the innate immune responses in AECs are regulated by HGF/c-Met and TGF-α/EGFR. We hypothesized that HGF/c-Met and TGF-α/EGFR would regulate innate immune

responses to influenza A virus infection in human AECs. We found that recombinant human HGF (rhHGF) and rhTGF-α stimulated primary human AECs to secrete IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) strongly and IL-6 and monocyte chemotactic protein 1 moderately. Influenza infection stimulated the secretion of IL-8 and GM-CSF by AECs plated on rat-tail collagen through EGFR activation likely by TGF-α released from AECs and through c-Met activated by HGF secreted from lung fibroblasts. HGF secretion by fibroblasts was stimulated by AEC production of prostaglandin E_2 during influenza infection. We conclude that HGF/c-Met and TGF-α/EGFR signaling enhances the innate immune responses by human AECs during influenza infections.

4.1416 **Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells**
Pallett, L.J. et al
Nature Med., ***21(6)***, *591-600 (2015)*

Infection with hepatitis B virus (HBV) results in disparate degrees of tissue injury: the virus can either replicate without pathological consequences or trigger immune-mediated necroinflammatory liver damage. We investigated the potential for myeloid-derived suppressor cells (MDSCs) to suppress T cell–mediated immunopathology in this setting. Granulocytic MDSCs (gMDSCs) expanded transiently in acute resolving HBV, decreasing in frequency prior to peak hepatic injury. In persistent infection, arginase-expressing gMDSCs (and circulating arginase) increased most in disease phases characterized by HBV replication without immunopathology, whilst L-arginine decreased. gMDSCs expressed liver-homing chemokine receptors and accumulated in the liver, their expansion supported by hepatic stellate cells. We provide *in vitro* and *ex vivo* evidence that gMDSCs potently inhibited T cells in a partially arginase-dependent manner. L-arginine–deprived T cells upregulated system L amino acid transporters to increase uptake of essential nutrients and attempt metabolic reprogramming. These data demonstrate the capacity of expanded arginase-expressing gMDSCs to regulate liver immunopathology in HBV infection.

4.1417 **Non-Aggregating Tau Phosphorylation by Cyclin-Dependent Kinase 5 Contributes to Motor Neuron Degeneration in Spinal Muscular Atrophy**
Miller, N. et al
J. Neurosci., ***35(15)***, *6038-6050 (2015)*

Mechanisms underlying motor neuron degeneration in spinal muscular atrophy (SMA), the leading inherited cause of infant mortality, remain largely unknown. Many studies have established the importance of hyperphosphorylation of the microtubule-associated protein tau in various neurodegenerative disorders, including Alzheimer's and Parkinson's diseases. However, tau phosphorylation in SMA pathogenesis has yet to be investigated. Here we show that tau phosphorylation on serine 202 (S202) and threonine 205 (T205) is increased significantly in SMA motor neurons using two SMA mouse models and human SMA patient spinal cord samples. Interestingly, phosphorylated tau does not form aggregates in motor neurons or neuromuscular junctions (NMJs), even at late stages of SMA disease, distinguishing it from other tauopathies. Hyperphosphorylation of tau on S202 and T205 is mediated by cyclin-dependent kinase 5 (Cdk5) in SMA disease condition, because tau phosphorylation at these sites is significantly reduced in Cdk5 knock-out mice; genetic knock-out of Cdk5 activating subunit p35 in an SMA mouse model also leads to reduced tau phosphorylation on S202 and T205 in the *SMA;p35*$^{-/-}$ compound mutant mice. In addition, expression of the phosphorylation-deficient tauS202A,T205A mutant alleviates motor neuron defects in a zebrafish SMA model *in vivo* and mouse motor neuron degeneration in culture, whereas expression of phosphorylation-mimetic tauS202E,T205E promotes motor neuron defects. More importantly, genetic knock-out of *tau* in SMA mice rescues synapse stripping on motor neurons, NMJ denervation, and motor neuron degeneration *in vivo*. Altogether, our findings suggest a novel mechanism for SMA pathogenesis in which hyperphosphorylation of non-aggregating tau by Cdk5 contributes to motor neuron degeneration.

4.1418 **CD81 Controls Immunity to Listeria Infection through Rac-Dependent Inhibition of Proinflammatory Mediator Release and Activation of Cytotoxic T Cells**
Martinez del Hoya, G., Ramirez-Huesca, M., Levy, S., Boucheix, C., Rubinstein, E., Minguito de la Escalera, M., Gonzalez-Cintado, L., Arvadin, C., Veiga, E., Yanez-Mo, M. and Sanchez-Madrid, F.
J. Immunol., ***194(12)***, *6090-6101 (2015)*

Despite recent evidence on the involvement of CD81 in pathogen binding and Ag presentation by dendritic cells (DCs), the molecular mechanism of how CD81 regulates immunity during infection remains to be elucidated. To investigate the role of CD81 in the regulation of defense mechanisms against microbial

infections, we have used the *Listeria monocytogenes* infection model to explore the impact of CD81 deficiency in the innate and adaptive immune response against this pathogenic bacteria. We show that $CD81^{-/-}$ mice are less susceptible than wild-type mice to systemic *Listeria* infection, which correlates with increased numbers of inflammatory monocytes and DCs in $CD81^{-/-}$ spleens, the main subsets controlling early bacterial burden. Additionally, our data reveal that CD81 inhibits Rac/STAT-1 activation, leading to a negative regulation of the production of TNF-α and NO by inflammatory DCs and the activation of cytotoxic T cells by splenic $CD8\alpha^{+}$ DCs. In conclusion, this study demonstrates that CD81–Rac interaction exerts an important regulatory role on the innate and adaptive immunity against bacterial infection and suggests a role for CD81 in the development of novel therapeutic targets during infectious diseases.

4.1419 Tweak regulates astrogliosis, microgliosis and skeletal muscle atrophy in a mouse model of amyotrophic lateral sclerosis

Bowerman, M., Salsac, C., Coque, E., Eiselt, E., Deschaumes, R.G., Brodovitch, A., Burkly, L.C., Scamps, F. and Raoul, C.

Hum. Mol. Genet., ***24(12)***, *3440-3456 (2015)*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that primarily affects motoneurons in the brain and spinal cord. Astrocyte and microglia activation as well as skeletal muscle atrophy are also typical hallmarks of the disease. However, the functional relationship between astrocytes, microglia and skeletal muscle in the pathogenic process remains unclear. Here, we report that the tumor necrosis factor-like weak inducer of apoptosis (Tweak) and its receptor Fn14 are aberrantly expressed in spinal astrocytes and skeletal muscle of $SOD1^{G93A}$ mice. We show that Tweak induces motoneuron death, stimulates astrocytic interleukin-6 release and astrocytic proliferation *in vitro*. The genetic ablation of *Tweak* in $SOD1^{G93A}$ mice significantly reduces astrocytosis, microgliosis and ameliorates skeletal muscle atrophy. The peripheral neutralization of Tweak through antagonistic anti-Tweak antibody ameliorates muscle pathology and notably, decreases microglial activation in $SOD1^{G93A}$ mice. Unexpectedly, none of these approaches improved motor function, lifespan and motoneuron survival. Our work emphasizes the multi-systemic aspect of ALS, and suggests that a combinatorial therapy targeting multiple cell types will be instrumental to halt the neurodegenerative process.

4.1420 Basic fibroblast growth factor-treated adipose tissue-derived mesenchymal stem cell infusion to ameliorate liver cirrhosis via paracrine hepatocyte growth factor

Tang, W-P., Akahoshi, T., Piao, J-S., Narahara, S., Murata, M., Kawano, T., Hamano, N., Ikeda, T. and Hashizume, M.

J. Gastroenterol. and Hepatol., ***30(6)***, *1065-1074 (2015)*

Background and Aim

Recent studies show that adipose tissue-derived mesenchymal stem cells have potential clinical applications. However, the mechanism has not been fully elucidated yet. Here, we investigated the effect of basic fibroblast growth factor-treated adipose tissue-derived mesenchymal stem cells infusion on a liver fibrosis rat model and elucidated the underlying mechanism.

Methods

Adipose tissue-derived mesenchymal stem cells were infused into carbon tetrachloride-induced hepatic fibrosis rats through caudal vein. Liver functions and pathological changes were assessed. A co-culture model was used to clarify the potential mechanism.

Results

Basic fibroblast growth factor treatment markedly improved the proliferation, differentiation, and hepatocyte growth factor expression ability of adipose tissue-derived mesenchymal stem cells. Although adipose tissue-derived mesenchymal stem cells infusion alone slightly ameliorated liver functions and suppressed fibrosis progression, basic fibroblast growth factor-treatment significantly enhanced the therapeutic effect in association with elevated hepatocyte growth factor expression. Moreover, double immunofluorescence staining confirmed that the infused cells located in fibrosis area. Furthermore, co-culture with adipose tissue-derived mesenchymal stem cell led to induction of hepatic stellate cell apoptosis and enhanced hepatocyte proliferation. However, these effects were significantly weakened by knockdown of hepatocyte growth factor. Mechanism investigation revealed that co-culture with adipose tissue-derived mesenchymal stem cells activated c-jun N-terminal kinase-p53 signaling in hepatic stellate cell and promoted apoptosis.

Conclusions

Basic fibroblast growth factor treatment enhanced the therapeutic effect of adipose tissue-derived mesenchymal stem cells, and secretion of hepatocyte growth factor from adipose tissue-derived

mesenchymal stem cells plays a critical role in amelioration of liver injury and regression of fibrosis.

4.1421 Image-based cell-resolved screening assays in flow
Cheung, M.C., McKenna, B., Wang, S.S., Wolf, D. and Erhlich, D.J.
*Cytometry Part A, **87(6)**, 541-548 (2015)*

A parallel microfluidic cytometer (PMC) is based on a one-dimensional (1D) scanning detector, a parallel array of flow channels, and new multiparameter analysis algorithms that operate on low-pixel-count 1D images. In this article, we explore a series of image-based live- and fixed-cell screening assays, including two NF-kB nuclear translocations and T-cell capping. We then develop a new multiparametric linear weighted classifier that achieves a Z' factor sufficient for scaled pharmaceutical discovery with Jurkat cells in suspension. We conclude that the PMC should have the throughput and statistical power to permit a new capability for image-based high-sample-number pharmaceutical screening with suspension samples

4.1422 Activated endothelial cells limit inflammatory response, but increase chemoattractant potential and bacterial clearance by human monocytes
Mancilla-Herrera, I., Alvarado-Moreno, J.A., Cerbulo-Vazquez, A., Prieto-Chavez. J.L., Ferat-Osorio, E., Lopez-Macias, C., Estrada-Parra, S., Isibasi, A. and Arriaga-Pizano, L.
*Cell Biol. Int., **39(6)**, 721-732 (2015)*

Inflammation is the normal immune response of vascularized tissues to damage and bacterial products, for which leukocyte transendothelial migration (TEM) is critical. The effects of cell-to-cell contact seen in both leukocyte and endothelial cells include cytoskeleton rearrangement, and dynamic expression of adhesion molecules and metalloproteinases. TEM induces expression of anti-apoptotic molecules, costimulatory molecules associated with antigen presentation, and pattern recognition receptors (PRR), such as TLR-4, in monocytes. However, little is known about how TLR-4 increment operates in monocytes during an inflammatory response. To understand it better, we used an in vitro model in which monocytes crossed a layer of IL-1β stimulated Human Umbilical Vein Endothelial Cells (HUVEC). After TEM, monocytes were tested for the secretion of inflammatory cytokines and chemokines, their phenotype (CD14, CD16, TLR-4 expression), and TLR-4 canonical [Nuclear Factor kappa B, (NF-κB) pathway] and non-canonical [p38, extracellular signal-regulated kinases (ERK) 1/2 pathway] signal transduction induced by lipopolysaccharide (LPS). Phagocytosis and bacterial clearance were also measured. There was diminished secretion of LPS-induced inflammatory cytokines (IL-1β, IL-6, and TNF-α) and higher secretion of chemokines (CXCL8/IL-8 and CCL2/MCP-1) in supernatant of TEM monocytes. These changes were accompanied by increases in TLR-4, CD14 (surfaces expression), p38, and ERK1/2 phosphorylated cytoplasmic forms, without affecting NF-κB activation. It also increased bacterial clearance after TEM by an O_2-independent mechanism. The data suggest that interaction between endothelial cells and monocytes fine-tunes the inflammatory response and promotes bacterial elimination.

4.1423 Age-Related Decline of Autocrine Pituitary Adenylate Cyclase-Activating Polypeptide Impairs Angiogenic Capacity of Rat Cerebromicrovascular Endothelial Cells
Banki, E., Sosnowska, D., Tucsek, Z., Gautam, T., Toth, P., Tarantini, S., Tamas, A., Helyes, Z., Reglodi, D., Sonntag, W.E., Csiszar, A. and Ungvari, Z.
*J. Gerontol. A Biol. Sci. Med. Sci., **70**, 665-674 (2015)*

Aging impairs angiogenic capacity of cerebromicrovascular endothelial cells (CMVECs) promoting microvascular rarefaction, but the underlying mechanisms remain elusive. PACAP is an evolutionarily conserved neuropeptide secreted by endothelial cells and neurons, which confers important antiaging effects. To test the hypothesis that age-related changes in autocrine PACAP signaling contributes to dysregulation of endothelial angiogenic capacity, primary CMVECs were isolated from 3-month-old (young) and 24-month-old (aged) Fischer 344 x Brown Norway rats. In aged CMVECs, expression of PACAP was decreased, which was associated with impaired capacity to form capillary-like structures, impaired adhesiveness to collagen (assessed using electric cell-substrate impedance sensing [ECIS] technology), and increased apoptosis (caspase3 activity) when compared with young cells. Overexpression of PACAP in aged CMVECs resulted in increased formation of capillary-like structures, whereas it did not affect cell adhesion. Treatment with recombinant PACAP also significantly increased endothelial tube formation and inhibited apoptosis in aged CMVECs. In young CMVECs shRNA knockdown of autocrine PACAP expression significantly impaired tube formation capacity, mimicking the aging phenotype. Cellular and mitochondrial reactive oxygen species production (dihydroethidium and MitoSox fluorescence, respectively) were increased in aged CMVECs and were unaffected by PACAP. Collectively,

PACAP exerts proangiogenic effects and age-related dysregulation of autocrine PACAP signaling may contribute to impaired angiogenic capacity of CMVECs in aging.

4.1424 Histone H3K9 demethylase JMJD1A modulates hepatic stellate cells activation and liver fibrosis by epigenetically regulating peroxisome proliferator-activated receptor γ
Jiang, Y., Wang, S., Zhao, Y., Lin, C., Zhong, F., Jin, L., He, F. and Wang, H.
*FASEB J., **29(5)**, 1830-1841 (2015)*

As a central event in liver fibrogenesis, hepatic stellate cell (HSC) transdifferentiation involves loss of regulation by adipogenic transcription factors such as peroxisome proliferator-activated receptor *γ*; (PPAR*γ*), which is epigenetically silenced during HSC activation. We hypothesized that JMJD1A, an H3K9 demethylase involved in adipogenic metabolism, could regulate PPAR*γ*. In human HSC cell line, rat primary HSCs, and carbontetrachloride-induced mouse liver fibrogenesis model, we down-regulated the expression of JMJD1A using small interfering or short hairpin RNAs, and overexpressed its wild-type and mutant. We analyzed the effects of JMJD1A manipulation on the histone di-methyl-H3K9 (H3k9me2) status of PPAR*γ* gene and the expression of PPAR*γ* and fibrosis markers using chromatin immunoprecipitation, real-time quantitative RT-PCR and Western blot, and also investigated the *in vitro* and *in vivo* consequences on liver fibrosis and necrosis by Masson or hematoxylin-eosin staining, respectively. JMJD1A knockdown in HSCs correlated with reinforced H3K9me2 in the PPAR*γ* gene promoter, and its down-regulation in both mRNA and protein led to increased expression of fibrosis markers, which could be consistently rescued by JMJD1A overexpression. Jmjd1a knockdown *in situ* resulted in significantly increased expression of *α*-smooth muscle actin ($P = 0.005$) and Col1a ($P = 0.036$), strengthened production of collagens ($P = 0.028$), and remarkably enhanced necrosis ($P = 0.007$) 4 weeks after treatment. This study suggests JMJD1A as a novel epigenetic regulator that modulates HSC activation and liver fibrosis through targeting PPAR*γ* gene expression.—Jiang, Y., Wang, S., Zhao, Y., Lin, C., Zhong, F., Jin, L., He, F., Wang, H. Histone H3K9 demethylase JMJD1A modulates hepatic stellate cells activation and liver fibrosis by epigenetically regulating peroxisome proliferator-activated receptor *γ*.

4.1425 Continuous Flow Microfluidic Bioparticle Concentrator
Martel, J.M., Smith, K.C., Dlamini, M., Pletchert, K., yang, J., karabacak, M., haber, D.A., Kapur, R. and Toner, M.
*Scientific Reports, **5**:11300 (2015)*

Innovative microfluidic technology has enabled massively parallelized and extremely efficient biological and clinical assays. Many biological applications developed and executed with traditional bulk processing techniques have been translated and streamlined through microfluidic processing with the notable exception of sample volume reduction or centrifugation, one of the most widely utilized processes in the biological sciences. We utilize the high-speed phenomenon known as inertial focusing combined with hydraulic resistance controlled multiplexed micro-siphoning allowing for the continuous concentration of suspended cells into pre-determined volumes up to more than 400 times smaller than the input with a yield routinely above 95% at a throughput of 240 ml/hour. Highlighted applications are presented for how the technology can be successfully used for live animal imaging studies, in a system to increase the efficient use of small clinical samples, and finally, as a means of macro-to-micro interfacing allowing large samples to be directly coupled to a variety of powerful microfluidic technologies.

4.1426 PTPRO-Associated Hepatic Stellate Cell Activation Plays a Critical Role in Liver Fibrosis
Zhang, X., Tan, Z., Wang, Y., Tang, J., Jiang, R., Hou, J., Zhuo, H., Wang, X., Ji, J., Qin, X. and Sun, B.
*Cell. Physiol. Biochem., **35**, 885-898 (2015)*

Background/Aims: PTPRO (protein tyrosine phosphatase, receptor type O) is implicated in diverse physiological and pathological processes in cancer and hepatic ischemia/reperfusion injury, although little is known about its role in hepatic fibrosis. *Methods:* Here, by using genetically deficient mice, we reported that PTPRO knockout ($PTPRO^{-/-}$) significantly attenuated liver injury, release of inflammatory factors, tissue remodeling, and liver fibrosis in two experimental mouse models of fibrogenesis induced by bile-duct ligation or carbon tetrachloride administration. *Results:* However, we proved that PTPRO expression was strongly downregulated in clinical and experimental liver fibrosis specimens. Further investigations revealed that stimulation of primary hepatic stellate cells (HSCs) and hepatocytes with specific activator platelet-derived growth factor (PDGF)-BB increased PTPRO transcription in HSCs but had the opposite effect in primary hepatocytes. More importantly, synthetic short hairpin RNA targeting PTPRO significantly neutralized PDGF-BB-induced HSC proliferation and myofibroblast marker expression

through downregulated phosphorylation of extracellular signal-regulated kinase (ERK) and AKT. *Conclusion:* These observations confirm that PTPRO plays a critical role in liver fibrogenesis by affecting PDGF signaling in HSC activation and might be developed into a feasible therapeutic approach for the treatment of chronic fibrotic liver diseases.

4.1427 High-Speed Discrimination and Sorting of Submicron Particles Using a Microfluidic Device
Rajauria, S., Axline, C., Gottstein, C. and Cleland, A.N.
Nano Lett., ***15(1)***, *469-475 (2015)*

The size- and fluorescence-based sorting of micro- and nanoscale particles suspended in fluid presents a significant and important challenge for both sample analysis and for manufacturing of nanoparticle-based products. Here, we demonstrate a disposable microfluidic particle sorter that enables high-throughput, on-demand counting and binary sorting of submicron particles and cells using either fluorescence or an electrically based determination of particle size. Size-based sorting uses a resistive pulse sensor integrated on-chip, whereas fluorescence-based discrimination is achieved using on-the-fly optical image capture and analysis. Following detection and analysis, the individual particles are deflected using a pair of piezoelectric actuators, directing the particles into one of two desired output channels; the main flow goes into a third waste channel. The integrated system can achieve sorting fidelities of better than 98%, and the mechanism can successfully count and actuate, on demand, more than 60 000 particles/min.

4.1428 Assessment of the Performance of Membrane Bioreactors Applied to the Treatment of Industrial Effluents Containing Poly(vinyl alcohol)
Blanco, L., Hermosilla, D., Blanco, A., Swinnen, N., Prieto, D. and Negro, C.
Ind. Eng. Chem. Res., ***54(20)***, *5442-5449 (2015)*

To assess and to optimize the treatment of effluents containing poly(vinyl alcohol) (PVA) from a poly(vinyl chloride) (PVC) production site, two aerobic membrane bioreactors (MBR) were run in parallel. PVA and total organics degradation, pH, temperature, oxygen consumption, organic loading rate, hydraulic retention time, and macro- and micronutrients contents were measured and optimized. Total removal of PVA, total nitrification, > 90% chemical oxygen demand (COD) removal, and >95% biological oxygen demand (BOD_5) reduction were achieved from a mixed culture of microorganisms after an adequate adaptation period. In addition, different bacteria cultures were assessed and analyzed in order to study PVA biodegradation: γ-proteobacteria and α-proteobacteria were the main groups of PVA-degrading bacteria. Membranes showed a good performance during the trials. Furthermore, permeability was completely recovered by chemical cleaning. After the assessed MBR treatment, the effluent fulfills appropriate water quality requirements for its post-treatment in a desalination unit in order to be reused within the PVC production processes.

4.1429 Clinical outcomes of a novel therapeutic vaccine with Tax peptide-pulsed dendritic cells for adult T cell leukaemia/lymphoma in a pilot study
Suehiro, Y. et al
Br. J. hematol., ***169(6)***, *356-367 (2015)*

Adult T cell leukaemia/lymphoma (ATL) is a human T cell leukaemia virus type-I (HTLV-I)-infected T cell malignancy with poor prognosis. We herein developed a novel therapeutic vaccine designed to augment an HTLV-I Tax-specific cytotoxic T lymphocyte (CTL) response that has been implicated in anti-ATL effects, and conducted a pilot study to investigate its safety and efficacy. Three previously treated ATL patients, classified as intermediate- to high-risk, were subcutaneously administered with the vaccine, consisting of autologous dendritic cells (DCs) pulsed with Tax peptides corresponding to the CTL epitopes. In all patients, the performance status improved after vaccination without severe adverse events, and Tax-specific CTL responses were observed with peaks at 16–20 weeks. Two patients achieved partial remission in the first 8 weeks, one of whom later achieved complete remission, maintaining their remission status without any additional chemotherapy 24 and 19 months after vaccination, respectively. The third patient, whose tumour cells lacked the ability to express Tax at biopsy, obtained stable disease in the first 8 weeks and later developed slowly progressive disease although additional therapy was not required for 14 months. The clinical outcomes of this pilot study indicate that the Tax peptide-pulsed DC vaccine is a safe and promising immunotherapy for ATL.

4.1430 Defects in optineurin- and myosin VI-mediated cellular trafficking in amyotrophic lateral sclerosis
Sundaramoorthy, V., Walker, A.K., Tan, V., Fifita, J.A., McCann, E.P., Williams, K.L., Blair, I.P.,

Guillemin, G.J., Frag, M.A. and Atkin, J.D.
Hum. Mol. Genet., ***24(13)***, *3830-3846 (2015)*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder primarily affecting motor neurons. Mutations in optineurin cause a small proportion of familial ALS cases, and wild-type (WT) optineurin is misfolded and forms inclusions in sporadic ALS patient motor neurons. However, it is unknown how optineurin mutation or misfolding leads to ALS. Optineurin acts an adaptor protein connecting the molecular motor myosin VI to secretory vesicles and autophagosomes. Here, we demonstrate that ALS-linked mutations p.Q398X and p.E478G disrupt the association of optineurin with myosin VI, leading to an abnormal diffuse cytoplasmic distribution, inhibition of secretory protein trafficking, endoplasmic reticulum (ER) stress and Golgi fragmentation in motor neuron-like NSC-34 cells. We also provide further insight into the role of optineurin as an autophagy receptor. WT optineurin associated with lysosomes and promoted autophagosome fusion to lysosomes in neuronal cells, implying that it mediates trafficking of lysosomes during autophagy in association with myosin VI. However, either expression of ALS mutant optineurin or small interfering RNA-mediated knockdown of endogenous optineurin blocked lysosome fusion to autophagosomes, resulting in autophagosome accumulation. Together these results indicate that ALS-linked mutations in optineurin disrupt myosin VI-mediated intracellular trafficking processes. In addition, in control human patient tissues, optineurin displayed its normal vesicular localization, but in sporadic ALS patient tissues, vesicles were present in a significantly decreased proportion of motor neurons. Optineurin binding to myosin VI was also decreased in tissue lysates from sporadic ALS spinal cords. This study therefore links several previously described pathological mechanisms in ALS, including defects in autophagy, fragmentation of the Golgi and induction of ER stress, to disruption of optineurin function. These findings also indicate that optineurin–myosin VI dysfunction is a common feature of both sporadic and familial ALS.

4.1431 Liver fibrosis occurs through dysregulation of MyD88-dependent innate B-cell activity
Thapa, M., Chinnadurai, r., Velazquez, V.M., Tedesco, D., Elrod, E., Han, J-H., Sharma, P., Ibegbu, C., Gewirtz, A., Anania, F., Pulendran, b., Suthar, M. and Grakoui, A.
Hepatology, ***61(6)***, *2067-2079 (2015)*

Chronic liver disease mediated by activation of hepatic stellate cells (HSCs) leads to liver fibrosis. Here, we postulated that the immune regulatory properties of HSCs might promote the profibrogenic activity of B cells. Fibrosis is completely attenuated in carbon tetrachloride–treated, B cell–deficient μMT mice, showing that B cells are required. The retinoic acid produced by HSCs augmented B-cell survival, plasma cell marker CD138 expression, and immunoglobulin G production. These activities were reversed following addition of the retinoic acid inhibitor LE540. Transcriptional profiling of fibrotic liver B cells revealed increased expression of genes related to activation of nuclear factor κ light chain enhancer of activated B cells, proinflammatory cytokine production, and CD40 signaling, suggesting that these B cells are activated and may be acting as inflammatory cells. Biological validation experiments also revealed increased activation (CD44 and CD86 expression), constitutive immunoglobulin G production, and secretion of the proinflammatory cytokines tumor necrosis factor-α, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α. Likewise, targeted deletion of B-cell-intrinsic myeloid differentiation primary response gene 88 signaling, an innate adaptor with involvement in retinoic acid signaling, resulted in reduced infiltration of migratory $CD11c^{+}$ dendritic cells and $Ly6C^{++}$ monocytes and, hence, reduced liver pathology. *Conclusion*: Liver fibrosis occurs through a mechanism of HSC-mediated augmentation of innate B-cell activity. These findings highlight B cells as important "first responders" of the intrahepatic immune environment.

4.1432 Rapid Identification by Surface-Enhanced Raman Spectroscopy of Cancer Cells at Low Concentrations Flowing in a Microfluidic Channel
Pallaoro, A., Hoonejani, M.R., Braun, G.B., Meinhart, C.D. and Moskovits, M.
ACS Nano, ***9(4)***, *4328-4336 (2015)*

Reliable identification and collection of cells from bodily fluids is of growing interest for monitoring patient response to therapy and for early detection of disease or its recurrence. We describe a detection platform that combines microfluidics with surface-enhanced Raman spectroscopy (SERS) for the identification of individual mammalian cells continuously flowing in a microfluidics channel. A mixture of cancerous and noncancerous prostate cells was incubated with SERS biotags (SBTs) developed and synthesized by us, then injected into a flow-focused microfluidic channel, which forces the cells into a single file. The spectrally rich SBTs are based on a silver nanoparticle dimer core labeled with a Raman-

active small reporter molecule paired with an affinity biomolecule, providing a unique barcode whose presence in a composite SERS spectrum can be deconvoluted. Individual cancer cells passing through the focused laser beam were correctly identified among a proportionally larger number of other cells by their Raman signatures. We examine two deconvolution strategies: principal component analysis and classical least-squares. The deconvolution strategies are used to unmix the overall spectrum to determine the relative contributions between two SBT barcodes, where one SBT barcode indicates neuropilin-1 overexpression, while a second SBT barcode is more universal and indicates unspecific binding to a cell's membrane. Highly reliable results were obtained for all of the cell mixture ratios tested, the lowest being 1 in 100 cells.

4.1433 Metallothionein-I/II Promotes Axonal Regeneration in the Central Nervous System
Siddiq, M.M., Hannila, S.S., Carmel, J.B., Bryson, J.B., Hou, J., Nikulina, E., Willis, M.R., Mellado, W., Richman, E.L., Hilaire, M., hart, R.P. and Filbin, M.T.
J. Biol. Chem., ***290(26)***, *16343-16356 (2015)*

The adult CNS does not spontaneously regenerate after injury, due in large part to myelin-associated inhibitors such as myelin-associated glycoprotein (MAG), Nogo-A, and oligodendrocyte-myelin glycoprotein. All three inhibitors can interact with either the Nogo receptor complex or paired immunoglobulin-like receptor B. A conditioning lesion of the sciatic nerve allows the central processes of dorsal root ganglion (DRG) neurons to spontaneously regenerate *in vivo* after a dorsal column lesion. After a conditioning lesion, DRG neurons are no longer inhibited by myelin, and this effect is cyclic AMP (cAMP)- and transcription-dependent. Using a microarray analysis, we identified several genes that are up-regulated both in adult DRGs after a conditioning lesion and in DRG neurons treated with cAMP analogues. One gene that was up-regulated under both conditions is metallothionein (MT)-I. We show here that treatment with two closely related isoforms of MT (MT-I/II) can overcome the inhibitory effects of both myelin and MAG for cortical, hippocampal, and DRG neurons. Intrathecal delivery of MT-I/II to adult DRGs also promotes neurite outgrowth in the presence of MAG. Adult DRGs from MT-I/II-deficient mice extend significantly shorter processes on MAG compared with wild-type DRG neurons, and regeneration of dorsal column axons does not occur after a conditioning lesion in MT-I/II-deficient mice. Furthermore, a single intravitreal injection of MT-I/II after optic nerve crush promotes axonal regeneration. Mechanistically, MT-I/II ability to overcome MAG-mediated inhibition is transcription-dependent, and MT-I/II can block the proteolytic activity of α-secretase and the activation of PKC and Rho in response to soluble MAG.

4.1434 Regulation of neuronal high-voltage activated CaV2 Ca2+ channels by the small GTPase RhoA
Rousset, M., Cens, T., menard, C., Bowerman, M., Bellis, M., Bruses, J., Raoul, C., Scaamps, F. and Charnet, P.
Neuropharmacol., ***97***, *201-209 (2015)*

High-Voltage-Activated (HVA) Ca^{2+} channels are known regulators of synapse formation and transmission and play fundamental roles in neuronal pathophysiology. Small GTPases of Rho and RGK families, via their action on both cytoskeleton and Ca^{2+} channels are key molecules for these processes. While the effects of RGK GTPases on neuronal HVA Ca^{2+} channels have been widely studied, the effects of RhoA on the HVA channels remains however elusive. Using heterologous expression in *Xenopus laevis* oocytes, we show that RhoA activity reduces Ba^{2+} currents through $Ca_V2.1$, $Ca_V2.2$ and $Ca_V2.3$ Ca^{2+} channels independently of $Ca_V\beta$ subunit. This inhibition occurs independently of RGKs activity and without modification of biophysical properties and global level of expression of the channel subunit. Instead, we observed a marked decrease in the number of active channels at the plasma membrane. Pharmacological and expression studies suggest that channel expression at the plasma membrane is impaired via a ROCK-sensitive pathway. Expression of constitutively active RhoA in primary culture of spinal motoneurons also drastically reduced HVA Ca^{2+} current amplitude. Altogether our data revealed that HVA Ca^{2+} channels regulation by RhoA might govern synaptic transmission during development and potentially contribute to pathophysiological processes when axon regeneration and growth cone kinetics are impaired.

4.1435 Herpes Simplex Virus 1 Reactivates from Autonomic Ciliary Ganglia Independently from Sensory Trigeminal Ganglia To Cause Recurrent Ocular Disease
Lee, S., Ives, A.M. and Bertke, A.S.
J. Virol., ***89(16)***, *8383-8391 (2015)*

Herpes simplex virus 1 (HSV-1) and HSV-2 establish latency in sensory and autonomic neurons after ocular or genital infection, but their recurrence patterns differ. HSV-1 reactivates from latency to cause recurrent orofacial disease, and while HSV-1 also causes genital lesions, HSV-2 recurs more efficiently in the genital region and rarely causes ocular disease. The mechanisms regulating these anatomical preferences are unclear. To determine whether differences in latent infection and reactivation in autonomic ganglia contribute to differences in HSV-1 and HSV-2 anatomical preferences for recurrent disease, we compared HSV-1 and HSV-2 clinical disease, acute and latent viral loads, and viral gene expression in sensory trigeminal and autonomic superior cervical and ciliary ganglia in a guinea pig ocular infection model. HSV-2 produced more severe acute disease, correlating with higher viral DNA loads in sensory and autonomic ganglia, as well as higher levels of thymidine kinase expression, a marker of productive infection, in autonomic ganglia. HSV-1 reactivated in ciliary ganglia, independently from trigeminal ganglia, to cause more frequent recurrent symptoms, while HSV-2 replicated simultaneously in autonomic and sensory ganglia to cause more persistent disease. While both HSV-1 and HSV-2 expressed the latency-associated transcript (LAT) in the trigeminal and superior cervical ganglia, only HSV-1 expressed LAT in ciliary ganglia, suggesting that HSV-2 is not reactivation competent or does not fully establish latency in ciliary ganglia. Thus, differences in replication and viral gene expression in autonomic ganglia may contribute to differences in HSV-1 and HSV-2 acute and recurrent clinical disease.

4.1436 **Imaging the immunological synapse between dendritic cells and T cells**
Markey, K.A., Gartlan, K.H., Kuns, R.D., MacDonald, K.P.A. and Hill, G.R.
J. Immunol. Methods, ***423****, 40-44 (2015)*

Immunological synapse formation between antigen-specific T cells and antigen presenting cells (APC) involves reorganization of the cellular cytoskeleton (polymerization of filamentous actin) and recruitment of adhesion molecules (e.g. LFA-1, ICAM-1). This engagement is critical for the generation of specific immune responses. Until recently, quantitative, high-throughput measurements of these interactions have not been possible. Instead, previous assessment was reliant on qualitative microscopy of live cells, where typically the APC is adhered to a surface and the suspended T cell is required to migrate to facilitate synapse formation. While this methodology can demonstrate the capacity for synapse formation, it cannot accommodate quantification of large numbers of interacting cell pairs, nor does it allow for statistically robust comparison between test conditions.
We have developed a method for assessing immunological synapse formation between purified ex vivo dendritic cells (DCs) and responder antigen-specific $CD4^+$ T cells using imaging flow cytometry, allowing us to quantify LFA-1 and f-actin rearrangement at the interface between DC/T cell pairs. This novel application of imaging flow cytometry represents a major advance in dendritic cell function and immunological synapse research as it facilitates quantitative, high throughput analysis of the interaction between live, ex vivo DC and T cells.

4.1437 **Endolysosomal Deficits Augment Mitochondria Pathology in Spinal Motor Neurons of Asymptomatic fALS Mice**
Xie, Y., Zhou, B., Lin, M-Y., Wang, S., Foust, K.D. and Sheng, Z-H.
Neuron, ***87****, 355-370 (2015)*

One pathological hallmark in ALS motor neurons (MNs) is axonal accumulation of damaged mitochondria. A fundamental question remains: does reduced degradation of those mitochondria by an impaired autophagy-lysosomal system contribute to mitochondrial pathology? We reveal MN-targeted progressive lysosomal deficits accompanied by impaired autophagic degradation beginning at asymptomatic stages in fALS-linked $hSOD1^{G93A}$ mice. Lysosomal deficits result in accumulation of autophagic vacuoles engulfing damaged mitochondria along MN axons. Live imaging of spinal MNs from the adult disease mice demonstrates impaired dynein-driven retrograde transport of late endosomes (LEs). Expressing dynein-adaptor snapin reverses transport defects by competing with $hSOD1^{G93A}$ for binding dynein, thus rescuing autophagy-lysosomal deficits, enhancing mitochondrial turnover, improving MN survival, and ameliorating the disease phenotype in $hSOD1^{G93A}$ mice. Our study provides a new mechanistic link for $hSOD1^{G93A}$-mediated impairment of LE transport to autophagy-lysosomal deficits and mitochondrial pathology. Understanding these early pathological events benefits development of new therapeutic interventions for fALS-linked MN degeneration.

4.1438 **Type 1 innate lymphoid cells contribute to the pathogenesis of chronic hepatitis B**
Yang, Z., Tang, T., Wei, X., Yang, S. and Tian, Z.
Innate Immun., ***21(6)****, 665-673 (2015)*

Innate lymphoid cells (ILCs) function in producing effector cytokines in response to pathogenic infections. However, the roles and related mechanisms of the ILC subpopulations, ILC1 and ILC2, which mirror Th1 and Th2 in adaptive immunity, remain unclear. In this study, we found the markedly elevated levels of the ILC1 transcription factor T-bet, the effector cytokine IFN-γ and the IL/receptor signaling molecules IL-12/IL-12R, which are indispensable for ILC1 differentiation, in the helper ILCs of chronic hepatitis B (CHB) patients. The elevated level of the ILC1 population was significantly associated with hepatic damage in CHB patients, and was not related to telbivudine treatment. In contrast, although we also observed elevated levels of ILC2-related factors, including IL-33, ST2, GATA3 and IL-13 in helper ILCs, the extent of elevation shown by each was lower than that shown by the ILC1-related factors. Furthermore, the activity of the ILC2s did not correlate with either HBV copies or liver damage. The findings of this study suggest potential pro-inflammatory roles for ILC1s in CHB pathogenesis, potentiating these cells and their related molecules as targets of diagnostic, prognostic and/or therapeutic strategies for hepatitis B.

4.1439 **Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T**
Burga, R.A., Thorn, M., Point, G.R., Guha, P., Nguyen, C.T., Licata, L.A., DeMatteo, R.P., ayala, A., Espat, N.J., Junghans, R.P. and Katz, S.C.
Cancer Immunol. Immunother., ***64****, 817-829 (2015)*

Chimeric antigen receptor-modified T cell (CAR-T) technology, a promising immunotherapeutic tool, has not been applied specifically to treat liver metastases (LM). While CAR-T delivery to LM can be optimized by regional intrahepatic infusion, we propose that liver CD11b+Gr-1+ myeloid-derived suppressor cells (L-MDSC) will inhibit the efficacy of CAR-T in the intrahepatic space. We studied anti-CEA CAR-T in a murine model of CEA+ LM and identified mechanisms through which L-MDSC expand and inhibit CAR-T function. We established CEA+ LM in mice and studied purified L-MDSC and responses to treatment with intrahepatic anti-CEA CAR-T infusions. L-MDSC expanded threefold in response to LM, and their expansion was dependent on GM-CSF, which was produced by tumor cells. L-MDSC utilized PD-L1 to suppress anti-tumor responses through engagement of PD-1 on CAR-T. GM-CSF, in cooperation with STAT3, promoted L-MDSC PD-L1 expression. CAR-T efficacy was rescued when mice received CAR-T in combination with MDSC depletion, GM-CSF neutralization to prevent MDSC expansion, or PD-L1 blockade. As L-MDSC suppressed anti-CEA CAR-T, infusion of anti-CEA CAR-T in tandem with agents targeting L-MDSC is a rational strategy for future clinical trials.

4.1440 **Genome-wide analysis of DNA methylation associated with HIV infection based on a pair of monozygotic twins**
Zhang, Y., Li, S-K., Tsui, S.K-W.
Genomics Data, ***6****, 12-15 82015)*

Alteration of DNA methylation in mammalian cells could be elicited by many factors, including viral infections [1]. HIV has shown the ability to interact with host cellular factors to change the methylation status of some genes [2], [3] and [4]. However, the change of the DNA methylation associated with HIV infection based on the whole genome has not been well illustrated. In this study, a unique pair of monozygotic twins was recruited: one of the twins was infected with HIV without further anti-retroviral therapy while the other one was healthy, which could be considered as a relatively ideal model for profiling the alterations of DNA methylation associated with HIV infection. Therefore, using methylated DNA immunoprecipitation–microarray method (MeDIP–microarray), we found the increased DNA methylation level in peripheral blood mononuclear cells from HIV infected twin compared to her normal sibling. Moreover, several distinguished differential methylation regions (DMRs) in HIV infected twin worth further study. The raw data has been deposited in Gene Expression Omnibus (GEO) datasets with reference number GSE68028.

4.1441 **17β-Estradiol influences in vitro response of aged rat splenic conventional dendritic cells to TLR4 and TLR7/8 agonists in an agonist specific manner**
Stojic-Vukanic, Z., Nacka-Aleksic, M., Bufan, b., Pilipovic, I., Arsenovic-Ranin, N., Djikic, J., Kosec, D. and Leposavic, G.
Int. Immunopharmacol., ***24****, 24-35 (2015)*

This study was undertaken considering that, despite the broad use of the unopposed estrogen replacement therapy in elderly women, data on estrogen influence on the functional capacity of dendritic cells (DCs),

and consequently immune response are limited. We examined the influence of 17β-estradiol on phenotype, cytokine secretory profile, and allostimulatory and polarizing capacity of splenic (OX62+) conventional DCs from 26-month-old (aged) Albino Oxford rats matured *in vitro* in the presence of LPS, a TLR4 agonist, and R848, a TLR7/8 agonist. In the presence of 17β-estradiol, DCs from aged rats exhibited an impaired ability to mature upon stimulation with LPS, as shown by the lower surface density of MHC II and costimulatory CD80 and CD86 molecules. 17β-Estradiol alone enhanced CD40 expression in OX62+ DCs without affecting the expression of other costimulatory molecules, thereby confirming that the expression of this molecule is regulated independently from the regulation of other costimulatory molecules. However, although R848 upregulated the expression of MHC II and CD80 and CD40 costimulatory molecules on DCs, 17β-estradiol diminished the effect of this TLR agonist only on MHC II expression. In conjunction, the previous findings suggest that LPS and R848 elicit changes in the expression of costimulatory molecules *via* triggering differential intracellular signaling pathways. Furthermore, 17β-estradiol diminished the stimulatory influence of both LPS- and R848-matured OX62+ DCs on allogeneic CD4+ T lymphocyte proliferation in a mixed lymphocyte reaction (MLR). Moreover, as shown in MLR, the exposure to 17β-estradiol during LPS- and R848-induced maturation diminished Th1- and enhanced Th17-driving capacity and reduced Th1-driving capacity of OX62+ DCs, respectively. This suggests that LPS and R848 affect not only the surface phenotype, but also functional characteristics of OX62+ DCs triggering distinct intracellular signaling pathways. Collectively, the findings indicate that estrogen directly acting on OX62+ DCs, may affect CD4+ lymphocyte-dependent immune response in aged female rats.

4.1442 Normal Human Lung Epithelial Cells Inhibit Transforming Growth Factor-β Induced Myofibroblast Differentiation via Prostaglandin E2

Epa, A.P:, Thatcher, T.H., Pollock, S.J., Wahl, L.A., Lyda, E., Kottmann, R.M., Phipps, R. and Sime, P.J.
*PloS One, **10(8)**, e0135266 (2015)*

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive disease with very few effective treatments. The key effector cells in fibrosis are believed to be fibroblasts, which differentiate to a contractile myofibroblast phenotype with enhanced capacity to proliferate and produce extracellular matrix. The role of the lung epithelium in fibrosis is unclear. While there is evidence that the epithelium is disrupted in IPF, it is not known whether this is a cause or a result of the fibroblast pathology. We hypothesized that healthy epithelial cells are required to maintain normal lung homeostasis and can inhibit the activation and differentiation of lung fibroblasts to the myofibroblast phenotype. To investigate this hypothesis, we employed a novel co-culture model with primary human lung epithelial cells and fibroblasts to investigate whether epithelial cells inhibit myofibroblast differentiation.

Measurements and Main Results

In the presence of transforming growth factor (TGF)-β, fibroblasts co-cultured with epithelial cells expressed significantly less α-smooth muscle actin and collagen and showed marked reduction in cell migration, collagen gel contraction, and cell proliferation compared to fibroblasts grown without epithelial cells. Epithelial cells from non-matching tissue origins were capable of inhibiting TGF-β induced myofibroblast differentiation in lung, keloid and Graves' orbital fibroblasts. TGF-β promoted production of prostaglandin (PG) E_2 in lung epithelial cells, and a PGE_2 neutralizing antibody blocked the protective effect of epithelial cell co-culture.

Conclusions

We provide the first direct experimental evidence that lung epithelial cells inhibit TGF-β induced myofibroblast differentiation and pro-fibrotic phenotypes in fibroblasts. This effect is not restricted by tissue origin, and is mediated, at least in part, by PGE_2. Our data support the hypothesis that the epithelium plays a crucial role in maintaining lung homeostasis, and that damaged and/ or dysfunctional epithelium contributes to the development of fibrosis.

4.1443 Nlrp6 promotes recovery after peripheral nerve injury independently of inflammasomes

Ydens, E., Demon, D., Lornet, g., De Winter, V., Timmerman, V., Lamkanfi, M. and Janssens, S.
*Journal of Neuroinflammation, **12**:143 (2015)*

Background

NOD-like receptors (Nlrs) are key regulators of immune responses during infection and autoimmunity. A subset of Nlrs assembles inflammasomes, molecular platforms that are activated in response to endogenous danger and microbial ligands and that control release of interleukin (IL)-1β and IL-18. However, their role in response to injury in the nervous system is less understood.

Methods
In this study, we investigated the expression profile of major inflammasome components in the peripheral nervous system (PNS) and explored the physiological role of different Nlrs upon acute nerve injury in mice.
Results
While in basal conditions, predominantly members of NOD-like receptor B (Nlrb) subfamily (NLR family, apoptosis inhibitory proteins (NAIPs)) and Nlrc subfamily (ICE-protease activating factor (IPAF)/NOD) are detected in the sciatic nerve, injury causes a shift towards expression of the Nlrp family. Sterile nerve injury also leads to an increase in expression of the Nlrb subfamily, while bacteria trigger expression of the Nlrc subfamily. Interestingly, loss of Nlrp6 led to strongly impaired nerve function upon nerve crush. Loss of the inflammasome adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and effector caspase-1 and caspase-11 did not affect sciatic nerve function, suggesting that Nlrp6 contributed to recovery after peripheral nerve injury independently of inflammasomes. In line with this, we did not detect release of mature IL-1β upon acute nerve injury despite potent induction of pro-IL-1β and inflammasome components Nlrp3 and Nlrp1. However, Nlrp6 deficiency was associated with increased pro-inflammatory extracellular regulated MAP kinase (ERK) signaling, suggesting that hyperinflammation in the absence of Nlrp6 exacerbated peripheral nerve injury.
Conclusions
Together, our observations suggest that Nlrp6 contributes to recovery from peripheral nerve injury by dampening inflammatory responses independently of IL-1β and inflammasomes.

4.1444 A novel multicolor immunostaining method using ethynyl deoxyuridine for analysis of in situ immunoproliferative response
Kitazawa, Y., Ueta, H., Hünig, T., Sawanobori, Y. and Matsuno, K.
Histochem. Cell Biol., ***144****, 195-208 (2015)*

Immune responses are generally accompanied by antigen presentation and proliferation and differentiation of antigen-specific lymphocytes (immunoproliferation), but analysis of these events in situ on tissue sections is very difficult. We have developed a new method of simultaneous multicolor immunofluorescence staining for immunohistology and flow cytometry using a thymidine analogue, 5-ethynyl-2′-deoxyuridine (EdU). Because of the small size of azide dye using click chemistry and elimination of DNA denaturation steps, EdU staining allowed for immunofluorescence staining of at least four colors including two different markers on a single-cell surface, which is impossible with the standard 5-bromo-2′-deoxyuridine method. By using two *rat* models, successfully detected parameters were the cluster of differentiation antigens including phenotypic and functional markers of various immune cells, histocompatibility complex antigens, and even some nuclear transcription factors. Proliferating cells could be further sorted and used for RT-PCR analysis. This method thus enables functional in situ time-kinetic analysis of immunoproliferative responses in a distinct domain of the lymphoid organs, which are quantitatively confirmed by flow cytometry.

4.1445 Adverse effects of stromal vascular fraction during regenerative treatment of the intervertebral disc: observations in a goat model
Detiger, S.E.L., Helder, M.N., Smit, T.H. and Hoogendoorn, RS.J.W.
Eur. Spine J., ***24****, 1992-2000 (2015)*

Stromal vascular fraction (SVF), an adipose tissue-derived heterogeneous cell mixture containing, among others, multipotent adipose stromal cells (ASCs) and erythrocytes, has proved beneficial for a wide range of applications in regenerative medicine. We sought to establish intervertebral disc (IVD) regeneration by injecting SVF intradiscally during a one-step surgical procedure in an enzymatically (Chondroitinase ABC; cABC) induced goat model of disc degeneration. Unexpectedly, we observed a severe inflammatory response that has not been described before, including massive lymphocyte infiltration, neovascularisation and endplate destruction. A second study investigated two main suspects for these adverse effects: cABC and erythrocytes within SVF. The same destructive response was observed in healthy goat discs injected with SVF, thereby eliminating cABC as a cause. Density gradient removal of erythrocytes and ASCs purified by culturing did not lead to adverse effects. Following these observations, we incorporated an extra washing step in the SVF harvesting protocol. In a third study, we applied this protocol in a one-step procedure to a goat herniation model, in which no adverse responses were observed either. However, upon intradiscal injection of an identically processed SVF mixture into our goat IVD degeneration model during a fourth study, the adverse effects surprisingly occurred again. Despite our quest for the responsible agent, we eventually could not identify the mechanism through which the observed destructive responses

occurred. Although we cannot exclude that the adverse effects are species-dependent or model-specific, we advertise caution with the clinical application of autologous SVF injections into the IVD until the responsible agent(s) are identified.

4.1446 Bioengineering mini functional thymic units with EAK16-II/EAKIIH6 self-assembling hydrogel
Tajima, A., Liu, W., Pradhan, I., Bertera, S., bagia, C., Trucco, M., Meng, W.S. and Fan, Y.
Clin. Immunol., ***160***, *82-89 (2015)*

Herein, we highlight the technical feasibility of generating a functional mini thymus with a novel hydrogel system, based on a peptide-based self-assembly platform that can induce the formation of 3-D thymic epithelial cell (TEC) clusters. Amphiphilic peptide EAK16-II co-assembled with its histidinylated analogue EAKIIH6 into beta-sheet fibrils. When adaptor complexes (recombinant protein A/G molecules loaded with both anti-His and anti-EpCAM IgGs) were added to the mix, TECs were tethered to the hydrogel and formed 3-D mini clusters. TECs bound to the hydrogel composites retained their molecular properties; and when transplanted into athymic nude mice, they supported the development of functional T-cells. These mini thymic units of TECs can be useful in clinical applications to reconstitute T-cell adaptive immunity.

4.1447 Recent Advances in Boar Sperm Cryopreservation: State of the Art and Current Perspectives
Yeste, M.
Reprod. Dom. Anim., ***50***, *Suppl. 2, 71-79 (2015)*

While sperm cryopreservation is the best technology to store boar semen for long-term periods, only 1% of all artificial inseminations (AI) conducted worldwide are made using frozen–thawed boar sperm. With the emergence of long-term extenders for liquid storage, the use of cryopreserved sperm in routine AI is less required. However, banks of boar semen contain cryopreserved sperm and planning inseminations in AI centres may benefit from the use of frozen–thawed semen. Therefore, there is an interest in the use of this technology to preserve boar sperm. In this regard, although the first attempts to cryopreserve boar semen date back to the seventies and this technology is still considered as optimal, some relevant improvements have been made in the last decade. After giving a general picture about boar sperm cryodamage, the present review seeks to shed light on these recent cryopreservation advances. These contributions regard to protein markers for predicting ejaculate freezability, sperm selection prior to start cryopreservation procedures, additives to freezing and thawing extenders, relevance of the AI-technique and insemination-to-ovulation interval. In conclusion, most of these progresses have allowed counteracting better boar sperm cryodamage and are thus considered as forward steps for this storage method. It is also worth noting that, despite being lower than fresh/extended semen, reproductive performance outcomes following AI with frozen-thawed boar sperm are currently acceptable.

4.1448 CD68 acts as a major gateway for malaria sporozoite liver infection
Cha, S-J., Park, K., Srinivasan, P., Schindler, C.W., van Rooijen, N., Stins, M. and Jacobs-Lorena, M.
J. Exp. Med., ***212(9)***, *1391-1403 (2015)*

After being delivered by the bite from an infected mosquito, *Plasmodium* sporozoites enter the blood circulation and infect the liver. Previous evidence suggests that Kupffer cells, a macrophage-like component of the liver blood vessel lining, are traversed by sporozoites to initiate liver invasion. However, the molecular determinants of sporozoite–Kupffer cell interactions are unknown. Understanding the molecular basis for this specific recognition may lead to novel therapeutic strategies to control malaria. Using a phage display library screen, we identified a peptide, P39, that strongly binds to the Kupffer cell surface and, importantly, inhibits sporozoite Kupffer cell entry. Furthermore, we determined that P39 binds to CD68, a putative receptor for sporozoite invasion of Kupffer cells that acts as a gateway for malaria infection of the liver.

4.1449 Selective Expression of the MAPK Phosphatase Dusp9/MKP-4 in Mouse Plasmacytoid Dendritic Cells and Regulation of IFN-β Production
Niedzielska, M. et al
J. Immunol., ***195(4)***, *1753-1762 (2015)*

Plasmacytoid dendritic cells (pDCs) efficiently produce large amounts of type I IFN in response to TLR7 and TLR9 ligands, whereas conventional DCs (cDCs) predominantly secrete high levels of the cytokines IL-10 and IL-12. The molecular basis underlying this distinct phenotype is not well understood. In this

study, we identified the MAPK phosphatase Dusp9/MKP-4 by transcriptome analysis as selectively expressed in pDCs, but not cDCs. We confirmed the constitutive expression of Dusp9 at the protein level in pDCs generated in vitro by culture with Flt3 ligand and ex vivo in sorted splenic pDCs. Dusp9 expression was low in $B220^{-}$ bone marrow precursors and was upregulated during pDC differentiation, concomitant with established pDC markers. Higher expression of Dusp9 in pDCs correlated with impaired phosphorylation of the MAPK ERK1/2 upon TLR9 stimulation. Notably, Dusp9 was not expressed at detectable levels in human pDCs, although these displayed similarly impaired activation of ERK1/2 MAPK compared with cDCs. Enforced retroviral expression of Dusp9 in mouse GM-CSF–induced cDCs increased the expression of TLR9-induced IL-12p40 and IFN-β, but not of IL-10. Conditional deletion of Dusp9 in pDCs was effectively achieved in $Dusp9^{flox/flox}$; CD11c-Cre mice at the mRNA and protein levels. However, the lack of Dusp9 in pDC did not restore ERK1/2 activation after TLR9 stimulation and only weakly affected IFN-β and IL-12p40 production. Taken together, our results suggest that expression of Dusp9 is sufficient to impair ERK1/2 activation and enhance IFN-β expression. However, despite selective expression in pDCs, Dusp9 is not essential for high-level IFN-β production by these cells.

4.1450 The Effect of Progestins on Tumor Necrosis Factor α-Induced Matrix Metalloproteinase-9 Activity and Gene Expression in Human Primary Amnion and Chorion Cells In Vitro

Allen, T.K., Feng, L., Nazzal, M., Grotegut, C.A., Buhimschi, I.A. and Murtha, A.P.

Anesth. Analg., ***120(5)****, 1085-1094 (2015)*

BACKGROUND: Current treatment modalities for preventing preterm premature rupture of membranes are limited, but progestins may play a role. Tumor necrosis factor α (TNFα) enhances matrix metalloproteinase-9 (MMP-9) gene expression and activity in fetal membranes, contributing to membrane weakening and rupture. We previously demonstrated that progestins attenuate TNFα-induced MMP-9 activity in a cytotrophoblast cell line. However, whether they have a similar effect in primary amnion and chorion cells of fetal membranes is unknown. In this study, we evaluated the effect of progestins on basal and TNFα-induced MMP-9 activity and gene expression in primary chorion and amnion cells harvested from the fetal membranes of term nonlaboring patients.
METHODS: Primary amnion and chorion cells were isolated from fetal membranes obtained from term uncomplicated nonlaboring patients following elective cesarean delivery (n = 11). Confluent primary amnion and chorion cell cultures were both pretreated with vehicle (control), progesterone (P4), 17α-hydroxyprogesterone caproate (17P), or medroxyprogesterone acetate (MPA) at 10^{-6} M concentration for 6 hours followed by stimulation with TNFα at 10 ng/mL for an additional 24 hours. Cell cultures pretreated with the vehicle only served as the unstimulated control and the vehicle stimulated with TNFα served as the stimulated control. Both controls were assigned a value of 100 units. Cell culture medium was harvested for MMP-9 enzymatic activity quantification using gelatin zymography. Total RNA was extracted for quantifying MMP-9 gene expression using real-time quantitative PCR. Basal MMP-9 activity and gene expression data were normalized to the unstimulated control. TNFα-stimulated MMP-9 activity and gene expression were normalized to the stimulated control. The primary outcome was the effect of progestins on TNFα-induced MMP-9 enzymatic activity in term human primary amnion and chorion cells in vitro. Secondary outcomes included the effect of progestin therapy on TNFα-induced MMP-9 gene expression and on basal MMP-9 activity and gene expression in primary amnion and chorion cells in vitro.
RESULTS: Primary cells were harvested from 11 patients. Compared with the unstimulated control, TNFα increased MMP-9 activity (P = 0.005 versus control in primary amnion cells and P < 0.001 versus control in primary chorion cells) and MMP-9 gene expression (P = 0.030 versus control in primary amnion cells, P < 0.001 versus control in primary chorion cells). Compared with the unstimulated controls, MPA, but not P4 or 17P, reduced basal MMP-9 activity [mean difference (95% CI) −49.6 (−81.9, −17.3) units, P = 0.001] and gene expression [mean difference (95% CI) −53.4 (−105.9, −0.9) units, P = 0.045] in primary amnion cells. Compared with the stimulated control, MPA also reduced TNFα-induced MMP-9 activity [mean difference (95% CI) −69.0 (−91.8, −46.3) units, P < 0.001] and gene expression [mean difference (95% CI) −86.0 (−120.7, −51.3) units, P < 0.001] in primary amnion cells. Progestin pretreatment had no significant effect on basal or TNFα-induced MMP-9 activity and gene expression in primary chorion cells.
CONCLUSIONS: The inhibitory effect of MPA on both basal and TNFα-induced MMP-9 activity and gene expression in primary amnion cells demonstrate a possible mechanism by which progestins may prevent fetal membrane weakening leading to preterm premature rupture of membranes.

4.1451 Axon stretch growth of adult primary motor neurons

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J. Neurochem., Suppl. 1, 333 (2015)

Growth of embryonic dorsal root ganglion (DRG) axons has been markedly enhanced by controlled in vitro stretching without the involvement of the growth cone. However limited work has been done on enhancement of axonal growth on adult motor neurons. The spinal cord was harvested from adult Sprague–Dawley rats (8– 12 weeks) by bilateral neural arch dissection, demyelinated and separated neurons from the homogenized spinal cord using Papain (36 U/mL in 6 mLs processing media) digestion and trituration. Motor neurons were isolated from the homogenate using an established 4 step Optiprep density gradient centrifugation and plated onto poly-D-lysine (100 mg/mL) coated glass and aclar substrate (at a cell density of 320–500 cells/mm3) within the axon stretch bioreactor. Cells were grown in the bioreactor using an optimized neuron culture media. The bioreactor consisted of three sections, which were assembled, autoclaved, plated with neurons and sealed. Nerve cells were grown under controlled temperature (37°C) and 5% CO2 atmosphere for 8 days prior to commencement of stretching of the axons. The axons were stretched using a motor driven device controlled by PIMikro- Move software, where the axons were subjected to stretching, resting and stretching in sequence at an incremental rate commencing at 0.5 mm per day in 2 lm increments with 500 ms resting between stretching. Stretching of axons was continued for up to 13 days. The amount of stretch was monitored using a microcamera mounted onto the objective of an inverted microscope. Cultured neurons typically developed axons and dendrites by 4 days and remained viable in excess of 21 days. Cells were identified as motor neurons using HB9, Islet-1 and Neurofilament-M antibodies. Early results indicate feasibility in using this tailored adult primary motor neuron protocol for axon stretch growth in vitro experiments.

4.1452 Myelin-associated glycoprotein modulates apoptosis of motoneurons during early postnatal development via NgR/p75NTR receptor-mediated activation of RhoA signaling pathways
Palandri, A., Salvador, V.R., Wojnacki, J., Vivinetto, A.L., Schnaar, R.L. and Lopez, P.H.H.
*Cell Death and Disease, **6**, e1876 (2015)*

Myelin-associated glycoprotein (MAG) is a minor constituent of nervous system myelin, selectively expressed on the periaxonal myelin wrap. By engaging multiple axonal receptors, including Nogo-receptors (NgRs), MAG exerts a nurturing and protective effect the axons it ensheaths. Pharmacological activation of NgRs has a modulatory role on $p75^{NTR}$-dependent postnatal apoptosis of motoneurons (MNs). However, it is not clear whether this reflects a physiological role of NgRs in MN development. NgRs are part of a multimeric receptor complex, which includes $p75^{NTR}$, Lingo-1 and gangliosides. Upon ligand binding, this multimeric complex activates RhoA/ROCK signaling in a $p75^{NTR}$-dependent manner. The aim of this study was to analyze a possible modulatory role of MAG on MN apoptosis during postnatal development. A time course study showed that *Mag*-null mice suffer a loss of MNs during the first postnatal week. Also, these mice exhibited increased susceptibility in an animal model of $p75^{NTR}$-dependent MN apoptosis induced by nerve-crush injury, which was prevented by treatment with a soluble form of MAG (MAG-Fc). The protective role of MAG was confirmed in *in vitro* models of $p75^{NTR}$-dependent MN apoptosis using the MN1 cell line and primary cultures. Lentiviral expression of shRNA sequences targeting NgRs on these cells abolished protection by MAG-Fc. Analysis of RhoA activity using a FRET-based RhoA biosensor showed that MAG-Fc activates RhoA. Pharmacological inhibition of $p75^{NTR}$/RhoA/ROCK pathway, or overexpression of a $p75^{NTR}$ mutant unable to activate RhoA, completely blocked MAG-Fc protection against apoptosis. The role of RhoA/ROCK signaling was further confirmed in the nerve-crush model, where pretreatment with ROCK inhibitor Y-27632 blocked the pro-survival effect of MAG-Fc. These findings identify a new protective role of MAG as a modulator of apoptosis of MNs during postnatal development by a mechanism involving the $p75^{NTR}$/RhoA/ROCK signaling pathway. Also, our results highlight the relevance of the nurture/protective effects of myelin on neurons.

4.1453 Inhibition of pancreatic stellate cell activity by adipose-derived stem cells
Yu, F-X., Su, L-F., Dai, C-L., Wang, DY., teng, Y-Y., Fu, J-H., Zhang, Q-Y. and Tang, Y-H.
*Hepatobiliary Pancreat. Dis. Inst., **14(2)**, 215-221 (2015)*

Background
Pancreatic stellate cells (PSCs) play a critical role in the development of pancreatic fibrosis. In this study we used a novel method to isolate and culture rat PSCs and then investigated the inhibitory effects of adipose-derived stem cells (ADSCs) on activation and proliferation of PSCs.
Methods
Pancreatic tissue was obtained from Sprague-Dawley rats for PSCs isolation. Transwell cell cultures were adopted for co-culture of ADSCs and PSCs. PSCs proliferation and apoptosis were determined using CCK-8 and flow cytometry, respectively. α-SMA expressions were analyzed using Western blotting. The levels of cytokines [nerve growth factor (NGF), interleukin-10 (IL-10) and transforming growth factor-β1

(TGF-β1)] in conditioned medium were detected by ELISA. Gene expression (MMP-2, MMP-9 and TIMP-1) was analyzed using qRT-PCR.
Results
This method produced $17.6\pm6.5\times10^3$ cells per gram of the body weight with a purity of 90%–95% and a viability of 92%–97%. Co-culture of PSCs with ADSCs significantly inhibited PSCs proliferation and induced PSCs apoptosis. Moreover, α-SMA expression was significantly reduced in PSCs+ADSCs compared with that in PSC-only cultures, while expression of fibrinolytic proteins (e.g., MMP-2 and MMP-9) was up-regulated and anti-fibrinolytic protein (TIMP-1) was down-regulated. In addition, NGF expression was up-regulated, but IL-10 and TGF-β1 expressions were down-regulated in the co-culture conditioned medium compared with those in the PSC-only culture medium.
Conclusions
This study provided an easy and reliable technique to isolate PSCs. The data demonstrated the inhibitory effects of ADSCs on the activation and proliferation of PSCs *in vitro*.

4.1454 **Spinal Cord Ischemia-Reperfusion Injury Induces Erythropoietin Receptor Expression**
Foley, L.S., Fullerton, D.A., Bennett, D.T., Freeman, K.A., Mares, J., Bell, M.T., Cleveland, J.C., Weyant, M.J., Meng, X., Puskas, F. and Reece, TS.B.
Ann. Thorac. Surg., ***100***, *41-46 (2015)*

Background
Paraplegia remains a devastating complication of aortic surgery, occurring in up to 20% of complex thoracoabdominal repairs. Erythropoietin (EPO) attenuates this injury in models of spinal cord ischemia. Upregulation of the beta-common receptor (βcR) subunit of the EPO receptor is associated with reduced damage in murine models of neural injury. This receptor activates anti-apoptotic pathways including signaling transducer and activator of transcription 3 (STAT3). We hypothesized that spinal cord ischemia-reperfusion injury upregulates the βcR subunit with a subsequent increase in activated STAT3.
Methods
Adult male C57/BL6 mice received an intraperitoneal injection of 0.5 mL of EPO (10 U/kg) or 0.9% saline after induction of anesthesia. Spinal cord ischemia was induced through sternotomy and 4-minute thoracic aortic cross-clamp. Sham mice underwent sternotomy without cross-clamp placement. Four groups were studied: ischemic and sham groups, each with and without EPO treatment. After 4 hours of reperfusion, spinal cords were harvested and homogenized. The βcR subunit expression and STAT3 activation were evaluated by immunoblot.
Results
Ischemia reperfusion increased βcR subunit expression in spinal cords of ischemia + saline and ischemia + EPO mice compared with shams (3.4 ± 1.39 vs 1.31 ± 0.3, $p = 0.01$ and 3.80 ± 0.58 vs 1.56 ± 0.32, $p = 0.01$). Additionally, both ischemic groups demonstrated increased STAT3 activation compared with shams (1.35 ± 0.14 vs 1.09 ± 0.07, $p = 0.01$ and 1.66 ± 0.35 vs 1.08 ± 0.17, $p = 0.02$).
Conclusions
Ischemia-reperfusion injury induces EPO receptor βcR subunit expression and early downstream anti-apoptotic signaling through STAT3 activation. Further investigation into the role of the βcR subunit is warranted to determine tissue protective functions of EPO. Elucidation of mechanisms involved in spinal cord protection is essential for reducing delayed paraplegia.
Delayed paraplegia resulting from spinal cord ischemia-reperfusion injury remains a devastating complication of complex aortic operations, occurring in up to 20% of thoracoabdominal repairs 1 and 2. Advances in operative technique, use of hypothermia and cerebrospinal fluid drainage have spared some patients from this unfortunate outcome over the last decade; however, these innovations have led to only minor reductions in its overall incidence [3]. Furthermore, there is currently no widely accepted pharmacologic treatment available that is proven to reduce this injury.
Neurologic damage after ischemia reperfusion occurs on a spectrum ranging from dense immediate postoperative paraplegia to delayed onset functional deterioration. This bimodal pattern of injury is due to an initial ischemic insult at the time of surgery followed by an inflammatory response during reperfusion, which accelerates neuronal loss and spinal cord dysfunction 4 and 5. Tissue hypoxia and metabolic stress activate the innate-adaptive immune response, characterized by proinflammatory cytokines, which amplify the injury, activate cell death pathways, and ultimately increase damage beyond the initial lesion [6]. Curtailing this pathophysiologic response has proven promising in translational brain and spinal cord injury research [7]. Erythropoietin (EPO) has emerged as a potential therapeutic tool, with anti-inflammatory and neuroprotective effects in murine models of ischemic stroke and contusive spinal cord injury [8]. Local cerebral production of EPO and its ability to cross the blood-brain barrier are important characteristics that highlight its potential as an endogenous neuroprotective cytokine. Erythropoietin has

been shown to improve functional outcomes and reduce neuronal loss in multiple animal models of ischemic injury, including in our own laboratory's murine model of spinal cord ischemia-reperfusion injury using a thoracic aortic cross-clamp 9 and 10. The gross functional benefits of EPO treatment have been described; however, its mechanisms remain unclear. Anti-inflammatory properties and activation of anti-apoptotic pathways, including that of signal transducer and activator of transcription 3 (STAT3) and B-cell lymphoma 2 protein (BCL-2, an inhibitor of cell death) are thought to mediate these effects. Recent investigations into tissue-protective properties of EPO have led to the identification of a unique receptor that specifically mediates these effects, distinct from the EPO receptor that mediates hematopoiesis [11]. This tissue-protective receptor is present in various organs including the brain, heart, and kidney, and is identifiable by a beta-common subunit (βcR or CD131) domain. The βcR subunit expression is characteristically low at baseline, and significantly induced by hypoxia and metabolic stress resulting in improved functional outcomes with EPO administration [12]. Expression of this receptor on injured cells precedes a local increase in tissue production of EPO temporally [13]. The tissue production of EPO lagging behind receptor expression results briefly in a relative deficit of EPO and creates a window of opportunity for therapeutic intervention, as seen in Figure 1[14].

4.1455 The pro-fibrotic and anti-inflammatory foam cell macrophage paradox

Thomas, A.C., Eijgelaar, W.J., daemen, M.J.A.P. and Newby, A.C.

*Genomics Data, **6**, 136-138 (2015)*

The formation of foamy macrophages by sequestering extracellular modified lipids is a key event in atherosclerosis. However, there is controversy about the effects of lipid loading on macrophage phenotype, with *in vitro* evidence suggesting either pro- or anti-inflammatory consequences. To investigate this *in vivo* we compared the transcriptomes of foamy and non-foamy macrophages that accumulate in experimental subcutaneous granulomas in fat-fed ApoE null mice or normal chow-fed wild-type mice, respectively. Consistent with previous studies in peritoneal macrophages from LDL receptor null mice (Spann et al., 2012 [1]), we found that anti-inflammatory LXR/RXR pathway genes were over-represented in the foamy macrophages, but there was no change in M1 or M2 phenotypic markers. Quite unexpectedly, however, we found that genes related to the induction of fibrosis had also been up-regulated (Thomas et al., 2015 [2]). The progression of the foamy macrophages along anti-inflammatory and pro-fibrotic pathways was confirmed using immunohistochemistry (described fully in our primary research article (Thomas et al., 2015 [2]). Here we provide additional details on production of the macrophages and their transcriptomic comparison, with the raw and processed microarray data deposited in GEO (accession number GSE70126). Our observations on these cells are indeed paradoxical, because foamy macrophages have long been implicated in promoting inflammation, extracellular matrix degradation and atherosclerotic plaque rupture, which must be provoked by additional local mediators. Our findings probably explain how very early macrophage-rich lesions maintain their structural integrity.

4.1456 MicroRNA125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells

Hyun, J., Wang, S., Kim, J., Kim, G.J. and Jung, Y.

*Scientific Reports, **5**:14135 (2015)*

Although chorionic plate-derived mesenchymal stem cells (CP-MSCs) were shown to promote liver regeneration, the mechanisms underlying the effect remain unclear. Hedgehog (Hh) signaling orchestrates tissue reconstruction in damaged liver. MSCs release microRNAs mediating various cellular responses. Hence, we hypothesized that microRNAs from CP-MSCs regulated Hh signaling, which influenced liver regeneration. Livers were obtained from carbon tetrachloride (CCl4)-treated rats transplanted with human CP-MSCs (Tx) or saline (non-Tx). Sonic Hh, one of Hh ligands, increased in CCl4-treated liver, whereas it decreased in CP-MSC-treated liver with CCl4. The expression of Hh-target genes was significantly downregulated in the Tx. Reduced expansion of progenitors and regressed fibrosis were observed in the liver of the Tx rats. CP-MSCs suppressed the expression of Hh and profibrotic genes in co-cultured LX2 (human hepatic stellate cell) with CP-MSCs. MicroRNA-125b targeting smo was retained in exosomes of CP-MSCs. CP-MSCs with microRNA-125b inhibitor failed to attenuate the expression of Hh signaling and profibrotic genes in the activated HSCs. Therefore, these results demonstrated that microRNA-125b from CP-MSCs suppressed the activation of Hh signaling, which promoted the reduced fibrosis, suggesting that microRNA-mediated regulation of Hh signaling contributed to liver regeneration by CP-MSCs.

4.1457 Inhibition of NOX2 reduces locomotor impairment, inflammation, and oxidative stress after spinal cord injury

Khayrullina, G., Bermudez, S and Byrnes, K.R.
*J. Neuroinflammation, **12**:172 (2015)*

Background
Spinal cord injury (SCI) results in the activation of the NADPH oxidase (NOX) enzyme, inducing production of reactive oxygen species (ROS). We hypothesized that the NOX2 isoform plays an integral role in post-SCI inflammation and functional deficits.
Methods
Moderate spinal cord contusion injury was performed in adult male mice, and flow cytometry, western blot, and immunohistochemistry were used to assess NOX2 activity and expression, inflammation, and M1/M2 microglia/macrophage polarization from 1 to 28 days after injury. The NOX2-specific inhibitor, gp91ds-tat, was injected into the intrathecal space immediately after impact. The Basso Mouse Scale (BMS) was used to assess locomotor function at 24 h post-injury and weekly thereafter.
Results
Our findings show that gp91ds-tat treatment significantly improved functional recovery through 28 days post-injury and reduced inflammatory cell concentrations in the injured spinal cord at 24 h and 7 days post-injury. In addition, a number of oxidative stress markers were reduced in expression at 24 h after gp91ds-tat treatment, which was accompanied by a reduction in M1 polarization marker expression.
Conclusion
Based on our findings, we now conclude that inhibition of NOX2 significantly improves outcome after SCI, most likely via acute reductions in oxidative stress and inflammation. NOX2 inhibition may therefore have true potential as a therapy after SCI.

4.1458 All-In-One: Advanced preparation of Human Parenchymal and Non-Parenchymal Liver Cells

Werner, M., Driftmann, S., Kleinehr, K., Kaiser, G.M., mathe, Z., treckmann, J-w., paul, A., Sibbe, K., Timm, J., Canbay, A., gerken, G., Schlaak, J.F. and Boering, R.
*PloS One, **10(9)**, e0138655 (2015)*

Background & Aims
Liver cells are key players in innate immunity. Thus, studying primary isolated liver cells is necessary for determining their role in liver physiology and pathophysiology. In particular, the quantity and quality of isolated cells are crucial to their function. Our aim was to isolate a large quantity of high-quality human parenchymal and non-parenchymal cells from a single liver specimen.
Methods
Hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, and stellate cells were isolated from liver tissues by collagenase perfusion in combination with low-speed centrifugation, density gradient centrifugation, and magnetic-activated cell sorting. The purity and functionality of cultured cell populations were controlled by determining their morphology, discriminative cell marker expression, and functional activity.
Results
Cell preparation yielded the following cell counts per gram of liver tissue: $2.0\pm0.4\times10^7$ hepatocytes, $1.8\pm0.5\times10^6$ Kupffer cells, $4.3\pm1.9\times10^5$ liver sinusoidal endothelial cells, and $3.2\pm0.5\times10^5$ stellate cells. Hepatocytes were identified by albumin (95.5±1.7%) and exhibited time-dependent activity of cytochrome P450 enzymes. Kupffer cells expressed CD68 (94.5±1.2%) and exhibited phagocytic activity, as determined with 1μm latex beads. Endothelial cells were $CD146^+$ (97.8±1.1%) and exhibited efficient uptake of acetylated low-density lipoprotein. Hepatic stellate cells were identified by the expression of α-smooth muscle actin (97.1±1.5%). These cells further exhibited retinol (vitamin A)-mediated autofluorescence.
Conclusions
Our isolation procedure for primary parenchymal and non-parenchymal liver cells resulted in cell populations of high purity and quality, with retained physiological functionality *in vitro*. Thus, this system may provide a valuable tool for determining liver function and disease.

4.1459 Single human sperm cryopreservation method using hollow-core agarose capsules

Araki, Y., Yao, T., Asayama, Y., matsuhisa, A. and Araki, Y.
*Fertility and Sterility, **104(4)**, 1004-1009 (2015)*

Objective: To develop an efficient cryopreservation method using a single sperm.

Design: Experimental study.
Setting: Laboratory of a private institute.
Patient(s): A fertile donor.
Intervention(s):We produced hollow-core capsules with agarose walls. A single human sperm was injected into each capsule as per the conventional intracytoplasmic sperm injection (ICSI) method. The capsules that contained the spermatozoa were cryopreserved on polycarbonate or nylon mesh sheets using nitrogen vapor. Before their use, the capsules were thawed and recovered. The motile spermatozoa in the capsules were counted.
Main Outcome Measure(s)
The recovery rates of the agarose capsules and the spermatozoa in these capsules after thawing and the mortality and survival rates of the spermatozoa.
Result(s)
The recovery rates of the capsules were 91.5% (75/82) using polycarbonate sheets (PS) and 98.3% (59/60) using mesh sheets (MS) after thawing. The recovered capsules were not at all damaged. The recovery rates of the spermatozoa were 91.5% (75/82) using PS and 96.7% (58/60) using MS. Sperm motility rates were 85.3% (64/75) and 82.8% (48/58), whereas the survival rates of the immotile spermatozoa by the hypoosmotic swelling test were 81.8% (9/11) and 50.0% (5/10); furthermore, the total survival rates of the spermatozoa were 97.3% (73/75) and 91.4% (53/58) using PS and MS, respectively. There was no significant difference between the results obtained using PS and MS.
Conclusion(s)
A cryopreservation method for a single sperm using an agarose capsule has been developed. The method is expected to be useful in ICSI treatment in patients with few spermatozoa.

4.1460 Pilot Study Evaluating Regulatory T Cell–Promoting Immunosuppression and Nonimmunogenic Donor Antigen Delivery in a Nonhuman Primate Islet Allotransplantation Model
Lei, J. et al
Am. J. Transplant., ***15(10)****, 2739-2749 (2015)*

The full potential of islet transplantation will only be realized through the development of tolerogenic regimens that obviate the need for maintenance immunosuppression. Here, we report an immunotherapy regimen that combines 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide (ECDI)-treated donor lymphoid cell infusion (ECDI-DLI) with thymoglobulin, anti-interleukin-6 receptor antibody and rapamycin to achieve prolonged allogeneic islet graft survival in a nonhuman primate (NHP) model. Prolonged graft survival is associated with Treg expansion, donor-specific T cell hyporesponsiveness and a transient absence of donor-specific alloantibody production during the period of graft survival. This regimen shows promise for clinical translation.

4.1461 Acute GVHD results in a severe DC defect that prevents T-cell priming and leads to fulminant cytomegalovirus disease in mice
Wikstrom, M., Fleming, P., Kuns, R.D., Schuster, I.S., Voigt, V., Miller, G., Clouston, A.D., Tey, S-K., Andoniou, C.E., Hill, G.R. and Degli-Esposti, M.A.
Blood, ***126(12)****, 1503-1514 (2015)*

Viral infection is a common, life-threatening complication after allogeneic bone marrow transplantation (BMT), particularly in the presence of graft-versus-host disease (GVHD). Using cytomegalovirus (CMV) as the prototypic pathogen, we have delineated the mechanisms responsible for the inability to mount protective antiviral responses in this setting. Although CMV infection was self-limiting after syngeneic BMT, in the presence of GVHD after allogeneic BMT, CMV induced a striking cytopathy resulting in universal mortality in conjunction with a fulminant necrotizing hepatitis. Critically, GVHD induced a profound dendritic cell (DC) defect that led to a failure in the generation of CMV-specific $CD8^+$ T-cell responses. This was accompanied by a defect in antiviral $CD8^+$ T cells. In combination, these defects dramatically limited antiviral T-cell responses. The transfer of virus-specific cells circumvented the DC defects and provided protective immunity, despite concurrent GVHD. These data demonstrate the importance of avoiding GVHD when reconstructing antiviral immunity after BMT, and highlight the mechanisms by which the adoptive transfer of virus-specific T cells overcome the endogenous defects in priming invoked by GVHD.

4.1462 Density-gradient centrifugation enables the purification of cultured corneal endothelial cells for cell therapy by eliminating senescent cells
Okumura, N., Kusakabe, A., Hirano, H., Inoue, R., Okazaki, Y., nakano, S., Kinoshita, S. and Koizumi, N.

*Scientific Reports, **5**:15005 (2015)*

The corneal endothelium is essential for maintaining corneal transparency; therefore, corneal endothelial dysfunction causes serious vision loss. Tissue engineering-based therapy is potentially a less invasive and more effective therapeutic modality. We recently started a first-in-man clinical trial of cell-based therapy for treating corneal endothelial dysfunction in Japan. However, the senescence of corneal endothelial cells (CECs) during the serial passage culture needed to obtain massive quantities of cells for clinical use is a serious technical obstacle preventing the push of this regenerative therapy to clinical settings. Here, we show evidence from an animal model confirming that senescent cells are less effective in cell therapy. In addition, we propose that density-gradient centrifugation can eliminate the senescent cells and purify high potency CECs for clinical use. This simple technique might be applicable for other types of cells in the settings of regenerative medicine.

4.1463 Platelet binding sites for factor VIII in relation to fibrin and phosphatidylserine
Gilbert, G.E., Novakovic, V.A., Shi, J., Rasmussen, J. and Pipe, S.W.
Blood, 126(10), 1237-1244 (2015)

Thrombin-stimulated platelets expose very little phosphatidylserine (PS) but express binding sites for factor VIII (fVIII), casting doubt on the role of exposed PS as the determinant of binding sites. We previously reported that fVIII binding sites are increased three- to sixfold when soluble fibrin (SF) binds the αIIbβ3 integrin. This study focuses on the hypothesis that platelet-bound SF is the major source of fVIII binding sites. Less than 10% of fVIII was displaced from thrombin-stimulated platelets by lactadherin, a PS-binding protein, and an fVIII mutant defective in PS-dependent binding retained platelet affinity. Therefore, PS is not the determinant of most binding sites. FVIII bound immobilized SF and paralleled platelet binding in affinity, dependence on separation from von Willebrand factor, and mediation by the C2 domain. SF also enhanced activity of fVIII in the factor Xase complex by two- to fourfold. Monoclonal antibody (mAb) ESH8, against the fVIII C2 domain, inhibited binding of fVIII to SF and platelets but not to PS-containing vesicles. Similarly, mAb ESH4 against the C2 domain, inhibited >90% of platelet-dependent fVIII activity vs 35% of vesicle-supported activity. These results imply that platelet-bound SF is a component of functional fVIII binding sites.

4.1464 Moderate- and high-intensity exhaustive exercise in the heat induce a similar increase in monocyte Hsp72
Periard, J.D., Ruell, P.A., Thompson, M.W. and Caillaud, C.
*Cell Stress and Chaperones, **20(6)**, 1037-1042 (2015)*

This study examined the relationship between exhaustive exercise in the heat at moderate and high intensities on the intracellular heat shock protein 72 (iHsp72) response. Twelve male subjects cycled to exhaustion at 60 and 75 % of maximal oxygen uptake in hot conditions (40 °C, 50 % RH). iHsp72 concentration was measured in monocytes before, at exhaustion and 24 h after exercise. Rectal temperature, heart rate and oxygen uptake were recorded during exercise. Volitional exhaustion occurred at 58.9 ± 12.1 and 27.3 ± 9.5 min ($P < 0.001$) and a rectal temperature of 39.8 ± 0.4 and 39.2 ± 0.6 °C ($P = 0.002$), respectively, for 60 and 75 %. The area under the curve above a rectal temperature of 38.5 °C was greater at 60 % (17.5 ± 6.6 °C min) than 75 % (3.4 ± 4.8 °C min; $P < 0.001$), whereas the rate of increase in rectal temperature was greater at 75 % (5.1 ± 1.7 vs. 2.2 ± 1.4 °C h^{-1}; $P < 0.001$). iHsp72 concentration increased similarly at exhaustion relative to pre-exercise ($P = 0.044$) and then increased further at 24 h ($P < 0.001$). Multiple regression analysis revealed no predictor variables associated with iHsp72 expression; however, a correlation was observed between exercise intensities for the increase in iHsp expression at exhaustion and 24 h ($P < 0.05$). These results suggest that iHsp72 expression increased in relation to the level of hyperthermia attained and sustained at 60 % and the higher metabolic rate and greater rate of increase in core temperature at 75 %, with the further increase in iHsp72 concentration 24 h after exercise reinforcing its role as a chaperone and cytoprotective agent.

4.1465 Increased Sensitivity to Binge Alcohol-Induced Gut Leakiness and Inflammatory Liver Disease in HIV Transgenic Rats
Banerjee, A., Abdelmegeed, M.A., jang, S. And Song, B-J.
*PloS One, **10(10)**, e0140498 (2015)*

The mechanisms of alcohol-mediated advanced liver injury in HIV-infected individuals are poorly understood. Thus, this study was aimed to investigate the effect of binge alcohol on the inflammatory liver disease in HIV transgenic rats as a model for simulating human conditions. Female wild-type (WT) or HIV transgenic rats were treated with three consecutive doses of binge ethanol (EtOH) (3.5 g/kg/dose oral gavages at 12-h intervals) or dextrose (Control). Blood and liver tissues were collected at 1 or 6-h following the last dose of ethanol or dextrose for the measurements of serum endotoxin and liver pathology, respectively. Compared to the WT, the HIV rats showed increased sensitivity to alcohol-mediated gut leakiness, hepatic steatosis and inflammation, as evidenced with the significantly elevated levels of serum endotoxin, hepatic triglycerides, histological fat accumulation and F4/80 staining. Real-time PCR analysis revealed that hepatic levels of toll-like receptor-4 (TLR4), leptin and the downstream target monocyte chemoattractant protein-1 (MCP-1) were significantly up-regulated in the HIV-EtOH rats, compared to all other groups. Subsequent experiments with primary cultured cells showed that both hepatocytes and hepatic Kupffer cells were the sources of the elevated MCP-1 in HIV-EtOH rats. Further, TLR4 and MCP-1 were found to be upregulated by leptin. Collectively, these results show that HIV rats, similar to HIV-infected people being treated with the highly active anti-retroviral therapy (HAART), are more susceptible to binge alcohol-induced gut leakiness and inflammatory liver disease than the corresponding WT, possibly due to additive or synergistic interaction between binge alcohol exposure and HIV infection. Based on these results, HIV transgenic rats can be used as a surrogate model to study the molecular mechanisms of many disease states caused by heavy alcohol intake in HIV-infected people on HAART.

4.1466 Gene expression of peripheral blood mononuclear cells is affected by cold exposure
Reynes, B., Garcia-Ruiz, E., Oliver, P. and Palou, A.
*Am. J. Physiol. Regul. Comp. Physiol., **309**, R824-R834 (2015)*

Because of the discovery of brown adipose tissue (BAT) in humans, there is increased interest in the study of induction of this thermogenic tissue as a basis to combat obesity and related complications. Cold exposure is one of the strongest stimuli able to activate BAT and to induce the appearance of brown-like (brite) adipocytes in white fat depots (browning process). We analyzed the potential of peripheral blood mononuclear cells (PBMCs) to reflect BAT and retroperitoneal white adipose tissue (rWAT) response to 1-wk cold acclimation (4°C) at different ages of rat development (1, 2, 4, and 6 mo). As expected, cold exposure increased fatty acid β-oxidation capacity in BAT and rWAT (increased *Cpt1a* expression), explaining increased circulating nonesterified free fatty acids and decreased adiposity. Cold exposure increased expression of the key thermogenic gene, *Ucp1*, in BAT and rWAT, but only in 1-mo-old animals. Additionally, other brown/brite markers were affected by cold during the whole developmental period studied in BAT. However, in rWAT, cold exposure increased studied markers mainly at early age. PBMCs did not express *Ucp1*, but expressed other brown/brite markers, which were cold regulated. Of particular interest, PBMCs reflected adipose tissue-increased *Cpt1a* mRNA expression in response to cold (in older animals) and browning induction occurring in rWAT of young animals (1 mo) characterized by increased *Cidea* expression and by the appearance of a high number of multilocular CIDE-A positive adipocytes. These results provide evidence pointing to PBMCs as an easily obtainable biological material to be considered to perform browning studies with minimum invasiveness.

4.1467 Liver Sinusoidal Endothelial Cells Escape Senescence by Loss of p19ARF
Kouldelkova, P., Weber, G. and Mikulits, W.
*PloS One, **10(11)**, e0142134 (2015)*

Liver sinusoidal endothelial cells (LSECs) represent a highly differentiated cell type that lines hepatic sinusoids. LSECs form a discontinuous endothelium due to fenestrations under physiological conditions, which are reduced upon chronic liver injury. Cultivation of rodent LSECs associates with a rapid onset of stress-induced senescence a few days post isolation, which limits genetic and biochemical studies *ex vivo*. Here we show the establishment of LSECs isolated from $p19^{ARF-/-}$ mice which undergo more than 50 cell doublings in the absence of senescence. Isolated $p19^{ARF-/-}$ LSECs display a cobblestone-like morphology and show the ability of tube formation. Analysis of DNA content revealed a stable diploid phenotype after long-term passaging without a gain of aneuploidy. Notably, $p19^{ARF-/-}$ LSECs express the endothelial markers CD31, vascular endothelial growth factor receptor (VEGFR)-2, VE-cadherin, von Willebrand factor, stabilin-2 and CD146 suggesting that these cells harbor and maintain an endothelial phenotype. In line, treatment with small molecule inhibitors against VEGFR-2 caused cell death, demonstrating the sustained ability of $p19^{ARF-/-}$ LSECs to respond to anti-angiogenic therapeutics. From these data we

conclude that loss of $p19^{ARF}$ overcomes senescence of LSECs, allowing immortalization of cells without losing endothelial characteristics. Thus, $p19^{ARF-/-}$ LSECs provide a novel cellular model to study endothelial cell biology.

4.1468 Non-Canonical Wnt Predominates in Activated Rat Hepatic Stellate Cells, Influencing HSC Survival and Paracrine Stimulation of Kupffer Cells
Corbett, L., Mann, J. and Mann,. D.A.
*PloS One, **10(11)**, e0142794 (2015)*

The Wnt system is highly complex and is comprised of canonical and non-canonical pathways leading to the activation of gene expression. Our aim was to examine changes in the expression of Wnt ligands and regulators during hepatic stellate cell (HSC) transdifferentiation and assess the relative contributions of the canonical and non-canonical Wnt pathways in fibrogenic activated HSC. The expression profile of Wnt ligands and regulators in HSC was not supportive for a major role for β-catenin-dependent canonical Wnt signalling, this verified by inability to induce Topflash reporter activity in HSC even when expressing a constitutive active β-catenin. We detected expression of Wnt5a in activated HSC which can signal via non-canonical mechanisms and showed evidence for non-canonical signalling in these cells involving phosphorylation of Dvl2 and pJNK. Stimulation of HSC or Kupffer cells with Wnt5a regulated HSC apoptosis and expression of TGF-β1 and MCP1 respectively. We were unable to confirm a role for β-catenin-dependent canonical Wnt in HSC and instead propose autocrine and paracrine functions for Wnts expressed by activated HSC via non-canonical pathways. The data warrant detailed investigation of Wnt5a in liver fibrosis.

4.1469 Type I IFN-mediated synergistic activation of mouse and human DC subsets by TLR agonists
Kreutz, M., Bakdash, G., Dolen, Y., Sköld, A.E., van Hout-Kuijer, M.A., de Vries, I.J.M. and Figdor, C.G.
*Eur. J. Immunol., **45(10)**, 2798-2909 (2015)*

Novel approaches of dendritic cell (DC) based cancer immunotherapy aim at harnessing the unique attributes of different DC subsets. Classical monocyte-derived DC vaccines are currently being replaced by either applying primary DCs or specifically targeting antigens and adjuvants to these subsets in vivo. Appropriate DC activation in both strategies is essential for optimal effect. For this purpose TLR agonists are favorable adjuvant choices, with TLR7 triggering being essential for inducing strong Th1 responses. However, mouse $CD8\alpha^+$ DCs, considered to be the major cross-presenting subset, lack TLR7 expression. Interestingly, this DC subset can respond to TLR7 ligand upon concurrent TLR3 triggering. Nevertheless, the mechanism underlying this synergy remains obscure. We now show that TLR3 ligation results in the production of IFN-α, which rapidly induces the expression of TLR7, resulting in synergistic activation. Moreover, we demonstrate that this mechanism conversely holds for plasmacytoid DCs that respond to TLR3 ligation when TLR7 pathway is mobilized. We further demonstrate that this mechanism of sharpening DC senses is also conserved in human $BDCA1^+$ DCs and plasmacytoid DCs. These findings have important implications for future clinical trials as it suggests that combinations of TLR ligands should be applied irrespective of initial TLR expression profiles on natural DC subsets for optimal stimulation.

4.1470 Dynamics and Transcriptomics of Skin Dendritic Cells and Macrophages in an Imiquimod-Induced, Biphasic Mouse Model of Psoriasis
Terhorst, D., Chelbi, R., Wohn, C., Malosse, C., Tamoutounour, S., Jorquera, A., bajenoff, M., Ddalod, M., Malissen, B. and Henri, S.
*J. Immunol., **195(10)**, 4953-4961 (2015)*

Psoriasis is a chronic inflammatory skin disease of unknown etiology. Previous studies showed that short-term, 5–7 d-long application of imiquimod (IMQ), a TLR7 agonist, to the skin of mice triggers a psoriasis-like inflammation. In the current study, by applying IMQ for 14 consecutive d, we established an improved mouse psoriasis-like model in that it recapitulated many of the clinical and cellular hallmarks observed in human patients during both the early-onset and the late-stable phase of psoriasis. Although macrophages and dendritic cells (DCs) have been proposed to drive the psoriatic cascade, their largely overlapping phenotype hampered studying their respective role. Based on our ability to discriminate Langerhans cells (LCs), conventional DCs, monocytes, monocyte-derived DCs, macrophages, and plasmacytoid DCs in the skin, we addressed their dynamics during both phases of our biphasic psoriasis-like model. Plasmacytoid DCs were not detectable during the whole course of IMQ treatment. During the early phase, neutrophils infiltrated the epidermis, whereas monocytes and monocyte-derived DCs were predominant in the dermis. During the late phase, LCs and macrophage numbers transiently increased in the epidermis and dermis,

respectively. LC expansion resulted from local proliferation, a conclusion supported by global transcriptional analysis. Genetic depletion of LCs permitted to evaluate their function during both phases of the biphasic psoriasis-like model and demonstrated that their absence resulted in a late phase that is associated with enhanced neutrophil infiltration. Therefore, our data support an anti-inflammatory role of LCs during the course of psoriasis-like inflammation.

4.1471 Brief Exercises Affect Gene Expression in Circulating Monocytes
Wang, D., Cai, F., Ge, J. and Yin, L.
Scand. J. Immunol., ***82(5)***, *429-235 (2015)*

We aimed to give a systematic hypothesis on the functions of exercise on circulating monocytes by identifying a discrete set of genes in circulating monocytes that were altered by exercise. The microarray expression profile of GSE51835 was downloaded from gene expression omnibus (GEO) database for the identification of differentially expressed genes (DEGs) using limma and affy packages in R language. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed for DEGs, followed by the construction of co-expression network and protein–protein interaction (PPI) network. The top 10 nodes in PPI network were screened, and subnetwork was constructed for the key genes identification. Totally, 35 DEGs, including 2 upregulated genes and 33 downregulated genes, were identified. The enriched GO terms were mainly linked to immune response and defence response, and the enriched KEGG pathways were mainly associated with natural killer cell-mediated cytotoxicity and graft-versus-host disease. Dual-specificity phosphatase 2 (*DUSP2*) was identified as a key node in the co-expression network. In the PPI network, CD247 module (*CD247*), chemokine (C-X-C motif) receptor 4 (*CXCR4*), granzyme B (*GZMB*) and perforin 1 (*PRF1*) were identified as key nodes. An important interaction, GZMB/PRF1, was detected. Five key genes, including *DUSP2*, *CD247*, *CXCR4*, *GZMB* and *PRF1*, and an interaction of GZMB/PRF1, were significant factors in the immune processes of circulating monocytes, which might be regulated by brief exercises, leading to the enhancement of immune function.

4.1472 Expression of Peroxiredoxin 1 After Traumatic Spinal Cord Injury in Rats
Huang, S., Liu, X., Zhang, J., Bao, g., Xu, G., Sun, Y., Shen, Q., Lian, M., Huang, Y. and Cui, Z.
Cell. Mol. Neurobiol., ***35(8)***, *1217-1226 (2015)*

Reactive astrogliosis and microgliosis after spinal cord injury (SCI) contribute to glial scar formation that impedes axonal regeneration. The mechanisms underlying reactive astrocyte and microglia proliferation upon injury remain partially understood. Peroxiredoxin 1 (PRDX1) is an antioxidant participating in cell proliferation, differentiation, and apoptosis. However, PRDX1 functions in SCI-induced astrocyte and microglia proliferation are unknown. In this study, we established an acute spinal cord contusion injury model in adult rats to investigate the potential role of PRDX1 during the pathological process of SCI. We found the palpable expression increase of PRDX1 after SCI by western blot and immunohistochemistry staining. Double immunofluorescence staining showed that PRDX1 expression mainly increased in astrocytes and microglia. In addition, PRDX1/proliferating cell nuclear antigen (PCNA) colocalized in astrocytes and microglia. Furthermore, PCNA expression also elevated after SCI, as well as was positively correlated with PRDX1 expression. In vitro, PRDX1 expression in primary rat spinal cord astrocytes and microglia changed in a concentration- and time-dependent manner according to LPS treatment. In addition, PRDX1 knockdown in astrocytes and microglia resulted in the decrease of PCNA expression after LPS stimulation, showing that PRDX1 promoted astrocyte and microglia proliferation after inflammation. Our results suggested that PRDX1 might play a crucial role in astrocyte and microglia proliferation after SCI.

4.1473 Characterizing Cellular Biophysical Responses to Stress by Relating Density, Deformability, and Size
Byun, S., Hecht, V.C. and Manalis, S.R.
Biophys. J., ***109(8)***

Cellular physical properties are important indicators of specific cell states. Although changes in individual biophysical parameters, such as cell size, density, and deformability, during cellular processes have been investigated in great detail, relatively little is known about how they are related. Here, we use a suspended microchannel resonator (SMR) to measure single-cell density, volume, and passage time through a narrow constriction of populations of cells subjected to a variety of environmental stresses. Osmotic stress significantly affects density and volume, as previously shown. In contrast to density and volume, the effect of an osmotic challenge on passage time is relatively small. Deformability, as determined by comparing

passage times for cells with similar volume, exhibits a strong dependence on osmolarity, indicating that passage time alone does not always provide a meaningful proxy for deformability. Finally, we find that protein synthesis inhibition, cell-cycle arrest, protein kinase inhibition, and cytoskeletal disruption result in unexpected relationships among deformability, density, and volume. Taken together, our results suggest that by measuring multiple biophysical parameters, one can detect unique characteristics that more specifically reflect cellular behaviors.

4.1474 Ubiquitin C-terminal hydrolase 1: A novel functional marker for liver myofibroblasts and a therapeutic target in chronic liver disease

Wilson, C.L., Murphy, L.B., Leslie, J., Kendrick, S., French, J., Fox, C.R., Sheerin, N.S., Fisher, A., Robinson, J.H., Tiniakos, D.G., Gray, D.A., Oakley, F. and Mann, D.A.
J. Hepatol., ***63***, *1421-1428 (2015)*

Background & Aims
Ubiquitination is a reversible protein modification involved in the major cellular processes that define cell phenotype and behaviour. Ubiquitin modifications are removed by a large family of proteases named deubiquitinases. The role of deubiquitinases in hepatic stellate cell (HSC) activation and their contribution to fibrogenesis are poorly defined. We have identified that the deubiquitinase ubiquitin C-terminal hydrolase 1 (UCHL1) is highly induced following HSC activation, determined its function in activated HSC and its potential as a therapeutic target for fibrosis.
Methods
Deubiquitinase expression was determined in day 0 and day 10 HSC. Increased UCHL1 expression was confirmed in human HSC and in an alcoholic liver disease (ALD) patient liver. The importance of UCHL1 in hepatic fibrosis was investigated in CCl_4 and bile duct ligation injured mice using a pharmacological inhibitor (LDN 57444). The effects of UCHL1 inhibition on HSC proliferation were confirmed by Western blot and 3H thymidine incorporation.
Results
Here we report that pharmacological inhibition of UCHL1 blocks progression of established fibrosis in CCl_4 injured mice. UCHL1 siRNA knockdown, LDN 57444 treatment, or HSC isolated from *UCHL1*$^{-/-}$ mice show attenuated proliferation in response to the mitogen, platelet-derived growth factor. Additionally, we observed changes in the phosphorylation of the cell cycle regulator retinoblastoma protein (Rb) in the absence of UCHL1 highlighting a potential mechanism for the reduced proliferative response.
Conclusions
UCHL1 expression is highly upregulated upon HSC activation and is involved in the regulation of HSC proliferation. This study highlights therapeutic opportunities for pharmacological targeting of UCHL1 in chronic liver disease.

4.1475 Long Non-coding RNA Growth Arrest-specific Transcript 5 (GAS5) Inhibits Liver Fibrogenesis through a Mechanism of Competing Endogenous RNA

Yu, F., Zheng, J., Mao, Y., Dong, P., Lu, Z., Li, G., Guo, C., Liu, Z. and Fan, X.
J. Biol. Chem., ***290(47)***, *28286-28298 (2015)*

Effective control of hepatic stellate cell (HSC) activation and proliferation is critical to the treatment of liver fibrosis. Long non-coding RNAs have been shown to play a pivotal role in the regulation of cellular processes. It has been reported that growth arrest-specific transcript 5 (GAS5) acts as a crucial mediator in the control of cell proliferation and growth. However, little is known about the role and underlying mechanism of GAS5 in liver fibrosis. In this study, our results indicated that GAS5 expression was reduced in mouse, rat, and human fibrotic liver samples and in activated HSCs. Overexpression of GAS5 suppressed the activation of primary HSCs *in vitro* and alleviated the accumulation of collagen in fibrotic liver tissues *in vivo*. We identified GAS5 as a target of microRNA-222 (miR-222) and showed that miR-222 could inhibit the expression of GAS5. Interestingly, GAS5 could also repress miR-222 expression. A pulldown assay further validated that GAS5 could directly bind to miR-222. As a competing endogenous RNAs, GAS5 had no effect on primary miR-222 expression. In addition, GAS5 was mainly localized in the cytoplasm. Quantitative RT-PCR further demonstrated that the copy numbers of GAS5 per cell are higher than those of miR-222. GAS5 increased the level of p27 protein by functioning as a competing endogenous RNA for miR-222, thereby inhibiting the activation and proliferation of HSCs. Taken together, a new regulatory circuitry in liver fibrosis has been identified in which RNAs cross-talk by competing for shared microRNAs. Our findings may provide a new therapeutic strategy for liver fibrosis.

4.1476 Dectin-1 Regulates Hepatic Fibrosis and Hepatocarcinogenesis by Suppressing TLR4 Signaling

Pathways
Seifert, L. et al
*Cell Reports, **13**, 1-13 (2015)*

Dectin-1 is a C-type lectin receptor critical in anti-fungal immunity, but Dectin-1 has not been linked to regulation of sterile inflammation or oncogenesis. We found that Dectin-1 expression is upregulated in hepatic fibrosis and liver cancer. However, Dectin-1 deletion exacerbates liver fibro-inflammatory disease and accelerates hepatocarcinogenesis. Mechanistically, we found that Dectin-1 protects against chronic liver disease by suppressing TLR4 signaling in hepatic inflammatory and stellate cells. Accordingly, *Dectin-1–/–* mice exhibited augmented cytokine production and reduced survival in lipopolysaccharide (LPS)-mediated sepsis, whereas Dectin-1 activation was protective. We showed that Dectin-1 inhibits TLR4 signaling by mitigating TLR4 and CD14 expression, which are regulated by Dectin-1-dependent macrophage colony stimulating factor (M-CSF) expression. Our study suggests that Dectin-1 is an attractive target for experimental therapeutics in hepatic fibrosis and neoplastic transformation. More broadly, our work deciphers critical cross-talk between pattern recognition receptors and implicates a role for Dectin-1 in suppression of sterile inflammation, inflammation-induced oncogenesis, and LPS-mediated sepsis.

4.1477 CD11chi Dendritic Cells Regulate Ly-6Chi Monocyte Differentiation to Preserve Immune-privileged CNS in Lethal Neuroinflammation
Kim, J.H., Choi, J.Y., Kim, S.B., Uyangaa, E., patil, A.M., Han, Y.W., park, S-Y., Lee, J.H., Kim, K. and Eo, S.K.
*Scientific Reports, **5**:17548 (2015)*

Although the roles of dendritic cells (DCs) in adaptive defense have been defined well, the contribution of DCs to T cell-independent innate defense and subsequent neuroimmunopathology in immune-privileged CNS upon infection with neurotropic viruses has not been completely defined. Notably, DC roles in regulating innate CD11b+Ly-6Chi monocyte functions during neuroinflammation have not yet been addressed. Using selective ablation of CD11chiPDCA-1int/lo DCs without alteration in CD11cintPDCA-1hi plasmacytoid DC number, we found that CD11chi DCs are essential to control neuroinflammation caused by infection with neurotropic Japanese encephalitis virus, through early and increased infiltration of CD11b+Ly-6Chi monocytes and higher expression of CC chemokines. More interestingly, selective CD11chi DC ablation provided altered differentiation and function of infiltrated CD11b+Ly-6Chi monocytes in the CNS through Flt3-L and GM-CSF, which was closely associated with severely enhanced neuroinflammation. Furthermore, CD11b+Ly-6Chi monocytes generated in CD11chi DC-ablated environment had a deleterious rather than protective role during neuroinflammation, and were more quickly recruited into inflamed CNS, depending on CCR2, thereby exacerbating neuroinflammation via enhanced supply of virus from the periphery. Therefore, our data demonstrate that CD11chi DCs provide a critical and unexpected role to preserve the immune-privileged CNS in lethal neuroinflammation via regulating the differentiation, function, and trafficking of CD11b+Ly-6Chi monocytes.

4.1478 Amelioration of Japanese encephalitis by blockage of 4-1BB signaling is coupled to divergent enhancement of type I/II IFN responses and Ly-6Chi monocyte differentiation
Kim, S.B., Choi, J.Y., Kim, J.H., Uyangaa, E., patil, A.M., park, S-Y., Lee, J.H., Kim, K., Han, Y.W. and Eo, S.K.
*J. Neuroinfammation, **12**:216 (2015)*

Background
Japanese encephalitis (JE), a neuroinflammation caused by zoonotic JE virus, is the major cause of viral encephalitis worldwide and poses an increasing threat to global health and welfare. To date, however, there has been no report describing the regulation of JE progression using immunomodulatory tools for developing therapeutic strategies. We tested whether blocking the 4-1BB signaling pathway would regulate JE progression using murine JE model.
Methods
Infected wild-type and 4-1BB-knockout (KO) mice were examined daily for mortality and clinical signs, and neuroinflammation in the CNS was evaluated by infiltration of inflammatory leukocytes and cytokine expression. In addition, viral burden, JEV-specific T cell, and type I/II IFN (IFN-I/II) innate responses were analyzed.
Results
Blocking the 4-1BB signaling pathway significantly increased resistance to JE and reduced viral burden in

extraneural tissues and the CNS, rather than causing a detrimental effect. In addition, treatment with 4-1BB agonistic antibody exacerbated JE. Furthermore, JE amelioration and reduction of viral burden by blocking the 4-1BB signaling pathway were associated with an increased frequency of IFN-II-producing NK and $CD4^+$ Th1 cells as well as increased infiltration of mature Ly-$6C^{hi}$ monocytes in the inflamed CNS. More interestingly, DCs and macrophages derived from 4-1BB KO mice showed potent and rapid IFN-I innate immune responses upon JEV infection, which was coupled to strong induction of PRRs (RIG-I, MDA5), transcription factors (IRF7), and antiviral ISG genes (ISG49, ISG54, ISG56). Further, the ablation of 4-1BB signaling enhanced IFN-I innate responses in neuron cells, which likely regulated viral spread in the CNS. Finally, we confirmed that blocking the 4-1BB signaling pathway in myeloid cells derived from hematopoietic stem cells (HSCs) played a dominant role in ameliorating JE. In support of this finding, HSC-derived leukocytes played a dominant role in generating the IFN-I innate responses in the host.
Conclusions
Blocking the 4-1BB signaling pathway ameliorates JE via divergent enhancement of IFN-II-producing NK and $CD4^+$ Th1 cells and mature Ly-$6C^{hi}$ monocyte infiltration, as well as an IFN-I innate response of myeloid-derived cells. Therefore, regulation of the 4-1BB signaling pathway with antibodies or inhibitors could be a valuable therapeutic strategy for the treatment of JE.

4.1479 Cation Homeostasis in Red Cells From Patients With Sickle Cell Disease Heterologous for HbS and HbC (HbSC Genotype)
Hannemann, A., Rees, D.C., Tewari, S. and Gibson, J.S.
*EBioMedicine, **2**, 1669-1676 (2015)*

Sickle cell disease (SCD) in patients of HbSC genotype is considered similar, albeit milder, to that in homozygous HbSS individuals — but with little justification. In SCD, elevated red cell cation permeability is critical as increased solute loss causes dehydration and encourages sickling. Recently, we showed that the KCl cotransporter (KCC) activity in red cells from HbSC patients correlated significantly with disease severity, but that in HbSS patients did not. Two transporters involved in red cell dehydration, the conductive channels P_{sickle} and the Gardos channel, behaved similarly in red cells from the two genotypes, but were significantly less active in HbSC patients. By contrast, KCC activity was quantitatively greater in HbSC red cells. Results suggest that KCC is likely to have greater involvement in red cell dehydration in HbSC patients, which could explain its association with disease severity in this genotype. This work supports the hypothesis that SCD in HbSC patients is a distinct disease entity to that in HbSS patients. Results suggest the possibility of designing specific treatments of particular benefit to HbSC patients and a rationale for the development of prognostic markers, to inform early treatment of children likely to develop more severe complications of the disease.

4.1480 Chronic Nicotine Exposure In Vivo and In Vitro Inhibits Vitamin B1 (Thiamin) Uptake by Pancreatic Acinar Cells
Srinivasan, P., Thrower, E.C., Loganaathan, G., Balamurugan, A.N., Subramanian, V.S., Gorelick, F.S. and Said, H.M.
*PloS One, **10(12)**, e0143575 (2015)*

Thiamin (vitamin B1), a member of the water-soluble family of vitamins, is essential for normal cellular functions; its deficiency results in oxidative stress and mitochondrial dysfunction. Pancreatic acinar cells (PAC) obtain thiamin from the circulation using a specific carrier-mediated process mediated by both thiamin transporters -1 and -2 (THTR-1 and THTR-2; encoded by the *SLC19A2* and *SLC19A3* genes, respectively). The aim of the current study was to examine the effect of chronic exposure of mouse PAC *in vivo* and human PAC *in vitro* to nicotine (a major component of cigarette smoke that has been implicated in pancreatic diseases) on thiamin uptake and to delineate the mechanism involved. The results showed that chronic exposure of mice to nicotine significantly inhibits thiamin uptake in murine PAC, and that this inhibition is associated with a marked decrease in expression of THTR-1 and THTR-2 at the protein, mRNA and hnRNAs level. Furthermore, expression of the important thiamin-metabolizing enzyme, thiamin pyrophosphokinase (TPKase), was significantly reduced in PAC of mice exposed to nicotine. Similarly, chronic exposure of cultured human PAC to nicotine (0.5 μM, 48 h) significantly inhibited thiamin uptake, which was also associated with a decrease in expression of THTR-1 and THTR-2 proteins and mRNAs. This study demonstrates that chronic exposure of PAC to nicotine impairs the physiology and the molecular biology of the thiamin uptake process. Furthermore, the study suggests that the effect is, in part, mediated through transcriptional mechanism(s) affecting the *SLC19A2* and *SLC19A3* genes.

4.1481 Patient-specific blood rheology in sickle-cell anaemia

Li, X., Du, E., lei, H., tang, Y-H., Dao, M., Suresh, S and karniadakis, G.E.
*Interface Focus, **6**, 20150065 (2015)*

Sickle-cell anaemia (SCA) is an inherited blood disorder exhibiting heterogeneous cell morphology and abnormal rheology, especially under hypoxic conditions. By using a multiscale red blood cell (RBC) model with parameters derived from patient-specific data, we present a mesoscopic computational study of the haemodynamic and rheological characteristics of blood from SCA patients with hydroxyurea (HU) treatment (on-HU) and those without HU treatment (off-HU). We determine the shear viscosity of blood in health as well as in different states of disease. Our results suggest that treatment with HU improves or worsens the rheological characteristics of blood in SCA depending on the degree of hypoxia. However, on-HU groups always have higher levels of haematocrit-to-viscosity ratio (HVR) than off-HU groups, indicating that HU can indeed improve the oxygen transport potential of blood. Our patient-specific computational simulations suggest that the HVR level, rather than the shear viscosity of sickle RBC suspensions, may be a more reliable indicator in assessing the response to HU treatment.

4.1482 Microencapsulated Pig Islet Xenotransplantation as an Alternative Treatment of Diabetes

Zhu, H., Yu, L., He, Y., Lyu, Y. and Wang, B.
*Tissue Engineering: Part B, **21(5)**, 474-489 (2015)*

Islet transplantation is emerging as an attractive option for the treatment of type 1 diabetes mellitus (T1DM). However, some major obstacles are needed to be overcome, including shortage of islet supply and excessive immunosuppressive therapy. Xenotransplantation of bioartificial pancreas (BAP) made of microencapsulated pig islets will effectively solve these problems. Before widespread application of this therapy, several important issues should be addressed to further improve pig islet viability and functionality, such as pig islet source, optimization of microcapsule preparation, cryopreservation of implant, selection of biocompatible material, and implant site, as well as prevention of xenoreaction and biosafety concern. It is hoped that improvements in these critical aspects will lead to wider human application of microencapsulation of pig islets.

4.1483 Evaluation of small noncoding RNAs in ex vivo stored human mature red blood cells: changes in noncoding RNA levels correlate with storage lesion events

Sarachana, T., Kulkarni, S. and Atreya, C.D.
*Transfusion, **55(11)**, 2672-2683 (2015)*

BACKGROUND
While biomarkers of storage lesions (SLs) for red blood cells (RBCs) abound, the physiologic consequences of SLs and associated important events are poorly understood. Previously we have identified differentially expressed regulatory small noncoding RNAs (ncRNAs) in stored RBCs, suggesting their role in the RBC SL process and their potential as quality biomarkers of stored RBCs.
STUDY DESIGN AND METHODS
Comprehensive ncRNA expression analysis of RBCs stored for up to 56 days was performed on RNAs collected from enriched mature RBCs on Days 0, 7, 14, 28, 42, and 56. Three known RBC SL processes, that is, mature RBCs' suicidal death (eryptosis), ATP loss, and changes in RBC indices, were correlated with differentially expressed ncRNAs to gain knowledge on the SL molecular processes.
RESULTS
The analysis identified four ncRNAs whose changes in the expression levels were correlated with the selected three SL processes. Differential expression on Days 14 and 28 of the four selected ncRNAs was confirmed by TaqMan quantitative reverse transcription–polymerase chain reaction analysis. Bioinformatics analysis identified potential targets and biologic functions of these ncRNAs. Overexpression of one such ncRNA, hsa-miR-196a, in a human erythroblast cell line confirmed its protective effects against the cell death and ATP loss.
CONCLUSION
Overall, this study demonstrates that changes in the levels of small ncRNAs of stored RBCs correlate with some of the SL events and thus they have the potential to serve as the storage quality markers.

4.1484 GDF10 is a signal for axonal sprouting and functional recovery after stroke

Li, S., Nie, E.H., Yin, Y., Benowitz, L.I., Tung, S., Vinters, H.V., Bahjat, F.R., Stenzel-Poore, M.P., Kawaguchi, R., Xoppala, G. and Carmichael, T.
*Nature Neuroscience, **18(12)**, 1737-1745 (2015)*

Stroke produces a limited process of neural repair. Axonal sprouting in cortex adjacent to the infarct is part of this recovery process, but the signal that initiates axonal sprouting is not known. Growth and differentiation factor 10 (GDF10) is induced in peri-infarct neurons in mice, non-human primates and humans. GDF10 promotes axonal outgrowth *in vitro* in mouse, rat and human neurons through TGFβRI and TGFβRII signaling. Using pharmacogenetic gain- and loss-of-function studies, we found that GDF10 produced axonal sprouting and enhanced functional recovery after stroke; knocking down GDF10 blocked axonal sprouting and reduced recovery. RNA sequencing from peri-infarct cortical neurons revealed that GDF10 downregulated PTEN, upregulated PI3 kinase signaling and induced specific axonal guidance molecules. Using unsupervised genome-wide association analysis of the GDF10 transcriptome, we found that it was not related to neurodevelopment, but may partially overlap with other CNS injury patterns. Thus, GDF10 is a stroke-induced signal for axonal sprouting and functional recovery.

4.1485 Hepatic CD206-positive macrophages express amphiregulin to promote the immunosuppressive activity of regulatory T cells in HBV infection
Dai, K., Huang, L., Sun, X., yang, L. and Gong, Z.
J. Leukoc. Biol., ***98(6)***, *1071-1080 (2015)*

Hepatitis B virus is a major cause of chronic liver inflammation worldwide. Innate and adaptive immune responses work together to restrain or eliminate hepatitis B virus in the liver. Compromised or failed adaptive immune response results in persistent virus replication and spread. How to promote antiviral immunity is a research focus for hepatitis B virus prevention and therapy. In this study, we investigated the role of macrophages in the regulation of antiviral immunity. We found that $F4/80^{+}CD206^{+}CD80^{lo/+}$ macrophages were a particular hepatic macrophage subset that expressed amphiregulin in our mouse hepatitis B virus infection model. $CD206^{+}$ macrophage-derived amphiregulin promoted the immunosuppressive activity of intrahepatic regulatory T cells, demonstrated by higher expression of CTLA-4, ICOS, and CD39, as well as stronger inhibition of antiviral function of $CD8^{+}$ T cells. Amphiregulin-neutralizing antibody diminished the effect of $CD206^{+}$ macrophages on regulatory T cells. In addition, we found that $CD206^{+}$ macrophage-derived amphiregulin activated mammalian target of rapamycin signaling in regulatory T cells, and this mammalian target of rapamycin activation was essential for promotion of regulatory T cell activity by $CD206^{+}$ macrophages. Adoptive transfer of $CD206^{+}$ macrophages into hepatitis B virus-infected mice increased cytoplasmic hepatitis B virus DNA in hepatocytes and also increased serum hepatitis B surface antigen. The antiviral activity of $CD8^{+}$ T cells was decreased after macrophage transfer. Therefore, our research indicated that amphiregulin produced by $CD206^{+}$ macrophages plays an important role in modulating regulatory T cell function and subsequently restrains the antiviral activity of $CD8^{+}$ T cells. Our study offers new insights into the immunomodulation in hepatitis B virus infection.

4.1486 lincRNA-p21 inhibits hepatic stellate cell activation and liver fibrogenesis via p21
Zheng, J., Dong, P., Mao, Y., Chen, S., Wu, X., Li, G., Lu, Z. and Yu, F.
FEBS J., ***282(24)***, *4810-4821 (2015)*

Long non-coding RNAs are involved in various biological processes and diseases. The biological role of long intergenic non-coding RNA-p21 (lincRNA-p21) in liver fibrosis remains unknown before this study. In this study, we observed marked reduction of lincRNA-p21 expression in mice liver fibrosis models and human cirrhotic liver. Over-expression of lincRNA-p21 suppressed activation of hepatic stellate cells (HSCs) *in vitro*. Lentivirus-mediated lincRNA-p21 transfer into mice decreased the severity of liver fibrosis *in vivo*. Additionally, lincRNA-p21 reversed the activation of HSCs to their quiescent phenotype. The mRNA levels of lincRNA-p21 and p21 were positively correlated. Our results show that over-expression of lincRNA-p21 promotes up-regulation of p21 at both the mRNA and protein levels. Furthermore, lincRNA-p21 inhibited cell-cycle progression and proliferation of primary HSCs through enhancement of p21 expression. Compared with healthy subjects, serum lincRNA-p21 levels were significantly lower in patients with liver cirrhosis, especially those with decompensation. These findings collectively indicate that lincRNA-p21 is a mediator of HSC activation, supporting its utility as a novel therapeutic target for liver fibrosis.

4.1487 Bioremediation strategies for removal of residual atrazine in the boreal groundwater zone
Nousiainen, A.O., Björklöf, K., Sagarkar, S., Lund Nielsen, J., Kapley, A. and Jørgensen, K.S.
Appl. Microbiol. Biotechnol., ***99(23)***, *10249-10259 (2015)*

Strategies for bioremediation of atrazine, a pesticide commonly polluting groundwater in low concentrations, were studied in two boreal nonagricultural soils. Atrazine was not mineralized in soil without bioremediation treatments. In biostimulation treatment with molasses, up to 52 % of atrazine was mineralized at 10 °C, even though the degradation gene copy numbers did not increase. Incubations with radioactively labeled atrazine followed by microautoradiographic analysis revealed that bioremediation strategies increased the relative proportion of active degraders from 0.3 up to 1.9 % of the total bacterial count. These results indicate that atrazine degradation might not solely be facilitated by *atzA*/*trzN*–*atzB* genes. In combined biostimulation treatment using citrate or molasses and augmentation with *Pseudomonas citronellolis* ADP or *Arthrobacter aurescens* strain TC1, up to 76 % of atrazine was mineralized at 30 °C, and the atrazine degradation gene numbers increased up to 10^7 copies g^{-1} soil. Clone libraries from passive samplers in groundwater monitoring wells revealed the presence of phylogenetic groups formerly shown to include atrazine degraders, and the presence of atrazine degradation genes *atzA* and *atzB*. These results show that the mineralization of low concentrations of atrazine in the groundwater zone at low temperatures is possible by bioremediation treatments.

4.1488 Myeloperoxidase–Hepatocyte–Stellate Cell Cross Talk Promotes Hepatocyte Injury and Fibrosis in Experimental Nonalcoholic Steatohepatitis

Pulli, B., Ali, M., Iwamoto, Y., Zeller, M.W.G., Schob, S., Linnoila, J.J. and Chen, J.W.
*Antioxidants & Redox Signaling, **23(16)**, 1255-1269 (2015)*

Aims: Myeloperoxidase (MPO), a highly oxidative enzyme secreted by leukocytes has been implicated in human and experimental nonalcoholic steatohepatitis (NASH), but the underlying mechanisms remain unknown. In this study, we investigated how MPO contributes to progression from steatosis to NASH. ***Results:*** In C57Bl/6J mice fed a diet deficient in methionine and choline to induce NASH, neutrophils and to a lesser extent inflammatory monocytes are markedly increased compared with sham mice and secrete abundant amounts of MPO. Through generation of HOCl, MPO directly causes hepatocyte death *in vivo*. *In vitro* experiments demonstrate mitochondrial permeability transition pore induction *via* activation of SAPK/JNK and PARP. MPO also contributes to activation of hepatic stellate cells (HSCs), the most important source of collagen in the liver. *In vitro* MPO-activated HSCs have an activation signature (MAPK and PI3K-AKT phosphorylation) and upregulate *COL1A1*, α-*SMA*, and *CXCL1*. MPO-derived oxidative stress also activates transforming growth factor β (TGF-β) *in vitro*, and TGF-β signaling inhibition with SB-431542 decreased steatosis and fibrosis *in vivo*. Conversely, congenital absence of MPO results in reduced hepatocyte injury, decreased levels of TGF-β, fewer activated HSCs, and less severe fibrosis *in vivo*. ***Innovation and Conclusion:*** Cumulatively, these findings demonstrate important cross talk between inflammatory myeloid cells, hepatocytes, and HSCs *via* MPO and establish MPO as part of a proapoptotic and profibrotic pathway of progression in NASH, as well as a potential therapeutic target to ameliorate this disease.

Lung-derived exosome uptake into and epigenetic modulation of marrow progenitor/stem and differentiated cells

Aliotta, J.M., Pereira, M., Sears, E.H., Dooner, M.S., Wen, S., Goldberg, L.R. and Quesenberry, P.J.
*J. Extracellular Vesicles, **4**:26166 (2015)*

Background: Our group has previously demonstrated that murine whole bone marrow cells (WBM) that internalize lung-derived extracellular vesicles (LDEVs) in culture express pulmonary epithelial cell–specific genes for up to 12 weeks. In addition, the lungs of lethally irradiated mice transplanted with lung vesicle–modulated marrow have 5 times more WBM-derived type II pneumocytes compared to mice transplanted with unmanipulated WBM. These findings indicate that extracellular vesicle modification may be an important consideration in the development of marrow cell–based cellular therapies. Current studies were performed to determine the specific marrow cell types that LDEV stably modify.
Methods: Murine WBM-derived stem/progenitor cells (Lin-/Sca-1+) and differentiated erythroid cells (Ter119+), granulocytes (Gr-1+) and B cells (CD19+) were cultured with carboxyfluorescein *N*-succinimidyl ester (CFSE)-labelled LDEV. LDEV+ cells (CFSE+) and LDEV− cells (CFSE−) were separated by flow cytometry and visualized by fluorescence microscopy, analyzed by RT-PCR or placed into long-term secondary culture. In addition, murine Lin-/Sca-1+ cells were cultured with CFSE-labelled LDEV isolated from rats, and RT-PCR analysis was performed on LDEV+ and–cells using species-specific primers for surfactant (rat/mouse hybrid co-cultures).
Results: Stem/progenitor cells and all of the differentiated cell types studied internalized LDEV in culture, but heterogeneously. Expression of a panel of pulmonary epithelial cell genes was higher in LDEV+cells compared to LDEV− cells and elevated expression of these genes persisted in long-term culture.

Rat/mouse hybrid co-cultures revealed only mouse-specific surfactant B and C expression in LDEV+ Lin-/Sca-1+cells after 4 weeks of culture, indicating stable de novo gene expression.
Conclusions: LDEV can be internalized by differentiated and more primitive cells residing in the bone marrow in culture and can induce stable de novo pulmonary epithelial cell gene expression in these cells for several weeks after internalization. The gene expression represents a transcriptional activation of the target marrow cells. These studies serve as the basis for determining marrow cell types that can be used for cell-based therapies for processes that injure the pulmonary epithelial surfaces.

4.1489 In vitro fertilization in pigs: New molecules and protocols to consider in the forthcoming years
Romar, R., Funahashi, H. and Coy, P.
*Theriogenology, **85**, 125-134 (2016)*

Assisted reproduction technology (ART) protocols are used in livestock for the improvement and preservation of their genetics and to enhance reproductive efficiency. In the case of pigs, the potential use of embryos for biomedicine is being followed with great interest by the scientific community. Owing to the physiological similarities with humans, embryos produced *in vitro* and many of those produced *in vivo* are used in research laboratories for the procurement of stem cells or the production of transgenic animals, sometimes with the purpose of using their organs for xenotransplantation. Several techniques are required for the production of an *in vitro*–derived embryo. These include *in vitro* oocyte maturation, sperm preparation, IVF, and further culture of the putative zygotes. Without doubt, among these technologies, IVF is still a critical limiting factor because of the well-known, but still unsolved, question of polyspermy. Despite the improvements made in the past decade, current IVF systems hardly reach 50% to 60% efficiency and any progression in porcine ARTs requires an unavoidable improvement in the monospermy rate. It is time, then, to learn from what happens under *in vivo* physiological conditions and to transfer this knowledge into ART. This review describes the latest advances in porcine IVF, from sperm preparation procedures to culture media supplements with special attention paid to molecules with a known or potential role in *in vivo* fertilization. Oviductal fluid is the natural medium in which fertilization takes place, and, in the near future, could become the definitive supplement for culture media, where it would help to solve many of the problems inherent in ARTs in swine and improve the quality of *in vitro*–derived porcine embryos.

4.1490 Synthetic and natural small molecule TLR4 antagonists inhibit motoneuron death in cultures from ALS mouse model
De Paola, M., Sestito, S.E., Mariani,A., Memo, C., Fanelli, R., Freschi, M., Bendotti, C., Calabrese, V. and Peri, F.
*Pharmacol. Res., **103**, 180-187 (2016)*

Increasing evidence indicates that inflammatory responses could play a critical role in the pathogenesis of motor neuron injury in amyotrophic lateral sclerosis (ALS). Recent findings have underlined the role of Toll-like receptors (TLRs) and the involvement of both the innate and adaptive immune responses in ALS pathogenesis. In particular, abnormal TLR4 signaling in pro-inflammatory microglia cells has been related to motoneuron degeneration leading to ALS. In this study the effect of small molecule TLR4 antagonists on in vitro ALS models has been investigated. Two different types of synthetic glycolipids and the phenol fraction extracted from commercial extra-virgin olive oil (EVOO) were selected since they efficiently inhibit TLR4 stimulus in HEK cells by interacting with the TLR4·MD-2 complex and CD14 co-receptor. Here, TLR4 antagonists efficiently protected motoneurons from LPS-induced lethality in spinal cord cultures, and inhibited the interleukine-1β production by LPS-stimulated microglia. In motoneurons/glia cocultures obtained from wild type or SOD1 G93A mice, motoneuron death induced by SOD1mut glia was counteracted by TLR4 antagonists. The release of nitric oxide by LPS treatment or SOD1mut glia was also inhibited by EVOO, suggesting that the action of this natural extract could be mainly related to the modulation of this inflammatory mediator.

4.1491 Reciprocal interaction among gasotransmitters in isolated pancreatic *β*-cells
Moustafa, A. and Habara, Y.
*Free Radical Biology and medicine, **90**, 47-58 (2016)*

We aimed to elucidate the interplay among the three well-known gas molecules, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S), and their effects on intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and insulin secretion in rat pancreatic *β*-cells. Immunofluorescence studies demonstrated the expression of constitutive enzymes that are responsible for the production of NO, CO and H_2S. CO and H_2S increased

NO production as indicated by the increase in diaminofluorescein-2 triazole fluorescence. NO and CO induced an elevation in the sulfane sulfur pool and concomitantly H_2S production. The NO- and CO-induced H_2S production was partially inhibited by hypotaurine, an H_2S scavenger. NO and H_2S produced CO production as revealed by a myoglobin assay. A calmodulin antagonist in the absence of extracellular Ca^{2+} significantly attenuated NO and H_2S production. NO and CO induced a $[Ca^{2+}]_i$ increase mainly via Ca^{2+} release from internal stores; however, H_2S induced a $[Ca^{2+}]_i$ increase via the influx of extracellular Ca^{2+}. NO dose-dependently stimulated basal insulin release but CO dose-dependently inhibited it. H_2S showed an insignificant effect on basal insulin secretion from freshly isolated pancreatic islets. Herein, we address for the first time the reciprocal and synergistic relation among gasotransmitters with diverse effects on basal insulin secretion that regulate β-cells functions and homeostasis.

4.1492 Transdermal toxicity of topically applied anticoagulant rodenticide warfarin in rats
Subota, V., Mirkov, I., Demenesku, J., Aleksandrov, A.P., Ninkov, M., Mileusnic, D., Kataranovski, D. and Kataranovski, M.
*Environmental Toxicology and Pharmacology, **41**, 232-2540 (2016)*

Occupational/accidental exposure data have showed hemorrhage as a result of transdermal exposure to warfarin, however, other effects are not known. In the present study, the impact of epicutaneous application of 10 μg or 100 μg of warfarin (three times, once a day) on peripheral blood polymorphonuclear (PMN) and mononuclear cells (PBMC) was examined in rats. Both doses resulted in prolongation of prothrombin time and changes in hematologic parameters. Increases in PMN intracellular myeloperoxidase (MPO) activity were seen at higher warfarin dose and both doses resulted in higher percentages of granular $CD11b^+$ cells. In contrast, a decrease in PMN TNF and IL-6 production (ELISA) and gene expression (RT-PCR) was observed. Epicutaneous application of warfarin resulted in decreased numbers of PBMC, higher numbers of mononuclear $CD11b^+$ cells, but without effect on PMBC cytokine production. The data obtained showed differential effects of transdermal exposure to warfarin depending on leukocyte type and activity.

4.1493 Early modulation of pro-inflammatory microglia by minocycline loaded nanoparticles confers long lasting protection after spinal cord injury
Papa, S. et al
*Biomaterials, **75**, 13-24 (2016)*

Many efforts have been performed in order to understand the role of recruited macrophages in the progression of spinal cord injury (SCI). Different studies revealed a pleiotropic effect played by these cells associated to distinct phenotypes (M1 and M2), showing a predictable spatial and temporal distribution in the injured site after SCI. Differently, the role of activated microglia in injury progression has been poorly investigated, mainly because of the challenges to target and selectively modulate them *in situ*. A delivery nanovector tool (poly-ε-caprolactone-based nanoparticles) able to selectively treat/target microglia has been developed and used here to clarify the temporal and spatial involvement of the pro-inflammatory response associated to microglial cells in SCI. We show that a treatment with nanoparticles loaded with minocycline, the latter a well-known anti-inflammatory drug, when administered acutely in a SCI mouse model is able to efficiently modulate the resident microglial cells reducing the pro-inflammatory response, maintaining a pro-regenerative milieu and ameliorating the behavioral outcome up to 63 days post injury. Furthermore, by using this selective delivery tool we demonstrate a mechanistic link between early microglia activation and M1 macrophages recruitment to the injured site via CCL2 chemokine, revealing a detrimental contribution of pro-inflammatory macrophages to injury progression after SCI.

4.1494 Inversion of hematocrit partition at microfluidic bifurcations
Shen, Z., Coupier, G., Kaoui, B., Polack, B., Harting, J., Misbah, C. and Podgorski, T.
*Microvascular Res., **105**, 40-46 (2016)*

Partitioning of red blood cells (RBCs) at the level of bifurcations in the microcirculatory system affects many physiological functions yet it remains poorly understood. We address this problem by using T-shaped microfluidic bifurcations as a model. Our computer simulations and in vitro experiments reveal that the hematocrit (ϕ_0) partition depends strongly on RBC deformability, as long as $\phi_0 < 20\%$ (within the normal range in microcirculation), and can even lead to complete deprivation of RBCs in a child branch. Furthermore, we discover a deviation from the Zweifach–Fung effect which states that the child branch with lower flow rate recruits less RBCs than the higher flow rate child branch. At small enough ϕ_0, we get the inverse scenario, and the hematocrit in the lower flow rate child branch is even higher than in the

parent vessel. We explain this result by an intricate up-stream RBC organization and we highlight the extreme dependence of RBC transport on geometrical and cell mechanical properties. These parameters can lead to unexpected behaviors with consequences on the microcirculatory function and oxygen delivery in healthy and pathological conditions.

4.1495 Molecular characterization and expression analysis of B cell activating factor from rock bream (Oplegnathus fasciatus)

Godahewa, G.I., Perera, N.C.N., Umasuthan, N., Wan, Q., Whang, I. and Lee, J.
*Development and Comparative Immunology, **55**, 1-11 (2016)*

B cell activating factor (BAFF) is a member of the tumor necrosis factor (TNF) ligand family. BAFF has been shown to induce survival and proliferation of lymphocytes. We characterized the gene encoding BAFF (*RbBAFF*) in rock bream (*Oplegnathus fasciatus*), and attempted to determine its biological functions upon immune responses. *In silico* analysis of *RbBAFF* demonstrated the presence of common TNF ligand family features, including a TNF domain, a D-E loop, and three cysteine residues that are crucial for trimer formation. Amino acid sequence alignment confirmed that *RbBAFF* and its homologs were conserved at secondary and tertiary levels. Transcriptional analysis indicated that *RbBAFF* mRNAs were ubiquitously expressed in wide array of tissues. The higher levels of constitutive expression were observed in the kidney, head kidney and spleen, suggesting an important physiological relationship with lymphocytes. Under pathological conditions, *RbBAFF* mRNA levels were significantly elevated. The role of *RbBAFF* in lymphocyte survival and proliferation was confirmed by MTT assays and flow cytometry. Recombinant RbBAFF protein (10 μg/mL) was able to prolong the survival and/or enhance the proliferation of rock bream lymphocytes by approximately 30%. Transcription of *IL-10* and *NFκB-1* was significantly stimulated by *RbBAFF*. Our findings provide further information regarding fish BAFF gene and its role in adaptive immunity.

4.1496 Activation of Kupffer Cells Is Associated with a Specific Dysbiosis Induced by Fructose or High Fat Diet in Mice

Ferrere, G., Leroux, A., Wrzosek, L., Puchols, V., Gaudin, F., Ciocan, D., Renoud, M-L., Naveau, S., Perlemuter, G. and Cassars, A-M.
*PloS One, **11(1)**, e0146177 (2016)*

The increase consumption of fructose in diet is associated with liver inflammation. As a specific fructan substrate, fructose may modify the gut microbiota which is involved in obesity-induced liver disease. Here, we aimed to assess whether fructose-induced liver damage was associated with a specific dysbiosis, especially in mice fed a high fat diet (HFD). To this end, four groups of mice were fed with normal and HFD added or not with fructose. Body weight and glucose sensitivity, liver inflammation, dysbiosis and the phenotype of Kupffer cells were determined after 16 weeks of diet. Food intake was increased in the two groups of mice fed with the HFD. Mice fed with HFD and fructose showed a higher infiltration of lymphocytes into the liver and a lower inflammatory profile of Kupffer cells than mice fed with the HFD without fructose. The dysbiosis associated with diets showed that fructose specifically prevented the decrease of Mouse intestinal bacteria in HFD fed mice and increased *Erysipelotrichi* in mice fed with fructose, independently of the amount of fat. In conclusion, fructose, used as a sweetener, induced a dysbiosis which is different in presence of fat in the diet. Consequently, the activation of Kupffer cells involved in mice model of HFD-induced liver inflammation was not observed in an HFD/fructose combined diet. These data highlight that the complexity of diet composition could highly impact the development of liver lesions during obesity. Specific dysbiosis associated with the diet could explain that the progressions of liver damage are different.

4.1497 HybriFree: a robust and rapid method for the development of monoclonal antibodies from different host species

Kivi, G., Teesalu, K., parik, J., Kontkar, E., Ustav Jr., M. and Männik, A.
*BMC Biotechnol., **16**:2 (2016)*

Background

The production of recombinant monoclonal antibodies in mammalian cell culture is of high priority in research and medical fields. A critical step in this process is the isolation of the antigen-binding domain sequences of antibodies possessing the desired properties. Many different techniques have been described to achieve this goal, but all have shortcomings; most techniques have problems with robustness, are time-consuming and costly, or have complications in the transfer from isolation to production phase. Here, we

report a novel HybriFree technology for the development of monoclonal antibodies from different species that is robust, rapid, inexpensive and flexible and can be used for the subsequent production of antibodies in mammalian cell factories.

Results

HybriFree technology is illustrated herein via detailed examples of isolating mouse, rabbit and chicken monoclonal antibody sequences from immunized animals. Starting from crude spleen samples, antigen capturing of specific B-cells is performed initially. cDNA of antibody variable domains is amplified from the captured cells and used a source material for simple and rapid restriction/ligation free cloning of expression vector library in order to produce scFv-Fc or intact IgG antibodies. The vectors can be directly used for screening purposes as well as for the subsequent production of the developed monoclonal antibodies in mammalian cell culture. The antibodies isolated by the method have been shown to be functional in different immunoassays, including ELISA, immunofluorescence and Western blot. In addition, we demonstrate that by using a modified method including a negative selection step, we can isolate specific antibodies targeting the desired epitope and eliminate antibodies directed to undesired off-targets.

Conclusions

HybriFree can be used for the reliable development of monoclonal antibodies and their subsequent production in mammalian cells. This simple protocol requires neither the culturing of B-cells nor single-cell manipulations, and only standard molecular biology laboratory equipment is needed. In principle, the method is applicable to any species for which antibody cDNA sequence information is available.

4.1498 Telomerase reverse transcriptase acts in a feedback loop with NF-κB pathway to regulate macrophage polarization in alcoholic liver disease

Wu, W-q., Yang, Y., Li, W-x., Cheng, Y-h., Li, X-f., Huang, C.,Meng, X-m., Wu, B-m., Liu, X-h., Zhang, L., Lv, X-w. and Li, J.

*Scientific Reports, **6**:18685 (2016)*

Activation of Kupffer cells (KCs) plays a central role in the pathogenesis of alcoholic liver disease (ALD). C57BL/6 mice fed EtOH-containing diet showed a mixed induction of hepatic classical (M1) and alternative (M2) macrophage markers. Since telomerase activation occurs at critical stages of myeloid and lymphoid cell activation, we herein investigated the role of telomerase reverse transcriptase (TERT), the determining factor of telomerase, in macrophage activation during ALD. In our study, TERT expression and telomerase activity (TA) were remarkably increased in liver tissue of EtOH-fed mice. Moreover, EtOH significantly up-regulated TERT in isolated KCs and RAW 264.7 cells and LPS induced TERT production in vitro. These data indicate that up-regulation of TERT may play a critical role in macrophages during ALD. Furthermore, loss- and gain-of-function studies suggested that TERT switched macrophages towards M1 phenotype by regulating NF-κB signaling, but had limited effect on M2 macrophages polarization in vitro. Additionally, PDTC, a chemical inhibitor of NF-κB, could dramatically down-regulate TERT expression and the hallmarks of M1 macrophages. Therefore, our study unveils the role of TERT in macrophage polarization and the cross-talk between TERT and p65, which may provide a possible explanation for the ethanol-mediated hepatic proinflammatory response and M1 macrophage polarization.

4.1499 Analyses of movement and contact of two nucleated cells using a gas-driven micropipette aspiration technique

Yang, H., Tong, C., Fu, C., Xu, Y., Liu, X., Chen, Q., Zhang, Y., Lü, S., Li, N. and Long, M.

*J. Immunol. Methods, **428**, 20-29 (2016)*

Adhesion between two nucleated cells undergoes specific significances in immune responses and tumor metastasis since cellular adhesive molecules usually express on two apposed cell membranes. However, quantification of the interactions between two nucleated cells is still challenging in microvasculature. Here distinct cell systems were used, including three types of human cells (Jurkat cell or PMN vs. MDA-MB-231 cell) and two kinds of murine native cells (PMN vs. liver sinusoidal endothelial cell). Cell movement, compression to, and relaxation from the counterpart cell were quantified using an in-house developed gas-driven micropipette aspiration technique (GDMAT). This assay is robust to quantify this process since cell movement and contact inside a pipette are independent of the repeated test cycles. Measured approaching or retraction velocity follows well a normal distribution, which is independent on the cycle period. Contact area or duration also fits a Gaussian distribution and moreover contact duration is linearly correlated with the cycle period. Cell movement is positively related to gas flux but negatively associated to medium viscosity. Cell adhesion tends to reach an equilibrium state with increase of cycle period or contact duration. These results further the understanding in the dynamics of cell movement and contact in

microvasculature.

4.1500 Endogenous adaptation to low oxygen modulates T-cell regulatory pathways in EAE
Esen, N., katyshev, V., Serkin, Z., Kaytsheva, S. and Dore-Duffy, P.
*J. Neuroinflammation, **13**:13 (2016)*

Background
In the brain, chronic inflammatory activity may lead to compromised delivery of oxygen and glucose suggesting that therapeutic approaches aimed at restoring metabolic balance may be useful. In vivo exposure to chronic mild normobaric hypoxia (10 % oxygen) leads to a number of endogenous adaptations that includes vascular remodeling (angioplasticity). Angioplasticity promotes tissue survival. We have previously shown that induction of adaptive angioplasticity modulates the disease pattern in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE). In the present study, we define mechanisms by which adaptation to low oxygen functionally ameliorates the signs and symptoms of EAE and for the first time show that tissue hypoxia may fundamentally alter neurodegenerative disease.
Methods
C57BL/6 mice were immunized with MOG, and some of them were kept in the hypoxia chambers (day 0) and exposed to 10 % oxygen for 3 weeks, while the others were kept at normoxic environment. Sham-immunized controls were included in both hypoxic and normoxic groups. Animals were sacrificed at pre-clinical and peak disease periods for tissue collection and analysis.
Results
Exposure to mild hypoxia decreased histological evidence of inflammation. Decreased numbers of cluster of differentiation (CD)4+ T cells were found in the hypoxic spinal cords associated with a delayed Th17-specific cytokine response. Hypoxia-induced changes did not alter the sensitization of peripheral T cells to the MOG peptide. Exposure to mild hypoxia induced significant increases in anti-inflammatory IL-10 levels and an increase in the number of spinal cord CD25+FoxP3+ T-regulatory cells.
Conclusions
Acclimatization to mild hypoxia incites a number of endogenous adaptations that induces an anti-inflammatory milieu. Further understanding of these mechanisms system may pinpoint possible new therapeutic targets to treat neurodegenerative disease.

4.1501 IgG-Immune Complexes Promote B Cell Memory by Inducing BAFF
Kang, S.A., Keener, A.B., Jones, S.Z., Benschop, R.J., Caro-Maldonado, A., Rathmell, J.C., Clarke, S.H., Matsushima, G.K., Whitmire, J.K. and Vilen, B.J.
*J. Immunol., **196(1)**, 196-206 (2016)*

Memory B cell responses are vital for protection against infections but must also be regulated to prevent autoimmunity. Cognate T cell help, somatic hypermutation, and affinity maturation within germinal centers (GCs) are required for high-affinity memory B cell formation; however, the signals that commit GC B cells to the memory pool remain unclear. In this study, we identify a role for IgG-immune complexes (ICs), FcγRs, and BAFF during the formation of memory B cells in mice. We found that early secretion of IgG in response to immunization with a T-dependent Ag leads to IC–FcγR interactions that induce dendritic cells to secrete BAFF, which acts at or upstream of Bcl-6 in activated B cells. Loss of CD16, hematopoietic cell–derived BAFF, or blocking IC:FcγR regions in vivo diminished the expression of Bcl-6, the frequency of GC and memory B cells, and secondary Ab responses. BAFF also contributed to the maintenance and/or expansion of the follicular helper T cell population, although it was dispensable for their formation. Thus, early Ab responses contribute to the optimal formation of B cell memory through IgG-ICs and BAFF. Our work defines a new role for FcγRs in GC and memory B cell responses.

4.1502 Semaphorin 7A Promotes Chemokine-Driven Dendritic Cell Migration
Van Rijn, A., paulis, L., te Riet, J., Vasauturo, A., Reinieren-Beeren, I., van der Schaaf, A., Kuipers, A.J., Schulte, L.P., Jongbloets, B.C., Pasterkamp, R.J., Figdor, C.G., van Spriel, A.B. and Buschow, S.I.
*J. Immunol., **196**, 459-468 (2016)*

Dendritic cell (DC) migration is essential for efficient host defense against pathogens and cancer, as well as for the efficacy of DC-based immunotherapies. However, the molecules that induce the migratory phenotype of DCs are poorly defined. Based on a large-scale proteome analysis of maturing DCs, we identified the GPI-anchored protein semaphorin 7A (Sema7A) as being highly expressed on activated

primary myeloid and plasmacytoid DCs in human and mouse. We demonstrate that Sema7A deficiency results in impaired chemokine CCL21-driven DC migration in vivo. Impaired formation of actin-based protrusions, resulting in slower three-dimensional migration, was identified as the mechanism underlying the DC migration defect. Furthermore, we show, by atomic force microscopy, that Sema7A decreases adhesion strength to extracellular matrix while increasing the connectivity of adhesion receptors to the actin cytoskeleton. This study demonstrates that Sema7A controls the assembly of actin-based protrusions that drive DC migration in response to CCL21.

4.1503 Induction and regulation of murine emphysema by elastin peptides
Sellami, M., meghraoui-Kheddar, A., Terryn, C., Fichel, C., Bouland, N., Diebold, M-D., Guenounou, M., Hery-Huynh, S. and Le Naour, R.
Am. J. Physiol. Lung Cell Mol. Physiol., ***310***, *L8-L23 (2016)*

Emphysema is the major component of chronic obstructive pulmonary disease (COPD). During emphysema, elastin breakdown in the lung tissue originates from the release of large amounts of elastase by inflammatory cells. Elevated levels of elastin-derived peptides (EP) reflect massive pulmonary elastin breakdown in COPD patients. Only the EP containing the GXXPG conformational motif with a type VIII β-turn are elastin receptor ligands inducing biological activities. In addition, the COOH-terminal glycine residue of the GXXPG motif seems a prerequisite to the biological activity. In this study, we endotracheally instilled C57BL/6J mice with GXXPG EP and/or COOH-terminal glycine deleted-EP whose sequences were designed by molecular dynamics and docking simulations. We investigated their effect on all criteria associated with the progression of murine emphysema. Bronchoalveolar lavages were recovered to analyze cell profiles by flow cytometry and lungs were prepared to allow morphological and histological analysis by immunostaining and confocal microscopy. We observed that exposure of mice to EP elicited hallmark features of emphysema with inflammatory cell accumulation associated with increased matrix metalloproteinases and desmosine expression and of remodeling of parenchymal tissue. We also identified an inactive COOH-terminal glycine deleted-EP that retains its binding-activity to EBP and that is able to inhibit the in vitro and in vivo activities of emphysema-inducing EP. This study demonstrates that EP are key actors in the development of emphysema and that they represent pharmacological targets for an alternative treatment of emphysema based on the identification of EP analogous antagonists by molecular modeling studies.

4.1504 Biotin-conjugated fusogenic liposomes for high-quality cell purification
Hersch, N., Wolters, B., Ungvari, Z., Gautam, T., Deshpande, D., Merkel, R., Cziszar, A., Hoffmann, B. and Cziszar, A.
J. Biomater. Appl., ***30(6)***, *846-856 (2016)*

Purification of defined cell populations from mixed primary cell sources is essential for many biomedical and biotechnological applications but often very difficult to accomplish due to missing specific surface markers. In this study, we developed a new approach for efficient cell population separation based on the specific membrane fusion characteristics of distinct cell types upon treatment with fusogenic liposomes. When such liposomes are conjugated with biotin, specific cell populations can be efficiently surface functionalized by biotin after liposomal treatment while other populations remain unlabeled. Due to the high affinity of biotin for avidin-like proteins, biotin functionalized cells are ideal targets for conjugation of e.g. avidin tagged magnetic beads, fluorophores or antibodies with bioanalytical relevance. Here, based on the differential biotinylation of distinct cell populations high quality separation of cardiac fibroblasts from myocytes, and cerebromicrovascular endothelial cells from fibroblasts was successfully established.

4.1505 Post-thaw ATP supplementation enhances cryoprotective effect of iodixanol in rat spermatozoa
Kim, S., Hooper, S., Agca, C. and Agca, Y.
Reproductive Biol.and Endocrinol., ***14***:*5 (2016)*

Background
Successful cryopreservation of rat spermatozoa from various strains still remains a challenge. The objective of this study was to determine if combinations of OptiPrep™ (iodixanol) and adenosine 5′-triphosphate (ATP) can improve rat sperm function during the cryopreservation procedure.
Methods
Epididymal rat spermatozoa were frozen under different OptiPrep™ concentrations (0, 1, 2, 3 or 4 %) and were diluted with media supplemented with or without 2 mM ATP after thawing. Post-thaw sperm motility, acrosomal membrane integrity (AMI) and mitochondrial membrane potential (MMP) were then evaluated.

In addition, the effect of different OptiPrep™ concentrations on fresh and cooled rat spermatozoa was tested via motility.

Results

There was no effect of OptiPrep™ on motility of fresh and cooled spermatozoa. The supplementation of 1 and 2 % OptiPrep™ increased motility of frozen spermatozoa at 10 min after thawing, while it did not improve motility of spermatozoa at 3 h after thawing in the absence of ATP. During incubation of thawed spermatozoa, the ATP addition protected time-dependent decrease in motility after thawing in OptiPrep™-treated samples. OptiPrep™ had no effect on AMI and MMP in frozen-thawed spermatozoa but combinations of OptiPrep™ and ATP improved MMP in frozen-thawed spermatozoa.

Conclusions

Iodixanol has cryoprotective effects during rat sperm freezing without any toxic effect. Moreover, the combinations of iodixanol and ATP have a beneficial role in maintaining function of frozen-thawed rat spermatozoa for long period of incubation post-thaw.

4.1506 Proteomic Analysis of Dynein-Interacting Proteins in Amyotrophic Lateral Sclerosis Synaptosomes Reveals Alterations in the RNA-Binding Protein Staufen1

Gershoni-Emek, N., Mazza, A., Chein, M., Gradus-Pery, T., Xiang, X., Wan, K., Sharan, R. and Perlson, E.
*Mol. Cell. Proteomics, **15**, 506-522 (2016)*

Synapse disruption takes place in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). However, the mechanistic understanding of this process is still limited. We set out to study a possible role for dynein in synapse integrity. Cytoplasmic dynein is a multisubunit intracellular molecule responsible for diverse cellular functions, including long-distance transport of vesicles, organelles, and signaling factors toward the cell center. A less well-characterized role dynein may play is the spatial clustering and anchoring of various factors including mRNAs in distinct cellular domains such as the neuronal synapse. Here, in order to gain insight into dynein functions in synapse integrity and disruption, we performed a screen for novel dynein interactors at the synapse. Dynein immunoprecipitation from synaptic fractions of the ALS model $mSOD1^{G93A}$ and wild-type controls, followed by mass spectrometry analysis on synaptic fractions of the ALS model $mSOD1^{G93A}$ and wild-type controls, was performed. Using advanced network analysis, we identified Staufen1, an RNA-binding protein required for the transport and localization of neuronal RNAs, as a major mediator of dynein interactions via its interaction with protein phosphatase 1–beta (PP1B). Both *in vitro* and *in vivo* validation assays demonstrate the interactions of Staufen1 and PP1B with dynein, and their colocalization with synaptic markers was altered as a result of two separate ALS-linked mutations: $mSOD1^{G93A}$ and $TDP43^{A315T}$. Taken together, we suggest a model in which dynein's interaction with Staufen1 regulates mRNA localization along the axon and the synapses, and alterations in this process may correlate with synapse disruption and ALS toxicity.

4.1507 Combination strategy of multi-layered surface camouflage using hyperbranched polyethylene glycol and immunosuppressive drugs for the prevention of immune reactions against transplanted porcine islets

Haque, M.R., Jeong, J-H. and Byun, Y.
*Biomaterials, **84**, 144-156 (2016)*

This study suggests a novel method of stabilizing fragile porcine islets to prevent the dissociation after isolation and reducing immune cell invasion in a combination therapy of ‘surface camouflaging’ and immunosuppressive drugs (FK506, Rapamycin, MR-1, anti-CD19 mAb, and Clodrosome®) to effectively alleviate overall immune reactions against xenotransplanted porcine islets. The surface camouflage of pancreatic islets using biocompatible materials improved stabilization of pancreatic islet and prevented the infiltration of immune cells. Firstly, the surface of porcine islets was camouflaged by SH-6-arm-PEG-lipid and gelatin-catechol (artificial extracellular matrix) in order to stabilize the fragile isolated islets. Secondly, three different PEG layers (6-arm-PEG-SH, 6-arm-PEG-catechol, and linear PEG-SH) were chemically conjugated onto the surface of the stabilized porcine islets. Both artificial extracellular matrix (artificial ECM) and PEGylation effectively covered the surface of porcine islets without increasing the size of the whole islet. In addition, the viability and functionality of the islets were not affected by this multi-layer surface modification. The multi-layer modification significantly reduced the attachment of human serum albumin, fibronectin, and immunoglobulin G in comparison to the control collagen surface. The combination effect of multi-layer PEGylation and cocktailed immunosuppressive drugs on the survival time of the transplanted islets was assessed in a xenogeneic porcine-to-mouse model. The median survival time (MST) of ‘artificial ECM + PEGylation' group was 4-fold increased compared to that of control group.

In addition, the MST of 'artificial ECM + PEGylation + drug' group was 2.16-fold increased, compared to the 'control + drug' group. In conclusion, we proposed a novel porcine islet transplantation protocol using surface multi-layer modification and cocktailed immunosuppressive drugs, for stabilization and immunoprotection against xenogeneic immune reactions.

4.1508 Co-culture of Hepatocytes and Kupffer Cells as an In Vitro Model of Inflammation and Drug-Induced Hepatotoxicity

Rose, K.A., Holman, N.S., Green, A.M., Andersen, M.E. and LeCluyse, E.L.
*J. Pharmaceut. Sci., **105**, 950-964 (2016)*

Immune-mediated drug-induced hepatotoxicity is often unrecognized as a potential mode of action due to the lack of appropriate *in vitro* models. We have established an *in vitro* rat donor-matched hepatocyte and Kupffer cell co-culture (HKCC) model to study immune-related responses to drug exposure. Optimal cell culture conditions were identified for the maintenance of co-cultures based on cell longevity, monolayer integrity, and cytokine response after lipopolysaccharide (LPS) exposure. Hepatocyte monocultures and HKCCs were then used to test a subset of compounds associated with hepatotoxic effects with or without LPS. Cytokine levels and metabolic activity (cytochrome P450 3A [Cyp3A]) were measured after a 48-h exposure to monitor endotoxin-induced changes in acute phase and functional end points. LPS-activated HKCCs, but not hepatocyte monocultures, treated with trovafloxacin or acetaminophen, compounds associated with immune-mediated hepatotoxicity, showed LPS-dependent decreases in interleukin-6 production with concomitant increases in Cyp3A activity. Differential endotoxin- and model-dependent alterations were observed in cytokine profiles and Cyp3A activity levels that corresponded to specific compounds. These results indicate the utility of the HKCC model system to discern compound-specific effects that may lead to enhanced or mitigate hepatocellular injury due to innate or adaptive immune responses.

4.1509 Pregnancy outcomes using stallion epididymal sperm stored at 5 °C for 24 or 48 hours before harvest

Stawicki, R.J., McDonnell, S.M., Giguere, S. and Turner, R.M.
*Theriogenology, **85**, 698-702 (2016)*

The cryopreservation of epididymal sperm can be useful in a variety of circumstances for ensuring genetic preservation of a valued stallion. Although early studies have reported pregnancy rates significantly lower than those achieved with cryopreserved ejaculated sperm, two recent studies report over 60% one-cycle pregnancy rates with epididymal sperm stored for 24 hours at 5 °C before harvest and cryopreservation. The aims of this study were to: (1) attempt to replicate the one-cycle pregnancy rate of over 60% using epididymal sperm cooled and stored within the epididymis for 24 hours before harvest and cryopreservation and (2) evaluate pregnancy outcome with sperm cooled and stored within the epididymis for 48 hours before sperm harvest and cryopreservation. Testicles were obtained from 13 stallions undergoing routine castration. The epididymides were stored at 5 °C for either 24 or 48 hours before sperm harvest and cryopreservation in an egg yolk and dimethylformamide-based freezing extender. Thirteen mares were bred on one cycle with cryopreserved epididymal sperm stored for 24 hours before harvest, and 10 of those 13 mares were also bred on a previous or subsequent cycle with samples from the same stallion that had been stored for 48 hours before harvest. Pregnancy occurred in 7 of the 13 inseminations of sperm stored for 24 hours before harvest, and in 4 of the 10 inseminations of sperm stored for 48 hours before harvest. The pregnancy rate using epididymal sperm stored for 24 hours before harvest is consistent with that of previous reports. In addition, these results provide evidence that pregnancies can be achieved when the epididymides are cooled and stored for 48 hours before sperm harvest and cryopreservation.

4.1510 Substitution of egg yolk by a cyclodextrin-cholesterol complex allows a reduction of the glycerol concentration into the freezing medium of equine sperm

Blommaert, D., Franck, T., Donnay, I., Lejeune, J-P., Detilleux, J. and Serteyn, D.
*Cryobiology, **72**, 27-32 (2016)*

The aim of this work was to completely replace the egg yolk a classical diluent for freezing equine semen by a cyclodextrin-cholesterol complex. At the same time, the reduction in the glycerol content used for cryopreservation and the incubation time between sperm and the freezing media were evaluated. Horse ejaculates were frozen with four different freezing extenders: a frozen reference medium (IF) containing egg yolk and 2.5% glycerol and media without egg yolk but supplemented with 1.5 mg 2-hydroxypropyl-beta-cyclodextrin cholesterol (HPβCD-C) complex and containing either 1% (G1), 2%

(G2) or 3% glycerol (G3). Three incubation times (90, 120 and 180 min) at 4 °C between the fresh semen and the different media were tested before freezing. Viability and motility analyses were performed with computer assisted semen analysis (CASA).
Results showed that the freezing media containing the HPβCD–C complex with 1%, 2% and 3% glycerol significantly improve the 3 in vitro parameters of post thawing semen quality (viability, progressive and total mobilities) compared to IF. The best improvement of the parameters was obtained with G1 medium and the longest contact time. The substitution of egg yolk by HPβCD–C complex allows the decrease of protein charge of the medium while favouring the cholesterol supply to membrane spermatozoa offering it a better resistance to osmotic imbalance and a better tolerance to the glycerol toxicity. Our results highlight that the egg yolk of an extender for the freezing of horse semen can be completely substituted by HPβCD–C complex.

4.1511 The Evolutionarily Conserved Tre2/Bub2/Cdc16 (TBC), Lysin Motif (LysM), Domain Catalytic (TLDc) Domain Is Neuroprotective against Oxidative Stress
Finelli, M.J., Sanchez-Pulido, L., Liu, K.X., Davies, K.E. and Oliver, P.L.
J. Biol. Chem., ***291(6)***, *2751-2763 (2016)*

Oxidative stress is a pathological feature of many neurological disorders; therefore, utilizing proteins that are protective against such cellular insults is a potentially valuable therapeutic approach. Oxidation resistance 1 (OXR1) has been shown previously to be critical for oxidative stress resistance in neuronal cells; deletion of this gene causes neurodegeneration in mice, yet conversely, overexpression of OXR1 is protective in cellular and mouse models of amyotrophic lateral sclerosis. However, the molecular mechanisms involved are unclear. OXR1 contains the Tre2/Bub2/Cdc16 (TBC), lysin motif (LysM), domain catalytic (TLDc) domain, a motif present in a family of proteins including TBC1 domain family member 24 (TBC1D24), a protein mutated in a range of disorders characterized by seizures, hearing loss, and neurodegeneration. The TLDc domain is highly conserved across species, although the structure-function relationship is unknown. To understand the role of this domain in the stress response, we carried out systematic analysis of all mammalian TLDc domain-containing proteins, investigating their expression and neuroprotective properties in parallel. In addition, we performed a detailed structural and functional study of this domain in which we identified key residues required for its activity. Finally, we present a new mouse insertional mutant of *Oxr1*, confirming that specific disruption of the TLDc domain *in vivo* is sufficient to cause neurodegeneration. Our data demonstrate that the integrity of the TLDc domain is essential for conferring neuroprotection, an important step in understanding the functional significance of all TLDc domain-containing proteins in the cellular stress response and disease.

4.1512 Characterization of an IgG monoclonal antibody targeted to both tissue cyst and sporocyst walls of Toxoplasma gondii
Gondim, L.F.P., Wolf, A., Vrhovec, M.G., pantchev, N., Bauer, C., langennayer, M.C., Bohne, W., Teifke, J.P., Dubey, J.P., Conratahs, F.J. and Schares, G.
Exp. Parasitol., ***163***, *46-56 (2016)*

Toxoplasma gondii infects animals habiting terrestrial and aquatic environments. Its oocysts and tissue cysts are important for the horizontal transmission of this parasite. The oocyst and tissue cyst walls are crucial for the ability of the parasite to persist in the environment or in animal tissues, respectively. However, the composition of these walls is not well understood. We report the generation of monoclonal antibodies directed against wall components using mice immunized with oocyst antigens of *T. gondii*. One monoclonal antibody (mAb) G1/19 reacted solely with *T. gondii* sporozoites. The respective antigen had a relative molecular weight (Mr) of 30 kDa. MAb G1/19 failed to react with sporozoites of any other coccidian parasite species tested (*Hammondia hammondi*, *Hammondia heydorni*, *Cystoisospora felis*, *Eimeria bovis*, *Sarcocystis* sp.). Another mAb, designated K8/15-15, recognized antigens in sporocyst walls of the parasite and in the walls of *in vivo* or *in vitro* produced tissue cysts, as demonstrated by immunofluorescence and immunoblot assays. Antigens of 80 to a high molecular weight protein of about 350 kDa Mr were recognized by this antibody using antigen extracts from sporocysts, and from *in vitro* or *in vivo* generated tissue cysts of the parasite. Tissue cyst and sporocyst walls of *H. hammondi* and *H. heydorni*, and tissue cysts of *Neospora caninum* were also recognized by mAb K8/15-15. Sporocyst walls of *C. felis* also reacted to this mAb. The cyst walls of *Sarcocystis* sp. and *Besnoitia besnoiti* were not recognized by mAb K8/15-15. Reactivity by a single mAb against *T. gondii* antigens in tissue cysts and sporocysts had not been reported previously. MAb K8/15-15 may be a practical tool for the identification of both cysts and sporocysts of the parasite, and may also be potentially employed in proteomic studies on the identification of new components of the cyst and sporocyst walls of *T. gondii*.

4.1513 Spatial distribution of osteoblast activating peptide in the rat stomach

Noreldin, A.E., Sogabe, M., Yamano, Y., Uehara, M., Mahdy, M.A.A, Elnasharty, M.A., Sayed-Ahmed, A., Warita, K. and Hosaka, Y.Z.

*Acta Histochemica, **118**, 109-117 (2016)*

Osteoblast activating peptide (OBAP) was previously reported to be expressed in the rat stomach and to have a vital role in osteogenesis, but its distribution in rat stomach has not been determined. Thus, the aim of the present study was to identify the cell types expressing OBAP in the rat stomach. The stomachs of twelve 10-to-11-week-old male Jc1:SD rats were used. Samples were collected for immunohistochemistry, immunoelectron microscopy and dot blot assay. Immunohistochemical investigation revealed that OBAP was distributed mainly in parietal cells without any expression in chief cells, X/A-like cells or enterochromaffin-like cells. Moreover, OBAP-immunopositive cells were observed mainly in the upper and lower parts of the gastric gland. Significantly high optical density of immunopositive cells was observed in the upper and lower gastric gland regions. The dot blot assay confirmed that OBAP is secreted by parietal cells and that it is present in the gastric gland lumen. Immunoelectron microscopy demonstrated that OBAP was confined to the mitochondrial inner membrane within parietal cells and that the number of mitochondria in the upper and lower parts of the gastric epithelium was significantly larger than the number in the middle part of the gastric epithelium. Based on the results, it was concluded that OBAP is mainly produced by mitochondria of parietal cells in the upper and lower parts of the gastric epithelium. Moreover, the presence of OBAP in the gastric gland lumen suggests an exocrine mechanism of release.

4.1514 Hepatic stellate cell transdifferentiation involves genome-wide remodeling of the DNA methylation landscape

Page, A., Paoli, P., Salvador, E.M., White, S., French, J. and Mann, J.

*J. Hepatol., **64**, 661-673 (2016)*

Background & Aims
DNA methylation (5-mC) is an epigenetic mark that is an established regulator of transcriptional repression with an important role in liver fibrosis. Currently, there is very little knowledge available as to how DNA methylation controls the phenotype of hepatic stellate cell (HSC), the key cell type responsible for onset and progression of liver fibrosis. Moreover, recently discovered DNA hydroxymethylation (5-hmC) is involved in transcriptional activation and its patterns are often altered in human diseases. The aim of this study is to investigate the role of DNA methylation/hydroxymethylation in liver fibrosis.
Methods
Levels of 5-mC and 5-hmC were assessed by slot blot in a range of animal liver fibrosis models and human liver diseases. Expression levels of TET and DNMT enzymes were measured by qRT-PCR and Western blotting. Reduced representation bisulfite sequencing (RRBS) method was used to examine 5-mC and 5-hmC patterns in quiescent and *in vivo* activated rat HSC.
Results
We demonstrate global alteration in 5-mC and 5-hmC and their regulatory enzymes that accompany liver fibrosis and HSC transdifferentiation. Using RRBS, we show exact genomic positions of changed methylation patterns in quiescent and *in vivo* activated rat HSC. In addition, we demonstrate that reduction in DNMT3a expression leads to attenuation of pro-fibrogenic phenotype in activated HSC.
Conclusions
Our data suggest that DNA 5-mC/5-hmC is a crucial step in HSC activation and therefore fibrogenesis. Changes in DNA methylation during HSC activation may bring new insights into the molecular events underpinning fibrogenesis and may provide biomarkers for disease progression as well as potential new drug targets.

4.1515 Determination and Characterization of Tetraspanin-Associated Phosphoinositide-4 Kinases in Primary and Neoplastic Liver Cells

Rombouts, K. and Carloni, V.

*Methods in Mol. Biol., **1376**, 203-212 (2016)*

Accumulating evidence implicates phosphoinositide 4-phosphate as a regulatory molecule in its own right recruiting specific effector proteins to cellular membranes. Here, we describe biochemical and immunocytochemical methods to evaluate tetraspanin-associated phosphoinositide-4 kinases activity in primary human hepatic stellate cells (hHSC) and neoplastic hepatoblastoma cells.

4.1516 **Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints**
Koyama, S. et al
*Nature Communications, **7**:10501 (2016)*

Despite compelling antitumour activity of antibodies targeting the programmed death 1 (PD-1): programmed death ligand 1 (PD-L1) immune checkpoint in lung cancer, resistance to these therapies has increasingly been observed. In this study, to elucidate mechanisms of adaptive resistance, we analyse the tumour immune microenvironment in the context of anti-PD-1 therapy in two fully immunocompetent mouse models of lung adenocarcinoma. In tumours progressing following response to anti-PD-1 therapy, we observe upregulation of alternative immune checkpoints, notably T-cell immunoglobulin mucin-3 (TIM-3), in PD-1 antibody bound T cells and demonstrate a survival advantage with addition of a TIM-3 blocking antibody following failure of PD-1 blockade. Two patients who developed adaptive resistance to anti-PD-1 treatment also show a similar TIM-3 upregulation in blocking antibody-bound T cells at treatment failure. These data suggest that upregulation of TIM-3 and other immune checkpoints may be targetable biomarkers associated with adaptive resistance to PD-1 blockade.

4.1517 **All-Trans Retinoic Acid Induces Expression of a Novel Intergenic Long Noncoding RNA in Adult rat Primary Hippocampal Neurons**
Kour, S. and Rath, P.C.
*J. Mol. Sci., **58(2)**, 266-276 (2016)*

Around 90 % of the mammalian genome undergoes pervasive transcription into various types of small and long regulatory noncoding RNAs, whereas only ∼1.5 % codes for proteins. Long noncoding RNAs (lncRNAs) constitute diverse classes of sense- and antisense transcripts that are abundantly expressed in the mammalian central nervous system (CNS) in cell type- and developmental stage-specific manners. They are implicated in brain development, differentiation, neuronal plasticity, and other cognitive functions. Mammalian brain requires the vitamin A metabolite all-*trans* retinoic acid (atRA) for its normal development, differentiation, and cell-fate determination. However, its role in adult brain function is less understood. Here, we report atRA-mediated transcriptional upregulation of endogenous expression of a novel long intergenic noncoding RNA-rat brain expressed (*LINC-RBE*) in cultured primary hippocampal neurons from adult rat. We have previously reported *LINC-RBE* as an intergenic, simple repeat sequence containing lncRNA highly expressed in the rat brain. This is a first-time report of involvement of atRA in transcriptional upregulation of lncRNA expression in rat hippocampal neurons. Therefore, it may be involved in regulation of brain function and disease.

4.1518 **Biophysical changes reduce energetic demand in growth factor–deprived lymphocytes**
Hecht, V.C., Sullivan, L.B., Kimmerling, R.J., Kim, D-H., Hosios, A.M., Stockslager, M.A., Stevens, M.M., Kang, J.H., Wirtz, D., Vander heiden, M.G. and Manalis, S.R.
*J. Cell Biol., **212(4)**, 439-447 (2016)*

Cytokine regulation of lymphocyte growth and proliferation is essential for matching nutrient consumption with cell state. Here, we examine how cellular biophysical changes that occur immediately after growth factor depletion promote adaptation to reduced nutrient uptake. After growth factor withdrawal, nutrient uptake decreases, leading to apoptosis. Bcl-x_L expression prevents cell death, with autophagy facilitating long-term cell survival. However, autophagy induction is slow relative to the reduction of nutrient uptake, suggesting that cells must engage additional adaptive mechanisms to respond initially to growth factor depletion. We describe an acute biophysical response to growth factor withdrawal, characterized by a simultaneous decrease in cell volume and increase in cell density, which occurs before autophagy initiation and is observed in both FL5.12 Bcl-x_L cells depleted of IL-3 and primary $CD8^+$ T cells depleted of IL-2 that are differentiating toward memory cells. The response reduces cell surface area to minimize energy expenditure while conserving biomass, suggesting that the biophysical properties of cells can be regulated to promote survival under conditions of nutrient stress.

4.1519 **Hepatic stellate cells regulate liver immunity to visceral leishmaniasis through P110δ-dependent induction and expansion of regulatory T cells in mice**
Khadem, F., Gao, X., Mou, Z., Jia, P., Movassagh, H., Onyilagha, C., Gounni, A.S., Wright, M.C. and Uzonna, J.E.
*Hepatology, **63(2)**, 620-632 (2016)*

Visceral leishmaniasis (VL) is associated with severe immune dysfunction and if untreated leads to death. Because the liver is one of the primary target organs in VL, unraveling the mechanisms governing the local hepatic immune response is important for understanding the immunopathogenesis of VL. We previously reported that mice with inactivating knockin mutation in the p110δ gene ($p110\delta^{D910A}$) are resistant to VL, due in part to impaired regulatory T-cell (Treg) expansion. In this study, we investigated the mechanism of this resistance by focusing on hepatic stellate cells (HSCs), which are known to regulate Treg induction and expansion. We show that HSCs are infected with *Leishmania donovani in vivo* and *in vitro* and that this infection leads to the production of interleukin-2, interleukin-6, and transforming growth factor-β, cytokines known to induce Tregs. We further demonstrate that *L. donovani* infection leads to expansion of HSCs in a p110δ-dependent manner and that this correlated with proliferation of hepatic Tregs *in vivo*. *In vitro* studies clearly show that *L. donovani*–infected HSCs induce $CD4^+$ T cells to become Tregs and expand Tregs in a p110δ-dependent manner. Targeted depletion of HSCs during infection caused a dramatic reduction in liver Treg numbers and proliferation, which was associated with a decrease in interleukin-10 production by hepatic T cells and a more efficient parasite control. *Conclusion*: These results demonstrate the critical role of HSCs in the pathogenesis of VL and suggest that the enhanced resistance of $p110\delta^{D910A}$ mice to *L. donovani* infection is due in part to impaired expansion and inability of their HSCs to induce and expand Tregs in the liver.

4.1520 Role of TGF-β signaling in differentiation of mesothelial cells to vitamin A-poor hepatic stellate cells in liver fibrosis

Li, Y., Lua, I., French, S.W. and Asahina, K.
Am. J. Physiol. Gastrointest. Liver Physiol., ***310****, G262-G272 (2016)*

Mesothelial cells (MCs) form a single layer of the mesothelium and cover the liver surface. A previous study demonstrated that, upon liver injury, MCs migrate inward from the liver surface and give rise to hepatic stellate cells (HSCs) in biliary fibrosis induced by bile duct ligation (BDL) or myofibroblasts in CCl_4-induced fibrosis. The present study analyzed the role of transforming growth factor-β (TGF-β) signaling in mesothelial-mesenchymal transition (MMT) and the fate of MCs during liver fibrosis and its regression. Deletion of TGF-β type II receptor (*Tgfbr2*) gene in cultured MCs suppressed TGF-β-mediated myofibroblastic conversion. Conditional deletion of *Tgfbr2* gene in MCs reduced the differentiation of MCs to HSCs and myofibroblasts in the BDL and CCl_4 models, respectively, indicating that the direct TGF-β signaling in MCs is responsible to MMT. After BDL and CCl_4 treatment, MC-derived HSCs and myofibroblasts were distributed near the liver surface and the thickness of collagen was increased in Glisson's capsule beneath the liver surface. Fluorescence-activated cell sorting analysis revealed that MC-derived HSCs and myofibroblasts store little vitamin A lipids and have fibrogenic phenotype in the fibrotic livers. MCs contributed to 1.4 and 2.0% of activated HSCs in the BDL and CCl_4 models, respectively. During regression of CCl_4-induced fibrosis, 20% of MC-derived myofibroblasts survived in the liver and deactivated to vitamin A-poor HSCs. Our data indicate that MCs participate in capsular fibrosis by supplying vitamin A-poor HSCs during a process of liver fibrosis and regression.

4.1521 Increases in core temperature counterbalance effects of haemoconcentration on blood viscosity during prolonged exercise in the heat

Buono, M.J., Krippes, T., Kolkhorst, F.W., Williams, A.T. and Cabrales, P.
Exp. Physiol., ***101(2)****, 332-342 (2016)*

Previous studies have reported that blood viscosity is significantly increased following exercise. However, these studies measured both pre- and postexercise blood viscosity at 37°C even though core and blood temperatures would be expected to have increased during the exercise. Consequently, the effect of exercise-induced hyperthermia on mitigating change in blood viscosity may have been missed. The purpose of this study was to isolate the effects of exercise-induced haemoconcentration and hyperthermia and to determine their combined effects on blood viscosity. Nine subjects performed 2 h of moderate-intensity exercise in the heat (37°C, 40% relative humidity), which resulted in significant increases from pre-exercise values for rectal temperature (from 37.11 ± 0.35 to 38.76 ± 0.13°C), haemoconcentration (haematocrit increased from 43.6 ± 3.6 to 45.6 ± 3.5%) and dehydration (change in body weight = −3.6 ± 0.7%). Exercise-induced haemoconcentration significantly ($P < 0.05$) increased blood viscosity by 9% (from 3.97 to 4.33 cP at 300 s^{-1}), whereas exercise-induced hyperthermia significantly decreased blood viscosity by 7% (from 3.97 to 3.69 cP at 300 s^{-1}). When both factors were considered together, there was no overall change in blood viscosity (from 3.97 to 4.03 cP at 300 s^{-1}). The effects of exercise-induced haemoconcentration, increased plasma viscosity and increased red blood cell aggregation,

all of which increased blood viscosity, were counterbalanced by increased red blood cell deformability (e.g. red blood cell membrane shear elastic modulus and elongation index) caused by the hyperthermia. Thus, blood viscosity remained unchanged following prolonged moderate-intensity exercise in the heat.

4.1522 Sonic hedgehog stimulates neurite outgrowth in a mechanical stretch model of reactive-astrogliosis
Berretta, A., Gowing, E.K., Jasoni, C.L. and Clarkson, A.N.
*Scientific Reports, **6**:21896 (2016)*

Although recovery following a stroke is limited, undamaged neurons under the right conditions can establish new connections and take on-board lost functions. Sonic hedgehog (Shh) signaling is integral for developmental axon growth, but its role after injury has not been fully examined. To investigate the effects of Shh on neuronal sprouting after injury, we used an in vitro model of glial scar, whereby cortical astrocytes were mechanically traumatized to mimic reactive astrogliosis observed after stroke. This mechanical trauma impaired neurite outgrowth from post-natal cortical neurons plated on top of reactive astrocytes. Addition of Shh to the media, however, resulted in a concentration-dependent increase in neurite outgrowth. This response was inhibited by cyclopamine and activated by oxysterol 20(S)-hydroxycholesterol, both of which modulate the activity of the Shh co-receptor Smoothened (Smo), demonstrating that Shh-mediated neurite outgrowth is Smo-dependent. In addition, neurite outgrowth was not associated with an increase in Gli-1 transcription, but could be inhibited by PP2, a selective inhibitor of Src family kinases. These results demonstrate that neurons exposed to the neurite growth inhibitory environment associated with a glial scar can be stimulated by Shh, with signaling occurring through a non-canonical pathway, to overcome this suppression and stimulate neurite outgrowth.

4.1523 8-Oxoguanine accumulation in mitochondrial DNA causes mitochondrial dysfunction and impairs neuritogenesis in cultured adult mouse cortical neurons under oxidative conditions
Leon, J., Sakumi. K., Castillo, E., Sheng, Z., Oka, S. and Makabeppy, Y.
*Scientific Reports, **6**:22086 (2016)*

Oxidative stress and mitochondrial dysfunction are implicated in aging-related neurodegenerative disorders. 8-Oxoguanine (8-oxoG), a common oxidised base lesion, is often highly accumulated in brains from patients with neurodegenerative disorders. MTH1 hydrolyses 8-oxo-2′-deoxyguanosine triphosphate (8-oxo-dGTP) to 8-oxo-dGMP and pyrophosphate in nucleotide pools, while OGG1 excises 8-oxoG paired with cytosine in DNA, thereby minimising the accumulation of 8-oxoG in DNA. Mth1/Ogg1-double knockout (TO-DKO) mice are highly susceptible to neurodegeneration under oxidative conditions and show increased accumulation of 8-oxoG in mitochondrial DNA (mtDNA) in neurons, suggesting that 8-oxoG accumulation in mtDNA causes mitochondrial dysfunction. Here, we evaluated the contribution of MTH1 and OGG1 to the prevention of mitochondrial dysfunction during neuritogenesis in vitro. We isolated cortical neurons from adult wild-type and TO-DKO mice and maintained them with or without antioxidants for 2 to 5 days and then examined neuritogenesis. In the presence of antioxidants, both TO-DKO and wild-type neurons exhibited efficient neurite extension and arborisation. However, in the absence of antioxidants, the accumulation of 8-oxoG in mtDNA of TO-DKO neurons was increased resulting in mitochondrial dysfunction. Cells also exhibited poor neurite outgrowth with decreased complexity of neuritic arborisation, indicating that MTH1 and OGG1 are essential for neuritogenesis under oxidative conditions.

4.1524 Adoptive transferred hepatic stellate cells attenuated drug-induced liver injury by modulating the rate of regulatory T cells/T helper 17 cells
Feng, M., Wang, Q., Jiang, z., Ding, J., Wang, H., Wang, M., Lu, L. and Guan, W.
*Clin. Immunol., **165**, 12-18 (2016)*

Our study showed that hepatic stellate cells (HSCs) promote the healing of the liver after drug-induced acute injury. However, the relevant mechanisms by which this is accomplished remain unclear. The objective of this study was to investigate the role of the adoptive transfer of HSCs in acute liver injury and the underlying mechanisms for healing. It was found that adoptive transfer of HSCs resulted in an increase in Tregs and a decrease in Th17 cells. Liver insult was consistently attenuated by HSC treatment. HSC cultured medium induced Tregs from naive T cells and suppressed the differentiation of Th17 cells. This study demonstrated that the adoptive transfer of HSCs protected the liver from drug-induced acute injury. Promoting the differentiation of Tregs and suppressing the development of Th17 cells are possibly involved in the protective effect of adoptive transfer of HSCs.

4.1525 **Identifying Effective Enzyme Activity Targets for Recombinant Class I and Class II Collagenase for Successful Human Islet Isolation**
Balamurugan, A.N., Green, M.L., Breite, A.G., Loganathan, G., Wilhelm, J.J., Tweed, B., Vargova, L., Lockbridge, A., Kuriti, M., Hughes, M.G., Williams, S-K., Hering, B.J., Dwulet, F.E. and McCarthy, R.C.
*Transplantation Direct, **2**:e54 (2016)*

Background: Isolation following a good manufacturing practice-compliant, human islet product requires development of a robust islet isolation procedure where effective limits of key reagents are known. The enzymes used for islet isolation are critical but little is known about the doses of class I and class II collagenase required for successful islet isolation.
Methods: We used a factorial approach to evaluate the effect of high and low target activities of recombinant class I (rC1) and class II (rC2) collagenase on human islet yield. Consequently, 4 different enzyme formulations with divergent C1:C2 collagenase mass ratios were assessed, each supplemented with the same dose of neutral protease. Both split pancreas and whole pancreas models were used to test enzyme targets (n = 20). Islet yield/g pancreas was compared with historical enzymes (n = 42).
Results: Varying the Wunsch (rC2) and collagen degradation activity (CDA, rC1) target dose, and consequently the C1:C2 mass ratio, had no significant effect on tissue digestion. Digestions using higher doses of Wunsch and CDA resulted in comparable islet yields to those obtained with 60% and 50% of those activities, respectively. Factorial analysis revealed no significant main effect of Wunsch activity or CDA for any parameter measured. Aggregate results from 4 different collagenase formulations gave 44% higher islet yield (>5000 islet equivalents/g) in the body/tail of the pancreas (n = 12) when compared with those from the same segment using a standard natural collagenase/protease mixture (n = 6). Additionally, islet yields greater than 5000 islet equivalents/g pancreas were also obtained in whole human pancreas.
Conclusions: A broader C1:C2 ratio can be used for human islet isolation than has been used in the past. Recombinant collagenase is an effective replacement for the natural enzyme and we have determined that high islet yield can be obtained even with low doses of rC1:rC2, which is beneficial for the survival of islets.

4.1526 **Bicyclic-Capped Histone Deacetylase 6 Inhibitors with Improved Activity in a Model of Axonal Charcot–Marie–Tooth Disease**
Shen, S., Benoy, V., bergman, J.A., Kalin, J.H., Frojuello, M., Vistoli, G., Haeck, W., Van Den Bosch, L. and Kozikowski, A.P.
*ACS Chem. Neurosci., **7(2)**, 240-258 (2016)*

Charcot–Marie–Tooth (CMT) disease is a disorder of the peripheral nervous system where progressive degeneration of motor and sensory nerves leads to motor problems and sensory loss and for which no pharmacological treatment is available. Recently, it has been shown in a model for the axonal form of CMT that histone deacetylase 6 (HDAC6) can serve as a target for the development of a pharmacological therapy. Therefore, we aimed at developing new selective and activity-specific HDAC6 inhibitors with improved biochemical properties. By utilizing a bicyclic cap as the structural scaffold from which to build upon, we developed several analogues that showed improved potency compared to tubastatin A while maintaining excellent selectivity compared to HDAC1. Further screening in N2a cells examining both the acetylation of α-tubulin and histones narrowed down the library of compounds to three potent and selective HDAC6 inhibitors. In mutant HSPB1-expressing DRG neurons, serving as an in vitro model for CMT2, these inhibitors were able to restore the mitochondrial axonal transport deficits. Combining structure-based development of HDAC6 inhibitors, screening in N2a cells and in a neuronal model for CMT2F, and preliminary ADMET and pharmacokinetic profiles, resulted in the selection of compound **23d** that possesses improved biochemical, functional, and druglike properties compared to tubastatin A.

4.1527 **Long-term Efficacy and Biocompatibility of Encapsulated Islet Transplantation With Chitosan-Coated Alginate Capsules in Mice and Canine Models of Diabetes**
Yang, H.K. et al
*Transplantation, **100(2)**, 334-343 (2016)*

Background: Clinical application of encapsulated islet transplantation is hindered by low biocompatibility of capsules leading to pericapsular fibrosis and decreased islet viability. To improve biocompatibility, we designed a novel chitosan-coated alginate capsules and compared them to uncoated alginate capsules.
Methods: Alginate capsules were formed by crosslinking with $BaCl_2$, then they were suspended in chitosan solution for 10 minutes at pH 4.5. Xenogeneic islet transplantation, using encapsulated porcine islets in 1,3-galactosyltransferase knockout mice, and allogeneic islet transplantation, using encapsulated canine

islets in beagles, were performed without immunosuppressants.
Results: The chitosan-alginate capsules showed similar pore size, islet viability, and insulin secretory function compared to alginate capsules, in vitro. Xenogeneic transplantation of chitosan-alginate capsules demonstrated a trend toward superior graft survival ($P = 0.07$) with significantly less pericapsular fibrosis (cell adhesion score: 3.77 ± 0.41 vs 8.08 ± 0.05; $P < 0.001$) compared to that of alginate capsules up to 1 year after transplantation. Allogeneic transplantation of chitosan-alginate capsules normalized the blood glucose level up to 1 year with little evidence of pericapsular fibrotic overgrowth on graft explantation.
Conclusions: The efficacy and biocompatibility of chitosan-alginate capsules were demonstrated in xenogeneic and allogeneic islet transplantations using small and large animal models of diabetes. This capsule might be a potential candidate applicable in the treatment of type 1 diabetes mellitus patients, and further studies in nonhuman primates are required.

4.1528 Ammonia produces pathological changes in human hepatic stellate cells and is a target for therapy of portal hypertension

Jalan, R., De Chiara, F., Balasubramaniyan, V., Andreola, F., Kheta, V., Malago, M., Pinzani, M., Mookerjee, R.P. and Rombouts, K.
*J. Hepatol., **64**, 823-833 (2016)*

Background & Aims
Hepatic stellate cells (HSCs) are vital to hepatocellular function and the liver response to injury. They share a phenotypic homology with astrocytes that are central in the pathogenesis of hepatic encephalopathy, a condition in which hyperammonemia plays a pathogenic role. This study tested the hypothesis that ammonia modulates human HSC activation *in vitro* and *in vivo*, and evaluated whether ammonia lowering, by using l-ornithine phenylacetate (OP), modifies HSC activation *in vivo* and reduces portal pressure in a bile duct ligation (BDL) model.
Methods
Primary human HSCs were isolated and cultured. Proliferation (BrdU), metabolic activity (MTS), morphology (transmission electron, light and immunofluorescence microscopy), HSC activation markers, ability to contract, changes in oxidative status (ROS) and endoplasmic reticulum (ER) were evaluated to identify effects of ammonia challenge (50 μM, 100 μM, 300 μM) over 24–72 h. Changes in plasma ammonia levels, markers of HSC activation, portal pressure and hepatic eNOS activity were quantified in hyperammonemic BDL animals, and after OP treatment.
Results
Pathophysiological ammonia concentrations caused significant and reversible changes in cell proliferation, metabolic activity and activation markers of hHSC *in vitro.* Ammonia also induced significant alterations in cellular morphology, characterised by cytoplasmic vacuolisation, ER enlargement, ROS production, hHSC contraction and changes in pro-inflammatory gene expression together with HSC-related activation markers such as α-SMA, myosin IIa, IIb, and PDGF-Rβ. Treatment with OP significantly reduced plasma ammonia (BDL 199.1 μmol/L ± 43.65 *vs.* BDL + OP 149.27 μmol/L ± 51.1, $p < 0.05$) and portal pressure (BDL 14 ± 0.6 *vs.* BDL + OP 11 ± 0.3 mmHg, $p < 0.01$), which was associated with increased eNOS activity and abrogation of HSC activation markers.
Conclusions
The results show for the first time that ammonia produces deleterious morphological and functional effects on HSCs *in vitro*. Targeting ammonia with the ammonia lowering drug OP reduces portal pressure and deactivates hHSC *in vivo*, highlighting the opportunity for evaluating ammonia lowering as a potential therapy in cirrhotic patients with portal hypertension.

4.1529 Complement receptor 3 mediates renal protection in experimental C3 glomerulopathy

Barbour, T.D., Ling, G.S., Ruseva, M.M., Fossati-Jimack, L., Cook, H.T., Botto, M. and Pickering, M.C.
*Kidney Int., **89**, 823-832 (2016)*

C3 glomerulopathy is a complement-mediated renal disease that is frequently associated with abnormalities in regulation of the complement alternative pathway. Mice with deficiency of factor H (*Cfh*–/–), a negative alternative pathway regulator, are an established experimental model of C3 glomerulopathy in which complement C3 fragments including iC3b accumulate along the glomerular basement membrane. Here we show that deficiency of complement receptor 3 (CR3), the main receptor for iC3b, enhances the severity of spontaneous renal disease in *Cfh*–/– mice. This effect was found to be dependent on CR3 expression on bone marrow–derived cells. CR3 also mediated renal protection outside the setting of factor H deficiency, as shown by the development of enhanced renal injury in CR3-deficient mice during accelerated nephrotoxic nephritis. The iC3b–CR3 interaction downregulated the proinflammatory cytokine

response of both murine and human macrophages to lipopolysaccharide stimulation *in vitro*, suggesting that the protective effect of CR3 on glomerular injury was mediated via modulation of macrophage-derived proinflammatory cytokines. Thus, CR3 has a protective role in glomerulonephritis and suggests that pharmacologic potentiation of the macrophage CR3 interaction with iC3b could be therapeutically beneficial.

4.1530 Controlled ice nucleation—Is it really needed for large-volume sperm cryopreservation?
Saragusty, J., Osmers, J-H. and Hildebrandt, T.B.
Theriogenology, ***85***, *1328-1333 (2016)*

Controlled ice nucleation (CIN) is an integral stage of slow freezing process when relatively large volumes (usually 1 mL or larger) of biological samples in suspension are involved. Without it, a sample will supercool to way below its melting point before ice crystals start forming, resulting in multiple damaging processes. In this study, we tested the hypothesis that when freezing large volumes by the directional freezing technique, a CIN stage is not needed. Semen samples collected from ten bulls were frozen in 2.5-mL HollowTubes in a split-sample manner with and without a CIN stage. Thawed samples were evaluated for viability, acrosome integrity, rate of normal morphology, and, using computer-aided sperm analysis system, for a wide range of motility parameters that were also evaluated after 3 hours of incubation at 37 °C. Analysis of the results found no difference between freezing with and without CIN stage in any and all of the 29 parameters compared ($P > 0.1$ for all). This similarity was maintained through 3 hours of incubation at 37 °C. Possibly, because of its structure, the directional freezing device promotes continuous ice nucleation so a specific CIN stage is no longer needed, thus reducing costs, energy use, and carbon footprint.

4.1531 Versican: a novel modulator of hepatic fibrosis
Bukong, T.N., Maurice, S.B., Chahai, B., Schaeffer, D.F. and Winwood, P.J.
Lab. Invest., ***96(3)***, *361-374 (2016)*

Little is known about the deposition and turnover of proteoglycans in liver fibrosis, despite their abundance in the extracellular matrix. Versican plays diverse roles in modulating cell behavior in other fibroproliferative diseases, but remains poorly described in the liver. Hepatic fibrosis was induced by carbon tetrachloride treatment of C57BL/6 mice over 4 weeks followed by recovery over a 28-day period. Primary mouse hepatic stellate cells (HSCs) were activated in culture and versican was transiently knocked down in human (LX2) and mouse HSCs. Expression of versican, A Disintegrin-like and Metalloproteinase with Thrombospondin-1 motifs (ADAMTS)-1, -4, -5, -8, -9, -15, and -20, and markers of fibrogenesis were studied using immunohistochemistry, real-time quantitative PCR, and western blotting. Immunohistochemistry showed increased expression of versican in cirrhotic human livers and the mouse model of fibrosis. Carbon tetrachloride treatment led to significant increases in versican expression and the proteoglycanases ADAMTS-5, -9, -15, and -20, alongside TNF-α, α-smooth muscle actin (α-SMA), collagen-1, and TGF-β expression. During recovery, expression of many of these genes returned to control levels. However, expression of ADAMTS-5, -8, -9, and -15 showed delayed increases in expression at 28 days of recovery, which corresponded with decreases in versican V0 and V1 cleavage products (G1-DPEAAE^{1401} and G1-DPEAAE^{441}). Activation of primary HSCs *in vitro* significantly increased versican, α-SMA, and collagen-1 expression. Transient knockdown of versican in HSCs led to decreases in markers of fibrogenesis and reduced cell proliferation, without inducing apoptosis. Versican expression increases during HSC activation and liver fibrosis, and proteolytic processing occurs during the resolution of fibrosis. Knockdown studies *in vitro* suggest a possible role of versican in modulating hepatic fibrogenesis.

4.1532 Mechanical tension applied to substrate films specifies location of neuritogenesis and promotes major neurite growth at the expense of minor neurite development
Feng, Z-Q., Franz, E.W., Leach, M.K., Winterroth, F., White, C.M., Rastogi, A., Gu, Z-Z. and Corey, J.M.
J. Biomed. Res. Part A, ***104(4)***, *966-974 (2016)*

One obstacle in neural repair is facilitating axon growth long enough to reach denervated targets. Recent studies show that axonal growth is accelerated by applying tension to bundles of neurites, and additional studies show that mechanical tension is critical to all neurite growth. However, no studies yet describe how individual neurons respond to tensile forces applied to cell bodies and neurites simultaneously; neither do any test motor neurons, a phenotype critical to neural repair. Here we examine the growth of dissociated motor neurons on stretchable substrates. E15 spinal motor neurons were cultured on poly-lactide-*co*-glycolide films stretched at 4.8, 9.6, or 14.3 mm day^{-1}. Morphological analysis revealed that substrate

stretching has profound effects on developing motor neurons. Stretching increases major neurite length; it also forces neuritogenesis to occur nearest poles of the cell closest to the sources of tension. Stretching also reduces the number of neurites per neuron. These data show that substrate stretching affects neuronal morphology by specifying locations on the cell where neuritogenesis occurs and favoring major neurite growth at the expense of minor neurites. These results serve as a building block for development of new techniques to control and improve the growth of neurons for nerve repair purposes.

4.1533 **Cyclic AMP and Polyamines Overcome Inhibition by Myelin-Associated Glycoprotein through eIF5A-Mediated Increases in p35 Expression and Activation of Cdk5**
He, H., Deng, K., Siddiz, M.M., Pyie, A., Mellado, W., Hannila, S.S. and Filbin, M.T.
J. Neurosci., ***36(10)***, *3079-3091 (2016)*

Inhibitory molecules associated with CNS myelin, such as myelin-associated glycoprotein (MAG), represent major obstacles to axonal regeneration following CNS injury. Our laboratory has shown that elevating levels of intracellular cAMP, via application of the nonhydrolyzable analog dibutyryl cAMP (dbcAMP), can block the inhibitory effects of MAG and myelin. We have also shown that elevation of cAMP results in upregulation of arginase I and increased polyamine synthesis. Treatment with putrescine or spermidine blocks myelin-mediated inhibition of neurite outgrowth, but the mechanism underlying this effect has not yet been elucidated. Here we show that cyclin-dependent kinase 5 (Cdk5) is required for dbcAMP and putrescine to overcome MAG-mediated inhibition. The ability of dbcAMP and putrescine to overcome inhibition by MAG is abolished in the presence of roscovitine, a Cdk inhibitor that has greater selectivity for Cdk5, and expression of dominant negative Cdk5 abolishes the ability of dbcAMP or putrescine to enhance neurite outgrowth in the presence of MAG. Importantly, dbcAMP and putrescine increase expression of p35, the neuron-specific activator of Cdk5, and rat DRG neurons transduced with HSV overexpressing p35 can overcome inhibition by MAG. The upregulation of p35 by putrescine is also reflected in increased localization of p35 to neurites and growth cones. Last, we show that putrescine upregulates p35 expression by serving as a substrate for hypusine modification of eIF5A, and that this hypusination is necessary for putrescine's ability to overcome inhibition by MAG. Our findings reveal a previously unknown mechanism by which polyamines may encourage regeneration after CNS injury.

4.1534 **Effector T-cell trafficking between the leptomeninges and the cerebrospinal fluid**
Schläger, C. et al
Nature, ***530***, *349-353 (2016)*

In multiple sclerosis, brain-reactive T cells invade the central nervous system (CNS) and induce a self-destructive inflammatory process. T-cell infiltrates are not only found within the parenchyma and the meninges, but also in the cerebrospinal fluid (CSF) that bathes the entire CNS tissue[1, 2]. How the T cells reach the CSF, their functionality, and whether they traffic between the CSF and other CNS compartments remains hypothetical[3, 4, 5, 6]. Here we show that effector T cells enter the CSF from the leptomeninges during Lewis rat experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. While moving through the three-dimensional leptomeningeal network of collagen fibres in a random Brownian walk, T cells were flushed from the surface by the flow of the CSF. The detached cells displayed significantly lower activation levels compared to T cells from the leptomeninges and CNS parenchyma. However, they did not represent a specialized non-pathogenic cellular sub-fraction, as their gene expression profile strongly resembled that of tissue-derived T cells and they fully retained their encephalitogenic potential. T-cell detachment from the leptomeninges was counteracted by integrins VLA-4 and LFA-1 binding to their respective ligands produced by resident macrophages. Chemokine signalling via CCR5/CXCR3 and antigenic stimulation of T cells in contact with the leptomeningeal macrophages enforced their adhesiveness. T cells floating in the CSF were able to reattach to the leptomeninges through steps reminiscent of vascular adhesion in CNS blood vessels, and invade the parenchyma. The molecular/cellular conditions for T-cell reattachment were the same as the requirements for detachment from the leptomeningeal milieu. Our data indicate that the leptomeninges represent a checkpoint at which activated T cells are licensed to enter the CNS parenchyma and non-activated T cells are preferentially released into the CSF, from where they can reach areas of antigen availability and tissue damage.

4.1535 **Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice**
Li, J., Sung, C.Y.J., Lee, N., Ni, Y., Pihlajamäki, J., Panagiotou, G. and El-Nezami, H.
PNAS, ***113(9)***, *E1306-E1315 (2016)*

The beneficial roles of probiotics in lowering the gastrointestinal inflammation and preventing colorectal

cancer have been frequently demonstrated, but their immunomodulatory effects and mechanism in suppressing the growth of extraintestinal tumors remain unexplored. Here, we adopted a mouse model and metagenome sequencing to investigate the efficacy of probiotic feeding in controlling s.c. hepatocellular carcinoma (HCC) and the underlying mechanism suppressing the tumor progression. Our result demonstrated that Prohep, a novel probiotic mixture, slows down the tumor growth significantly and reduces the tumor size and weight by 40% compared with the control. From a mechanistic point of view the down-regulated IL-17 cytokine and its major producer Th17 cells, whose levels decreased drastically, played critical roles in tumor reduction upon probiotics feeding. Cell staining illustrated that the reduced Th17 cells in the tumor of the probiotic-treated group is mainly caused by the reduced frequency of migratory Th17 cells from the intestine and peripheral blood. In addition, shotgun-metagenome sequencing revealed the crosstalk between gut microbial metabolites and the HCC development. Probiotics shifted the gut microbial community toward certain beneficial bacteria, including *Prevotella* and *Oscillibacter*, that are known producers of antiinflammatory metabolites, which subsequently reduced the Th17 polarization and promoted the differentiation of antiinflammatory Treg/Tr1 cells in the gut. Overall, our study offers novel insights into the mechanism by which probiotic treatment modulates the microbiota and influences the regulation of the T-cell differentiation in the gut, which in turn alters the level of the proinflammatory cytokines in the extraintestinal tumor microenvironment.

4.1536 MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression

Hyun, J., Wang, S., Kim, J., Rao, K.M., park, S.Y., Chung, I., Ha, C-S., Kim, S-W., Yun, Y.H: and Jung, Y.

*Nature Communications, **7**:10993 (2016)*

Hedgehog (Hh) signalling regulates hepatic fibrogenesis. MicroRNAs (miRNAs) mediate various cellular processes; however, their role in liver fibrosis is unclear. Here we investigate regulation of miRNAs in chronically damaged fibrotic liver. MiRNA profiling shows that expression of miR-378 family members (miR-378a-3p, miR-378b and miR-378d) declines in carbon tetrachloride (CCl_4)-treated compared with corn-oil-treated mice. Overexpression of miR-378a-3p, directly targeting Gli3 in activated hepatic stellate cells (HSCs), reduces expression of Gli3 and profibrotic genes but induces *gfap*, the inactivation marker of HSCs, in CCl_4-treated liver. Smo blocks transcriptional expression of miR-378a-3p by activating the p65 subunit of nuclear factor-κB (NF-κB). The hepatic level of miR-378a-3p is inversely correlated with the expression of Gli3 in tumour and non-tumour tissues in human hepatocellular carcinoma. Our results demonstrate that miR-378a-3p suppresses activation of HSCs by targeting Gli3 and its expression is regulated by Smo-dependent NF-κB signalling, suggesting miR-378a-3p has therapeutic potential for liver fibrosis.

4.1537 Methods for Isolation and Purification of Murine Liver Sinusoidal Endothelial Cells: A Systematic Review

Meyer, J., Gonelle-Gispert. C., Morel, P. and Bühler, L.

*PloS One, **11(3)**, E0151945 (2016)*

To study the biological functions of liver sinusoidal endothelial cells (LSEC) and to identify their interplay with blood or liver cells, techniques allowing for the isolation and purification of LSEC have been developed over the last decades. The objective of the present review is to summarize and to compare the efficiency of existing methods for isolating murine LSEC. Toward this end, the MEDLINE database was searched for all original articles describing LSEC isolation from rat and mouse livers. Out of the 489 publications identified, 23 reported the main steps and outcomes of the procedure and were included in our review. Here, we report and analyse the technical details of the essential steps of the techniques used for LSEC isolation. The correlations between the prevalence of some steps and the efficiency of LSEC isolation were also identified. We found that centrifugal elutriation, selective adherence and, more recently, magnetic-activated cell sorting were used for LSEC purification. Centrifugal elutriation procured high yields of pure LSEC (for rats 30–141.9 million cells for 85–98% purities; for mice 9–9.25 million cells for >95% purities), but the use of this method remained limited due to its high technical requirements. Selective adherence showed inconsistent results in terms of cell yields and purities in rats (5–100 million cells for 73.7–95% purities). In contrast, magnetic-activated cell sorting allowed for the isolation of highly pure LSEC, but overall lower cell yields were reported (for rats 10.7 million cells with 97.6% purity; for mice 0.5–9 million cells with 90–98% purities). Notably, the controversies regarding the accuracy of several phenotypic markers for LSEC should be considered and their use for both magnetic sorting and characterization remain doubtful. It appears that more effort is needed to refine and standardize the

procedure for LSEC isolation, with a focus on the identification of specific antigens. Such a procedure is required to identify the molecular mechanisms regulating the function of LSEC and to improve our understanding of their role in complex cellular processes in the liver.

4.1538 TLR4-Dependent Secretion by Hepatic Stellate Cells of the Neutrophil-Chemoattractant CXCL1 Mediates Liver Response to Gut Microbiota

Bigorgne, A.E., John, B., ebrahimkhani, M.R., Shimizu-Albergine, M., Campbell, J.S. and Crispe, I.N:
*PloS One, **11(3)**, e0151063 (2016)*

Background & Aims
The gut microbiota significantly influences hepatic immunity. Little is known on the precise mechanism by which liver cells mediate recognition of gut microbes at steady state. Here we tested the hypothesis that a specific liver cell population was the sensor and we aimed at deciphering the mechanism by which the activation of TLR4 pathway would mediate liver response to gut microbiota.
Methods
Using microarrays, we compared total liver gene expression in WT versus TLR4 deficient mice. We performed *in situ* localization of the major candidate protein, CXCL1. With an innovative technique based on cell sorting, we harvested enriched fractions of KCs, LSECs and HSCs from the same liver. The cytokine secretion profile was quantified in response to low levels of LPS (1ng/mL). Chemotactic activity of stellate cell-derived CXCL1 was assayed in vitro on neutrophils upon TLR4 activation.
Results
TLR4 deficient liver had reduced levels of one unique chemokine, CXCL1 and subsequent decreased of neutrophil counts. Depletion of gut microbiota mimicked TLR4 deficient phenotype, i.e., decreased neutrophils counts in the liver. All liver cells were responsive to low levels of LPS, but hepatic stellate cells were the major source of chemotactic levels of CXCL1. Neutrophil migration towards secretory hepatic stellate cells required the TLR4 dependent secretion of CXCL1.
Conclusions
Showing the specific activation of TLR4 and the secretion of one major functional chemokine—CXCL1, the homolog of human IL-8-, we elucidate a new mechanism in which Hepatic Stellate Cells play a central role in the recognition of gut microbes by the liver at steady state.

4.1539 Microfluidic fabrication of bioactive microgels for rapid formation and enhanced differentiation of stem cell spheroids

Siltanen, C., Yaghoobi, M., haque, A., You, J., Lowen, J., Soleimani, M. and Revzin, A.
*Acta Biomaterialia, **34**, 125-132 (2016)*

A major challenge in tissue engineering is to develop robust protocols for differentiating ES and iPS cells to functional adult tissues at a clinically relevant scale. The goal of this study is to develop a high throughput platform for generating bioactive, stem cell-laden microgels to direct differentiation in a well-defined microenvironment. We describe a droplet microfluidics system for fabricating microgels composed of polyethylene glycol and heparin, with tunable geometric, mechanical, and chemical properties, at kHz rates. Heparin-containing hydrogel particles sequestered growth factors Nodal and FGF-2, which are implicated in specifying pluripotent cells to definitive endoderm. Mouse ESCs were encapsulated into heparin microgels with a single dose of Nodal and FGF-2, and expressed high levels of endoderm markers Sox17 and FoxA2 after 5 days. These results highlight the use of microencapsulation for tailoring the stem cell microenvironment to promote directed differentiation, and may provide a straightforward path to large scale bioprocessing in the future.

4.1540 Establishment of a Drug-Induced, Bile Acid–Dependent Hepatotoxicity Model Using HepaRG Cells

Susukida, T., Sekine, S., Nozaki, M., Tokizono, M., Oizumi, K., Horie, T. and Ito, K.
*J. Pharmaceut. Sci., **105**, 1550-1560 (2016)*

Bile acid (BA) retention within hepatocytes is an underlying mechanism of cholestatic drug-induced liver injury (DILI). We previously developed an assay using sandwich-cultured human hepatocytes (SCHHs) to evaluate drug-induced hepatocyte toxicity accompanying intracellular BA accumulation. However, due to shortcomings commonly associated with the use of primary human hepatocytes (e.g., limited availability, lot-to-lot variability, and high cost), we examined if the human hepatic stem cell line, HepaRG, might also be applicable to our assay system. Consequently, mRNA expression levels of human BA efflux and uptake transporters were lower in HepaRG cells than in SCHHs but higher than in HepG2 human hepatoma cells. Nevertheless, HepaRG cells and SCHHs showed similar toxicity responses to 22 selected drugs, including

cyclosporine A (CsA). CsA (10 μM) was cytotoxic toward HepaRG cells in the presence of BAs and also reduced the biliary efflux rate of [^{3}H]taurocholic acid from 38.5% to 19.2%. Therefore, HepaRG cells are useful for the evaluation of BA-dependent drug toxicity caused by biliary BA efflux inhibition. Regardless, the prediction accuracy for cholestatic DILI risk was poor for HepaRG cells versus SCHHs, suggesting that our DILI model system requires further improvements to increase the utility of HepaRG cells as a preclinical screening tool.

4.1541 Long noncoding RNAs expressed in human hepatic stellate cells form networks with extracellular matrix proteins

Zhou, C., York, S.R., Chen, J.Y., Pondick, J.V., Motola, D.L., Chung, R.T. and Mullen, A.C.
*Genome Medicine, **8**:31 (2016)*

Background
Hepatic fibrosis is the underlying cause of cirrhosis and liver failure in nearly every form of chronic liver disease, and hepatic stellate cells (HSCs) are the primary cell type responsible for fibrosis. Long noncoding RNAs (lncRNAs) are increasingly recognized as regulators of development and disease; however, little is known about their expression in human HSCs and their function in hepatic fibrosis.
Methods
We performed RNA sequencing and *ab initio* assembly of RNA transcripts to define the lncRNAs expressed in human HSC myofibroblasts. We analyzed chromatin immunoprecipitation data and expression data to identify lncRNAs that were regulated by transforming growth factor beta (TGF-β) signaling, associated with super-enhancers and restricted in expression to HSCs compared with 43 human tissues and cell types. Co-expression network analyses were performed to discover functional modules of lncRNAs, and principle component analysis and K-mean clustering were used to compare lncRNA expression in HSCs with other myofibroblast cell types.
Results
We identified over 3600 lncRNAs that are expressed in human HSC myofibroblasts. Many are regulated by TGF-β, a major fibrotic signal, and form networks with genes encoding key components of the extracellular matrix (ECM), which is the substrate of the fibrotic scar. The lncRNAs directly regulated by TGF-β signaling are also enriched at super-enhancers. More than 400 of the lncRNAs identified in HSCs are uniquely expressed in HSCs compared with 43 other human tissues and cell types and HSC myofibroblasts demonstrate different patterns of lncRNA expression compared with myofibroblasts originating from other tissues. Co-expression analyses identified a subset of lncRNAs that are tightly linked to collagen genes and numerous proteins that regulate the ECM during formation of the fibrotic scar. Finally, we identified lncRNAs that are induced during progression of human liver disease.
Conclusions
lncRNAs are likely key contributors to the formation and progression of fibrosis in human liver disease.

4.1542 The HSV-1 Latency-Associated Transcript Functions to Repress Latent Phase Lytic Gene Expression and Suppress Virus Reactivation from Latently Infected Neurons

Nicoll, M.P., Hann, W., Shivkumar, M., harmann, L.E.R., Connor, V., Coleman, H.M., Proenca, J.T. and Efstathiou, S.
*PloS Pathogens, **12(4)**, e1005539 (2016)*

Herpes simplex virus 1 (HSV-1) establishes life-long latent infection within sensory neurons, during which viral lytic gene expression is silenced. The only highly expressed viral gene product during latent infection is the latency-associated transcript (LAT), a non-protein coding RNA that has been strongly implicated in the epigenetic regulation of HSV-1 gene expression. We have investigated LAT-mediated control of latent gene expression using chromatin immunoprecipitation analyses and LAT-negative viruses engineered to express firefly luciferase or β-galactosidase from a heterologous lytic promoter. Whilst we were unable to determine a significant effect of LAT expression upon heterochromatin enrichment on latent HSV-1 genomes, we show that reporter gene expression from latent HSV-1 genomes occurs at a greater frequency in the absence of LAT. Furthermore, using luciferase reporter viruses we have observed that HSV-1 gene expression decreases during long-term latent infection, with a most marked effect during LAT-negative virus infection. Finally, using a fluorescent mouse model of infection to isolate and culture single latently infected neurons, we also show that reactivation occurs at a greater frequency from cultures harbouring LAT-negative HSV-1. Together, our data suggest that the HSV-1 LAT RNA represses HSV-1 gene expression in small populations of neurons within the mouse TG, a phenomenon that directly impacts upon the frequency of reactivation and the maintenance of the transcriptionally active latent reservoir.

4.1543 Characterization of hepatic stellate cells, portal fibroblasts, and mesothelial cells in normal and fibrotic livers

Lua, I., Li, Y., Zagory, J.A., Wang, K.S., French, S.W., Sevigny, J. and Asahina, K.
J. Hepatol., ***64***, *1137-1146 (2016)*

Background & Aims
Contribution of hepatic stellate cells (HSCs), portal fibroblasts (PFs), and mesothelial cells (MCs) to myofibroblasts is not fully understood due to insufficient availability of markers and isolation methods. The present study aimed to isolate these cells, characterize their phenotypes, and examine their contribution to myofibroblasts in liver fibrosis.
Methods
Liver fibrosis was induced in Collagen1a1-green fluorescent protein (*Col1a1*^{GFP}) mice by bile duct ligation (BDL), 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet, or CCl_4 injections. Combining vitamin A (VitA) lipid autofluorescence and expression of GFP and glycoprotein M6a (GPM6A), we separated HSCs, PFs, and MCs from normal and fibrotic livers by fluorescence-activated cell sorting (FACS).
Results
Normal *Col1a1*^{GFP} livers broadly expressed GFP in HSCs, PFs, and MCs. Isolated VitA+ HSCs expressed reelin, whereas VitA−GFP+GPM6A− PFs expressed ectonucleoside triphosphate diphosphohydrolase-2 and elastin. VitA−GFP+GPM6A+ MCs expressed keratin 19, mesothelin, and uroplakin 1b. Transforming growth factor (TGF)-β1 treatment induced the transformation of HSCs, PFs, and MCs into myofibroblasts in culture. TGF-β1 suppressed cyclin D1 mRNA expression in PFs but not in HSCs and MCs. In biliary fibrosis, PFs adjacent to the bile duct expressed α-smooth muscle actin. FACS analysis revealed that HSCs are the major source of GFP+ myofibroblasts in the injured *Col1a1*^{GFP} mice after DDC or CCl_4 treatment. Although PFs partly contributed to GFP+ myofibroblasts in the BDL model, HSCs were still dominant source of myofibroblasts.
Conclusion
HSCs, PFs, and MCs have distinct phenotypes, and PFs partly contribute to myofibroblasts in the portal triad in biliary fibrosis.

4.1544 CCL2–CCR2 signaling promotes hepatic ischemia/reperfusion injury

Zhang, J., Xu, P., Song, P., Wang, H., Zhang, Y., Hu, Q., Wang, G., Zhang, S., Yu, Q., Billiar, T.R., Wang, C. and Zhang, J.
J.Surg. Res., ***15***, *352-362 (2016)*

Background
Liver ischemia/reperfusion (I/R) injury is a type of uncontrolled inflammatory cascade in which neutrophils, an early infiltrating immune cell population, elicit significant tissue damage. However, the precise mechanism for neutrophil recruitment and infiltration remains to be fully characterized.
Methods
A hepatic partial I/R model was reproduced in wild-type, $CCL2^{-/-}$ and $CCR2^{-/-}$ mice. Tissue damage was evaluated by serum enzyme analysis, hematoxylin–eosin staining, and cytokine production measurement. Mobilization of neutrophils from the bone marrow and subsequent infiltration into the liver were measured by flow cytometry. C-C motif chemokine receptor 2 (CCR2) expression on neutrophils and C-C motif chemokine ligand 2 (CCL2) chemotaxis were measured using flow cytometry. The cellular source of CCL2 in the liver was determined by deleting specific cell groups and performing intracellular staining.
Results
Liver damage was ameliorated, and neutrophil recruitment and accumulation were decreased in both $CCL2^{-/-}$ and $CCR2^{-/-}$ mice compared with wild-type mice. Neutrophils displayed upregulated expression of CCR2 during I/R, and these cells were required for CCL2-induced chemotaxis. Depletion of Kupffer cells protected the liver from I/R injury. Furthermore, genetic ablation of CCL2 reduced liver injury, as demonstrated by decreases in the levels of alanine aminotransferase and aspartate aminotransferase and subsequent reductions in neutrophil recruitment and accumulation.
Conclusions
Kupffer cells secrete CCL2 to promote CCR2-expressing neutrophil recruitment from the bone marrow and subsequent infiltration into the liver during I/R. These findings reveal a novel pro-inflammatory role of cell-mediated CCL2–CCR2 interactions during this sterile insult.

4.1545 Caspase 6 has a protective role in SOD1G93A transgenic mice

Hogg, M.C., Mitchem., M.R., König, H-G. and Prehn, J.H.M.
Biochim. Biophys. Acta, ***1862***, *1063-1073 (2016)*

In amyotrophic lateral sclerosis (ALS), it has been suggested that the process of neurodegeneration starts at the neuromuscular junction and is propagated back along axons towards motor neurons. Caspase-dependent pathways are well established as a cause of motor neuron death, and recent work in other disease models indicated a role for caspase 6 in axonal degeneration. Therefore we hypothesised that caspase 6 may be involved in motor neuron death in ALS. To investigate the role of caspase 6 in ALS we profiled protein levels of caspase-6 throughout disease progression in the ALS mouse model $SOD1^{G93A}$; this did not reveal differences in caspase 6 levels during disease. To investigate the role of caspase 6 further we generated a colony with $SOD1^{G93A}$ transgenic mice lacking *caspase 6*. Analysis of the transgenic $SOD1^{G93A}$; $Casp6^{-/-}$ revealed an exacerbated phenotype with motor dysfunction occurring earlier and a significantly shortened lifespan when compared to transgenic $SOD1^{G93A}$; $Casp6^{+/+}$ mice. Immunofluorescence analysis of the neuromuscular junction revealed no obvious difference between *caspase* $6^{+/+}$ and *caspase* $6^{-/-}$ in non-transgenic mice, while the $SOD1^{G93A}$ transgenic mice showed severe degeneration compared to non-transgenic mice in both genotypes. Our data indicate that *caspase-6* does not exacerbate ALS pathogenesis, but may have a protective role.

4.1546 Olfactory Ensheathing Cells Express α7 Integrin to Mediate Their Migration on Laminin
Ingram, N.T., Khankan, R.R. and Phelps, P.E.
*PloS One, **11(4)**, e0153394 (2016)*

The unique glia located in the olfactory system, called olfactory ensheathing cells (OECs), are implicated as an attractive choice for transplantation therapy following spinal cord injury because of their pro-regenerative characteristics. Adult OECs are thought to improve functional recovery and regeneration after injury by secreting neurotrophic factors and making cell-to-cell contacts with regenerating processes, but the mechanisms are not well understood. We show first that α7 integrin, a laminin receptor, is highly expressed at the protein level by OECs throughout the olfactory system, i.e., in the olfactory mucosa, olfactory nerve, and olfactory nerve layer of the olfactory bulb. Then we asked if OECs use the α7 integrin receptor directly to promote neurite outgrowth on permissive and neutral substrates, in vitro. We co-cultured α7+/+ and α7lacZ/lacZ postnatal cerebral cortical neurons with α7+/+ or α7lacZ/lacZ OECs and found that genotype did not effect the ability of OECs to enhance neurite outgrowth by direct contact. Loss of α7 integrin did however significantly decrease the motility of adult OECs in transwell experiments. Twice as many α7+/+ OECs migrated through laminin-coated transwells compared to α7+/+ OECs on poly-L-lysine (PLL). This is in contrast to α7lacZ/lacZ OECs, which showed no migratory preference for laminin substrate over PLL. These results demonstrate that OECs express α7 integrin, and that laminin and its α7 integrin receptor contribute to adult OEC migration in vitro and perhaps also in vivo.

4.1547 Blockage of indoleamine 2,3-dioxygenase regulates Japanese encephalitis via enhancement of type I/II IFN innate and adaptive T-cell responses
Kim, S.B. Choi, J.Y., Uyangaa, E., patil, A.M., Hossain, F.M.A., Hur, J., park, S-Y., Lee, J-H., Kim, K. and Eo, S.K.
*Journal of Neuroinflammation, **13**:79 (2016)*

Background
Japanese encephalitis (JE), a leading cause of viral encephalitis, is characterized by extensive neuroinflammation following infection with neurotropic JE virus (JEV). Indoleamine 2,3-dioxygenase (IDO) has been identified as an enzyme associated with immunoregulatory function. Although the regulatory role of IDO in viral replication has been postulated, the in vivo role of IDO activity has not been fully addressed in neurotropic virus-caused encephalitis.
Methods
Mice in which IDO activity was inhibited by genetic ablation or using a specific inhibitor were examined for mortality and clinical signs after infection. Neuroinflammation was evaluated by central nervous system (CNS) infiltration of leukocytes and cytokine expression. IDO expression, viral burden, JEV-specific T-cell, and type I/II interferon (IFN-I/II) innate responses were also analyzed.
Results
Elevated expression of IDO activity in myeloid and neuron cells of the lymphoid and CNS tissues was closely associated with clinical signs of JE. Furthermore, inhibition of IDO activity enhanced resistance to JE, reduced the viral burden in lymphoid and CNS tissues, and resulted in early and increased CNS infiltration by Ly-$6C^{hi}$ monocytes, NK, $CD4^+$, and $CD8^+$ T-cells. JE amelioration in IDO-ablated mice was also associated with enhanced NK and JEV-specific T-cell responses. More interestingly, IDO ablation induced rapid enhancement of type I IFN (IFN-I) innate responses in $CD11c^+$ dendritic cells (DCs),

including conventional and plasmacytoid DCs, following JEV infection. This enhanced IFN-I innate response in IDO-ablated $CD11c^+$ DCs was coupled with strong induction of PRRs (RIG-I, MDA5), transcription factors (IRF7, STAT1), and antiviral ISG genes (Mx1, Mx2, ISG49, ISG54, ISG56). IDO ablation also enhanced the IFN-I innate response in neuron cells, which may delay the spread of virus in the CNS. Finally, we identified that IDO ablation in myeloid cells derived from hematopoietic stem cells (HSCs) dominantly contributed to JE amelioration and that HSC-derived leukocytes played a key role in the enhanced IFN-I innate responses in the IDO-ablated environment.

Conclusions

Inhibition of IDO activity ameliorated JE via enhancement of antiviral IFN-I/II innate and adaptive T-cell responses and increased CNS infiltration of peripheral leukocytes. Therefore, our data provide valuable insight into the use of IDO inhibition by specific inhibitors as a promising tool for therapeutic and prophylactic strategies against viral encephalitis caused by neurotropic viruses.

4.1548 Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease

Flach, A-C., Litke, T., Strauss, J., Haberl, M., Gomez, C.C., reindl, M., Saiz, A., Fehling, H-J., Wienands, J., Odoardi, F., Lühder, F. and Flügel, A.

*PNAS, **113(12)**, 3323-3328 (2016)*

Multiple sclerosis (MS) is caused by T cells that are reactive for brain antigens. In experimental autoimmune encephalomyelitis, the animal model for MS, myelin-reactive T cells initiate the autoimmune process when entering the nervous tissue and become reactivated upon local encounter of their cognate CNS antigen. Thereby, the strength of the T-cellular reactivation process within the CNS tissue is crucial for the manifestation and the severity of the clinical disease. Recently, B cells were found to participate in the pathogenesis of CNS autoimmunity, with several diverse underlying mechanisms being under discussion. We here report that B cells play an important role in promoting the initiation process of CNS autoimmunity. Myelin-specific antibodies produced by autoreactive B cells after activation in the periphery diffused into the CNS together with the first invading pathogenic T cells. The antibodies accumulated in resident antigen-presenting phagocytes and significantly enhanced the activation of the incoming effector T cells. The ensuing strong blood–brain barrier disruption and immune cell recruitment resulted in rapid manifestation of clinical disease. Therefore, myelin oligodendrocyte glycoprotein (MOG)-specific autoantibodies can initiate disease bouts by cooperating with the autoreactive T cells in helping them to recognize their autoantigen and become efficiently reactivated within the immune-deprived nervous tissue.

4.1549 Herpes Simplex Virus and Interferon Signaling Induce Novel Autophagic Clusters in Sensory Neurons

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*J. Virol., **90(9)**, 4706-4719 (2016)*

Herpes simplex virus 1 (HSV-1) establishes lifelong infection in the neurons of trigeminal ganglia (TG), cycling between productive infection and latency. Neuronal antiviral responses are driven by type I interferon (IFN) and are crucial to controlling HSV-1 virulence. Autophagy also plays a role in this neuronal antiviral response, but the mechanism remains obscure. In this study, HSV-1 infection of murine TG neurons triggered unusual clusters of autophagosomes, predominantly in neurons lacking detectable HSV-1 antigen. Treatment of neurons with IFN-β induced a similar response, and cluster formation by infection or IFN treatment was dependent upon an intact IFN-signaling pathway. The autophagic clusters were decorated with both ISG15, an essential effecter of the antiviral response, and p62, a selective autophagy receptor. The autophagic clusters were not induced by rapamycin or starvation, consistent with a process of selective autophagy. While clusters were triggered by other neurotropic herpesviruses, infection with unrelated viruses failed to induce this response. Following ocular infection in vivo, clusters formed exclusively in the infected ophthalmic branch of the TG. Taken together, our results show that infection with HSV and antiviral signaling in TG neurons produce an unorthodox autophagic response. This autophagic clustering is associated with antiviral signaling, the presence of viral genome, and the absence of HSV protein expression and may therefore represent an important neuronal response to HSV infection and the establishment of latency.

4.1550 MyD88 Mediates Instructive Signaling in Dendritic Cells and Protective Inflammatory Response during Rickettsial Infection

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Infect. Immun., ***84(4)***, *883-893 (2016)*

Spotted fever group rickettsiae cause potentially life-threatening infections throughout the world. Several members of the Toll-like receptor (TLR) family are involved in host response to rickettsiae, and yet the mechanisms by which these TLRs mediate host immunity remain incompletely understood. In the present study, we found that host susceptibility of MyD88$^{-/-}$ mice to infection with Rickettsia conorii or Rickettsia australis was significantly greater than in wild-type (WT) mice, in association with severely impaired bacterial clearance *in vivo*. R. australis-infected MyD88$^{-/-}$ mice showed significantly lower expression levels of gamma interferon (IFN-γ), interleukin-6 (IL-6), and IL-1β, accompanied by significantly fewer inflammatory infiltrates of macrophages and neutrophils in infected tissues, than WT mice. The serum levels of IFN-γ, IL-12, IL-6, and granulocyte colony-stimulating factor were significantly reduced, while monocyte chemoattractant protein 1, macrophage inflammatory protein 1α, and RANTES were significantly increased in infected MyD88$^{-/-}$ mice compared to WT mice. Strikingly, R. australis infection was incapable of promoting increased expression of MHC-II^{high} and production of IL-12p40 in MyD88$^{-/-}$ bone marrow-derived dendritic cells (BMDCs) compared to WT BMDCs, although costimulatory molecules were upregulated in both types of BMDCs. Furthermore, the secretion levels of IL-1β by Rickettsia-infected BMDCs and in the sera of infected mice were significantly reduced in MyD88$^{-/-}$ mice compared to WT controls, suggesting that *in vitro* and *in vivo* production of IL-1β is MyD88 dependent. Taken together, our results suggest that MyD88 signaling mediates instructive signals in DCs and secretion of IL-1β and type 1 immune cytokines, which may account for the protective inflammatory response during rickettsial infection.

4.1551 Isolation of Conventional Dendritic Cells from Mouse Lungs

Van de Laar, L., Guilliams, M. and Tavernier, S.
Methods in Mol. Biol., ***1423***, *139-152 (2016)*

The lungs are in direct contact with the environment. Separated only by a thin layer of mucosa, the lung immune system is being exposed to dangers like pathogens, allergens, or pollutants. The lung dendritic cells form an elaborate network at the basolateral side of the epithelium and continuously sample antigens from the airway lumen. The conventional dendritic cells (cDCs) in the lung can be subdivided into two distinct subsets based on their ontogeny and are described to have distinct immunological functions. High-quality ex vivo isolation of these cells is required for experiments such as functional assays, transfer experiments, or transcriptomics and is crucial to further our knowledge concerning these subpopulations. In this chapter we describe a protocol for the isolation of both CD103^{+} and CD11b^{+} cDCs. In our protocol we compare different methods of cell isolation. We propose that the optimal isolation technique is based on the number of cells needed and the type of experiment that will be performed. If low cell numbers are required, simple flow cytometry-assisted cell sorting (FACS) is sufficient. In the case of high cell numbers that will be lysed or fixed upon sorting, positive selection of CD11c^{+} cells followed by FACS can be utilized. Purification of cDCs through gradient selection and subsequent sorting is found to be optimal for experiments that require large amount of cells for functional assays.

4.1552 Negative regulation of the hepatic fibrogenic response by suppressor of cytokine signaling 1

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Cytokine, ***82***, *58-69 (2016)*

Suppressor of cytokine signaling 1 (SOCS1) is an indispensable regulator of IFNγ signaling and has been implicated in the regulation of liver fibrosis. However, it is not known whether SOCS1 mediates its anti-fibrotic functions in the liver directly, or via modulating IFNγ, which has been implicated in attenuating hepatic fibrosis. Additionally, it is possible that SOCS1 controls liver fibrosis by regulating hepatic stellate cells (HSC), a key player in fibrogenic response. While the activation pathways of HSCs have been well characterized, the regulatory mechanisms are not yet clear. The goals of this study were to dissociate IFNγ-dependent and SOCS1-mediated regulation of hepatic fibrogenic response, and to elucidate the regulatory functions of SOCS1 in HSC activation. Liver fibrosis was induced in Socs1−/−Ifng−/− mice with dimethylnitrosamine or carbon tetrachloride. Ifng−/− and C57BL/6 mice served as controls. Following fibrogenic treatments, Socs1−/−Ifng−/− mice showed elevated serum ALT levels and increased liver fibrosis compared to Ifng−/− mice. The latter group showed higher ALT levels and fibrosis than C57BL/6 controls. The livers of SOCS1-deficient mice showed bridging fibrosis, which was associated with

increased accumulation of myofibroblasts and abundant collagen deposition. SOCS1-deficient livers showed increased expression of genes coding for smooth muscle actin, collagen, and enzymes involved in remodeling the extracellular matrix, namely matrix metalloproteinases and tissue inhibitor of metalloproteinases. Primary HSCs from SOCS1-deficient mice showed increased proliferation in response to growth factors such as HGF, EGF and PDGF, and the fibrotic livers of SOCS1-deficient mice showed increased expression of the Pdgfb gene. Taken together, these data indicate that SOCS1 controls liver fibrosis independently of IFNγ and that part of this regulation may occur via regulating HSC proliferation and limiting growth factor availability.

4.1553 Single and double layer centrifugation improve the quality of cryopreserved bovine sperm from poor quality ejaculates

Gloria, A., Carluccio, A., Wegher, L., Robbe, D., Befacchia, G. and Contri, A.
*J. Animal Science and Biotechnology, **7**:30 (2016)*

Background
Density gradient centrifugation was reported as a technique of semen preparation in assisted reproductive techniques in humans and animals. This technique was found to be efficient in improving semen quality after harmful techniques such as cryopreservation. Recently a modified technique, single layer centrifugation, was proposed as a technique providing a large amount of high quality spermatozoa, and this treatment was performed before conservation. Single layer centrifugation has been studied prevalently in stallions and in boars, but limited data were available for bulls. Occasionally bulls are known to experience a transient reduction in semen quality, thus techniques that allow improvement in semen quality could be applied in this context. The aim of this study was the evaluation of single layer and double layer centrifugation by the use of iodixanol, compared with conventional centrifugation and non-centrifuged semen, on the sperm characteristics during the cryopreservation process in bulls with normal and poor semen quality.

4.1554 Whole Blood RNA as a Source of Transcript-Based Nutrition- and Metabolic Health-Related Biomarkers

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*PloS One, **11(5)**, e0155361 (2016)*

Blood cells are receiving an increasing attention as an easily accessible source of transcript-based biomarkers. We studied the feasibility of using mouse whole blood RNA in this context. Several paradigms were studied: (i) metabolism-related transcripts known to be affected in rat tissues and peripheral blood mononuclear cells (PBMC) by fasting and upon the development of high fat diet (HFD)-induced overweight were assessed in whole blood RNA of fasted rats and mice and of HFD-fed mice; (ii) retinoic acid (RA)-responsive genes in tissues were assessed in whole blood RNA of control and RA-treated mice; (iii) lipid metabolism-related transcripts previously identified in PBMC as potential biomarkers of metabolic health in a rat model were assessed in whole blood in an independent model, namely retinoblastoma haploinsufficient (Rb+/-) mice. Blood was collected and stored in RNAlater® at -80°C until analysis of selected transcripts by real-time RT-PCR. Comparable changes with fasting were detected in the expression of lipid metabolism-related genes when RNA from either PBMC or whole blood of rats or mice was used. HFD-induced excess body weight and fat mass associated with expected changes in the expression of metabolism-related genes in whole blood of mice. Changes in gene expression in whole blood of RA-treated mice reproduced known transcriptional actions of RA in hepatocytes and adipocytes. Reduced expression of Fasn, Lrp1, Rxrb and Sorl1 could be validated as early biomarkers of metabolic health in young Rb+/- mice using whole blood RNA. Altogether, these results support the use of whole blood RNA in studies aimed at identifying blood transcript-based biomarkers of nutritional/metabolic status or metabolic health. Results also support reduced expression of Fasn, Lrp1, Rxrb and Sorl1 in blood cells at young age as potential biomarkers of metabolic robustness.

4.1555 Iso-acoustic focusing of cells for size-insensitive acousto-mechanical phenotyping

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*Nature Communications, **7**:11556 (2016)*

Mechanical phenotyping of single cells is an emerging tool for cell classification, enabling assessment of effective parameters relating to cells' interior molecular content and structure. Here, we present iso-acoustic focusing, an equilibrium method to analyze the effective acoustic impedance of single cells in continuous flow. While flowing through a microchannel, cells migrate sideways, influenced by an acoustic

field, into streams of increasing acoustic impedance, until reaching their cell-type specific point of zero acoustic contrast. We establish an experimental procedure and provide theoretical justifications and models for iso-acoustic focusing. We describe a method for providing a suitable acoustic contrast gradient in a cell-friendly medium, and use acoustic forces to maintain that gradient in the presence of destabilizing forces. Applying this method we demonstrate iso-acoustic focusing of cell lines and leukocytes, showing that acoustic properties provide phenotypic information independent of size.

4.1556 Unique microbial-derived volatile organic compounds in portal venous circulation in murine non-alcoholic fatty liver disease

Reid, D.T., McDonald, B., Khalid, T., Vo, T., Schenck, L.P., Surette, M.G., Beck, P.L., Reimer, R.A., Robert, C.S., Rioux, K.P. and Eksteen, B.
*Biochim. Biophys. Acta, **1862**, 1337-1344 (2016)*

Background and aims
Non-alcoholic fatty liver disease is now the leading liver disease in North America. The progression of non-alcoholic fatty liver disease to the inflammatory condition, non-alcoholic steatohepatitis is complex and currently not well understood. Intestinal microbial dysbiosis has been implicated in the development of non-alcoholic fatty liver disease and progression of non-alcoholic steatohepatitis. Volatile organic compounds are byproducts of microbial metabolism in the gut that may enter portal circulation and have hepatotoxic effects contributing to the pathogenesis of non-alcoholic steatohepatitis. To test this hypothesis, we measured volatile organic compounds in cecal luminal contents and portal venous blood in a mouse model of non-alcoholic steatohepatitis.
Methods
Gas chromatography–mass spectrometry analysis was conducted on cecal content and portal vein blood for volatile organic compound detection from mice fed a methionine and choline deficient diet, which induces non-alcoholic steatohepatitis. The colonic microbiome was studied by 16S rRNA gene amplification using the Illumina MiSeq platform.
Results
Sixty-eight volatile organic compounds were detected in cecal luminal content, a subset of which was also present in portal venous blood. Importantly, differences in portal venous volatile organic compounds were associated with diet-induced steatohepatitis establishing a biochemical link between gut microbiota-derived volatile organic compounds and increased susceptibility to non-alcoholic steatohepatitis.
Conclusion
Our model creates a novel tool to further study the role of gut-derived volatile organic compounds in the pathogenesis of non-alcoholic steatohepatitis.

4.1557 RNA-Seq following PCR-based sorting reveals rare cell transcriptional signatures

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*BMC Genomics, **17**:361 (2016)*

Background
Rare cell subtypes can profoundly impact the course of human health and disease, yet their presence within a sample is often missed with bulk molecular analysis. Single-cell analysis tools such as FACS, FISH-FC and single-cell barcode-based sequencing can investigate cellular heterogeneity; however, they have significant limitations that impede their ability to identify and transcriptionally characterize many rare cell subpopulations.
Results
PCR-activated cell sorting (PACS) is a novel cytometry method that uses single-cell TaqMan PCR reactions performed in microfluidic droplets to identify and isolate cell subtypes with high-throughput. Here, we extend this method and demonstrate that PACS enables high-dimensional molecular profiling on TaqMan-targeted cells. Using a random priming RNA-Seq strategy, we obtained high-fidelity transcriptome measurements following PACS sorting of prostate cancer cells from a heterogeneous population. The sequencing data revealed prostate cancer gene expression profiles that were obscured in the unsorted populations. Single-cell expression analysis with PACS was subsequently used to confirm a number of the differentially expressed genes identified with RNA sequencing.
Conclusions
PACS requires minimal sample processing, uses readily available TaqMan assays and can isolate cell subtypes with high sensitivity. We have now validated a method for performing next-generation sequencing on mRNA obtained from PACS isolated cells. This capability makes PACS well suited for transcriptional profiling of rare cells from complex populations to obtain maximal biological insight into

cell states and behaviors.

4.1558 Expansion and Hepatic Differentiation of Adult Blood-Derived CD34+ Progenitor Cells and Promotion of Liver Regeneration After Acute Injury
Hu, M., Li, S., Menon, S., Liu, B., Hu, M.S., Longaker, M.T. and Lorentz, H.P.
*Stem Cells Translational Medicine, **5**, 723-732 (2016)*

The low availability of functional hepatocytes has been an unmet demand for basic scientific research, new drug development, and cell-based clinical applications for decades. Because of the inability to expand hepatocytes in vitro, alternative sources of hepatocytes are a focus of liver regenerative medicine. We report a new group of blood-derived $CD34^+$ progenitor cells (BDPCs) that have the ability to expand and differentiate into functional hepatocyte-like cells and promote liver regeneration. BDPCs were obtained from the peripheral blood of an adult mouse with expression of surface markers CD34, CD45, Sca-1, c-kit, and Thy1.1. BDPCs can proliferate in vitro and differentiate into hepatocyte-like cells expressing hepatocyte markers, including CK8, CK18, CK19, α-fetoprotein, integrin-β1, and A6. The differentiated BDPCs (dBDPCs) also display liver-specific functional activities, such as glycogen storage, urea production, and albumin secretion. dBDPCs have cytochrome P450 activity and express specific hepatic transcription factors, such as hepatic nuclear factor 1α. To demonstrate liver regenerative activity, dBDPCs were injected into mice with severe acute liver damage caused by a high-dose injection of carbon tetrachloride (CCl_4). dBDPC treatment rescued the mice from severe acute liver injury, increased survival, and induced liver regeneration. Because of their ease of access and application through peripheral blood and their capability of rapid expansion and hepatic differentiation, BDPCs have great potential as a cell-based therapy for liver disease.

4.1559 Analysis of Dendritic Cell Function Using Clec9A-DTR Transgenic Mice
Tetlak, P. and Ruedl, C.
*Methods in Mol. Biol., **1423**, 275-289 (2016)*

The Clec9A-diphtheria toxin receptor (DTR) transgenic mouse strain provides a robust animal model to study the function of lymphoid organ-resident $CD8^+$ dendritic cells (DCs) and nonlymphoid organ-specific $CD103^+$ DCs in infectioous diseases and inflammation. Here we describe some basic protocols for $CD8^+/CD103^+$ DC isolation, for their in vivo depletion, and for their characterization by multi-color flow cytometry analysis. As an example for in vivo functional characterization of this DC subset, we present here the experimental cerebral malaria model. Furthermore, we illustrate advantages and pitfalls of the Clec9A-DTR system.

4.1560 Producing megakaryocytes from a human peripheral blood source
Ivetic, N., Nazi, I., Karim, N., Clare, R., Smith, J.W., Moore, J.C., Hope, K.J., Kelton, J.G. and Arnold, D.M.
*Transfusion, **56(5)**, 1066-1074 (2016)*

BACKGROUND
Cultured megakaryocytes could prove useful in the study of human diseases, but it is difficult to produce sufficient numbers for study. We describe and evaluate the use of an expansion process to develop mature megakaryocytes from peripheral blood–derived human hematopoietic stem and progenitor cells (HSPCs).
STUDY DESIGN AND METHODS
HSPCs (CD34+) were isolated from peripheral blood by positive selection and expanded using an optimal CD34+ expansion supplement. We evaluated megakaryocyte growth, maturation, and morphology in response to thrombopoietin (TPO) stimulation using flow cytometry and electron microscopy. TPO demonstrated a dose-dependent stimulatory effect on both megakaryocyte number and maturation.
RESULTS
From 90 to 120 mL of unmanipulated peripheral blood, we isolated a mean of 1.5×10^5 HSPCs (1.5×10^3 cells/mL of whole blood). HSPCs expanded nine-fold after a 4-day culture using an expansion supplement. Expanded cells were cultured for an additional 8 days with TPO (20 ng/mL), which resulted in a 2.9-fold increase in megakaryocytic cells where 83% of live cells expressed CD41a+, a marker of megakaryocyte commitment, and 50% expressed CD42b+, a marker for megakaryocyte maturation. The expanded HSPCs responded to TPO stimulation to yield more than 1.0×10^6 megakaryocytes. This cell number was sufficient for morphologic studies that demonstrated these expanded HSPCs produced mature polyploid megakaryocytes capable of forming proplatelet extensions.
CONCLUSIONS

Peripheral blood HSPCs can be expanded and differentiated into functional, mature megakaryocytes, a finding that supports the use of this process to study inherent platelet (PLT) production disorders as well as study factors that impair normal PLT production.

4.1561 ResolvinD1 reduces apoptosis and inflammation in primary human alveolar epithelial type 2 cells
Xie, W., Wang, H., Liu, Q., Li, Y., Wang, J., Yao, S. and Wu, Q.
Lab. Invest., ***96(5)***, *526-536 (2016)*

Lung epithelial apoptosis and inflammatory responses are important pathological processes in many pulmonary disorders. ResolvinD1 (RvD1), generated in inflammatory resolution processes, reduces inflammatory responses in animal models of lung diseases. The aim of this study was to investigate whether RvD1 attenuates apoptosis and proinflammatory responses in primary human alveolar epithelial type 2 cells (AEC2 cells) that are exposed to lipopolysaccharide (LPS) *in vitro*. We examined the percentage of apoptotic AEC2 cells by flow cytometry. The expression levels of cytokines and chemokines were determined by ELISA and microarray. The expression levels of molecular signaling modulators were evaluated by western blot. LPS-stimulated AEC2 cells pretreated with RvD1 exhibited a statistically significant reduction in apoptosis. The pretreatment of LPS-stimulated cells with RvD1 stimulated the phosphorylation of AKT and prevented the cleavage of caspase-3, the upregulation of Bax, and the downregulation of Bcl-2. The antiapoptotic effects of RvD1 were abrogated upon pretreatment with a PI3K inhibitor. In addition, RvD1 reduced the release of cytokines and chemokines, and inhibited the degradation and phosphorylation of IκB-α in LPS-stimulated AEC2 cells. RvD1 reduces apoptosis of LPS-exposed AEC2 cells by inducing the phosphorylation of AKT and attenuates the inflammatory response by suppressing the degradation and phosphorylation of IκB-α.

4.1562 Striatopallidal Neuron NMDA Receptors Control Synaptic Connectivity, Locomotor, and Goal-Directed Behaviors
Lambot, L., Rodriguez, E.C., Houtteman, D., Li, Y., Schiffmann, S.N., Gall, D. and de Kerchove d'Exaerde, A.
J. Neurosci., ***36(18)***, *4976-4992 (2016)*

The basal ganglia (BG) control action selection, motor programs, habits, and goal-directed learning. The striatum, the principal input structure of BG, is predominantly composed of medium-sized spiny neurons (MSNs). Arising from these spatially intermixed MSNs, two inhibitory outputs form two main efferent pathways, the direct and indirect pathways. Striatonigral MSNs give rise to the activating, direct pathway MSNs and striatopallidal MSNs to the inhibitory, indirect pathway (iMSNs). BG output nuclei integrate information from both pathways to fine-tune motor procedures and to acquire complex habits and skills. Therefore, balanced activity between both pathways is crucial for harmonious functions of the BG. Despite the increase in knowledge concerning the role of glutamate NMDA receptors (NMDA-Rs) in the striatum, understanding of the specific functions of NMDA-R iMSNs is still lacking. For this purpose, we generated a conditional knock-out mouse to address the functions of the NMDA-R in the indirect pathway. At the cellular level, deletion of GluN1 in iMSNs leads to a reduction in the number and strength of the excitatory corticostriatopallidal synapses. The subsequent scaling down in input integration leads to dysfunctional changes in BG output, which is seen as reduced habituation, delay in goal-directed learning, lack of associative behavior, and impairment in action selection or skill learning. The NMDA-R deletion in iMSNs causes a decrease in the synaptic strength of striatopallidal neurons, which in turn might lead to a imbalanced integration between direct and indirect MSN pathways, making mice less sensitive to environmental change. Therefore, their ability to learn and adapt to the environment-based experience was significantly affected.

4.1563 Type I and III IFNs Produced by Plasmacytoid Dendritic Cells in Response to a Member of the Flaviviridae Suppress Cellular Immune Responses
Reid, E., Juleff, N., Windsor, M., Gubbins, S., Roberts, L., Morgan, s., Meyers, G., Perez-Martin, E., Tchilian, E., Charleston, B. and Seago, J.
J. Immunol., ***196(10)***, *4214-4226 (2016)*

The pestivirus noncytopathic bovine viral diarrhea virus (BVDV) can suppress IFN production in the majority of cell types in vitro. However, IFN is detectable in serum during acute infection in vivo for ~5–7 d, which correlates with a period of leucopoenia and immunosuppression. In this study, we demonstrate that a highly enriched population of bovine plasmacytoid dendritic cells (DCs) produced IFN in response to BVDV in vitro. We further show that the majority of the IFN produced in response to infection both in

vitro and in vivo is type III IFN and acid labile. Further, we show IL-28B (IFN-λ3) mRNA is induced in this cell population in vitro. Supernatant from plasmacytoid DCs harvested postinfection with BVDV or recombinant bovine IFN-α or human IL-28B significantly reduced $CD4^+$ T cell proliferation induced by *tubercle bacillus* Ag 85–stimulated monocyte-derived DCs. Furthermore, these IFNs induced IFN-stimulated gene expression predominantly in monocyte-derived DCs. IFN-treated immature DCs derived from murine bone marrow also had a reduced capacity to stimulate T cell proliferative responses to *tubercle bacillus* Ag 85. Immature DCs derived from either source had a reduced capacity for Ag uptake following IFN treatment that is dose dependent. Immunosuppression is a feature of a number of pestivirus infections; our studies suggest type III IFN production plays a key role in the pathogenesis of this family of viruses. Overall, in a natural host, we have demonstrated a link between the induction of type I and III IFN after acute viral infection and transient immunosuppression.

4.1564 VEGF-sdf1 recruitment of CXCR7+ bone marrow progenitors of liver sinusoidal endothelial cells promotes rat liver regeneration

Delever, L.D., Wang, X. and Wang, L.

Am. J. Physiol. Gastrointest. Liver Physiol., ***310(9)****, G739-G746 (2016)*

In liver injury, recruitment of bone marrow (BM) progenitors of liver sinusoidal endothelial cells (sprocs) is necessary for normal liver regeneration. Hepatic vascular endothelial growth factor (VEGF) is a central regulator of the recruitment process. We examine whether stromal cell-derived factor 1 [sdf1, or CXC ligand 12 (CXCL12)] acts downstream from VEGF to mediate recruitment of BM sprocs, what the sdf1 receptor type [CXC receptor (CXCR)-4 or CXCR7] is on sprocs, and whether sdf1 signaling is required for normal liver regeneration. Studies were performed in the rat partial hepatectomy model. Tracking studies of BM sprocs were performed in wild-type Lewis rats that had undergone BM transplantation from transgenic enhanced green fluorescent protein-positive Lewis rats. Knockdown studies were performed using antisense oligonucleotides (ASOs). Expression of sdf1 doubles in liver and liver sinusoidal endothelial cells (LSECs) after partial hepatectomy. Upregulation of sdf1 expression increases proliferation of sprocs in the BM, mobilization of $CXCR7^+$ BM sprocs to the circulation, and engraftment of $CXCR7^+$ BM sprocs in the liver and promotes liver regeneration. Knockdown of hepatic VEGF with ASOs decreases hepatic sdf1 expression and plasma sdf1 levels. When the effect of VEGF knockdown on sdf1 is offset by infusion of sdf1, VEGF knockdown-induced impairment of BM sproc recruitment after partial hepatectomy is completely attenuated and liver regeneration is normalized. These data demonstrate that the VEGF-sdf1 pathway regulates recruitment of $CXCR7^+$ BM sprocs to the hepatic sinusoid after partial hepatectomy and is required for normal liver regeneration.

4.1565 Olfactory Ensheathing Cell Transplantation after a Complete Spinal Cord Transection Mediates Neuroprotective and Immunomodulatory Mechanisms to Facilitate Regeneration

Khankan, R.R., Griffis, K.G., Haggerty-Skeans, J.R., Zhong, H., Roy, R.R., Edgerton, V.R. and Phelps, P.E.

J. Neurosci., ***36(23)****, 6269-6286 (2016)*

Multiple neural and peripheral cell types rapidly respond to tissue damage after spinal cord injury to form a structurally and chemically inhibitory scar that limits axon regeneration. Astrocytes form an astroglial scar and produce chondroitin sulfate proteoglycans (CSPGs), activate microglia, and recruit blood-derived immune cells to the lesion for debris removal. One beneficial therapy, olfactory ensheathing cell (OEC) transplantation, results in functional improvements and promotes axon regeneration after spinal cord injury. The lack of an OEC-specific marker, however, has limited the investigation of mechanisms underlying their proregenerative effects. We compared the effects of enhanced green fluorescent protein-labeled fibroblast (FB) and OEC transplants acutely after a complete low-thoracic spinal cord transection in adult rats. We assessed the preservation of neurons and serotonergic axons, the levels of inhibitory CSPGs and myelin debris, and the extent of immune cell activation between 1 and 8 weeks postinjury. Our findings indicate that OECs survive longer than FBs post-transplantation, preserve axons and neurons, and reduce inhibitory molecules in the lesion core. Additionally, we show that OECs limit immune-cell activation and infiltration, whereas FBs alter astroglial scar formation and increase immune-cell infiltration and concomitant secondary tissue damage. Administration of cyclosporine-A to enhance graft survival demonstrated that immune suppression can augment OEC contact-mediated protection of axons and neurons during the first 2 weeks postinjury. Collectively, these data suggest that OECs have neuroprotective and immunomodulatory mechanisms that create a supportive environment for neuronal survival and axon regeneration after spinal cord injury.

4.1566 β1-C121W Is Down But Not Out: Epilepsy-Associated Scn1b-C121W Results in a Deleterious Gain-of-Function

Kruger, L.C., O'Malley, H.A., Hull, J.M., Kleeman, A., Patino, G.A.and Isom, L.L.
J. Neurosci., ***36(23)****, 6213-6224 (2016)*

Voltage-gated sodium channel (VGSC) β subunits signal through multiple pathways on multiple time scales. In addition to modulating sodium and potassium currents, β subunits play nonconducting roles as cell adhesion molecules, which allow them to function in cell–cell communication, neuronal migration, neurite outgrowth, neuronal pathfinding, and axonal fasciculation. Mutations in *SCN1B*, encoding VGSC β1 and β1B, are associated with epilepsy. Autosomal-dominant *SCN1B-C121W*, the first epilepsy-associated VGSC mutation identified, results in genetic epilepsy with febrile seizures plus (GEFS+). This mutation has been shown to disrupt both the sodium-current-modulatory and cell-adhesive functions of β1 subunits expressed in heterologous systems. The goal of this study was to compare mice heterozygous for *Scn1b-C121W* (*Scn1b*$^{+/W}$) with mice heterozygous for the *Scn1b-null* allele (*Scn1b*$^{+/-}$) to determine whether the C121W mutation results in loss-of-function *in vivo*. We found that *Scn1b*$^{+/W}$ mice were more susceptible than *Scn1b*$^{+/-}$ and *Scn1b*$^{+/+}$ mice to hyperthermia-induced convulsions, a model of pediatric febrile seizures. β1-C121W subunits are expressed at the neuronal cell surface *in vivo*. However, despite this, β1-C121W polypeptides are incompletely glycosylated and do not associate with VGSC α subunits in the brain. β1-C121W subcellular localization is restricted to neuronal cell bodies and is not detected at axon initial segments in the cortex or cerebellum or at optic nerve nodes of Ranvier of *Scn1b*$^{W/W}$ mice. These data, together with our previous results showing that β1-C121W cannot participate in *trans*-homophilic cell adhesion, lead to the hypothesis that *SCN1B-C121W* confers a deleterious gain-of-function in human GEFS+ patients.

4.1567 Activation of hepatic stellate cell in Pten null liver injury model

He, L., Gubbins, J., Peng, Z., Medina, V., Fei, F., Ashina, K., Wang, J., Kahn, M., Rountree, C.B. and Stiles, B.L.
Fibrogenesis & Tissue Repair, ***9****:8 (2016)*

Background
Hepatic fibrosis is a prominent pathological feature associated with chronic liver disease including non-alcoholic hepatosteatosis (NASH), and a precursor for liver cancer development. We previously reported that PTEN loss in the liver, which leads to hyperactivated liver insulin signaling results in NASH development. Here we used the same mouse model to study the progression from steatosis to fibrosis.
Results
The *Pten* null livers develop progressive liver fibrosis as indicated by Sirius Red staining and increased expression of collagen I, Timp 1, SMAα, and p75NTR. Consistently, hepatic stellate cells (HSCs) isolated from *Pten* null livers are readily activated when compared with that from mice with intact PTEN. Deletion of AKT2, the downstream target of PTEN signal, blocked NASH development, and alleviated fibrosis. HSCs from the *Pten/Akt2* double null mice are quiescent like those isolated from the control livers. Our analysis shows that the activation of HSCs does not depend on the intrinsic signals regulated by PI3K/AKT, the target of PTEN, but does depend on steatosis and injury to the liver. During the progression of liver fibrosis in the *Pten* null model, Wnt ligands and signaling receptor are induced, concurrent with the reduction of sFRP5, a Wnt antagonist. We showed that treatment of HSCs with Wnt receptor antagonist blocks the observed morphological changes when HSCs undergo activation in culture. This signal appears to be mediated by β-catenin, as manipulating β-catenin signaling alters marker gene expressions of HSC activation.
Conclusions
Wnt/β-catenin activation serves as an important mediator for fibrosis development resulting from NASH using a mouse model where NASH is mimicked by PTEN loss.

4.1568 Assessment of human platelet survival in the NOD/SCID mouse model: technical considerations

Fuhrmann, J., Jouni, R., Alex, J., Zöllner, H., Wesche, J., Greinacher, A. and Bakchoul, T.
Transfusion, ***56****, 1370-1376 (82016)*

BACKGROUND
The NOD/SCID mouse model is a unique and sophisticated method to study the survival of human platelets (PLTs) in vivo. Meanwhile, several research groups adopted this model to analyze a wide range of PLT antibodies. Differences exist between the research groups regarding the method of PLT injection, the amount and route of antibody injection, and the preparation of blood samples collected from the animal,

making it difficult to compare results between studies.
STUDY DESIGN AND METHODS
We compared the survival of human PLTs infused into NOD/SCID mice via the tail vein or the retro-orbital plexus. The percentage of circulating human PLTs in the mouse circulation was determined by flow cytometry. Murine blood samples were prepared using two different methods: 1) direct fixation of whole blood samples and 2) isolation of PLTs by density gradient centrifugation.
RESULTS
Recovery of human PLTs after tail vein injection was comparable to retro-orbital injection (13% vs. 11% of all circulating PLTs, $p = 0.401$). However, the survival rate of tail vein–infused PLTs was higher than that of retro-orbitally injected PLTs (median PLT survival after 5 hr 84% vs. 56%, $p = 0.025$). Moreover, we observed that determination of circulating human PLTs in directly fixed murine whole blood samples shows better reproducibility compared to the density gradient centrifugation method.
CONCLUSIONS
Tail vein injection of human PLTs into the NOD/SCID mice is superior to retro-orbital injection in terms of human PLT survival. Direct fixation of whole blood samples allows better reproducibility of results compared to the density gradient centrifugation method.

4.1569 The Human Pancreas as a Source of Protolerogenic Extracellular Matrix Scaffold for a New-generation Bioartificial Endocrine Pancreas
Peloso, A. et al
*Annals of Surgery, **264(1)**, 169-179 (2016)*

Objectives: Our study aims at producing acellular extracellular matrix scaffolds from the human pancreas (hpaECMs) as a first critical step toward the production of a new-generation, fully human-derived bioartificial endocrine pancreas. In this bioartificial endocrine pancreas, the hardware will be represented by hpaECMs, whereas the software will consist in the cellular compartment generated from patient's own cells.
Background: Extracellular matrix (ECM)-based scaffolds obtained through the decellularization of native organs have become the favored platform in the field of complex organ bioengineering. However, the paradigm is now switching from the porcine to the human model.
Methods: To achieve our goal, human pancreata were decellularized with Triton-based solution and thoroughly characterized. Primary endpoints were complete cell and DNA clearance, preservation of ECM components, growth factors and stiffness, ability to induce angiogenesis, conservation of the framework of the innate vasculature, and immunogenicity. Secondary endpoint was hpaECMs' ability to sustain growth and function of human islet and human primary pancreatic endothelial cells.
Results: Results show that hpaECMs can be successfully and consistently produced from human pancreata and maintain their innate molecular and spatial framework and stiffness, and vital growth factors. Importantly, hpaECMs inhibit human naïve $CD4^+$ T-cell expansion in response to polyclonal stimuli by inducing their apoptosis and promoting their conversion into regulatory T cells. hpaECMs are cytocompatible and supportive of representative pancreatic cell types.
Discussion: We, therefore, conclude that hpaECMs has the potential to become an ideal platform for investigations aiming at the manufacturing of a regenerative medicine-inspired bioartificial endocrine pancreas.

4.1570 Isolating High Islet Mass Even from Alcoholic Pancreatitis Pancreases Intended for Clinical Islet Auto-Transplantation: Improved Strategies to Human Islet Isolation Technique
Balamurugan, A., Loganathan, G., Tweed, B., Tucker, W., Mokshagundam, S., Williams, S.and Hughes, M,
*Am. J. Transplant., **16(S3)**, abstract 81, 405-798 (2016)*

It has been reported in the literature that after pancreatic resection with planned islet auto-transplantation (IAT), islets isolated from alcoholic pancreatitis (AP) pancreases resulted in lower islet yields and in some cases, failed isolations (*J Am Coll Surg. 2013 Apr;216:59: Should pancreatectomy with islet cell autotransplantation in patients with chronic alcoholic pancreatitis be abandoned?*). Isolating islets from AP pancreases poses multiple challenges (alterations in duct structure and accumulation of fibrotic bundles) which make it difficult to obtain high islet yield. We have developed new approaches to maximize the islet yield from pancreases with AP. The modified islet isolation process includes 1) Dosing the collagenase enzyme based on severity of fibrosis instead of using the standard (brain-dead donor pancreas) one full vial approach. 2) Adequate delivery of tissue dissociation enzyme throughout the pancreas by using both intraductal and parenchymal injection methods 3) Judging the quality of distention

after sectioning the pancreases and perform additional enzyme distention 4) Warm enzyme recirculation if digestion time is prolonged for >30minutes. Etiology of chronic pancreatitis (CP) was alcoholism (n=4), and idiopathic (n=3). Islets were isolated according to the clinical transplantation protocols. AP cases were compared with idiopathic patients. The new enzyme mixture (VitaCyte CIzyme HA+ Serva Neutral Protease) was used for pancreas digestion. Islet purification was done with iodixanol density gradients. The final islet product was tested for sterility and viability. Transplant outcome was monitored by c-peptide measurements. The average trimmed pancreas weight was 75 ± 9 grams. The average transplanted islet mass was 4,593 ± 728 IEQ/kg (nonalcoholic cases: 5,728 ± 278) and the average IEQ/gram pancreas was 4,487 ± 1,158 (nonalcoholic: 5,788 ± 1,055). The average infused tissue volume was 9.3 ± 6.2 cc. The average islet viability was >88%. Positive c-peptide secretion was monitored in all patients one month after transplantation.
Our results indicated that it was possible to obtain >4,000 IEQ/gram from AP pancreases and transplant >4500 IEQ/kg of patient body weight. Modified islet isolation approaches were effective in maximizing islet yield.

4.1571 Attenuated viral hepatitis in Trem1−/− mice is associated with reduced inflammatory activity of neutrophils

Kozik, J-H., Trautmann, T., Carambia, A., Preti, M., Lütgehetmann, M., Krech, T., Wiegard, C., Heeren, J. and Herkel, J:
*Scientific Reports, **6**:28556 (2016)*

TREM1 (Triggering Receptor Expressed on Myeloid Cells 1) is a pro-inflammatory receptor expressed by phagocytes, which can also be released as a soluble molecule (sTREM1). The roles of TREM1 and sTREM1 in liver infection and inflammation are not clear. Here we show that patients with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection manifest elevated serum levels of sTREM1. In mice, experimental viral hepatitis induced by infection with Lymphocytic Choriomeningitis Virus (LCMV)-WE was likewise associated with increased sTREM1 in serum and urine, and with increased TREM1 and its associated adapter molecule DAP12 in the liver. Trem1−/− mice showed accelerated clearance of LCMV-WE and manifested attenuated liver inflammation and injury. TREM1 expression in the liver of wild-type mice was mostly confined to infiltrating neutrophils, which responded to LCMV by secretion of CCL2 and TNF-α, and release of sTREM1. Accordingly, the production of CCL2 and TNF-α was decreased in the livers of LCMV-infected Trem1−/− mice, as compared to LCMV-infected wildtype mice. These findings indicate that TREM1 plays a role in viral hepatitis, in which it seems to aggravate the immunopathology associated with viral clearance, mainly by increasing the inflammatory activity of neutrophils.

4.1572 EPIGENETIC FACTORS IN THE EFFECTS OF NEONATAL ALCOHOL EXPOSURE ON HYPOTHALAMIC MICROGLIA IN RATS

Chastain, L., Shrivastava, P., Cabrera, M. and Sarkar, D.K.
*Alcoholism: Clin. Exp. Res., **40(S1)**, abstract 256, 16A-247A*

Microglia are the brain's resident immune cells, and experimental evidence shows that microglia over-activation may play a role in the detrimental effects of alcohol on the hypothalamus, but the mechanism for this is unknown. These studies sought to (i) characterize the effects of developmental alcohol exposure onmicroglia activation and (ii) investigate the effects of alcohol on epigenetic factors in microglia using a rat model for fetal alcohol exposure. Neonatal rat pups (third trimester human equivalent) were fed by oral gavage a milk formula containing 11.34%ethanol (vol/vol), yielding a total daily ethanol dose of 2.5 g/kg (AF), or isocaloric control (PF), or they were left in the litter with themother (AD) for 5 days (postnatal days 2–6). Two hours after the last feeding, the pups were sacrificed and hypothalamus was dissected.Whole hypothalamic tissue was used for quantification of RNA transcripts involved in inflammation using quantitative real time PCR. In another set of animals, microglia was purified from hypothalamic tissue by differential gradient centrifugation using OptiPrep gradient.Microglia characterization and comparison between AF, PF, and AD pups was performed by flow cytometry. In the gene expression study, AF pups showed a significant increase in pro-inflammatory transcripts including monocyte chemoattractant protein-1 (MCP-1) and colony stimulating factor 1 receptor (CSFR1) compared to AD controls, indicating increased pro-inflammatory gene expression as a result of neonatal alcohol exposure. Flow cytometry characterization of purified microglia showed a significant increase in microglia-specific calcium-binding protein IBA1 and chemokine interferon gamma (IFN-c) in AF pups compared to AD pups. As these results confirmed an increase in microglia activation and pro-inflammatory gene expression in the hypothalamus after neonatal alcohol exposure, the effects of neonatal

alcohol on epigenetic regulators of gene expression were also investigated. AF pups showed a significant decrease in transcriptional repressors methyl CpG binding protein 2 (MeCP2) and histone deacetylase 1 (HDAC1) proteins compared to PF and AD pups. These results suggest that neonatal alcohol activates microglia possibly by decreasing transcriptional repressors, revealing a possible epigenetic mechanism for the adverse effects of alcohol on hypothalamic inflammation. (Supported by NIH grants F32AA023434 and R37AA008757).

4.1573 Rickettsia australis Activates Inflammasome in Human and Murine Macrophages
Smalley, C., Bechelli, J., Rockx-Brouwer, D., Saito, T., Azar, S.R., Ismali, N., Walker, D.H. and Fang, R.
*PloS One, **11(6)**, e0157231 (2016)*

Rickettsiae actively escape from vacuoles and replicate free in the cytoplasm of host cells, where inflammasomes survey the invading pathogens. In the present study, we investigated the interactions of Rickettsia australis with the inflammasome in both mouse and human macrophages. R. australis induced a significant level of IL-1β secretion by human macrophages, which was significantly reduced upon treatment with an inhibitor of caspase-1 compared to untreated controls, suggesting caspase-1-dependent inflammasome activation. Rickettsia induced significant secretion of IL-1β and IL-18 in vitro by infected mouse bone marrow-derived macrophages (BMMs) as early as 8–12 h post infection (p.i.) in a dose-dependent manner. Secretion of these cytokines was accompanied by cleavage of caspase-1 and was completely abrogated in BMMs deficient in caspase-1/caspase-11 or apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), suggesting that R. australis activate the ASC-dependent inflammasome. Interestingly, in response to the same quantity of rickettsiae, NLRP3-/- BMMs significantly reduced the secretion level of IL-1β compared to wild type (WT) controls, suggesting that NLRP3 inflammasome contributes to cytosolic recognition of R. australis in vitro. Rickettsial load in spleen, but not liver and lung, of R. australis-infected NLRP3-/- mice was significantly greater compared to WT mice. These data suggest that NLRP3 inflammasome plays a role in host control of bacteria in vivo in a tissue-specific manner. Taken together, our data, for the first time, illustrate the activation of ASC-dependent inflammasome by R. australis in macrophages in which NLRP3 is involved.

4.1574 ITGAV and ITGA5 diversely regulate proliferation and adipogenic differentiation of human adipose derived stem cells
Morandi, E.M., Verstappen, R., Zwierzina, M.E., Geley, S., Pierer, G. and Ploner, C.
*Scientific Reports, **6**, 28889 (2016)*

The fate of human adipose tissue stem cells (ASCs) is largely determined by biochemical and mechanical cues from the extracellular matrix (ECM), which are sensed and transmitted by integrins. It is well known that specific ECM constituents influence ASC proliferation and differentiation. Nevertheless, knowledge on how individual integrins regulate distinct processes is still limited. We performed gene profiling of 18 alpha integrins in sorted ASCs and adipocytes, identifying downregulations of RGD-motif binding integrins integrin-alpha-V (ITGAV) and integrin-alpha-5 (ITGA5), upregulation of laminin binding and leukocyte-specific integrins and individual regulations of collagen and LDV-receptors in differentiated adipocytes in-vivo. Gene function analyses in in-vitro cultured ASCs unraveled differential functions of ITGA5 and ITGAV. Knockdown of ITGAV, but not ITGA5 reduced proliferation, caused p21Cip1 induction, repression of survivin and specific regulation of Hippo pathway mediator TAZ. Gene knockdown of both integrins promoted adipogenic differentiation, while transgenic expression impaired adipogenesis. Inhibition of ITGAV using cilengitide resulted in a similar phenotype, mimicking loss of pan-ITGAV expression using RNAi. Herein we show ASC specific integrin expression patterns and demonstrate distinct regulating roles of both integrins in human ASCs and adipocyte physiology suggesting a negative impact of RDG-motif signaling on adipogenic differentiation of ASCs via ITGA5 and ITGAV.

4.1575 Expanding the tools for identifying mononuclear phagocyte subsets in swine: Reagents to porcine CD11c and XCR1
Deloizy, C., Bouguyon, E., Fossum, E., Sebo, P., Osicka, R., Bole, A., Pierres, M., Biacchesi, S., Salod, M., Bogen, B., Bertho, N. and Schwartz-Cornil, I.
*Development. Comp. Immunol., **65**, 31-40 (2016)*

Pig is a domestic species of major importance in the agro-economy and in biomedical research. Mononuclear phagocytes (MNP) are organized in subsets with specialized roles in the orchestration of the immune response and new tools are awaited to improve MNP subset identification in the pig. We cloned

pig CD11c cDNA and generated a monoclonal antibody to pig CD11c which showed a pattern of expression by blood and skin MNP subsets similar to humans. We also developed a porcine XCL1-mCherry dimer which specifically reacted with the XCR1-expressing dendritic cell subset of the type 1 lineage in blood and skin. These original reagents will allow the efficient identification of pig MNP subsets to study their role in physiological and pathological processes and also to target these cells in novel intervention and vaccine strategies for veterinary applications and preclinical evaluations.

4.1576 Free Fatty Acids Differentially Downregulate Chemokines in Liver Sinusoidal Endothelial Cells: Insights into Non-Alcoholic Fatty Liver Disease
McMahan, R.H., Porsche, C.E., Edwards, M.G. and Rosen, H.R.
*PloS One, **11(7)**, e015917 (2016)*

Non-alcoholic fatty liver disease is a prevalent problem throughout the western world. Liver sinusoidal endothelial cells (LSEC) have been shown to play important roles in liver injury and repair, but their role in the underlying pathogenetic mechanisms of non-alcoholic fatty liver disease remains undefined. Here, we evaluated the effects of steatosis on LSEC gene expression in a murine model of non-alcoholic fatty liver disease and an immortalized LSEC line. Using microarray we identified distinct gene expression profiles following exposure to free fatty acids. Gene pathway analysis showed a number of differentially expressed genes including those involved in lipid metabolism and signaling and inflammation. Interestingly, in contrast to hepatocytes, fatty acids led to decreased expression of pro-inflammatory chemokines including CCL2 (MCP-1), CXCL10 and CXCL16 in both primary and LSEC cell lines. Chemokine downregulation translated into a significant inhibition of monocyte migration and LSECs isolated from steatotic livers demonstrated a similar shift towards an anti-inflammatory phenotype. Overall, these pathways may represent a compensatory mechanism to reverse the liver damage associated with non-alcoholic fatty liver disease.

4.1577 Nasal delivery of chitosan-coated poly(lactide-co-glycolide)-encapsulated honeybee (Apis mellifera) venom promotes Th 1-specific systemic and local intestinal immune responses in weaned pigs
Lee, J-A., Kim, Y-M., Kim, T-H., Lee, S-H., Lee, C-A., Cho, C-W., Jeon, J-w., Park, J-k., Kim, S-K., Jung, B-G. and Lee, B-J.
*Vet. Immunol. Immunopathol., **178**, 99-106 (2016)*

Nasal delivery is a convenient and acceptable route for drug administration, and has been shown to elicit a much more potent local and systemic response compared with other drug delivery routes. We previously demonstrated that rectal administration of poly(lactide-co-glycolide)-encapsulated honeybee venom (P-HBV) could enhance systemic Th 1-specific immune responses. We therefore synthesized chitosan-coated P-HBV (CP-HBV) and then evaluated the immune-boosting efficacy of nasally administered CP-HBV on systemic and local intestinal immunity compared with non-chitosan-coated P-HBV. The nasally delivered CP-HBV effectively enhanced Th 1-specific responses, eliciting a significant increase in the $CD3^+CD4^+CD8^-$ Th cell population, lymphocyte proliferation capacity, and expression of Th 1 cytokines (IFN-γ, IL-12, and IL-2) in peripheral blood mononuclear cells. Furthermore, these immune-boosting effects persisted up to 21 days post CP-HBV administration. Nasal administration of CP-HBV also led to an increase of not only the $CD4^+$ Th 1 and IFN-γ secreting $CD4^+$ Th 1 cell population but also Th 1-specific cytokines and transcription factors, including IL-12, IFN-γ, STAT4, and T-bet, in isolated mononuclear cells from the spleen and ileum.

4.1578 Exosome-mediated activation of toll-like receptor 3 in stellate cells stimulates interleukin-17 production by γδ T cells in liver fibrosis
Seo, W., Eun, H., Kim, S.Y., Yi, H-S., Lee, Y-S., park, S-H., jang, M-J., Jo, E., Kim, S.C., Han, Y-M., Park, K-G. and Jeong, W-I.
*Hepatology, **64(2)**, 616-631 (2016)*

During liver injury, hepatocytes secrete exosomes that include diverse types of self-RNAs. Recently, self-noncoding RNA has been recognized as an activator of Toll-like receptor 3 (TLR3). However, the roles of hepatic exosomes and TLR3 in liver fibrosis are not yet fully understood. Following acute liver injury and early-stage liver fibrosis induced by a single or 2-week injection of carbon tetrachloride (CCl_4), increased interleukin (IL)-17A production was detected primarily in hepatic γδ T cells in wild-type (WT) mice. However, liver fibrosis and IL-17A production by γδ T cells were both significantly attenuated in TLR3 knockout (KO) mice compared with WT mice. More interestingly, IL-17A-producing γδ T cells were in close contact with activated hepatic stellate cells (HSCs), suggesting a role for HSCs in IL-17A production

by γδ T cells. *In vitro* treatments with exosomes derived from CCl_4-treated hepatocytes significantly increased the expression of IL-17A, IL-1β, and IL-23 in WT HSCs but not in TLR3 KO HSCs. Furthermore, IL-17A production by γδ T cells was substantially increased upon coculturing with exosome-treated WT HSCs or conditioned medium from TLR3-activated WT HSCs. However, similar increases were not detected when γδ T cells were cocultured with exosome-treated HSCs from IL-17A KO or TLR3 KO mice. Using reciprocal bone marrow transplantation between WT and TLR3 KO mice, we found that TLR3 deficiency in HSCs contributed to decreased IL-17A production by γδ T cells, as well as liver fibrosis. *Conclusion*: In liver injury, the exosome-mediated activation of TLR3 in HSCs exacerbates liver fibrosis by enhancing IL-17A production by γδ T cells, which might be associated with HSC stimulation by unknown self-TLR3 ligands from damaged hepatocytes. Therefore, TLR3 might be a novel therapeutic target for liver fibrosis.

4.1579 The respiratory DC/macrophage network at steady-state and upon influenza infection in the swine biomedical model

Maisonnasse, P., Bouguyon, E., Piton, G., Ezquerra, A., Urien, C., Deloizy, C., Bourge, M., leplat, J-J., Simon, G., Chevalier, C., Vincent-Naulleau, S., Crisci, E., Montoya, M., Schwartz-Cornil, I. and bertho, N.
Mucosal Immunol., ***9(4)***, *835-849 (2016)*

Human and mouse respiratory tracts show anatomical and physiological differences, which will benefit from alternative experimental models for studying many respiratory diseases. Pig has been recognized as a valuable biomedical model, in particular for lung transplantation or pathologies such as cystic fibrosis and influenza infection. However, there is a lack of knowledge about the porcine respiratory immune system. Here we segregated and studied six populations of pig lung dendritic cells (DCs)/macrophages (Mθs) as follows: conventional DCs (cDC) 1 and cDC2, inflammatory monocyte-derived DCs (moDCs), monocyte-derived Mθs, and interstitial and alveolar Mθs. The three DC subsets present migratory and naive T-cell stimulation capacities. As observed in human and mice, porcine cDC1 and cDC2 were able to induce T-helper (Th)1 and Th2 responses, respectively. Interestingly, porcine moDCs increased in the lung upon influenza infection, as observed in the mouse model. Pig cDC2 shared some characteristics observed in human but not in mice, such as the expression of FCεRIα and Langerin, and an intra-epithelial localization. This work, by unraveling the extended similarities of the porcine and human lung DC/Mθ networks, highlights the relevance of pig, both as an exploratory model of DC/Mθ functions and as a model for human inflammatory lung pathologies.

4.1580 Posttranscriptional control of NLRP3 inflammasome activation in colonic macrophages

Filardy, A.A., He, J., bennink, J., Yewdell, J. and Kelsall, B.L.
Mucosal Immunol., ***9(4)***, *850-858 (2016)*

Colonic macrophages (cMPs) are important for intestinal homeostasis as they kill microbes and yet produce regulatory cytokines. Activity of the NLRP3 (nucleotide-binding leucine-rich repeat-containing pyrin receptor 3) inflammasome, a major sensor of stress and microorganisms that results in pro-inflammatory cytokine production and cell death, must be tightly controlled in the intestine. We demonstrate that resident cMPs are hyporesponsive to NLRP3 inflammasome activation owing to a remarkable level of posttranscriptional control of NLRP3 and pro-interleukin-1β (proIL-1β) protein expression, which was also seen for tumor necrosis factor-α and IL-6, but lost during experimental colitis. Resident cMPs rapidly degraded NLRP3 and proIL-1β proteins by the ubiquitin/proteasome system. Finally, blocking IL-10R-signaling *in vivo* enhanced NLRP3 and proIL-1β protein but not mRNA levels in resident cMPs, implicating a role for IL-10 in environmental conditioning of cMPs. These data are the first to show dramatic posttranscriptional control of inflammatory cytokine production by a relevant tissue-derived macrophage population and proteasomal degradation of proIL-1β and NLRP3 as a mechanism to control inflammasome activation, findings which have broad implications for our understanding of intestinal and systemic inflammatory diseases.

4.1581 Characterization of the Expression and Function of the C-Type Lectin Receptor CD302 in Mice and Humans Reveals a Role in Dendritic Cell Migration

Lo, T-H., Silveira, P.A., Fromm, P.D., verma, N.D., Vu, P.A., Kupresanin, F., Adam, R., Kato, M., Cogger, V.C., Clark, G.J. and hart, D.N.J.
J. Immunol., ***197(3)***, *885-898 (2016)*

C-type lectin receptors play important roles in immune cell interactions with the environment. We described CD302 as the simplest, single domain, type I C-type lectin receptor and showed it was expressed

mainly on the myeloid phagocytes in human blood. CD302 colocalized with podosomes and lamellopodia structures, so we hypothesized that it played a role in cell adhesion or migration. In this study, we used mouse models to obtain further insights into CD302 expression and its potential immunological function. Mouse CD302 transcripts were, as in humans, highest in the liver, followed by lungs, lymph nodes (LN), spleen, and bone marrow. In liver, CD302 was expressed by hepatocytes, liver sinusoidal endothelial cells, and Kupffer cells. A detailed analysis of CD302 transcription in mouse immune cells revealed highest expression by myeloid cells, particularly macrophages, granulocytes, and myeloid dendritic cells (mDC). Interestingly, 2.5-fold more CD302 was found in migratory compared with resident mDC populations and higher CD302 expression in mouse M1 versus M2 macrophages was also noteworthy. CD302 knockout (CD302KO) mice were generated. Studies on the relevant immune cell populations revealed a decrease in the frequency and numbers of migratory mDC within CD302KO LN compared with wild-type LN. In vitro studies showed CD302KO and wild-type DC had an equivalent capacity to undergo maturation, prime T cells, uptake Ags, and migrate toward the CCL19/CCL21 chemokines. Nevertheless, CD302KO migratory DC exhibited reduced in vivo migration into LN, confirming a functional role for CD302 in mDC migration.

4.1582 IL4-10 Fusion Protein Is a Novel Drug to Treat Persistent Inflammatory Pain

Eijkelkamp, N., Steen-Louws, C., Hartgring, S.A.Y., Willemen, H.L.D.M., Prado, J., Lafeber, F.P.J.G., Heijnen, C.J., Hack, C.E., van Roon, J.A.G., and Kavelaars, A.
J. Neurosci., ***36(28)****, 7353-7363 (2016)*

Chronic pain is a major clinical problem that is difficult to treat and requires novel therapies. Although most pain therapies primarily target neurons, neuroinflammatory processes characterized by spinal cord and dorsal root ganglion production of proinflammatory cytokines play an important role in persistent pain states and represent potential therapeutic targets. Anti-inflammatory cytokines are attractive candidates to regulate aberrant neuroinflammatory processes, but the therapeutic potential of these cytokines as stand-alone drugs is limited. Their optimal function requires concerted actions with other regulatory cytokines, and their relatively small size causes rapid clearance. To overcome these limitations, we developed a fusion protein of the anti-inflammatory cytokines interleukin 4 (IL4) and IL10. The IL4-10 fusion protein is a 70 kDa glycosylated dimeric protein that retains the functional activity of both cytokine moieties. Intrathecal administration of IL4-10 dose-dependently inhibited persistent inflammatory pain in mice: three IL4-10 injections induced full resolution of inflammatory pain in two different mouse models of persistent inflammatory pain. Both cytokine moieties were required for optimal effects. The IL4-10 fusion protein was more effective than the individual cytokines or IL4 plus IL10 combination therapy and also inhibited allodynia in a mouse model of neuropathic pain. Mechanistically, IL4-10 inhibited the activity of glial cells and reduced spinal cord and dorsal root ganglion cytokine levels without affecting paw inflammation. In conclusion, we developed a novel fusion protein with improved efficacy to treat pain, compared with wild-type anti-inflammatory cytokines. The IL4-10 fusion protein has potential as a treatment for persistent inflammatory pain.

4.1583 Antitumor effect of antiplatelet agents in gastric cancer cells: an in vivo and in vitro study

Mikami, J., Kurokawa, Y., Takahashi, T., Miyazaki, Y., Yamasaki, M., Miyata, H., nakajima, K., Takiguchi, S., Mori, M. and Doki, Y.
Gastric Cancer, ***19****, 817-826 (2016)*

Background
The antitumor effects of antiplatelet agents in gastric cancer cells are not well known. In this study, the possibility of gastric cancer treatment with an antiplatelet agent, mainly aspirin, was examined both in vivo and in vitro.
Methods
For in vivo experiments, tumor-bearing mice were treated by an antiplatelet antibody or aspirin, and the tumor growth was compared. For in vitro experiments, human gastric cancer cell lines were used to confirm the cancer cell growth and inhibition by reducing the platelet count or using aspirin. We also examined several cytokines by using an ELISA assay and conducted microRNA microarray analysis of MKN-45 tumor cells to determine the influence of platelets or aspirin.
Results
In vivo experiments showed that tumor growth was inhibited by halving the circulating platelet count by using an antiplatelet antibody or peroral daily aspirin. In vitro experiments showed that the proliferation rates of gastric cancer cell lines were increased after coincubation with platelets and that the effect was inhibited by aspirin. Although the expression of interleukin-6, platelet-derived growth factor, transforming

growth factor-β, and prostaglandin E2 did not correlate with tumor growth inhibition by aspirin, seven microRNAs showed altered expression in cancer cells in response to coincubation with platelets or addition of aspirin. Cells transfected with mir-4670-5p showed a significant increase in proliferation compared to negative control cells.
Conclusions
Our study showed that platelets increased the proliferation of gastric cancer cells and that this increase was inhibited by antiplatelet antibody or aspirin. Mir-4670-5p may play an important role in these responses.

4.1584 Sperm is epigenetically programmed to regulate gene transcription in embryos
Teperek, M., Simeone, A., Gaggioli, V., Miyamoto, K., Allen, G.E., Erkek, S., Kwon, T., marcotte, E.M., Zegerman, P., Bradshaw, C.R., Peters, A.H.M., Gurdon, J.B. and Jullien, J.
Genome Res., ***26***, *1034-1046 (2016)*

For a long time, it has been assumed that the only role of sperm at fertilization is to introduce the male genome into the egg. Recently, ideas have emerged that the epigenetic state of the sperm nucleus could influence transcription in the embryo. However, conflicting reports have challenged the existence of epigenetic marks on sperm genes, and there are no functional tests supporting the role of sperm epigenetic marking on embryonic gene expression. Here, we show that sperm is epigenetically programmed to regulate embryonic gene expression. By comparing the development of sperm- and spermatid-derived frog embryos, we show that the programming of sperm for successful development relates to its ability to regulate transcription of a set of developmentally important genes. During spermatid maturation into sperm, these genes lose H3K4me2/3 and retain H3K27me3 marks. Experimental removal of these epigenetic marks at fertilization de-regulates gene expression in the resulting embryos in a paternal chromatin-dependent manner. This demonstrates that epigenetic instructions delivered by the sperm at fertilization are required for correct regulation of gene expression in the future embryos. The epigenetic mechanisms of developmental programming revealed here are likely to relate to the mechanisms involved in transgenerational transmission of acquired traits. Understanding how parental experience can influence development of the progeny has broad potential for improving human health.

4.1585 Muscle specific nucleus ambiguus neurons isolation and culturing
Hernandez-Morato, I., Pitman, M.J. and Sharma, S.
J. Neuroscience Methods, ***273***, *33-39 (2016)*

Background
Peripheral nerve injury leads to a regenerative state. However, the reinnervation process is highly non-selective. Growing axons are often misrouted and establish aberrant synapsis to abductor or adductor muscles. Determining the complex properties of abductor and adductor motoneurons in a neuron culture, may lay the groundwork for future studies on axon guidance, leading to a clinical treatment for a selective reinnervation.
New method
In the present study we develop a neuron culture protocol to isolate recurrent laryngeal nerve abductor and adductor motoneurons in order to study their unique properties.
Comparison with existing methods the best period to perform the present protocol for postnatal rat cranial motoneurons isolation was determined. In addition, the method allows identification of specific motoneurons from other primary motoneurons and interneurons within brainstem.
Conclusion
The present protocol will allow investigators to perform targeted and novel studies of the mechanisms of peripheral nerve regeneration.

4.1586 The effects of urine concentration, and cushion centrifugation to remove urine, on the quality of cool-stored stallion sperm
Voge, J., Varner, D.D., Blanchard, T.L., Meschini, M., Turner, C., Teague, S.R., Brinsko, S.P. and Love, C.C.
Theriogenology, ***86***, *1294-1298 (2016)*

Urine-contaminated stallion semen is a clinical problem due to a variety of causes. The effect of the level of urine contamination on the longevity of sperm quality has not been evaluated. The aim of this study was to determine the effects of urine concentration level (0%, 10%, 20%, 30%, and 40%) and cushioned centrifugation and resuspension of the sperm pellet in fresh extender, on measures of sperm quality, immediately after semen collection (T_0), after 1 hour of storage at room temperature (T_1), and after

24 hours of cooled storage (T_{24}). In general, most sperm quality measures declined with increasing urine concentration starting at T_0. Cushioned centrifugation (CC), but not simple dilution, generally maintained sperm quality at T_{24} as compared with T_1. At T_{24}, total sperm motility was higher in all urine-contaminated CC samples compared with uncentrifuged samples ($P < 0.05$); sperm viability was lower in CC than uncentrifuged at a urine concentration of 20%, but higher at 30% and 40% ($P < 0.05$); and DNA quality was decreased (higher % cells outside the main population) in all urine concentrations ($P < 0.05$). Immediate extension in semen extender, followed by cushioned centrifugation and resuspension of the sperm pellet in fresh extender, provided the best option for preserving sperm quality of urospermic semen.

4.1587 Hepatic immunophenotyping for streptozotocin-induced hyperglycemia in mice

Lee, Y-S., Eun, H.S., Kim, S.Y., Jeong, J-M., Seo, W., Byun, J-S., Jeong, W-i. and Yi, H-S.
*Scientific Reports, **6**:30656 (2016)*

Emerging evidence revealed that diabetes induces abnormal immune responses that result in serious complications in organs. However, the effect of hyperglycemia on hepatic immunity remains obscure. We evaluated the population and function of hepatic immune cells in streptozotocin (STZ)-induced hyperglycemic mice. CC chemokine receptor 2 (CCR2)-knockout mice and mice with a depletion of regulatory T cells (DEREG) were used to investigate the migration and role of regulatory T cells (Tregs) in hyperglycemic mice. The inflammatory cytokines and hepatic transaminase levels were significantly increased in the hyperglycemic mice. The population and number of infiltrating monocytes, granulocytes, and Tregs were enhanced in the livers of the hyperglycemic mice. Hepatic monocytes other than macrophages showed the increased expression of inflammatory cytokines and chemokines in the hyperglycemic mice. The CCR2 knockout and DEREG chimeric mice exhibited increased populations of activated T cells and neutrophils compared to the WT chimeric mice, which promoted hepatic inflammation in the hyperglycemic mice. The migration of CCR2 knockout Tregs into the liver was significantly reduced compared to the WT Tregs. We demonstrated that hyperglycemia contributes to increase in infiltrating monocytes and Tregs, which are associated with hepatic immune dysfunction in mice. CCR2-mediated migration of Tregs regulates hyperglycemia-induced hepatic inflammation.

4.1588 Leucine Zipper-bearing Kinase promotes axon growth in mammalian central nervous system neurons

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*Scientific Reports, **6**:31482 (2016)*

Leucine Zipper-bearing Kinase (LZK/MAP3K13) is a member of the mixed lineage kinase family with high sequence identity to Dual Leucine Zipper Kinase (DLK/MAP3K12). While DLK is established as a key regulator of axonal responses to injury, the role of LZK in mammalian neurons is poorly understood. By gain- and loss-of-function analyses in neuronal cultures, we identify LZK as a novel positive regulator of axon growth. LZK signals specifically through MKK4 and JNKs among MAP2Ks and MAPKs respectively in neuronal cells, with JNK activity positively regulating LZK protein levels. Neuronal maturation or activity deprivation activates the LZK-MKK4-JNK pathway. LZK and DLK share commonalities in signaling, regulation, and effects on axon extension. Furthermore, LZK-dependent regulation of DLK protein expression and the lack of additive effects on axon growth upon co-manipulation suggest complex functional interaction and cross-regulation between these two kinases. Together, our data support the possibility for two structurally related MAP3Ks to work in concert to mediate axonal responses to external insult or injury in mammalian CNS neurons.

4.1589 Splenectomy enhances the therapeutic effect of adipose tissue-derived mesenchymal stem cell infusion on cirrhosis rats

Tang, W-P., Akahoshi, T., Piao, J-S., narahara, S., Murata, M., Kawano, T., Hamano, N., Ikeda, T. and Hashizume, M.
*Liver Int., **36(8)**, 1151-1159 (2016)*

Background & Aims
Clinical studies suggest that splenectomy improves liver function in cirrhotic patients, but the influence of splenectomy on stem cell transplantation is poorly understood. This study investigated the effect of splenectomy on stem cell infusion and elucidated its mechanism.
Methods
Rat adipose tissue-derived mesenchymal stem cells were infused into cirrhosis rats with or without splenectomy, followed by the assessment of the *in vivo* distribution of stem cells and pathological changes.

Stromal cell-derived factor-1 and hepatocyte growth factor expression were also investigated in splenectomized cirrhosis patients and rats.

Results

Splenectomy, prior to cell infusion, improved liver function and suppressed fibrosis progression more efficiently than cell infusion alone in the experimental cirrhosis model. Stromal cell-derived factor-1 and hepatocyte growth factor levels after splenectomy were increased in patients and rats. These upregulated cytokines significantly facilitated stem cell motility, migration and proliferation *in vitro*. C-X-C chemokine receptor type 4 neutralization weakened the promotion of cell migration by these cytokines. The infused cells integrated into liver fibrosis septa and participated in regeneration more efficiently in splenectomized rats. Direct coculture with stem cells led to inhibition of hepatic stellate cell proliferation. In addition, hepatocyte growth factor induced hepatic stellate cell apoptosis via the c-jun N-terminal kinase-p53 pathway.

Conclusions

Splenectomy prior to cell infusion enhanced the therapeutic effect of stem cells on cirrhosis, which involved upregulation of stromal cell-derived factor-1 and hepatocyte growth factor after splenectomy.

4.1590 TPL-2 Regulates Macrophage Lipid Metabolism and M2 Differentiation to Control TH2-Mediated Immunopathology

Kannan, Y., Perez-Lloret, J., Li, Y., Entwistle, L.J., Khoury, H., Papoutsopoulou, S., Mahmood, R., Mansour, N.R., Huang, S.C-C., Pearce, E.J., de Carvalho, L.P.S., Ley, S.C. and Wilson, M.S.
*PloS Pathogens, **12(8)**, e1005783 (2016)*

Persistent TH2 cytokine responses following chronic helminth infections can often lead to the development of tissue pathology and fibrotic scarring. Despite a good understanding of the cellular mechanisms involved in fibrogenesis, there are very few therapeutic options available, highlighting a significant medical need and gap in our understanding of the molecular mechanisms of TH2-mediated immunopathology. In this study, we found that the Map3 kinase, TPL-2 (Map3k8; Cot) regulated TH2-mediated intestinal, hepatic and pulmonary immunopathology following Schistosoma mansoni infection or S. mansoni egg injection. Elevated inflammation, TH2 cell responses and exacerbated fibrosis in Map3k8–/–mice was observed in mice with myeloid cell-specific (LysM) deletion of Map3k8, but not CD4 cell-specific deletion of Map3k8, indicating that TPL-2 regulated myeloid cell function to limit TH2-mediated immunopathology. Transcriptional and metabolic assays of Map3k8–/–M2 macrophages identified that TPL-2 was required for lipolysis, M2 macrophage activation and the expression of a variety of genes involved in immuno-regulatory and pro-fibrotic pathways. Taken together this study identified that TPL-2 regulated TH2-mediated inflammation by supporting lipolysis and M2 macrophage activation, preventing TH2 cell expansion and downstream immunopathology and fibrosis.

4.1591 PAK proteins and YAP-1 signalling downstream of integrin beta-1 in myofibroblasts promote liver fibrosis

Martin, K., Pritchett, J., Llewellym, J., Mullan, A.F., Athwal, V.S., Dobie, R., Harvey, E., Zeef, L., Farrow, S., Streuli, C., Henderson, N.C., Friedman, S.L., Hanley, N.A. and Hanley, K.P.
*Nature Communications, **7**:12502 (2016)*

Fibrosis due to extracellular matrix (ECM) secretion from myofibroblasts complicates many chronic liver diseases causing scarring and organ failure. Integrin-dependent interaction with scar ECM promotes pro-fibrotic features. However, the pathological intracellular mechanism in liver myofibroblasts is not completely understood, and further insight could enable therapeutic efforts to reverse fibrosis. Here, we show that integrin beta-1, capable of binding integrin alpha-11, regulates the pro-fibrotic phenotype of myofibroblasts. Integrin beta-1 expression is upregulated in pro-fibrotic myofibroblasts *in vivo* and is required *in vitro* for production of fibrotic ECM components, myofibroblast proliferation, migration and contraction. Serine/threonine-protein kinase proteins, also known as P21-activated kinase (PAK), and the mechanosensitive factor, Yes-associated protein 1 (YAP-1) are core mediators of pro-fibrotic integrin beta-1 signalling, with YAP-1 capable of perpetuating integrin beta-1 expression. Pharmacological inhibition of either pathway *in vivo* attenuates liver fibrosis. PAK protein inhibition, in particular, markedly inactivates the pro-fibrotic myofibroblast phenotype, limits scarring from different hepatic insults and represents a new tractable therapeutic target for treating liver fibrosis.

4.1592 Elevated interleukin-27 levels in human neonatal macrophages regulate indoleamine dioxygenase in a STAT-1 and STAT-3-dependent manner

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and Robinson, C.M.
Immunology, ***149(1)***, *35-47 (2016)*

Microbial infections are a major cause of infant mortality as a result of limitations in immune defences. Interleukin-27 (IL-27) is a heterodimeric cytokine produced primarily by leucocytes and is immunosuppressive toward lymphocytes and leucocytes. Our laboratory demonstrated that human neonatal macrophages express IL-27 more abundantly than adult macrophages. Similarly in mice, IL-27 expression is elevated early in life and maintained through infancy. To determine IL-27-regulated mechanisms that may limit immunity, we evaluated the expression of a number of genes in response to this cytokine in primary human neonatal macrophages. Indoleamine 2,3-dioxygenase (IDO) gene expression was increased dose-responsively by IL-27. We have previously demonstrated inhibition of T-cell proliferation and cytokine production by neonatal macrophage-generated IL-27, and IDO is often implicated in this negative regulation. An increase in IDO protein was demonstrated by immunofluorescence microscopy and was consistent with increased enzyme activity following treatment with IL-27. Inclusion of a soluble receptor to neutralize endogenous IL-27, decreased IDO expression and activity compared with untreated macrophages. In response to IL-27, neonatal macrophages phosphorylate signal transdcuer and activator of transcription 1 (STAT-1) and STAT-3. Both transcription factors are recruited to the IDO regulatory region. STAT-3 dominates during steady-state regulation by lower levels of endogenous IL-27 production. A shift to enhanced STAT-1 recruitment occurs during increased levels of exogenously supplied IL-27. These data suggest an interesting interplay of STAT-1 and STAT-3 to regulate IDO activity and immunosuppression in response to different levels of IL-27 in the microenvironment of the immune response that may further our understanding of this interesting cytokine.

4.1593 OX40+ Regulatory T Cells in Cutaneous Squamous Cell Carcinoma Suppress Effector T-Cell Responses and Associate with Metastatic Potential
Lai, C., August, S., Albibas, A., Behar, R., Cho, S-Y., Polak, M.E., Theaker, J., MacLeod, A.S., French, R.R., Glennie, M.J., Al-Shamkani, A. and Healy, E.
Clin. Cancer Res., ***22(16)***, *4236-4248 (2016)*

Purpose: Cutaneous squamous cell carcinoma (cSCC) is the most common human cancer with metastatic potential. Despite T cells accumulating around cSCCs, these tumors continue to grow and persist. To investigate reasons for failure of T cells to mount a protective response in cSCC, we focused on regulatory T cells (Tregs) as this suppressive population is well represented among the infiltrating lymphocytes.
Experimental Design: Flow cytometry was conducted on cSCC lymphocytes and *in vitro* functional assays were performed using sorted tumoral T cells. Lymphocyte subsets in primary cSCCs were quantified immunohistochemically.
Results: $FOXP3^+$ Tregs were more frequent in cSCCs than in peripheral blood ($P < 0.0001$, $n = 86$ tumors). Tumoral Tregs suppressed proliferation of tumoral effector $CD4^+$ ($P = 0.005$, $n = 10$ tumors) and $CD8^+$ T cells ($P = 0.043$, $n = 9$ tumors) and inhibited IFNγ secretion by tumoral effector T cells ($P = 0.0186$, $n = 11$ tumors). The costimulatory molecule OX40 was expressed predominantly on tumoral Tregs ($P < 0.0001$, $n = 15$ tumors) and triggering OX40 with an agonist anti-OX40 antibody overcame the suppression exerted by Tregs, leading to increased tumoral effector $CD4^+$ lymphocyte proliferation ($P = 0.0098$, $n = 10$ tumors). Tregs and $OX40^+$ lymphocytes were more abundant in primary cSCCs that metastasized than in primary cSCCs that had not metastasized ($n = 48$ and $n = 49$ tumors, respectively).
Conclusions: Tregs in cSCCs suppress effector T-cell responses and are associated with subsequent metastasis, suggesting a key role for Tregs in cSCC development and progression. OX40 agonism reversed the suppressive effects of Tregs *in vitro*, suggesting that targeting OX40 could benefit the subset of cSCC patients at high risk of metastasis.

4.1594 Oxygen-Purged Microfluidic Device to Enhance Cell Viability in Photopolymerized PEG Hydrogel Microparticles
Xia, B., Krutkramelis, K and Oakey, J.
Biomacromolecules, ***17(7)***, *2459-2465 (2016)*

Encapsulating cells within biocompatible materials is a widely used strategy for cell delivery and tissue engineering. While cells are commonly suspended within bulk hydrogel-forming solutions during gelation, substantial interest in the microfluidic fabrication of miniaturized cell encapsulation vehicles has more recently emerged. Here, we utilize multiphase microfluidics to encapsulate cells within photopolymerized picoliter-volume water-in-oil droplets at high production rates. The photoinitiated polymerization of

polyethylene glycol diacrylate (PEGDA) is used to continuously produce solid particles from aqueous liquid drops containing cells and hydrogel forming solution. It is well understood that this photoinitiated addition reaction is inhibited by oxygen. In contrast to bulk polymerization in which ambient oxygen is rapidly and harmlessly consumed, allowing the polymerization reaction to proceed, photopolymerization within air permeable polydimethylsiloxane (PDMS) microfluidic devices allows oxygen to be replenished by diffusion as it is depleted. This sustained presence of oxygen and the consequential accumulation of peroxy radicals produce a dramatic effect upon both droplet polymerization and post-encapsulation cell viability. In this work we employ a nitrogen microjacketed microfluidic device to purge oxygen from flowing fluids during photopolymerization. By increasing the purging nitrogen pressure, oxygen concentration was attenuated, and increased post-encapsulation cell viability was achieved. A reaction-diffusion model was used to predict the cumulative intradroplet concentration of peroxy radicals, which corresponded directly to post-encapsulation cell viability. The nitrogen-jacketed microfluidic device presented here allows the droplet oxygen concentration to be finely tuned during cell encapsulation, leading to high post-encapsulation cell viability.

4.1595 **Identification of proliferative and mature β-cells in the islets of Langerhans**
Bader, E. et al
*Nature, **535**, 430-434 (2016)*

Insulin-dependent diabetes is a complex multifactorial disorder characterized by loss or dysfunction of β-cells. Pancreatic β-cells differ in size, glucose responsiveness, insulin secretion and precursor cell potential[1, 2, 3, 4, 5]; understanding the mechanisms that underlie this functional heterogeneity might make it possible to develop new regenerative approaches. Here we show that *Fltp* (also known as *Flattop* and *Cfap126*), a Wnt/planar cell polarity (PCP) effector and reporter gene[6], acts as a marker gene that subdivides endocrine cells into two subpopulations and distinguishes proliferation-competent from mature β-cells with distinct molecular, physiological and ultrastructural features. Genetic lineage tracing revealed that endocrine subpopulations from Fltp-negative and -positive lineages react differently to physiological and pathological changes. The expression of Fltp increases when endocrine cells cluster together to form polarized and mature 3D islet mini-organs[7, 8, 9]. We show that 3D architecture and Wnt/PCP ligands are sufficient to trigger β-cell maturation. By contrast, the Wnt/PCP effector Fltp is not necessary for β-cell development, proliferation or maturation. We conclude that 3D architecture and Wnt/PCP signalling underlie functional β-cell heterogeneity and induce β-cell maturation. The identification of Fltp as a marker for endocrine subpopulations sheds light on the molecular underpinnings of islet cell heterogeneity and plasticity and might enable targeting of endocrine subpopulations for the regeneration of functional β-cell mass in diabetic patients.

4.1596 **Progesterone, Inflammatory Cytokine (TNF-α), and Oxidative Stress (H2O2) Regulate Progesterone Receptor Membrane Component 1 Expression in Fetal Membrane Cells**
Meng, Y., Murtha, A.P. and Feng, L.
*Reproductive Sciences, **23(9)**, 1168-1178 (2016)*

Progesterone receptor membrane component 1 (PGRMC1) is an important novel mediator of progesterone (P4) function in fetal membrane cells. We demonstrated previously that PGRMC1 is differentially expressed in fetal membranes among pregnancy subjects and diminished in preterm premature rupture of membrane subjects. In the current study, we aim to elucidate whether PGRMC1 expression is regulated by P4, tumor necrosis factor α (TNF-α), and H_2O_2 in fetal membrane cells. Primary cultured membrane cells were serum starved for 24 hours followed by treatments of P4, 17 hydroxyprogesterone caproate, and medroxyprogesterone 17 acetate (MPA) at 10^{-7} mol/L with ethanol as vehicle control; TNF-α at 10, 20, and 50 ng/mL with phosphate-buffered saline (PBS) as control; and H_2O_2 at 10 and 100 μmol/L with culture media as control for 24, 48, and 72 hours. The messenger RNA (mRNA) and protein expression of PGRMC1 was quantified using polymerase chain reaction and Western blotting, respectively. We found that PGRMC1 protein expression was regulated by MPA, TNF-α, and H_2O_2 in a dose-dependent manner. This regulation is also specific to the type of cell (amnion, chorion, or decidua). The upregulation of PGRMC1 by MPA might be mediated through glucocorticoid receptor (GR) demonstrated using amnion and chorion cells model with GR knockdown by specific small interfering RNA transfection. The mRNA expression of PGRMC1 was decreased by H_2O_2 (100 μmol/L) treatment in amnion cells, which might ultimately result in downregulation of PGRMC1 protein as our data demonstrated. None of other treatments changed PGRMC1 mRNA level in these cells. We conclude that these stimuli act as regulatory factors of PGRMC1 in a cell-specific manner.

4.1597 Early steatohepatitis in hyperlipidemic mice with endothelial-specific gain of TRPC3 function precedes changes in aortic atherosclerosis

Smedlund, K., Dube, P. and Vazquez, G.

*Physiol. Genomics, **48**, 644-649 (2016)*

Nonalcoholic fatty liver disease (NAFLD) and its more advanced form nonalcoholic steatohepatitis (NASH) are the most common chronic liver diseases in developed countries. Moreover, NAFLD and NASH are considerable risk factors for atherosclerosis, the most frequent vascular pathology in these and other metabolic diseases. Despite this strong connection, current knowledge of the relationship between NAFLD/NASH and atherosclerosis is scarce. Recently, we studied hyperlipidemic Apoe knockout mice with endothelial-specific gain of transient receptor potential canonical 3 channel function (TgESTRPC3/ApoeKO) and found that these animals had increased burden of advanced aortic atherosclerosis (16 wk on high-fat diet) compared with nontransgenic ApoeKO littermate controls (non-Tg/ApoeKO), whereas early lesions (10 wk on high-fat diet) were not different. Here, we report that at the early stage when differences in aortic atherosclerosis are not yet manifest, the livers of TgESTRPC3/ApoeKO mice show steatosis, fibrosis, and altered hepatic enzymes compared with non-Tg/ApoeKO animals. Because differences in liver pathology were noticeable long before differences in atherosclerosis were evident, our studies suggest that TRPC3-related endothelial mechanisms that promote steatohepatitis may also contribute to atherosclerosis progression. In vitro, downregulation of TRPC3 in liver sinusoid endothelial cells reduces their susceptibility to endoplasmic reticulum stress-induced apoptosis, suggesting that a proapoptotic effect of TRPC3 may add to other fibrogenic factors in vivo. These novel findings show a positive association between augmented expression of an endothelial TRPC channel, development of early steatohepatitis, and atherosclerotic burden in a hyperlipidemic mouse model of NAFLD fed conventional Western-type diet.

4.1598 Cellular normoxic biophysical markers of hydroxyurea treatment in sickle cell disease

Hosseini, P., Abidi, S.Z., Du, E., papageorgiou, D.P., Choi, Y., Park, Y., Higgins, J.M., Kato, G.J., Suresh, S., Dao, M., Yaqoob, Z. and So, P.T.C.

*PNAS, **113(34)**, 9527-9532 (2016)*

Hydroxyurea (HU) has been used clinically to reduce the frequency of painful crisis and the need for blood transfusion in sickle cell disease (SCD) patients. However, the mechanisms underlying such beneficial effects of HU treatment are still not fully understood. Studies have indicated a weak correlation between clinical outcome and molecular markers, and the scientific quest to develop companion biophysical markers have mostly targeted studies of blood properties under hypoxia. Using a common-path interferometric technique, we measure biomechanical and morphological properties of individual red blood cells in SCD patients as a function of cell density, and investigate the correlation of these biophysical properties with drug intake as well as other clinically measured parameters. Our results show that patient-specific HU effects on the cellular biophysical properties are detectable at normoxia, and that these properties are strongly correlated with the clinically measured mean cellular volume rather than fetal hemoglobin level.

4.1599 CCR5 ameliorates Japanese encephalitis via dictating the equilibrium of regulatory CD4+Foxp3+ T and IL-17+CD4+ Th17 cells

Kim, J.H., Patil, A.M., Choi, J.Y., Kim, S.B., Uyangaa, E., Hossain, F.M.A., Park, S-Y., Lee, J.H. and Eo, S.K.

*J. Neuroinflammation, **13**:223 (2016)*

Background

CCR5 is a CC chemokine receptor involved in the migration of effector leukocytes including macrophages, NK, and T cells into inflamed tissues. Also, the role of CCR5 in $CD4^{+}Foxp3^{+}$ regulatory T cell (Treg) homing has recently begun to grab attention. Japanese encephalitis (JE) is defined as severe neuroinflammation of the central nervous system (CNS) following infection with mosquito-borne flavivirus JE virus. However, the potential contribution of CCR5 to JE progression via mediating $CD4^{+}Foxp3^{+}$ Treg homing has not been investigated.

Methods

Infected wild-type ($Ccr5^{+/+}$) and CCR5-deficient ($Ccr5^{-/-}$) mice were examined daily for mortality and clinical signs, and neuroinflammation in the CNS was evaluated by infiltration of inflammatory leukocytes and cytokine expression. In addition, viral burden, NK- and JEV-specific T cell responses were analyzed. Adoptive transfer of $CCR5^{+}CD4^{+}Foxp3^{+}$ Tregs was used to evaluate the role of Tregs in JE progression.

Results
CCR5 ablation exacerbated JE without altering viral burden in the extraneural and CNS tissues, as manifested by increased CNS infiltration of Ly-6C^{hi} monocytes and Ly-6G^{hi} granulocytes. Compared to Ccr5$^{+/+}$ mice, Ccr5$^{-/-}$ mice unexpectedly showed increased responses of IFN-γ^{+}NK and CD8^{+} T cells in the spleen, but not CD4^{+} T cells. More interestingly, CCR5-ablation resulted in a skewed response to IL-17^{+}CD4^{+} Th17 cells and correspondingly reduced CD4^{+}Foxp3^{+} Tregs in the spleen and brain, which was closely associated with exacerbated JE. Our results also revealed that adoptive transfer of sorted CCR5^{+}CD4^{+}Foxp3^{+} Tregs into Ccr5$^{-/-}$ mice could ameliorate JE progression without apparently altering the viral burden and CNS infiltration of IL-17^{+}CD4^{+} Th17 cells, myeloid-derived Ly-6C^{hi} monocytes and Ly-6G^{hi} granulocytes. Instead, adoptive transfer of CCR5^{+}CD4^{+}Foxp3^{+} Tregs into Ccr5$^{-/-}$ mice resulted in increased expression of anti-inflammatory cytokines (IL-10 and TGF-β) in the spleen and brain, and transferred CCR5^{+} Tregs were found to produce IL-10.
Conclusions
CCR5 regulates JE progression via governing timely and appropriate CNS infiltration of CD4^{+}Foxp3^{+} Tregs, thereby facilitating host survival. Therefore, this critical and extended role of CCR5 in JE raises possible safety concerns regarding the use of CCR5 antagonists in human immunodeficiency virus (HIV)-infected individuals who inhabit regions in which both HIV and flaviviruses, such as JEV and West Nile virus, are endemic.

4.1600 The Deficiency of Indoleamine 2,3-Dioxygenase Aggravates the CCl4-Induced Liver Fibrosis in Mice
Ogiso, H., Ito, H., Ando, T., Arioka, Y., Kanbe, A., Ando, K., Ishikawa, T., Saito, K., Hara, A., Moriwaki, H., Shimizu, M. and Seishima, M.
*PloS One, **11(9)**, e0162183 (2016)*

In the present study, we examined the role of indoleamine 2,3-dioxygenase (IDO) in the development of CCl4-induced hepatic fibrosis. The liver fibrosis induced by repetitive administration with CCl4 was aggravated in IDO-KO mice compared to WT mice. In IDO-KO mice treated with CCl4, the number of several inflammatory cells and the expression of pro-inflammatory cytokines increased in the liver. In the results, activated hepatic stellate cells (HSCs) and fibrogenic factors on HSCs increased after repetitive CCl4 administration in IDO-KO mice compared to WT mice. Moreover, the treatment with l-tryptophan aggravated the CCl4-induced hepatic fibrosis in WT mice. Our findings demonstrated that the IDO deficiency enhanced the inflammation in the liver and aggravated liver fibrosis in repetitive CCl4-treated mice.

4.1601 Rabbit M1 and M2 macrophages can be induced by human recombinant GM-CSF and M-CSF
Yamane, K. and Leung, K-P.
*FEBS Open Bio, **6(9)**, 945-953 (2016)*

Macrophages can change their phenotype in response to environmental cues. Polarized macrophages are broadly classified into two groups: classical activated M1 and alternative activated M2. Characterization of human macrophages has been widely studied, but polarized macrophages in rabbits have not been characterized. We characterized rabbit macrophages that were polarized using human recombinant GM-CSF and M-CSF. GM-CSF-treated macrophages had higher mRNA expression of proinflammatory cytokines (M1 phenotype) than did the M-CSF-treated counterpart. By contrast, high levels of TGF-β and IL-10 expression (M2 phenotype) were found in M-CSF-treated macrophages. The present study may be useful to understand roles of polarized macrophages in rabbit disease models.

4.1602 An optimized method for obtaining adult rat spinal cord motor neurons to be used for tissue culture
Brinn, M., O'Neill, K., Musgrave, I., Freeman, B.J.C., Henneberg, M. and Kumaratilake, J.
*J. Neurosci. Methods, **273**, 128-137 (2016)*

Background
There is a paucity of detailed methods describing how to harvest and process motor neurons obtained from the adult rat spinal cord.
New method
Removal of intra-cardiac perfusion step. The spinal cord is extruded intact from the rat in under 60 s post-decapitation then processed without differentiation of ventral and dorsal regions. The temperature during processing was maintained at room temperature (22 °C) except during the Papain processing step where the temperature was increased to 30 °C.
Results

Cell debris interfered with the counting of cells at the time of plating. Also, cell types could not be identified since they appear rounded structures with no projections. Cell viability counts reduced to 91% and 63% from day 7 to day 14 and days 7–28 respectively. Red blood cell counts in stepped density gradient layers 2 and 3 were low.
Comparison with existing method(s)
No requirement for intra-cardiac perfusion. No requirement to cool to 4 °C post harvesting, No requirement for specialized substrates. Reduces processing time by at least 2 h and reduces the potential for processing errors through a reduction in complexity. Procedures are also explained suitable for those new to the culture of primary adult motor neurons.
Conclusions
Cell viability counts indicate that removal of the perfusion step has a minimal effect on the viability of the cultured nerve cells, which may be due to the reduction in the spinal cord harvesting time and the inclusion of Hibernate based media during extrusion and processing.

4.1603 Latency Entry of Herpes Simplex Virus 1 Is Determined by the Interaction of Its Genome with the Nuclear Environment

Ali Maroui, M., Calle, A., Cohen, C., Streichenberger, N., Texier, P., Takissian, J., Rousseau, A., Poccardi, N., Welsch, J., Corpet, A., Schaeffer, L., labetoulle, M. and Lomonte, P.
*PloS Pathogens, **12(9)**, e1005834 (2016)*

Herpes simplex virus 1 (HSV-1) establishes latency in trigeminal ganglia (TG) sensory neurons of infected individuals. The commitment of infected neurons toward the viral lytic or latent transcriptional program is likely to depend on both viral and cellular factors, and to differ among individual neurons. In this study, we used a mouse model of HSV-1 infection to investigate the relationship between viral genomes and the nuclear environment in terms of the establishment of latency. During acute infection, viral genomes show two major patterns: replication compartments or multiple spots distributed in the nucleoplasm (namely "multiple-acute"). Viral genomes in the "multiple-acute" pattern are systematically associated with the promyelocytic leukemia (PML) protein in structures designated viral DNA-containing PML nuclear bodies (vDCP-NBs). To investigate the viral and cellular features that favor the acquisition of the latency-associated viral genome patterns, we infected mouse primary TG neurons from wild type (wt) mice or knock-out mice for type 1 interferon (IFN) receptor with wt or a mutant HSV-1, which is unable to replicate due to the synthesis of a non-functional ICP4, the major virus transactivator. We found that the inability of the virus to initiate the lytic program combined to its inability to synthesize a functional ICP0, are the two viral features leading to the formation of vDCP-NBs. The formation of the "multiple-latency" pattern is favored by the type 1 IFN signaling pathway in the context of neurons infected by a virus able to replicate through the expression of a functional ICP4 but unable to express functional VP16 and ICP0. Analyses of TGs harvested from HSV-1 latently infected humans showed that viral genomes and PML occupy similar nuclear areas in infected neurons, eventually forming vDCP-NB-like structures. Overall our study designates PML protein and PML-NBs to be major cellular components involved in the control of HSV-1 latency, probably during the entire life of an individual.

4.1604 Estradiol enhances capacity of TLR-matured splenic dendritic cells to polarize CD4+ lymphocytes into IL-17/GM-CSF-producing cells in vitro

Stojic-Vukanic, Z., Bufan, B., Pilipovic, I., Vujnovic, I., Nacka-Aleksic, M., Petrovic, R., Arsenovic-Ranin, N. and Leposavic, G.
*Int. Immunopharmacol., **40**, 244-253 (2016)*

There are little data on modulatory effects of estrogens on rat dendritic cell (DC) responses to inflammatory stimuli, and consequently their ability to activate and polarize CD4+ T lymphocyte-mediated immune responses. Splenic conventional DCs from young female Albino Oxford rats were activated *in vitro* with LPS (TLR4 agonist) or R848 (TLR7/8 agonist) in the presence and absence of 17β-estradiol (E2), and their allostimulatory and CD4+ lymphocyte polarizing ability in mixed leukocyte culture (MLC) were studied. Irrespective of the E2 presence, LPS and R848 up-regulated the expression of MHC II on DCs, so they exhibited enhanced allostimulatory capacity in co-culture with CD4+ lymphocytes. On the other hand, E2 promoted stimulatory action of both TLRs on OX62+ DC IL-23 production, augmented their stimulatory effects on IL-6 and IL-1β production, but diminished their enhancing effects on the expression IL-10 and IL-27 by DCs. Consequently, in MLC, OX62+ DCs activated/matured in the co-presence of E2 and either LPS or R848 increased the levels of IL-17, the signature Th17 cell cytokine, when compared with those activated/matured in the absence of E2. GM-CSF levels were also increased in these MLC. Given that the expression of IL-7 mRNA was diminished in DCs activated/matured in the co-

presence of E2 and TLR, this increase most likely did not reflect enhanced differentiation of Th cells producing GM-CSF only (Th-GM).

4.1605 Hepatocyte Toll-Like Receptor 5 Promotes Bacterial Clearance and Protects Mice Against High-Fat Diet–Induced Liver Disease

Etienne-Mesmin, L., Vijay-Kumar, M., Gewirtz, A.T. and Chassaing, B.
*Cell. Mol. Gastroenterol. Hepatol., **2**, 584-604 (2016)*

Background & Aims
Innate immune dysfunction can promote chronic inflammatory diseases of the liver. For example, mice lacking the flagellin receptor Toll-like receptor 5 (TLR5) show microbial dysbiosis and predisposition to high-fat diet (HFD)-induced hepatic steatosis. The extent to which hepatocytes play a direct role in detecting bacterial products in general, or flagellin in particular, is poorly understood. In the present study, we investigated the role of hepatocyte TLR5 in recognizing flagellin, policing bacteria, and protecting against liver disease.
Methods
Mice were engineered to lack TLR5 specifically in hepatocytes ($TLR5^{\Delta Hep}$) and analyzed relative to sibling controls ($TLR5^{fl/fl}$). TLR5 messenger RNA levels, responses to exogenous flagellin, elimination of circulating motile bacteria, and susceptibility of liver injury (concanavalin A, carbon tetrachloride, methionine- and choline-deficient diet, and HFD) were measured.
Results
$TLR5^{\Delta Hep}$ expressed similar levels of TLR5 as $TLR5^{fl/fl}$ in all organs examined, except in the liver, which showed a 90% reduction in TLR5 levels, indicating that hepatocytes accounted for the major portion of TLR5 expression in this organ. $TLR5^{\Delta Hep}$ showed impairment in responding to purified flagellin and clearing flagellated bacteria from the liver. Although $TLR5^{\Delta Hep}$ mice did not differ markedly from sibling controls in concanavalin A or carbon tetrachloride–induced liver injury models, they showed exacerbated disease in response to a methionine- and choline-deficient diet and HFD. Such predisposition of $TLR5^{\Delta Hep}$ to diet-induced liver pathology was associated with increased expression of proinflammatory cytokines, which was dependent on the Nod-like-receptor C4 inflammasome and rescued by microbiota ablation.
Conclusions
Hepatocyte TLR5 plays a critical role in protecting liver against circulating gut bacteria and against diet-induced liver disease.

4.1606 Blocking Notch signal in myeloid cells alleviates hepatic ischemia reperfusion injury by repressing the activation of NF-κB through CYLD

Yu, H-C., Bai, L., Yang, Z-X., Qin, H-Y., Tao, K-S., Han, H. and Dou, K-F.
*Scientific Reports, **6**:32226 (2016)*

Ischemia-reperfusion (I/R) is a major reason of hepatocyte injury during liver surgery and transplantation. Myeloid cells including macrophages and neutrophils play important roles in sustained tissue inflammation and damage, but the mechanisms regulating myeloid cells activity have been elusive. In this study, we investigate the role of Notch signaling in myeloid cells during hepatic I/R injury by using a mouse model of myeloid specific conditional knockout of RBP-J. Myeloid-specific RBP-J deletion alleviated hepatic I/R injury. RBP-J deletion in myeloid cells decreased hepatocytes apoptosis after hepatic I/R injury. Furthermore, myeloid-specific RBP-J deletion led to attenuated inflammation response in liver after I/R injury. Consistently, Notch blockade reduced the production of inflammatory cytokines by macrophages in vitro. We also found that blocking Notch signaling reduced NF-κB activation and increased cylindromatosis (CYLD) expression and knockdown of CYLD rescued reduction of inflammatory cytokines induced by Notch blockade in macrophages during I/R injury in vitro. On the other hand, activation of Notch signaling in macrophages led to increased inflammatory cytokine production and NF-κB activation and decreased CYLD expression in vitro. These data suggest that activation of Notch signaling in myeloid cells aggravates I/R injury, by enhancing the inflammation response by NF-κB through down regulation of CYLD.

4.1607 ICER is requisite for Th17 differentiation

Yoshida, N., Comte, D., Mizui, M., Otomo, K., Rosetti, F., Mayadas, T.N., Crispin, J.C., Bradley, S.J., Koga, T., Kono, M., karampetsou, M.P., Kyttaris, V.C., Tenbrock, K. and Ysokos, G.C:
*Nature Communications, **7**:12993 (2016)*

Inducible cAMP early repressor (ICER) has been described as a transcriptional repressor isoform of the

cAMP response element modulator (CREM). Here we report that ICER is predominantly expressed in Th17 cells through the IL-6–STAT3 pathway and binds to the Il17a promoter, where it facilitates the accumulation of the canonical enhancer RORγt. In vitro differentiation from naive ICER/CREM-deficient CD4+ T cells to Th17 cells is impaired but can be rescued by forced overexpression of ICER. Consistent with a role of Th17 cells in autoimmune and inflammatory diseases, ICER/CREM-deficient B6.lpr mice are protected from developing autoimmunity. Similarly, both anti-glomerular basement membrane-induced glomerulonephritis and experimental encephalomyelitis are attenuated in ICER/CREM-deficient mice compared with their ICER/CREM-sufficient littermates. Importantly, we find ICER overexpressed in CD4+ T cells from patients with systemic lupus erythematosus. Collectively, our findings identify a unique role for ICER, which affects both organ-specific and systemic autoimmunity in a Th17-dependent manner.

4.1608 Identification of early gene expression changes in primary cultured neurons treated with topoisomerase I poisons

Rossi, S.L., Lumpkin, C.J., Harris, A.W., Holbrook, J., Gentillon, C., McVahan, S.M., Wang, W. and Butchbach, M.E.R.

Biochem. Biophys. Res. Comm., ***479***, *319-324 (2016)*

Topoisomerase 1 (TOP1) poisons like camptothecin (CPT) are currently used in cancer chemotherapy but these compounds can have damaging, off-target effects on neurons leading to cognitive, sensory and motor deficits. To understand the molecular basis for the enhanced sensitivity of neurons to CPT, we examined the effects of compounds that inhibit TOP1—CPT, actinomycin D (ActD) and β-lapachone (β-Lap)—on primary cultured rat motor (MN) and cortical (CN) neurons as well as fibroblasts. Neuronal cells expressed higher levels of *Top1* mRNA than fibroblasts but transcript levels are reduced in all cell types after treatment with CPT. Microarray analysis was performed to identify differentially regulated transcripts in MNs in response to a brief exposure to CPT. Pathway analysis of the differentially expressed transcripts revealed activation of ERK and JNK signaling cascades in CPT-treated MNs. Immediate-early genes like *Fos*, *Egr-1* and *Gadd45b* were upregulated in CPT-treated MNs. *Fos* mRNA levels were elevated in all cell types treated with CPT; *Egr-1*, *Gadd45b* and *Dyrk3* transcript levels, however, increased in CPT-treated MNs and CNs but decreased in CPT-treated fibroblasts. These transcripts may represent new targets for the development of therapeutic agents that mitigate the off-target effects of chemotherapy on the nervous system.

4.1609 Characterization of brevetoxin (PbTx-3) exposure in neurons of the anoxia-tolerant freshwater turtle (Trachemys scripta)

Cocilova, C.C. and Milton, S.L.

Quatic Toxicology, ***180***, *115-122 (2016)*

Harmful algal blooms are increasing in frequency and extent worldwide and occur nearly annually off the west coast of Florida where they affect both humans and wildlife. The dinoflagellate *Karenia brevis* is a key organism in Florida red tides that produces a suite of potent neurotoxins collectively referred to as the brevetoxins (PbTx). Brevetoxins bind to and open voltage gated sodium channels (VGSC), increasing cell permeability in excitable cells and depolarizing nerve and muscle tissue. Exposed animals may thus show muscular and neurological symptoms including head bobbing, muscle twitching, paralysis, and coma; large HABs can result in significant morbidity and mortality of marine life, including fish, birds, marine mammals, and sea turtles. Brevetoxicosis however is difficult to treat in endangered sea turtles as the physiological impacts have not been investigated and the magnitude and duration of brevetoxin exposure are generally unknown. In this study we used the freshwater turtle *Trachemys scripta* as a model organism to investigate the effects of the specific brevetoxin PbTx-3 in the turtle brain. Primary turtle neuronal cell cultures were exposed to a range of PbTx-3 concentrations to determine excitotoxicity. Agonists and antagonists of voltage-gated sodium channels and downstream targets were utilized to confirm the toxin's mode of action. We found that turtle neurons are highly resistant to PbTx-3; while cell viability decreased in a dose dependent manner across PbTx-3 concentrations of 100–2000 nM, the EC_{50} was significantly higher than has been reported in mammalian neurons. PbTx-3 exposure resulted in significant Ca^{2+} influx, which could be fully abrogated by the VGSC antagonist tetrodotoxin, NMDA receptor blocker MK-801, and tetanus toxin, indicating that the mode of action in turtle neurons is the same as in mammalian cells. As both turtle and mammalian VGSCs have a high affinity for PbTx-3, we suggest that the high resistance of the turtle neuron to PbTx-3 may be related to its ability to withstand anoxic depolarization. The ultimate goal of this work is to design treatment protocols for sea turtles exposed to red tides worldwide.

4.1610 Acoustic Cell Manipulation
Lenshof, A., Johannesson, C., Evander, M., Nilsson, J. and Laurel, T.
Microtechnology for Cell Manipulation and Sorting, 129-173 (2016)

This chapter reviews recent developments in the field of acoustic manipulation and processing of cells in microfluidic systems and gives an overview of different acoustofluidic operating modalities. Continuous flow-based acoustophoresis and acoustic trapping are key areas of interest. In view of the topic of this publication we have limited this chapter to mainly cover acoustofluidic work that concerns cell handling and cell-based studies. A focus is therefore maintained on developments that demonstrate how microscale acoustofluidic systems can be designed to solve unmet needs in the everyday work of life science laboratories related to cell biology or clinically relevant research.

4.1611 End Sequence Analysis Toolkit (ESAT) expands the extractable information from single-cell RNA-seq data
Alan Derr, Chaoxing Yang, Rapolas Zilionis, Alexey Sergushichev, David M. Blodgett, Sambra Redick, Rita Bortell, Jeremy Luban, David M. Harlan, Sebastian Kadener, Dale L. Greiner, Allon Klein, Maxim N. Artyomov, and Manuel Garber
*Genome Res., **26**, 1397-1410 (2016)*

RNA-seq protocols that focus on transcript termini are well suited for applications in which template quantity is limiting. Here we show that, when applied to end-sequencing data, analytical methods designed for global RNA-seq produce computational artifacts. To remedy this, we created the End Sequence Analysis Toolkit (ESAT). As a test, we first compared end-sequencing and bulk RNA-seq using RNA from dendritic cells stimulated with lipopolysaccharide (LPS). As predicted by the telescripting model for transcriptional bursts, ESAT detected an LPS-stimulated shift to shorter 3′-isoforms that was not evident by conventional computational methods. Then, droplet-based microfluidics was used to generate 1000 cDNA libraries, each from an individual pancreatic islet cell. ESAT identified nine distinct cell types, three distinct β-cell types, and a complex interplay between hormone secretion and vascularization. ESAT, then, offers a much-needed and generally applicable computational pipeline for either bulk or single-cell RNA end-sequencing.

4.1612 Clinical Islet Isolation
Hawthorne, W.J., Williams, L. and Chew, Y.V.
*Advances in Exp. Med., **938**, 89-122 (2016)*

The overarching success of islet transplantation relies on the success in the laboratory to isolate the islets. This chapter focuses on the processes of human islet cell isolation and the ways to optimally provide islet cells for transplantation. The major improvements in regards to the choice of enzyme type, way the digested pancreas tissue is handled to best separate islets from the acinar and surrounding tissues, the various methods of purification of the islets, their subsequent culture and quality assurance to improve outcomes to culminate in safe and effective islet transplantation will be discussed. After decades of improvements, islet cell isolation and transplantation now clearly offer a safe, effective and feasible therapeutic treatment option for an increasing number of patients suffering from type 1 diabetes specifically for those with severe hypoglycaemic unawareness.

4.1613 Miscellaneous Pathogens
Austin, B. and Austin, D.A.
Bacterial Fish Pathogens, 603-642 (2016)

Pseudoalteromonas piscicida, Pseudoalteromonas undina, Shewanella putrefaciens, Arcobacter cryaerophilus, Halomonas (=Deleya) cupida, Acinetobacter sp., *Moraxella* sp., *Moritella marina, Moritella viscosa, Mycoplasma mobile, Myxococcus piscicola, Aquaspirillum* sp., *Janthinobacterium lividum, Pasteurella skyensis, Piscirickettsia salmonis,* Rickettsia-like organisms, *Streptobacillus,* '*Candidatus* Arthromitus', '*Candidatus* Branchiomonas cysticola', '*Candidatus* Clavochlamydia salmonicola', '*Candidatus* Piscichlamydia salmonis' and '*Candidatus* Renichlamydia lutjani' have been associated with fish diseases. *Moritella viscosa* has been recovered from winter ulcer disease (= skin lesions) in Atlantic salmon with pathogenicity mechanisms reflecting the presence of extracellular products. Protection has been achieved with an adjuvanted formalin inactivated whole cell vaccine. *Piscirickettsia salmonis* is an obligate parasite, which has been associated with coho salmon syndrome, Huito disease and salmonid rickettsial septicaemia. Good protection was recorded by use of a formalised

whole cell suspension. '*Candidatus*' are uncultured organisms, which may be visualised in pathological material.

4.1614 **HMGB1 Activates Proinflammatory Signaling via TLR5 Leading to Allodynia**
Das, N., Dewan, V., Grace, P. et al
*Cell Reports, **17**, 1128-1140 (2016)*

Infectious and sterile inflammatory diseases are correlated with increased levels of high mobility group box 1 (HMGB1) in tissues and serum. Extracellular HMGB1 is known to activate Toll-like receptors (TLRs) 2 and 4 and RAGE (receptor for advanced glycation endproducts) in inflammatory conditions. Here, we find that TLR5 is also an HMGB1 receptor that was previously overlooked due to lack of functional expression in the cell lines usually used for studying TLR signaling. HMGB1 binding to TLR5 initiates the activation of NF-κB signaling pathway in a MyD88-dependent manner, resulting in proinflammatory cytokine production and pain enhancement in vivo. Biophysical and in vitro results highlight an essential role for the C-terminal tail region of HMGB1 in facilitating interactions with TLR5. These results suggest that HMGB1-modulated TLR5 signaling is responsible for pain hypersensitivity.

4.1615 **Helicobacter suis affects the health and function of porcine gastric parietal cells**
Zhang, G., Ducatelle, R., Mihi, B., Smet, A., Flahou, B. and Haesebrouk, F.
*Vet. Res., **47**:10 (2016)*

The stomach of pigs at slaughter age is often colonized by *Helicobacter* (*H.*) *suis*, which is also the most prevalent gastric non-*H. pylori Helicobacter* (NHPH) species in humans. It is associated with chronic gastritis, gastric ulceration and other gastric pathological changes in both hosts. Parietal cells are highly specialized, terminally differentiated epithelial cells responsible for gastric acid secretion and regulation. Dysfunction of these cells is closely associated with gastric pathology and disease. Here we describe a method for isolation and culture of viable and responsive parietal cells from slaughterhouse pigs. In addition, we investigated the interactions between *H. suis* and gastric parietal cells both in *H. suis*-infected six-month-old slaughter pigs, as well as in our in vitro parietal cell model. A close interaction of *H. suis* and parietal cells was observed in the fundic region of stomachs from *H. suis* positive pigs. The bacterium was shown to be able to directly interfere with cultured porcine parietal cells, causing a significant impairment of cell viability. Transcriptional levels of Atp4a, essential for gastric acid secretion, showed a trend towards an up-regulation in *H. suis* positive pigs compared to *H. suis*-negative pigs. In addition, sonic hedgehog, an important factor involved in gastric epithelial differentiation, gastric mucosal repair, and stomach homeostasis, was also significantly up-regulated in *H. suis* positive pigs. In conclusion, this study describes a successful approach for the isolation and culture of porcine gastric parietal cells. The results indicate that *H. suis* affects the viability and function of this cell type.

4.1616 **Non-invasive epicutaneous vaccine against Respiratory Syncytial Virus: Preclinical proof of concept**
Herve, P-L., Descamps, D., Deloizy, C., Dhelft, V., Laubreton, D., Bouguyon, E., Boukadiri, A., Dubuquoy, C., Larcher, T., Benhamou, P-H., Benhamou, P-H., Eleouët, J.F., bertho, N., Mondoulet, L. and Riffault, S.
*J. Controlled Release, **243**, 146-159 (2016)*

To put a Respiratory Syncytial Virus (RSV) vaccine onto the market, new vaccination strategies combining scientific and technical innovations need to be explored. Such a vaccine would also need to be adapted to the vaccination of young children that are the principal victims of acute RSV infection. In the present project, we describe the development and the preclinical evaluation of an original epicutaneous RSV vaccine that combines two technologies: Viaskin® epicutaneous patches as a delivery platform and RSV N-nanorings (N) as a subunit antigen. Such a needle-free vaccine may have a better acceptability for the vaccination of sensible population such as infants since it does not require any skin preparation. Moreover, this self-applicative vaccine would overcome some issues associated to injectable vaccines such as the requirement of sterile medical devices, the need of skilled health-care professionals and the necessity of stringent store conditions. Here, we demonstrate that Viaskin® patches loaded with a formulation containing N-nanorings (Viaskin®-N) are highly immunogenic in mice and promotes a Th1/Th17 oriented immune response. More importantly, Viaskin®-N epicutaneous vaccine confers a high level of protection against viral replication upon RSV challenge in mice, without exacerbating clinical symptoms. In swine, which provides the best experimental model for the transcutaneous passage of drug/antigen in human skin, we have shown that GFP fluorescent N-nanorings, delivered epicutaneously with Viaskin® patches, are taken up by epidermal Langerhans cells. We have also demonstrated that

Viaskin®-N induced a significant RSV N-specific T-cell response in pig. In conclusion, Viaskin®-N epicutaneous vaccine seems efficient to protect against RSV infection in animal model.

4.1617 **Histamine H3 receptor activation stimulates calcium mobilization in a subpopulation of rat striatal neurons in primary culture, but not in synaptosomes**
Rivera-Ramirez, N., Montejo-Lopez, W., Lopez-Mendez. M-C., Guerrero-Hernandez, A., Molina-Hernandez, A., Garcia-Hernandez-U. and Arias-Montano, J-A.
Neurochem. Int., ***101***, *38-47 (2016)*

The histamine H_3 receptor (H_3R) is abundantly expressed in the Central Nervous System where it regulates several functions pre and postsynaptically. H_3Rs couple to $G\alpha_{i/o}$ proteins and trigger or modulate several intracellular signaling pathways, including the cAMP/PKA pathway and the opening of N- and P/Q-type voltage-gated Ca^{2+} channels. In transfected cells, activation of the human H_3R of 445 amino acids (hH_3R_{445}) results in phospholipase C (PLC) stimulation and release of Ca^{2+} from intracellular stores. In this work we have studied whether H_3R activation induces Ca^{2+} mobilization from intracellular stores in native systems, either isolated nerve terminals (synaptosomes) or neurons in primary culture. In rat striatal synaptosomes H_3R activation induced inositol 1,4,5-trisphosphate (IP_3) formation but failed to increase the intracellular calcium concentration ($[Ca^{2+}]_i$). In striatal primary cultures H_3R activation resulted in IP_3 formation and increased the $[Ca^{2+}]_i$ in 18 out of 70 cells that responded with an elevation in the $[Ca^{2+}]_i$ to membrane depolarization with KCl (100 mM) as evaluated by microfluorometry. Confocal microscopy studies corroborated the increase in $[Ca^{2+}]i$ induced by H_3R activation in a fraction of those cells that were responsive to membrane depolarization. These results indicate that H_3R activation stimulates the $PLC/IP_3/Ca^{2+}$ pathway but only in a subpopulation of striatal neurons.

4.1618 **National Institutes of Health–Sponsored Clinical Islet Transplantation Consortium Phase 3 Trial: Manufacture of a Complex Cellular Product at Eight Processing Facilities**
Ricordi, C., Goldstein, J.S., Balamurugan, A.N. et al
Diabetes, ***65(11)***, *3418-3428 (2016)*

Eight manufacturing facilities participating in the National Institutes of Health–sponsored Clinical Islet Transplantation (CIT) Consortium jointly developed and implemented a harmonized process for the manufacture of allogeneic purified human pancreatic islet (PHPI) product evaluated in a phase 3 trial in subjects with type 1 diabetes. Manufacturing was controlled by a common master production batch record, standard operating procedures that included acceptance criteria for deceased donor organ pancreata and critical raw materials, PHPI product specifications, certificate of analysis, and test methods. The process was compliant with Current Good Manufacturing Practices and Current Good Tissue Practices. This report describes the manufacturing process for 75 PHPI clinical lots and summarizes the results, including lot release. The results demonstrate the feasibility of implementing a harmonized process at multiple facilities for the manufacture of a complex cellular product. The quality systems and regulatory and operational strategies developed by the CIT Consortium yielded product lots that met the prespecified characteristics of safety, purity, potency, and identity and were successfully transplanted into 48 subjects. No adverse events attributable to the product and no cases of primary nonfunction were observed.

4.1619 **Plasticity and Aggregation of Juvenile Porcine Islets in Modified Culture: Preliminary Observations**
Weegman, B.P., Taylor, M.J., Baicu, S.C., Mueller, K., O'Brien, T.D., Wilson, J. and Papas, K.K.
Cell Transplant., ***25***, *1763-1775 (2016)*

Diabetes is a major health problem worldwide, and there is substantial interest in developing xenogeneic islet transplantation as a potential treatment. The potential to relieve the demand on an inadequate supply of human pancreata is dependent upon the efficiency of techniques for isolating and culturing islets from the source pancreata. Porcine islets are favored for xenotransplantation, but mature pigs (>2 years) present logistic and economic challenges, and young pigs (3-6 months) have not yet proven to be an adequate source. In this study, islets were isolated from 20 juvenile porcine pancreata (~3 months; 25 kg Yorkshire pigs) immediately following procurement or after 24 h of hypothermic machine perfusion (HMP) preservation. The resulting islet preparations were characterized using a battery of tests during culture in silicone rubber membrane flasks. Islet biology assessment included oxygen consumption, insulin secretion, histopathology, and in vivo function. Islet yields were highest from HMP-preserved pancreata (2,242 ± 449 IEQ/g). All preparations comprised a high proportion (>90%) of small islets (<100 μm), and purity was on average 63 ± 6%. Morphologically, islets appeared as clusters on day 0, loosely disaggregated structures at day 1, and transitioned to aggregated structures comprising both exocrine and endocrine cells by day 6.

Histopathology confirmed both insulin and glucagon staining in cultures and grafts excised after transplantation in mice. Nuclear staining (Ki-67) confirmed mitotic activity consistent with the observed plasticity of these structures. Metabolic integrity was demonstrated by oxygen consumption rates = 175 ± 16 nmol/min/mg DNA, and physiological function was intact by glucose stimulation after 6-8 days in culture. In vivo function was confirmed with blood glucose control achieved in nearly 50% (8/17) of transplants. Preparation and culture of juvenile porcine islets as a source for islet transplantation require specialized conditions. These immature islets undergo plasticity in culture and form fully functional multicellular structures. Further development of this method for culturing immature porcine islets is expected to generate small pancreatic tissue-derived organoids termed "pancreatites," as a therapeutic product from juvenile pigs for xenotransplantation and diabetes research.

4.1620 Prior voluntary wheel running attenuates neuropathic pain
Grace, P.M., Fabisiak, T.J., Green-Fulgham, S.M., Anderson, N.D., Strand, K.A., Kwilasz, A.J., Galer, E.L., Walker, F.R., Greenwood, B.N., Maier, S.F., Fleshner, M. and Watkins, L.R.
*Pain, **157**, 2012-2023 (2016)*

Exercise is known to exert a systemic anti-inflammatory influence, but whether its effects are sufficient to protect against subsequent neuropathic pain is underinvestigated. We report that 6 weeks of voluntary wheel running terminating before chronic constriction injury (CCI) prevented the full development of allodynia for the ~3-month duration of the injury. Neuroimmune signaling was assessed at 3 and 14 days after CCI. Prior exercise normalized ipsilateral dorsal spinal cord expression of neuroexcitatory interleukin (IL)-1β production and the attendant glutamate transporter GLT-1 decrease, as well as expression of the disinhibitory P2X4R-BDNF axis. The expression of the macrophage marker Iba1 and the chemokine CCL2 (MCP-1), and a neuronal injury marker (activating transcription factor 3), was attenuated by prior running in the ipsilateral lumbar dorsal root ganglia. Prior exercise suppressed macrophage infiltration and/or injury site proliferation, given decreased presence of macrophage markers Iba1, iNOS (M1), and Arg-1 (M2; expression was time dependent). Chronic constriction injury–driven increases in serum proinflammatory chemokines were suppressed by prior running, whereas IL-10 was increased. Peripheral blood mononuclear cells were also stimulated with lipopolysaccharide ex vivo, wherein CCI-induced increases in IL-1β, nitrite, and IL-10 were suppressed by prior exercise. Last, unrestricted voluntary wheel running, beginning either the day of, or 2 weeks after, CCI, progressively reversed neuropathic pain. This study is the first to investigate the behavioral and neuroimmune consequences of regular exercise terminating before nerve injury. This study suggests that chronic pain should be considered a component of "the diseasome of physical inactivity," and that an active lifestyle may prevent neuropathic pain.

4.1621 The long non-coding RNA Morrbid regulates Bim and short-lived myeloid cell lifespan
Kotzin, J.J. et al
*Nature, **537(7619)**, 239-243 (2016)*

Neutrophils, eosinophils and 'classical' monocytes collectively account for about 70% of human blood leukocytes and are among the shortest-lived cells in the body[1, 2]. Precise regulation of the lifespan of these myeloid cells is critical to maintain protective immune responses and minimize the deleterious consequences of prolonged inflammation[1, 2]. However, how the lifespan of these cells is strictly controlled remains largely unknown. Here we identify a long non-coding RNA that we termed *Morrbid*, which tightly controls the survival of neutrophils, eosinophils and classical monocytes in response to pro-survival cytokines in mice. To control the lifespan of these cells, *Morrbid* regulates the transcription of the neighbouring pro-apoptotic gene, *Bcl2l11* (also known as *Bim*), by promoting the enrichment of the PRC2 complex at the *Bcl2l11* promoter to maintain this gene in a poised state. Notably, *Morrbid* regulates this process in *cis*, enabling allele-specific control of *Bcl2l11* transcription. Thus, in these highly inflammatory cells, changes in *Morrbid* levels provide a locus-specific regulatory mechanism that allows rapid control of apoptosis in response to extracellular pro-survival signals. As *MORRBID* is present in humans and dysregulated in individuals with hypereosinophilic syndrome, this long non-coding RNA may represent a potential therapeutic target for inflammatory disorders characterized by aberrant short-lived myeloid cell lifespan.

4.1622 Fetal liver endothelium regulates the seeding of tissue-resident macrophages
Rantakari, P., Jäppinen, N., Lokka, E., Mokkala, E., Gerke, H., Peuhu, E., Ivaska, J., Elima, K., Auvinen, K. and Salmi, M.

Nature, ***538(7625)***, *392-396 (2016)*

Macrophages are required for normal embryogenesis, tissue homeostasis and immunity against microorganisms and tumours[1, 2, 3, 4]. Adult tissue-resident macrophages largely originate from long-lived, self-renewing embryonic precursors and not from haematopoietic stem-cell activity in the bone marrow[4, 5]. Although fate-mapping studies have uncovered a great amount of detail on the origin and kinetics of fetal macrophage development in the yolk sac and liver[6, 7, 8, 9, 10, 11], the molecules that govern the tissue-specific migration of these cells remain completely unknown. Here we show that an endothelium-specific molecule, plasmalemma vesicle-associated protein (PLVAP), regulates the seeding of fetal monocyte-derived macrophages to tissues in mice. We found that PLVAP-deficient mice have completely normal levels of both yolk-sac- and bone-marrow-derived macrophages, but that fetal liver monocyte-derived macrophage populations were practically missing from tissues. Adult PLVAP-deficient mice show major alterations in macrophage-dependent iron recycling and mammary branching morphogenesis. PLVAP forms diaphragms in the fenestrae of liver sinusoidal endothelium during embryogenesis, interacts with chemoattractants and adhesion molecules and regulates the egress of fetal liver monocytes to the systemic vasculature. Thus, PLVAP selectively controls the exit of macrophage precursors from the fetal liver and, to our knowledge, is the first molecule identified in any organ as regulating the migratory events during embryonic macrophage ontogeny.

4.1623 **Visceral motor neuron diversity delineates a cellular basis for nipple- and pilo-erection muscle control**
Furlan, A., La Manno, G., Lübke, M., Häring, M., Abdo, H., Hochgerner, H., Kupari, J., Usoskin, D., Airaksinen, M.S., Oliver, G., Linnarsson, S. and Ernfors, P.
Nature Neurosci., ***19(10)***, *1331-1340 (2016)*

Despite the variety of physiological and target-related functions, little is known regarding the cellular complexity in the sympathetic ganglion. We explored the heterogeneity of mouse stellate and thoracic ganglia and found an unexpected variety of cell types. We identified specialized populations of nipple- and pilo-erector muscle neurons. These neurons extended axonal projections and were born among other neurons during embryogenesis, but remained unspecialized until target organogenesis occurred postnatally. Target innervation and cell-type specification was coordinated by an intricate acquisition of unique combinations of growth factor receptors and the initiation of expression of concomitant ligands by the nascent erector muscles. Overall, our results provide compelling evidence for a highly sophisticated organization of the sympathetic nervous system into discrete outflow channels that project to well-defined target tissues and offer mechanistic insight into how diversity and connectivity are established during development.

4.1624 **CCL2, but not its receptor, is essential to restrict immune privileged central nervous system-invasion of Japanese encephalitis virus via regulating accumulation of CD11b+ Ly-6Chi monocytes**
Kim, J.H., Patil, A.M., Choi, J.Y., Kim, S.B., Uyangaa, E., Hossain, F.M.A., Park, S-Y., Lee, J.H., Kim, K. and Eo, S.K.
Immunology, ***149(2)***, *186-203 (2016)*

Japanese encephalitis virus (JEV) is a re-emerging zoonotic flavivirus that poses an increasing threat to global health and welfare due to rapid changes in climate and demography. Although the CCR2–CCL2 axis plays an important role in trafficking $CD11b^{+}$ $Ly\text{-}6C^{hi}$ monocytes to regulate immunopathological diseases, little is known about their role in monocyte trafficking during viral encephalitis caused by JEV infection. Here, we explored the role of CCR2 and its ligand CCL2 in JE caused by JEV infection using CCR2- and CCL2-ablated murine models. Somewhat surprisingly, the ablation of CCR2 and CCL2 resulted in starkly contrasting susceptibility to JE. CCR2 ablation induced enhanced resistance to JE, whereas CCL2 ablation highly increased susceptibility to JE. This contrasting regulation of JE progression by CCR2 and CCL2 was coupled to central nervous system (CNS) infiltration of $Ly\text{-}6C^{hi}$ monocytes and $Ly\text{-}6G^{hi}$ granulocytes. There was also enhanced expression of CC and CXC chemokines in the CNS of CCL2-ablated mice, which appeared to induce CNS infiltration of these cell populations. However, our data revealed that contrasting regulation of JE in CCR2- and CCL2-ablated mice was unlikely to be mediated by innate natural killer and adaptive T-cell responses. Furthermore, CCL2 produced by haematopoietic stem cell-derived leucocytes played a dominant role in CNS accumulation of $Ly\text{-}6C^{hi}$ monocytes in infected bone marrow chimeric models, thereby exacerbating JE progression. Collectively, our data indicate that CCL2 plays an essential role in conferring protection against JE caused by JEV infection. In addition, blockage of CCR2, but not CCL2, will aid in the development of strategies for

prophylactics and therapeutics of JE.

4.1625 VOLIN and KJON—Two novel hyperdiploid myeloma cell lines
Våtsveen, T., Børset, M., Dikic, A., Tian, E., Micci, F., Lid, A.H.B., Meza-Zepeda, L.A., Coward, E., Waage, A., Sundan, A., Kuehl, W.M. and Holien, T.
*Genes, Chromosomes & Cancer, **55(11)**, 890-901 (2016)*

Multiple myeloma can be divided into two distinct genetic subgroups: hyperdiploid (HRD) or nonhyperdiploid (NHRD) myeloma. Myeloma cell lines are important tools to study myeloma cell biology and are commonly used for preclinical screening and testing of new drugs. With few exceptions human myeloma cell lines are derived from NHRD patients, even though about half of the patients have HRD myeloma. Thus, there is a need for cell lines of HRD origin to enable more representative preclinical studies. Here, we present two novel myeloma cell lines, VOLIN and KJON. Both of them were derived from patients with HRD disease and shared the same genotype as their corresponding primary tumors. The cell lines' chromosomal content, genetic aberrations, gene expression, immunophenotype as well as some of their growth characteristics are described. Neither of the cell lines was found to harbor immunoglobulin heavy chain translocations. The VOLIN cell line was established from a bone marrow aspirate and KJON from peripheral blood. We propose that these unique cell lines may be used as tools to increase our understanding of myeloma cell biology.

4.1626 Cell sources for in vitro human liver cell culture models
Zeilinger, K., Freyer, N., Damm, G., Seehofer, D. and Knöspel, F.
*Exp. Biol. Med., **241(15)**, 1684-1698 (2016)*

In vitro liver cell culture models are gaining increasing importance in pharmacological and toxicological research. The source of cells used is critical for the relevance and the predictive value of such models. Primary human hepatocytes (PHH) are currently considered to be the gold standard for hepatic *in vitro* culture models, since they directly reflect the specific metabolism and functionality of the human liver; however, the scarcity and difficult logistics of PHH have driven researchers to explore alternative cell sources, including liver cell lines and pluripotent stem cells. Liver cell lines generated from hepatomas or by genetic manipulation are widely used due to their good availability, but they are generally altered in certain metabolic functions. For the past few years, adult and pluripotent stem cells have been attracting increasing attention, due their ability to proliferate and to differentiate into hepatocyte-like cells *in vitro*. However, controlling the differentiation of these cells is still a challenge. This review gives an overview of the major human cell sources under investigation for *in vitro* liver cell culture models, including primary human liver cells, liver cell lines, and stem cells. The promises and challenges of different cell types are discussed with a focus on the complex 2D and 3D culture approaches under investigation for improving liver cell functionality *in vitro*. Finally, the specific application options of individual cell sources in pharmacological research or disease modeling are described.

4.1627 Sevelamer Improves Steatohepatitis, Inhibits Liver and Intestinal Farnesoid X Receptor (FXR), and Reverses Innate Immune Dysregulation in a Mouse Model of Non-alcoholic Fatty Liver Disease
McGettigan, B.M., McMahan, R.H., Luo, Y., Wang, X.X., Orlicky, D.J., Porsche, C., Levi, M. and Rosen, H.R.
*J. Biol. Chem., **291(44)**, 23058-23067 (2016)*

Bile acid sequestrants are synthetic polymers that bind bile acids in the gut and are used to treat dyslipidemia and hyperphosphatemia. Recently, these agents have been reported to lower blood glucose and increase insulin sensitivity by altering bile acid signaling pathways. In this study, we assessed the efficacy of sevelamer in treating mice with non-alcoholic fatty liver disease (NAFLD). We also analyzed how sevelamer alters inflammation and bile acid signaling in NAFLD livers. Mice were fed a low-fat or Western diet for 12 weeks followed by a diet-plus-sevelamer regimen for 2 or 12 weeks. At the end of treatment, disease severity was assessed, hepatic leukocyte populations were examined, and expression of genes involved in farnesoid X receptor (FXR) signaling in the liver and intestine was analyzed. Sevelamer treatment significantly reduced liver steatosis and lobular inflammation. Sevelamer-treated NAFLD livers had notably fewer pro-inflammatory infiltrating macrophages and a significantly greater fraction of alternatively activated Kupffer cells compared with controls. Expression of genes involved in FXR signaling in the liver and intestine was significantly altered in mice with NAFLD as well as in those treated with sevelamer. In a mouse model of NAFLD, sevelamer improved disease and counteracted innate immune cell dysregulation in the liver. This study also revealed a dysregulation of FXR signaling in the

liver and intestine of NAFLD mice that was counteracted by sevelamer treatment.

4.1628 A Single-Cell Transcriptomic Map of the Human and Mouse Pancreas Reveals Inter- and Intra-cell Population Structure
Baron, M., Veres, A., Wolock, S.L. et al
*Cell Systems, **3(4)**, 346-360 (2016)*

Although the function of the mammalian pancreas hinges on complex interactions of distinct cell types, gene expression profiles have primarily been described with bulk mixtures. Here we implemented a droplet-based, single-cell RNA-seq method to determine the transcriptomes of over 12,000 individual pancreatic cells from four human donors and two mouse strains. Cells could be divided into 15 clusters that matched previously characterized cell types: all endocrine cell types, including rare epsilon-cells; exocrine cell types; vascular cells; Schwann cells; quiescent and activated stellate cells; and four types of immune cells. We detected subpopulations of ductal cells with distinct expression profiles and validated their existence with immuno-histochemistry stains. Moreover, among human beta- cells, we detected heterogeneity in the regulation of genes relating to functional maturation and levels of ER stress. Finally, we deconvolved bulk gene expression samples using the single-cell data to detect disease-associated differential expression. Our dataset provides a resource for the discovery of novel cell type-specific transcription factors, signaling receptors, and medically relevant genes.

4.1629 Adult human pancreas-derived cells expressing stage-specific embryonic antigen 4 differentiate into Sox9-expressing and Ngn3-expressing pancreatic ducts in vivo
Lee, S., Lee, C.M. and Kim, S.C.
*Stem Cell Res. & Ther., **7**:162 (2016)*

Background
Tissue-specific stem/progenitor cells are found in various adult tissues and may have the capacity for lineage-specific differentiation, facilitating applications in autologous transplantation. Stage-specific embryonic antigen 4 (SSEA-4), an early embryonic glycolipid antigen, is expressed in cells derived from adult human pancreas exocrine tissue. Here, we examined the characteristics and lineage-specific differentiation capacity of SSEA-4$^+$ cells.
Methods
Human adult partial pancreas tissues were obtained from different donors and cultured in vitro. SSEA-4$^+$ and CA19-9$^+$ cells were isolated from adult human pancreas exocrine cells using magnetic-activated cell sorting, and gene expression was validated by quantitative polymerase chain reaction. To confirm in-vivo differentiation, SSEA-4$^+$ and CA19-9$^+$ cells were transplanted into the dorsal subcutaneous region of mice. Finally, morphological features of differentiated areas were confirmed by immunostaining and morphometric analysis.
Results
SSEA-4-expressing cells were detected in isolated pancreas exocrine cells from adult humans. These SSEA-4$^+$ cells exhibited coexpression of CA19-9, a marker of pancreatic duct cells, but not amylase expression, as shown by immunostaining and flow cytometry. SSEA-4$^+$ cells exhibited higher relative expression of *Oct4*, *Nanog*, *Klf4*, *Sox2*, and *c-Myc* mRNAs than CA19-9$^+$ cells. Pancreatic intralobular ducts (PIDs) were generated from SSEA-4$^+$ or CA19-9$^+$ cells in vivo at 5 weeks after transplantation. However, newly formed PIDs from CA19-9$^+$ cells were less abundant and showed an incomplete PID morphology. In contrast, newly formed PIDs from SSEA-4$^+$ cells were abundant in the transplanted area and showed a crowded morphology, typical of PIDs. Sox9 and Ngn3, key transcription factors associated with pancreatic development and regeneration, were expressed in PIDs from SSEA-4$^+$ cells.
Conclusions
SSEA-4-expressing cells in the adult human pancreas may have the potential for regeneration of the pancreas and may be used as a source of stem/progenitor cells for pancreatic cell lineage-specific differentiation.

4.1630 Red blood cell phase separation in symmetric and asymmetric microchannel networks: effect of capillary dilation and inflow velocity
Clavica, F., Homsy, A., jeandupeux, L. and Obrist, D.
*Scientific Reports, **6**:36763 (2016)*

The non-uniform partitioning or phase separation of red blood cells (RBCs) at a diverging bifurcation of a microvascular network is responsible for RBC heterogeneity within the network. The mechanisms

controlling RBC heterogeneity are not yet fully understood and there is a need to improve the basic understanding of the phase separation phenomenon. In this context, in vitro experiments can fill the gap between existing in vivo and in silico models as they provide better controllability than in vivo experiments without mathematical idealizations or simplifications inherent to in silico models. In this study, we fabricated simple models of symmetric/asymmetric microvascular networks; we provided quantitative data on the RBC velocity, line density and flux in the daughter branches. In general our results confirmed the tendency of RBCs to enter the daughter branch with higher flow rate (Zweifach-Fung effect); in some cases even inversion of the Zweifach-Fung effect was observed. We showed for the first time a reduction of the Zweifach-Fung effect with increasing flow rate. Moreover capillary dilation was shown to cause an increase of RBC line density and RBC residence time within the dilated capillary underlining the possible role of pericytes in regulating the oxygen supply.

4.1631 An optimized method for mouse liver sinusoidal endothelial cell isolation

Meyer, J., Lacotte, S., Morel, P., Gonelle-Gispert, C. and Bühler, L.
Exp. Cell Res., ***349***, *291-301 (2016)*

The objective of the present study was to develop an accurate and reproducible method for liver sinusoidal endothelial cell (LSEC) isolation in mice. Non-parenchymal cells were isolated using a modified two-step collagenase digestion combined with Optiprep density gradient centrifugation. LSEC were further purified using two prevalent methods, short-term selective adherence and CD146+ magnetic-activated cell sorting (MACS), and compared in terms of cell yield, viability and purity to our purification technique using CD11b cell depletion combined with long-term selective adherence. LSEC purification using our technique allowed to obtain 7.07±3.80 million LSEC per liver, while CD146+ MACS and short-term selective adherence yielded 2.94±1.28 and 0.99±0.66 million LSEC, respectively. Purity of the final cell preparation reached 95.10±2.58% when using our method. In contrast, CD146+ MACS and short-term selective adherence gave purities of 86.75±3.26% and 47.95±9.82%, respectively. Similarly, contamination by non-LSEC was the lowest when purification was performed using our technique, with a proportion of contaminating macrophages of only 1.87±0.77%. Further, isolated cells analysed by scanning electron microscopy presented typical LSEC fenestrations organized in sieve plates, demonstrating that the technique allowed to isolate *bona fide* LSEC. In conclusion, we described a reliable and reproducible technique for the isolation of high yields of pure LSEC in mice. This protocol provides an efficient method to prepare LSEC for studying their biological functions.

4.1632 Development of a toolbox to dissect host-endosymbiont interactions and protein trafficking in the trypanosomatid Angomonas deanei

Morales, J., Kokkori, S., Weidauer, D., Chapman, J., Goltsman, E., Rokhsar, D., Grossman, A.R. and Nowack, E.C.M.
BMC Evolutionary Biol., ***16****:247 (2016)*

Background
Bacterial endosymbionts are found across the eukaryotic kingdom and profoundly impacted eukaryote evolution. In many endosymbiotic associations with vertically inherited symbionts, highly complementary metabolic functions encoded by host and endosymbiont genomes indicate integration of metabolic processes between the partner organisms. While endosymbionts were initially expected to exchange only metabolites with their hosts, recent evidence has demonstrated that also host-encoded proteins can be targeted to the bacterial symbionts in various endosymbiotic systems. These proteins seem to participate in regulating symbiont growth and physiology. However, mechanisms required for protein targeting and the specific endosymbiont targets of these trafficked proteins are currently unexplored owing to a lack of molecular tools that enable functional studies of endosymbiotic systems.
Results
Here we show that the trypanosomatid *Angomonas deanei*, which harbors a β-proteobacterial endosymbiont, is readily amenable to genetic manipulation. Its rapid growth, availability of full genome and transcriptome sequences, ease of transfection, and high frequency of homologous recombination have allowed us to stably integrate transgenes into the *A. deanei* nuclear genome, efficiently generate null mutants, and elucidate protein localization by heterologous expression of a fluorescent protein fused to various putative targeting signals. Combining these novel tools with proteomic analysis was key for demonstrating the routing of a host-encoded protein to the endosymbiont, suggesting the existence of a specific endosymbiont-sorting machinery in *A. deanei*.
Conclusions
After previous reports from plants, insects, and a cercozoan amoeba we found here that also in *A. deanei*,

i.e. a member of a fourth eukaryotic supergroup, host-encoded proteins can be routed to the bacterial endosymbiont. This finding adds further evidence to our view that the targeting of host proteins is a general strategy of eukaryotes to gain control over and interact with a bacterial endosymbiont. The molecular resources reported here establish *A. deanei* as a time and cost efficient reference system that allows for a rigorous dissection of host-symbiont interactions that have been, and are still being shaped over evolutionary time. We expect this system to greatly enhance our understanding of the biology of endosymbiosis.

4.1633 Engineering Genetically-Encoded Mineralization and Magnetism via Directed Evolution
Liu, X., Lopez, P.A., Giessen, T.W., Gilers, M., Way, J.C. and Silver, P.A.
*Scientific Reports, **6**:38019 (2016)*

Genetically encoding the synthesis of functional nanomaterials such as magnetic nanoparticles enables sensitive and non-invasive biological sensing and control. Via directed evolution of the natural iron-sequestering ferritin protein, we discovered key mutations that lead to significantly enhanced cellular magnetism, resulting in increased physical attraction of ferritin-expressing cells to magnets and increased contrast for cellular magnetic resonance imaging (MRI). The magnetic mutants further demonstrate increased iron biomineralization measured by a novel fluorescent genetic sensor for intracellular free iron. In addition, we engineered Escherichia coli cells with multiple genomic knockouts to increase cellular accumulation of various metals. Lastly to explore further protein candidates for biomagnetism, we characterized members of the DUF892 family using the iron sensor and magnetic columns, confirming their intracellular iron sequestration that results in increased cellular magnetization.

4.1634 The Role of Progesterone and a Novel Progesterone Receptor, Progesterone Receptor Membrane Component 1, in the Inflammatory Response of Fetal Membranes to Ureaplasma parvum Infection
Feng, L., Ransom, C.E., Nazzai, M., Allen, T.K., Li, Y-J., Truong, T., Potts, L.C., Seed, P.-C. and Murtha, A.P.
*PloS One, **11(12)**, e0168102 (2016)*

Ureaplasma parvum (*U. parvum*) is gaining recognition as an important pathogen for chorioamnionitis and preterm premature rupture of membranes. We aimed to investigate the roles of progesterone (P4) and a novel progesterone receptor, progesterone receptor membrane component 1 (PGRMC1), in the response of fetal membranes to *U. parvum.* Fetal membrane cells (amnion, chorion and decidua) were isolated and confirmed to be free of *Mycoplasmataceae*. Cells were treated with *U. parvum* ($5x10^6$ CFU), and adherence was quantified by qPCR. Amnion and chorion cells were transfected with scrambled siRNA or validated PGRMC1 siRNA for 72h. Cells were then treated with *U. parvum* for 4h with or without pretreatment with P4 (10^{-7} M) or ethanol for 1h. Interleukin-8 (IL-8), matrix metalloproteinase 9 (MMP9) and cyclooxygenase (COX-2) mRNA expression were quantified by qRT-PCR. Culture medium was harvested and analyzed for IL-8 and prostaglandin (PGE_2) secretion by ELISA and MMP9 activity by zymography. *U. parvum* had a mean adherence of 15.0±0.6%, 16.9± 3.7% and 4.7±0.3% in cultured amnion, chorion and decidua cells, respectively. Exposure to *U. parvum* elicited significant inflammatory responses including induction of IL-8, COX-2, PGE2 and MMP9. A possible role of PGRMC1 was identified in the inhibition of *U. parvum*-stimulated COX-2 and MMP9 mRNA expression in chorion cells and MMP9 activity in amnion cells. On the other hand, it might enhance the *U. parvum*-stimulated IL-8 protein secretion in amnion cells. P4, mediated through PGRMC1, significantly inhibited *U. Parvum*-induced MMP9 mRNA and COX-2 mRNA expression in chorion cells. P4 appeared to attenuate *U. parvum* induced IL-8 mRNA expression in chorion cells, but this P4 effect might not mediated through PGRMC1. In summary, *U. parvum* preferentially adheres to and induces inflammatory responses in chorion and amnion cells. P4 and PGRMC1 appear to differentially modulate the inflammatory responses induced by *U. parvum* among amnion and chorion cells.

4.1635 Separation of Bacteria, Protozoa and Carbon Nanotubes by Density Gradient Centrifugation
Mortimer, M., Petersen, E.J., Buchholz, B.A. and Holden, P.A:
*Nanomaterials, **6**:181 (2016)*

Sustainable production and use of carbon nanotube (CNT)-enabled materials require efficient assessment of CNT environmental hazards, including the potential for CNT bioaccumulation and biomagnification in environmental receptors. Microbes, as abundant organisms responsible for nutrient cycling in soil and water, are important ecological receptors for studying the effects of CNTs. Quantification of CNT association with microbial cells requires efficient separation of CNT-associated cells from individually

dispersed CNTs and CNT agglomerates. Here, we designed, optimized, and demonstrated procedures for separating bacteria (*Pseudomonas aeruginosa*) from unbound multiwall carbon nanotubes (MWCNTs) and MWCNT agglomerates using sucrose density gradient centrifugation. We demonstrate separation of protozoa (*Tetrahymena thermophila*) from MWCNTs, bacterial agglomerates, and protozoan fecal pellets by centrifugation in an iodixanol solution. The presence of MWCNTs in the density gradients after centrifugation was determined by quantification of ^{14}C-labeled MWCNTs; the recovery of microbes from the density gradient media was confirmed by optical microscopy. Protozoan intracellular contents of MWCNTs and of bacteria were also unaffected by the designed separation process. The optimized methods contribute to improved efficiency and accuracy in quantifying MWCNT association with bacteria and MWCNT accumulation in protozoan cells, thus supporting improved assessment of CNT bioaccumulation.

4.1636 Broncho Alveolar Dendritic Cells and Macrophages Are Highly Similar to Their Interstitial Counterparts

Maisonnasse, P., Bordet, E., Bouuguyon, E. and Bertho, N.
*PloS One, **11(12)**, e0167315 (2016)*

In human medicine, bronchoalveolar lavage is the main non-traumatic procedure allowing an insight into the respiratory Dendritic Cells (DC) and Macrophages populations. However, it has never been demonstrated in a relevant model that alveolar DC subpopulations were comparable to their interstitial counterparts. In a precedent work we observed that respiratory pig DC and Macrophages were more similar to the human ones than to the mouse ones. In the present work, thanks to our animal model, we were able to collect the rare bronchoalveolar DC and compare them to their interstitial counterparts. We observed that DC presented very similar gene-expression patterns in the alveolar and interstitial compartments, validating the study of human bronchoalveolar DC as surrogate of their interstitium counterparts.

4.1637 Mincle Signaling Promotes Con A Hepatitis

Greco, S.H. et al
*J. Immunol., **197(7)**, 2816-2827 (2016)*

Con A hepatitis is regarded as a T cell–mediated model of acute liver injury. Mincle is a C-type lectin receptor that is critical in the immune response to mycobacteria and fungi but does not have a well-defined role in preclinical models of non-pathogen–mediated inflammation. Because Mincle can ligate the cell death ligand SAP130, we postulated that Mincle signaling drives intrahepatic inflammation and liver injury in Con A hepatitis. Acute liver injury was assessed in the murine Con A hepatitis model using C57BL/6, Mincle$^{-/-}$, and Dectin-1$^{-/-}$ mice. The role of C/EBPβ and hypoxia-inducible factor-1α (HIF-1α) signaling was assessed using selective inhibitors. We found that Mincle was highly expressed in hepatic innate inflammatory cells and endothelial cells in both mice and humans. Furthermore, sterile Mincle ligands and Mincle signaling intermediates were increased in the murine liver in Con A hepatitis. Most significantly, Mincle deletion or blockade protected against Con A hepatitis, whereas Mincle ligation exacerbated disease. Bone marrow chimeric and adoptive transfer experiments suggested that Mincle signaling in infiltrating myeloid cells dictates disease phenotype. Conversely, signaling via other C-type lectin receptors did not alter disease course. Mechanistically, we found that Mincle blockade decreased the NF-κβ–related signaling intermediates C/EBPβ and HIF-1α, both of which are necessary in macrophage-mediated inflammatory responses. Accordingly, Mincle deletion lowered production of nitrites in Con A hepatitis and inhibition of both C/EBPβ and HIF-1α reduced the severity of liver disease. Our work implicates a novel innate immune driver of Con A hepatitis and, more broadly, suggests a potential role for Mincle in diseases governed by sterile inflammation.

4.1638 A Critical Role for P2X7 Receptor–Induced VCAM-1 Shedding and Neutrophil Infiltration during Acute Lung Injury

Mishra, A., Guo, Y., Zhang, L., More, S., Weng, T., Chintagari, R., Huang, C., Liang, Y., Pushparaj, S., Gou, D., Breshears, M. and Liu, L.
*J. Immunol., **197(7)**, 2828-2837 (2016)*

Pulmonary neutrophils are the initial inflammatory cells that are recruited during lung injury and are crucial for innate immunity. However, pathological recruitment of neutrophils results in lung injury. The objective of this study is to determine whether the novel neutrophil chemoattractant, soluble VCAM-1 (sVCAM-1), recruits pathological levels of neutrophils to injury sites and amplifies lung inflammation

during acute lung injury. The mice with $P2X_7$ receptor deficiency, or treated with a $P2X_7$ receptor inhibitor or anti–VCAM-1 Abs, were subjected to a clinically relevant two-hit LPS and mechanical ventilation–induced acute lung injury. Neutrophil infiltration and lung inflammation were measured. Neutrophil chemotactic activities were determined by a chemotaxis assay. VCAM-1 shedding and signaling pathways were assessed in isolated lung epithelial cells. Ab neutralization of sVCAM-1 or deficiency or antagonism of $P2X_7R$ reduced neutrophil infiltration and proinflammatory cytokine levels. The ligands for sVCAM-1 were increased during acute lung injury. sVCAM-1 had neutrophil chemotactic activities and activated alveolar macrophages. VCAM-1 is released into the alveolar airspace from alveolar epithelial type I cells through $P2X_7$ receptor–mediated activation of the metalloproteinase ADAM-17. In conclusion, sVCAM-1 is a novel chemoattractant for neutrophils and an activator for alveolar macrophages. Targeting sVCAM-1 provides a therapeutic intervention that could block pathological neutrophil recruitment, without interfering with the physiological recruitment of neutrophils, thus avoiding the impairment of host defenses.

4.1639 Blomia tropicalis–Specific TCR Transgenic Th2 Cells Induce Inducible BALT and Severe Asthma in Mice by an IL-4/IL-13–Dependent Mechanism
Chua, Y.L. et al
J. Immunol., ***197(10)***, *3771-3781 (2016)*

Previous studies have highlighted the importance of lung-draining lymph nodes in the respiratory allergic immune response, whereas the lung parenchymal immune system has been largely neglected. We describe a new in vivo model of respiratory sensitization to *Blomia tropicalis*, the principal asthma allergen in the tropics, in which the immune response is focused on the lung parenchyma by transfer of Th2 cells from a novel TCR transgenic mouse, specific for the major *B. tropicalis* allergen Blo t 5, that targets the lung rather than the draining lymph nodes. Transfer of highly polarized transgenic CD4 effector Th2 cells, termed BT-II, followed by repeated inhalation of Blo t 5 expands these cells in the lung >100-fold, and subsequent Blo t 5 challenge induced decreased body temperature, reduction in movement, and a fall in specific lung compliance unseen in conventional mouse asthma models following a physiological allergen challenge. These mice exhibit lung eosinophilia; smooth muscle cell, collagen, and goblet cell hyperplasia; hyper IgE syndrome; mucus plugging; and extensive inducible BALT. In addition, there is a fall in total lung volume and forced expiratory volume at 100 ms. These pathophysiological changes were substantially reduced and, in some cases, completely abolished by administration of neutralizing mAbs specific for IL-4 and IL-13 on weeks 1, 2, and 3. This IL-4/IL-13–dependent inducible BALT model will be useful for investigating the pathophysiological mechanisms that underlie asthma and the development of more effective drugs for treating severe asthma.

4.1640 Kinetic analysis of internalization of white spot syndrome virus by haemocyte subpopulations of penaeid shrimp, Litopenaeus vannamei (Boone), and the outcome for virus and cell
Tuan, V.V., De Gryse, G.M.A., Thuong, K.V., Bossier, P. and Nauwynck, H.J.
J. Fish Diseases, ***39(12)****, 1477-1493 (2016)*

Little is known about the innate antiviral defence of shrimp haemocytes. In this context, the haemocytes of penaeid shrimp *Litopenaeus vannamei* (Boone) were separated by iodixanol density gradient centrifugation into five subpopulations (sub): sub 1 (hyalinocytes), sub 2 and 3 (prohyalinocytes), sub 4 (semigranulocytes) and sub 5 (granulocytes) and exposed to beads, white spot syndrome virus (WSSV) and ultraviolet (UV)-killed WSSV. In a first experiment, the uptake of beads, white spot syndrome virus (WSSV) and UV-killed WSSV by these different haemocyte subpopulations was investigated using confocal microscopy. Only haemocytes of sub 1, 4 and 5 were internalizing beads, WSSV and UV-killed WSSV. Beads were engulfed by a much larger percentage of cells (91.2 in sub 1; 84.1 in sub 4 and 58.1 in sub 5) compared to WSSV (9.6 in sub 1; 10.5 in sub 4 and 7.9 in sub 5) and UV-killed WSSV (12.9 in sub 1; 13.3 in sub 4; and 11.8 in sub 5). In a second experiment, it was shown that upon internalization, WSS virions lost their envelope most probably by fusion with the cellular membrane of the endosome (starting between 30 and 60 min post-inoculation) and that afterwards the capsid started to become disintegrated (from 360 min post-inoculation). Expression of new viral proteins was not observed. Incubation of haemocyte subpopulations with WSSV but not with UV-killed WSSV and polystyrene beads resulted in a significant drop in haemocyte viability. To find the underlying mechanism, a third experiment was performed in which haemocyte subpopulations were exposed to a short WSSV DNA fragment (VP19) and CpG ODNs. These small DNA fragments induced cell death. In conclusion, WSSV is efficiently internalized by hyalinocytes, semigranulocytes and granulocytes, after which the virus loses its envelope; as soon as the capsids start to disintegrate, cell death is activated, which in part may be explained by the

exposure of viral DNA to cellular-sensing molecules.

4.1641 Mesenchymal stromal cell-derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells
Wen, S., Dooner, M., Cheng, Y., Papa, E., Del Tatto, M., Pereira, M., Deng, Y., Goldberg, L., Aliotta, J., Chatterjee, D., Stewart, C., Carpanetto, A., Collino, F., Bruno, S., Camussi, G. and Quesenberry, P.
*Leukemia, **30(12)**, 2221-2231 (2016)*

Mesenchymal stromal cells (MSCs) have been shown to reverse radiation damage to marrow stem cells. We have evaluated the capacity of MSC-derived extracellular vesicles (MSC-EVs) to mitigate radiation injury to marrow stem cells at 4 h to 7 days after irradiation. Significant restoration of marrow stem cell engraftment at 4, 24 and 168 h post irradiation by exposure to MSC-EVs was observed at 3 weeks to 9 months after transplant and further confirmed by secondary engraftment. Intravenous injection of MSC-EVs to 500cGy exposed mice led to partial recovery of peripheral blood counts and restoration of the engraftment of marrow. The murine hematopoietic cell line, FDC-P1 exposed to 500cGy, showed reversal of growth inhibition, DNA damage and apoptosis on exposure to murine or human MSC-EVs. Both murine and human MSC-EVs reverse radiation damage to murine marrow cells and stimulate normal murine marrow stem cell/progenitors to proliferate. A preparation with both exosomes and microvesicles was found to be superior to either microvesicles or exosomes alone. Biologic activity was seen in freshly isolated vesicles and in vesicles stored for up to 6 months in 10% dimethyl sulfoxide at −80 °C. These studies indicate that MSC-EVs can reverse radiation damage to bone marrow stem cells.

4.1642 Heme drives hemolysis-induced susceptibility to infection via disruption of phagocyte functions
Martins, R. et al
*Nature Immunol., **17(12)**, 1361-1372 (2016)*

Hemolysis drives susceptibility to bacterial infections and predicts poor outcome from sepsis. These detrimental effects are commonly considered to be a consequence of heme-iron serving as a nutrient for bacteria. We employed a Gram-negative sepsis model and found that elevated heme levels impaired the control of bacterial proliferation independently of heme-iron acquisition by pathogens. Heme strongly inhibited phagocytosis and the migration of human and mouse phagocytes by disrupting actin cytoskeletal dynamics via activation of the GTP-binding Rho family protein Cdc42 by the guanine nucleotide exchange factor DOCK8. A chemical screening approach revealed that quinine effectively prevented heme effects on the cytoskeleton, restored phagocytosis and improved survival in sepsis. These mechanistic insights provide potential therapeutic targets for patients with sepsis or hemolytic disorders.

4.1643 Evolution of Osteocrin as an activity-regulated factor in the primate brain
Ataman, B. et al
*Nature, **539(7628)**, 242-247 (2016)*

Sensory stimuli drive the maturation and function of the mammalian nervous system in part through the activation of gene expression networks that regulate synapse development and plasticity. These networks have primarily been studied in mice, and it is not known whether there are species- or clade-specific activity-regulated genes that control features of brain development and function. Here we use transcriptional profiling of human fetal brain cultures to identify an activity-dependent secreted factor, Osteocrin (OSTN), that is induced by membrane depolarization of human but not mouse neurons. We find that *OSTN* has been repurposed in primates through the evolutionary acquisition of DNA regulatory elements that bind the activity-regulated transcription factor MEF2. In addition, we demonstrate that *OSTN* is expressed in primate neocortex and restricts activity-dependent dendritic growth in human neurons. These findings suggest that, in response to sensory input, *OSTN* regulates features of neuronal structure and function that are unique to primates.

4.1644 Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs
Cummings, R.J., Barbet, G., Bongers, G., hartmann, B.M., Gettler, K., Muniz, L., Furtado, G.C., Cho, J., Lira, S.A. and Blander, J.M.
*Nature, **539(7630)**, 565-569 (2016)*

Recognition and removal of apoptotic cells by professional phagocytes, including dendritic cells and macrophages, preserves immune self-tolerance and prevents chronic inflammation and autoimmune pathologies[1, 2]. The diverse array of phagocytes that reside within different tissues, combined with the

necessarily prompt nature of apoptotic cell clearance, makes it difficult to study this process *in situ*. The full spectrum of functions executed by tissue-resident phagocytes in response to homeostatic apoptosis, therefore, remains unclear. Here we show that mouse apoptotic intestinal epithelial cells (IECs), which undergo continuous renewal to maintain optimal barrier and absorptive functions[3], are not merely extruded to maintain homeostatic cell numbers[4], but are also sampled by a single subset of dendritic cells and two macrophage subsets within a well-characterized network of phagocytes in the small intestinal lamina propria[5, 6]. Characterization of the transcriptome within each subset before and after *in situ* sampling of apoptotic IECs revealed gene expression signatures unique to each phagocyte, including macrophage-specific lipid metabolism and amino acid catabolism, and a dendritic-cell-specific program of regulatory $CD4^+$ T-cell activation. A common 'suppression of inflammation' signature was noted, although the specific genes and pathways involved varied amongst dendritic cells and macrophages, reflecting specialized functions. Apoptotic IECs were trafficked to mesenteric lymph nodes exclusively by the dendritic cell subset and served as critical determinants for the induction of tolerogenic regulatory $CD4^+$ T-cell differentiation. Several of the genes that were differentially expressed by phagocytes bearing apoptotic IECs overlapped with susceptibility genes for inflammatory bowel disease[7]. Collectively, these findings provide new insights into the consequences of apoptotic cell sampling, advance our understanding of how homeostasis is maintained within the mucosa and set the stage for development of novel therapeutics to alleviate chronic inflammatory diseases such as inflammatory bowel disease.

4.1645 Strategies for Processing Semen from Subfertile Stallions for Cooled Transport
Varner, D.D.
Vet. Clin. Equine, ***32(3)****, 547-560 (2016)*

Simple dilution of semen in extender is generally satisfactory for cooled transport of semen if certain guidelines are applied.
Subfertility following insemination with cool-transported semen can be associated with different inciting factors.
Concentration of sperm in semen can be achieved by filtration or centrifugation procedures. Currently, centrifugation is most commonly applied.
Centrifugal fractionation of semen (also termed density gradient centrifugation) can be used to enhance sperm quality but recovery rates can be low, thereby necessitating low-dose insemination techniques for breeding purposes.

4.1646 Molecular immunology profiles of monkeys following xenografting with the islets and heart of α-1,3-galactosyltransferase knockout pigs
Ock, S.A., Lee, J., Oh, K.B., Hwang, S., Yun, I.J., Ahn, C.,, Chee, H.k., Kim, H., park, J.B., Kim, S.J., Im, G-S.and Park, E.W.
Xenotransplantation, ***23(5)****, 357-369 (2016)*

Effective immunosuppression strategies and genetically modified animals have been used to prevent hyperacute and acute xenograft rejection; however, the underlying mechanisms remain unknown. In this study, we evaluated the expression of a comprehensive set of immune system-related genes (89 genes, including five housekeeping genes) in the blood of cynomolgus monkeys (~5 yr old) used as graft recipients, before and after the xenografting of the islets and heart from single and double α-1,3-galactosyltransferase (GalT) knockout (KO) pigs (<6 weeks old). The immunosuppressive regimen included administration of cobra venom factor, anti-thymocyte globulin, rituximab, and anti-CD154 monoclonal antibodies to recipients before and after grafting. Islets were xenografted into the portal vein in type 1 diabetic monkeys, and the heart was xenografted by heterotopic abdominal heart transplantation. Genes from recipient blood were analyzed using RT^2 profiler PCR arrays and the web-based RT^2 profiler PCR array software v.3.5. Recipients treated with immunosuppressive agents without grafting showed significant downregulation of *CCL5*, *CCR4*, *CCR6*, *CD4*, *CD40LG*, *CXCR3*, *FASLG*, *CXCR3*, *FOXP3*, *GATA3*, *IGNG*, *L10*, *IL23A*, *TRAF6*, *MAPK8*, *MIF*, *STAT4*, *TBX21*, *TLR3*, *TLR7*, and *TYK2* and upregulation of *IFNGR1*; thus, genes involved in protection against viral and bacterial infection were downregulated, confirming the risk of infection. Notably, C3-level control resulted in xenograft failure within 2 days because of a 7- to 11-fold increase in all xenotransplanted models. Islet grafting using single GalT-KO pigs resulted in upregulation of *CXCL10* and *MX1*, early inflammation, and acute rejection-associated signals at 2 days after xenografting. We observed at least 5-fold upregulation in recipients transplanted with islets grafts from single (*MX1*) or double (*C3*, *CCR8*, *IL6*, *IL13*, *IRF6*, *CXCL10*, and *MX1*) GalT-KO pigs after 77 days; single GalT-KO incurred early losses owing to immune attacks. Our results suggest that this novel, simple, non-invasive, and time-efficient procedure (requiring only 1.5 ml

blood) for evaluating graft success, minimizing immune rejection, and blocking infection.

4.1647 Ethanol Exposures During the Developmental Period Increase Microglia Sensitivity to a Stress Challenge During Adulthood: A Possible Cause for the Stress Hyperresponse in Fetal Alcohol Exposed Offspring

Sarkar, D., Chastain, L., Cabrera, M. and Shrivastava , P.
*Neurosychopharmacology, **41**, S136, abstract M33 (2016)*

Background: In the healthy central nervous system (CNS), microglia, the immune cells of the CNS, quickly detect a stimulus, become activated and elicit the proper response. Once the stimulus is removed and homeostasis is restored, microglia revert to their inactive ramified state and returns to their surveillance duties. Recently it has been shown that a single exposure to Escherichia coli (E. Coli) activates microglia in rat neonates, and causes long-term alterations in microglia response and behavior in adulthood. No previous investigation on the long-term effects of ethanol induced microglia activation during the neonatal period in rats, a period equivalent to the third trimester of human pregnancy, has been reported. In this study, we investigate the effects of neonatal ethanol on microglia and their influence in regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to an immune stress challenge during adulthood.
Methods: Neonatal rat pups were fed by oral gavage a milk formula containing 11.34% ethanol (vol/vol), yielding a total daily ethanol dose of 2.5 g/kg (AF), or isocaloric control (PF), or they were left in the litter with the mother (AD) for 5 days (postnatal days 2-6). Some of the AF rats additionally received a minocycline pre-treatment one hour prior to the first feeding on each day by subcutaneous injection of minocycline solution (45µg/kg bodyweight). Two hours after the last feeding, some of the pups were sacrificed and hypothalamus was dissected for microglia separation by differential gradient centrifugation using OptiPrep gradient and characterization by measuring production of various activation markers and cytokines. The rest of the pups were maintained in the controlled condition until 90 days of age when they were used for immune stress challenge (Lipopolysaccharide, LPS; 100ug/kg bodyweight i.p.) and microglia characterization and HPA axis responses by measuring plasma levels of corticosterone and adrenocorticotrophin (ACTH). Animal surgery and care were performed in accordance with institutional guidelines and complied with the National Institutes of Health policy. Results: We found binge-like ethanol exposures during the postnatal period increased microglial activation markers (e.g., IBA1, CX3CR1), inflammatory cytokine (e.g., TNF-α), an inflammatory signal receptor (TLR-4), and inflammatory cell signaling molecules (IKBA) in microglia isolated from the hypothalamus. Comparable results were also obtained following a known activator of microglia, LPS. In association with the microglial activation, we found increased proopiomelanocortin (POMC) neuronal (a neuronal population known to regulate the HPA axis function) apoptosis in the arcuate nucleus of the hypothalamus. Treatment with minocycline, an inhibitor of microglia activation, blocked ethanol effect on POMC neuronal apoptosis. Neonatal ethanol also promotes the adult response of the microglia to an immune challenge. In addition, neonatal ethanol increased the response of the HPA axis, plasma corticosterone and ACTH, following LPS challenge, while neonatal minocycline prevented ethanol action on the stress hormone responses. Neonatal ethanol altered the expression of various proteins related to Dnmts, HDACs and MeCp2 and increased histone acetylation and decreased DNA methylation in microglia.
Conclusions: These data suggest that neonatal exposure to a high dose of ethanol activates microglia by producing various inflammatory cytokines leading to killing of POMC neurons and a deficiency in feedback regulatory control over the stress axis function. Additionally, neonatal ethanol exposures increase microglia sensitivity to the subsequent stimuli by possibly altering epigenetic mechanisms. In conclusion, this work provides important insights into the effects of both immediate and long-term effects of ethanol induced microglia activation during third trimester gestation. In our model, ethanol induced microglia activation leads to long-term alterations in microglia responses to subsequent stimuli that contribute to changes in HPA response in adulthood.

4.1648 FREE

4.1649 Reducing neuroinflammation by delivery of IL-10 encoding lentivirus from multiple-channel bridges

Margul, D.J. et al
*Bioeng. Translational Med., **1**, 136-148 (2016)*

The spinal cord is unable to regenerate after injury largely due to growth-inhibition by an inflammatory response to the injury that fails to resolve, resulting in secondary damage and cell death. An approach that prevents inhibition by attenuating the inflammatory response and promoting its resolution through the

transition of macrophages to anti-inflammatory phenotypes is essential for the creation of a growth permissive microenvironment. Viral gene delivery to induce the expression of anti-inflammatory factors provides the potential to provide localized delivery to alter the host inflammatory response. Initially, we investigated the effect of the biomaterial and viral components of the delivery system to influence the extent of cell infiltration and the phenotype of these cells. Bridge implantation reduces antigen-presenting cell infiltration at day 7, and lentivirus addition to the bridge induces a transient increase in neutrophils in the spinal cord at day 7 and macrophages at day 14. Delivery of a lentivirus encoding IL-10, an anti-inflammatory factor that inhibits immune cell activation and polarizes the macrophage population towards anti-inflammatory phenotypes, reduced neutrophil infiltration at both day 7 and day 28. Though IL-10 lentivirus did not affect macrophages number, it skewed the macrophage population toward an anti-inflammatory M2 phenotype and altered macrophage morphology. Additionally, IL-10 delivery resulted in improved motor function, suggesting reduced secondary damage and increased sparing. Taken together, these results indicate that localized expression of anti-inflammatory factors, such as IL-10, can modulate the inflammatory response following spinal cord injury, and may be a key component of a combinatorial approach that targets the multiple barriers to regeneration and functional recovery.

4.1650 Effect of GDNF on Morphology, Proliferation, and Phagocytic Activity of Rat Neonatal Cortex Isolated Microglia
Zhuravleva, M., Rizvanov, A. and Mukhamedshina, Y.
BioNanoSci., ***6***, *379-383 (2016)*

Microglia are the main defenders of the central nervous system and at the same time are involved in the pathogenesis of various neurological disorders. Microglia hyperactivity or phagocytic impairment exacerbates degenerative processes in nervous tissue leading to further loss of function. A variety of factors and cytokines may modify microglia function. In our study, it was shown that glial cell line-derived neurotrophic factor (GDNF), a well-known neuroprotective molecule, decreases phagocytic activity of microglia in vitro model of spinal cord injury. Recombinant adenovirus encoding GDNF (Ad5-GDNF) transfected microglia have shown the same effect and can be potentially used as a therapeutic agent in case of neurotrauma due to its debris phagocytic and GDNF-associated neuroprotective role.

4.1651 Dendritic cell sphingosine-1-phosphate lyase regulates thymic egress
Zamora-Pineda, J., Kumar, A., Suh, J.H., Zhang, M. and Saba, J.D.
J. Exp. Med., ***213(12)***, *2773-2791 (2016)*

T cell egress from the thymus is essential for adaptive immunity and involves chemotaxis along a sphingosine-1-phosphate (S1P) gradient. Pericytes at the corticomedullary junction produce the S1P egress signal, whereas thymic parenchymal S1P levels are kept low through S1P lyase (SPL)–mediated metabolism. Although SPL is robustly expressed in thymic epithelial cells (TECs), in this study, we show that deleting SPL in $CD11c^+$ dendritic cells (DCs), rather than TECs or other stromal cells, disrupts the S1P gradient, preventing egress. Adoptive transfer of peripheral wild-type DCs rescued the egress phenotype of DC-specific SPL knockout mice. These studies identify DCs as metabolic gatekeepers of thymic egress. Combined with their role as mediators of central tolerance, DCs are thus poised to provide homeostatic regulation of thymic export.

4.1652 Motor neuron mitochondrial dysfunction in spinal muscular atrophy
Miller, N., Shi, H., Zelikovich, A.S. and Ma, Y-C.
Hum. Mol. Genet., ***25(16)***, *3395-3406 (2016)*

Spinal muscular atrophy (SMA), the leading genetic cause of infant mortality, predominantly affects high metabolic tissues including motor neurons, skeletal muscles and the heart. Although the genetic cause of SMA has been identified, mechanisms underlying tissue-specific vulnerability are not well understood. To study these mechanisms, we carried out a deep sequencing analysis of the transcriptome of spinal motor neurons in an SMA mouse model, in which we unexpectedly found changes in many genes associated with mitochondrial bioenergetics. Importantly, functional measurement of mitochondrial activities showed decreased basal and maximal mitochondrial respiration in motor neurons from SMA mice. Using a reduction-oxidation sensitive GFP and fluorescence sensors specifically targeted to mitochondria, we found increased oxidative stress level and impaired mitochondrial membrane potential in motor neurons affected by SMA. In addition, mitochondrial mobility was impaired in SMA disease conditions, with decreased retrograde transport but no effect on anterograde transport. We also found significantly increased fragmentation of the mitochondrial network in primary motor neurons from SMA mice, with no

change in mitochondria density. Electron microscopy study of SMA mouse spinal cord revealed mitochondria fragmentation, edema and concentric lamellar inclusions in motor neurons affected by the disease. Intriguingly, these functional and structural deficiencies in the SMA mouse model occur during the presymptomatic stage of disease, suggesting a role in initiating SMA. Altogether, our findings reveal a critical role for mitochondrial defects in SMA pathogenesis and suggest a novel target for improving tissue health in the disease.

4.1653 Platelet-borne complement proteins and their role in platelet–bacteria interactions
Arbesu, I., Bucsaiova, M., Fisher, M.B. and mannhalter, C.
*J. Thrombosis and Haemostasis, **14**, 2241-2252 (2016)*

Background
The role of platelets in immune defense is increasingly being recognized. Platelets bind complement proteins from plasma, initiate complement activation, and interact with bacteria. However, the contribution of platelets to complement-mediated defense against bacterial infections is not known in detail.
Objectives
To assess platelet interactions with Escherichia coli strains, and evaluate the contributions of platelet complement proteins to host defense.
Methods
We studied the cell–cell interactions of a pathogenic and a non-pathogenic E. coli strain with platelet concentrates, washed platelets and manually isolated platelets by flow cytometry and ELISA. The presence of complement proteins and complement RNA in megakaryocytes and platelets was analyzed by PCR, RT-PCR, confocal microscopy, and western blotting.
Results
Incubation with E. coli leads to platelet activation, as indicated by the expression of CD62P and CD63 on the platelet surface. RNA and protein analyses show that megakaryocytes and platelets contain complement C3, and that platelet C3 migrates differently on polyacrylamide gels than plasmatic C3. Activation of platelets by bacteria leads to translocation of C3 to the cell surface. This translocation is not induced by thrombin receptor activating peptide or lipopolysaccharide. Interaction of platelets with E. coli occurs even in the absence of plasma proteins, and is independent of platelet toll-like receptor 4 and $\alpha_{2b}\beta_3$ (glycoprotein IIbIIIa).
Conclusion
Platelets contain a specific form of C3. Importantly, they can modulate immune defense against bacteria by enhancing plasmatic complement activation.

The Warburg Effect Mediator Pyruvate Kinase M2 Expression and Regulation in the Retina
Rajala, R.V.S., Rajala, A., Kooker, C., Wang, Y. and Anderson, R.E.
*Scientific Reports, **6**:37727 (2016)*

The tumor form of pyruvate kinase M2 (PKM2) undergoes tyrosine phosphorylation and gives rise to the Warburg effect. The Warburg effect defines a pro-oncogenic metabolism switch such that cancer cells take up more glucose than normal tissue and favor incomplete oxidation of glucose, even in the presence of oxygen. Retinal photoreceptors are highly metabolic and their energy consumption is equivalent to that of a multiplying tumor cell. In the present study, we found that PKM2 is the predominant isoform in both rod- and cone-dominant retina, and that it undergoes a light-dependent tyrosine phosphorylation. We also discovered that PKM2 phosphorylation is signaled through photobleaching of rhodopsin. Our findings suggest that phosphoinositide 3-kinase activation promotes PKM2 phosphorylation. Light and tyrosine phosphorylation appear to regulate PKM2 to provide a metabolic advantage to photoreceptor cells, thereby promoting cell survival.

4.1654 Isolation of Platelet-Derived Extracellular Vesicles
Aatonen, M., Valkonen, S., Böing, A., Yuana, Y., Nieuwland, R. and Siljander, P.
*Methods in Mol. Biol., **1545**, 177188 (2017)*

Platelets participate in several physiological functions, including hemostasis, immunity, and development. Additionally, platelets play key roles in arterial thrombosis and cancer progression. Given this plethora of functions, there is a strong interest of the role of platelet-derived (extracellular) vesicles (PDEVs) as functional mediators and biomarkers. Moreover, the majority of the blood-borne EVs are thought to originate from either platelets or directly from the platelet precursor cells, the megakaryocytes, which reside in the bone marrow. To circumvent confusion, we use the term PDEVs for both platelet-derived

and/or megakaryocyte-derived EVs. PDEVs can be isolated from blood or from isolated platelets after activation. In this chapter, we describe all commonly used PDEV isolation methods from blood and prepurified platelets.

4.1655 Association of a novel GABRG2 splicing variation and a PTGS2/COX-2 single nucleotide polymorphism with Taiwanese febrile seizures
Hung, K-L., Liang, J-S., Wang, J-S., Chen, H-J., Lin, J-J. and Lu, J-F.
Epilepsy Res., ***129***, *1-7 (2017)*

Abstract: Febrile seizure (FS) is the most common type of convulsion in infants and young children. The occurrence of FS in a subset of children with febrile illness suggested genetic factors may have an important effect on the predisposition of the disease. Using targeted next generation sequencing (NGS), a novel splicing variation (NM_198903.2:c.1249-1G>T) was identified in the γ-aminobutyric acid type A (GABA-A) receptor γ2 subunit (*GABRG2*) gene of a FS patient. To investigate possible association of FS with single nucleotide polymorphisms (SNPs) in prostaglandin-endoperoxide synthase-2 (*prostaglandin G/H synthase-2*; *PTGS2*/*cyclooxygenase-2*; *COX2*) gene involving in thermoregulatory pathway, eight SNPs, rs689465, rs689466, rs20417, rs13306038, rs201931599, rs689470, rs4648306 and rs4648308, along with 2 previously reported variations in *IL1RN* (86-bp VNTR) and *IL10* (rs1900872) were genotyped and utilized for case-control association studies on 35 FS and 31 non-FS controls. A single SNP (rs689466) localized at 5′-1192 of the *PTGS2* gene exhibited significant association with FS ($p = 0.045$) based on case-control allelic association analyses. A significant decrease in the frequency of the G allele in FS (0.357) was observed compared to that in controls (0.536) with an estimated odds ratio (OR) of 0.48 (95% CI, 0.23–0.99) for the G versus A allele. Using case-control genotypic association analysis, the −1192 A allele is most likely to confer susceptibility to FS by a recessive action model ($p = 0.045$, pointwise empirical p value (EMP1) = 0.049). The association of SNPs in *PTGS2*, in addition to *IL6*, *IL-6 receptor* (*IL6R*) and *prostaglandin E receptor 3* (*PTGER3*) in prior reports, with FS suggests their possible action in concert to modulate phenotypes in FS as well as the involvement of thermoregulatory pathway in pathogenesis of FS.

4.1656 Reactive gamma-ketoaldehydes as novel activators of hepatic stellate cells in vitro
Longato, L., Andreola, F., Davies, S.S., Roberts, J.L., Fusai, G., Pinzani, M., Moore, K. and Rombouts, K.
Free Radical Biol. Med., ***102***, *162-173 (2017)*

Aims
Products of lipid oxidation, such as 4-hydroxynonenal (4-HNE), are key activators of hepatic stellate cells (HSC) to a pro-fibrogenic phenotype. Isolevuglandins (IsoLG) are a family of acyclic γ-ketoaldehydes formed through oxidation of arachidonic acid or as by-products of the cyclooxygenase pathway. IsoLGs are highly reactive aldehydes which are efficient at forming protein adducts and cross-links at concentrations 100-fold lower than 4-hydroxynonenal. Since the contribution of IsoLGs to liver injury has not been studied, we synthesized 15-E_2-IsoLG and used it to investigate whether IsoLG could induce activation of HSC.
Results
Primary human HSC were exposed to 15-E_2-IsoLG for up to 48 h. Exposure to 5 μM 15-E_2-IsoLG in HSCs promoted cytotoxicity and apoptosis. At non-cytotoxic doses (50 pM-500 nM) 15-E_2-IsoLG promoted HSC activation, indicated by increased expression of α-SMA, sustained activation of ERK and JNK signaling pathways, and increased mRNA and/or protein expression of cytokines and chemokines, which was blocked by inhibitors of JNK and NF-kB. In addition, IsoLG promoted formation of reactive oxygen species, and induced an early activation of ER stress, followed by autophagy. Inhibition of autophagy partially reduced the pro-inflammatory effects of IsoLG, suggesting that it might serve as a cytoprotective response.
Innovation
This study is the first to describe the biological effects of IsoLG in primary HSC, the main drivers of hepatic fibrosis.
Conclusions
IsoLGs represent a newly identified class of activators of HSC *in vitro*, which are biologically active at concentrations as low as 500 pM, and are particularly effective at promoting a pro-inflammatory response and autophagy.

4.1657 Identification of axon growth promoters in the secretome of the deer antler velvet
Pita-Thomas, W., Barroso-Garcia, G., Moral, V., Hackett, A.R., Cavalli, V. and Nieto-Diaz, M.

Neuroscience, ***340***, *333-344 (2017)*

Every spring, deer cast their old antlers and initiate a regeneration process, which yields a new set of antlers of up to 1 m in length. Over the course of three months, branches of the trigeminal nerve, originating from the frontal skull, innervate velvet, a modified skin that covers the regenerating antler. The rate of growth of these axons reaches up to 2 cm per day making them the fastest regenerating axons in adult mammals. Here, we aim to identify the factors secreted by velvet that promote such high speed axon growth. Our experiments with cultures of adult rat trigeminal neurons demonstrate that conditioned medium harvested from velvet organotypic cultures has greater axon growth-promoting properties than a medium conditioned by normal skin. The axon growth-promoting effects of velvet act synergistically with the extracellular matrix (ECM) protein laminin, a component of the basal lamina present in the deer antler. Our proteomic analyses identified several axon growth promoters in the velvet-conditioned medium (VCM), including soluble proteins such as nerve growth factor (NGF) and apolipoprotein A-1, as well as matrix extracellular proteins, such as periostin and SPARC. Additional *in vitro* analyses allowed us to determine that a synergic relationship between periostin and NGF may contribute to neurite growth-promoting effects of velvet secretome. A combinatorial approach using these factors may promote regeneration at high speeds in patients with peripheral neuropathies.

4.1658 **Death of Monocytes through Oxidative Burst of Macrophages and Neutrophils: Killing in Trans**
Ponath, V. and Kaina, B.
PloS One, ***12(1)***, *e0170347 (2017)*

Monocytes and their descendants, macrophages, play a key role in the defence against pathogens. They also contribute to the pathogenesis of inflammatory diseases. Therefore, a mechanism maintaining a balance in the monocyte/macrophage population must be postulated. Our previous studies have shown that monocytes are impaired in DNA repair, rendering them vulnerable to genotoxic stress while monocyte-derived macrophages are DNA repair competent and genotoxic stress-resistant. Based on these findings, we hypothesized that monocytes can be selectively killed by reactive oxygen species (ROS) produced by activated macrophages. We also wished to know whether monocytes and macrophages are protected against their own ROS produced following activation. To this end, we studied the effect of the ROS burst on DNA integrity, cell death and differentiation potential of monocytes. We show that monocytes, but not macrophages, stimulated for ROS production by phorbol-12-myristate-13-acetate (PMA) undergo apoptosis, despite similar levels of initial DNA damage. Following co-cultivation with ROS producing macrophages, monocytes displayed oxidative DNA damage, accumulating DNA single-strand breaks and a high incidence of apoptosis, reducing their ability to give rise to new macrophages. Killing of monocytes by activated macrophages, termed *killing in trans*, was abolished by ROS scavenging and was also observed in monocytes co-cultivated with ROS producing activated granulocytes. The data revealed that monocytes, which are impaired in the repair of oxidised DNA lesions, are vulnerable to their own ROS and ROS produced by macrophages and granulocytes and support the hypothesis that this is a mechanism regulating the amount of monocytes and macrophages in a ROS-enriched inflammatory environment.

4.1659 **Steatosis induced CCL5 contributes to early-stage liver fibrosis in nonalcoholic fatty liver disease progress**
Li, B-H., He, F-P., Yang, X., Chen, Y-W. and Fan, J-G.
Translational Res., ***180***, *103-117 (2017)*

The rapidly increasing prevalence of nonalcoholic fatty liver disease (NAFLD) has become one of the major public health threats in China and worldwide. However, during the development of NAFLD, the key mechanism underlying the progression of related fibrosis remains unclear, which greatly impedes the development of optimal NAFLD therapy. In the current study, we were endeavored to characterize a proinflammatory cytokine, CCL5, as a major contributor for fibrosis in NAFLD. The results showed that CCL5 was highly expressed in fatty liver and NASH patients. In NAFLD rats induced by 8-week-HFD, CCL5 and its receptor, CCR5, were significantly up-regulated and liver fibrosis exclusively occurred in this group. In addition, we showed that hepatocytes are the major source contributing to this CCL5 elevation. Interestingly, a CCL5 inhibitor Met-CCL5, significantly decreased liver fibrosis but not hepatic steatosis. Using a cell model of hepatic steatosis, we found that the conditioned medium of lipid-overloaded hepatocytes (Fa2N-4 cells) which produced excessive CCL5 stimulated the profibrotic activities of hepatic stellate cells (LX-2) as manifested by increased migration rate, proliferation and collagen production of LX-2 cells. CCL5 knockdown in Fa2N-4 cells, Met-CCL5 or CCR5 antibody treatment on LX-2 cells all significantly inhibited the conditioned medium of FFA-treated Fa2N-4 cells to

exert stimulatory effects on LX-2 cells. Consistently, the conditioned medium of Fa2N-4 cells with CCL5 over-expression significantly enhanced migration rate, cell proliferation and collagen production of LX-2 cells. All these results support that CCL5 produced by steatotic hepatocytes plays an essential role in fibrotic signaling machinery of NAFLD. In addition, we were able to identify C/EBP-β as the up-stream regulator of CCL5 gene transcription in hepatocytes treated with free fatty acid (FFA). Our data strongly supported that CCL5 plays a pivotal regulatory role in hepatic fibrosis during NAFLD, which constitutes a novel and exciting observation that may call for potential future development of specific CCL5-targeted NAFLD therapy.

4.1660 **A Proof-of-Concept for Epigenetic Therapy of Tissue Fibrosis: Inhibition of Liver Fibrosis Progression by 3-Deazaneplanocin A**
Zeybel, M. et al
Molecular Therapy, ***25(1)***, *218-231 (2017)*

The progression of fibrosis in chronic liver disease is dependent upon hepatic stellate cells (HSCs) transdifferentiating to a myofibroblast-like phenotype. This pivotal process is controlled by enzymes that regulate histone methylation and chromatin structure, which may be targets for developing anti-fibrotics. There is limited pre-clinical experimental support for the potential to therapeutically manipulate epigenetic regulators in fibrosis. In order to learn if epigenetic treatment can halt the progression of pre-established liver fibrosis, we treated mice with the histone methyltransferase inhibitor 3-deazaneplanocin A (DZNep) in a naked form or by selectively targeting HSC-derived myofibroblasts via an antibody-liposome-DZNep targeting vehicle. We discovered that DZNep treatment inhibited multiple histone methylation modifications, indicative of a broader specificity than previously reported. This broad epigenetic repression was associated with the suppression of fibrosis progression as assessed both histologically and biochemically. The anti-fibrotic effect of DZNep was reproduced when the drug was selectively targeted to HSC-derived myofibroblasts. Therefore, the in vivo modulation of HSC histone methylation is sufficient to halt progression of fibrosis in the context of continuous liver damage. This discovery and our novel HSC-targeting vehicle, which avoids the unwanted effects of epigenetic drugs on parenchymal liver cells, represents an important proof-of-concept for epigenetic treatment of liver fibrosis.

4.1661 **Single-cell barcoding and sequencing using droplet microfluidics**
Zilionis, R., Nainys, J., Veres, A., Savova, v., Zemmeour, D., Klein, A.M. and Mazautis, L.
Nature Protocol., ***12(1)***, *44-73 (2017)*

Single-cell RNA sequencing has recently emerged as a powerful tool for mapping cellular heterogeneity in diseased and healthy tissues, yet high-throughput methods are needed for capturing the unbiased diversity of cells. Droplet microfluidics is among the most promising candidates for capturing and processing thousands of individual cells for whole-transcriptome or genomic analysis in a massively parallel manner with minimal reagent use. We recently established a method called inDrops, which has the capability to index >15,000 cells in an hour. A suspension of cells is first encapsulated into nanoliter droplets with hydrogel beads (HBs) bearing barcoding DNA primers. Cells are then lysed and mRNA is barcoded (indexed) by a reverse transcription (RT) reaction. Here we provide details for (i) establishing an inDrops platform (1 d); (ii) performing hydrogel bead synthesis (4 d); (iii) encapsulating and barcoding cells (1 d); and (iv) RNA-seq library preparation (2 d). inDrops is a robust and scalable platform, and it is unique in its ability to capture and profile >75% of cells in even very small samples, on a scale of thousands or tens of thousands of cells.

4.1662 **Sirtuin 2 aggravates postischemic liver injury by deacetylating mitogen-activated protein kinase phosphatase-1**
Wang, J., Koh, H-W., Zhou, L., Bae, U-J., Lee, H-S., Bang, I.H., Ka, S-O., Oh, S-H., Bae, E.J. and Park, B-H.
Hepatology, ***65(1)***, *225-236 (2017)*

Sirtuin 2 (Sirt2) is known to negatively regulate anoxia-reoxygenation injury in myoblasts. Because protein levels of Sirt2 are increased in ischemia-reperfusion (I/R)-injured liver tissues, we examined whether Sirt2 is protective or detrimental against hepatic I/R injury. We overexpressed Sirt2 in the liver of C57BL/6 mice using a Sirt2 adenovirus. Wild-type and Sirt2 knockout mice were subjected to a partial (70%) hepatic ischemia for 45 minutes, followed by various periods of reperfusion. In another set of experiments, wild-type mice were pretreated intraperitoneally with AGK2, a Sirt2 inhibitor. Isolated

hepatocytes and Kupffer cells from wild-type and Sirt2 knockout mice were subjected to hypoxia-reoxygenation injury to determine the *in vitro* effects of Sirt2. Mice subjected to I/R injury showed typical patterns of hepatocellular damage. Prior injection with Sirt2 adenovirus aggravated liver injury, as demonstrated by increases in serum aminotransferases, prothrombin time, proinflammatory cytokines, hepatocellular necrosis and apoptosis, and neutrophil infiltration relative to control virus-injected mice. Pretreatment with AGK2 resulted in significant improvements in serum aminotransferase levels and histopathologic findings. Similarly, experiments with Sirt2 knockout mice also revealed reduced hepatocellular injury. The molecular mechanism of Sirt2's involvement in this aggravation of hepatic I/R injury includes the deacetylation and inhibition of mitogen-activated protein kinase phosphatase-1 and consequent activation of mitogen-activated protein kinases. *Conclusion:* Sirt2 is an aggravating factor during hepatic I/R injury.

4.1663 Synaptopodin is regulated by aromatase activity

Fester, L., Zhou, L., Ossig, C., labitzke, J., Bläute, C., Bader, M., Vollmer, G., jarry, H. and Rune, G.M..
J. Neurochem., ***140***, *126-139 (2017)*

Locally synthesized estradiol plays an important role in synaptic plasticity in the hippocampus. We have previously shown that in hippocampal neurons, activity of the enzyme aromatase, which converts testosterone into estradiol, is reduced via Ca^{2+}-dependent phosphorylation. Synaptopodin is a highly estrogen responsive protein, and it has been shown that it is an important regulator of synaptic plasticity, mediated by its close association with internal calcium stores. In this study, we show that the expression of synaptopodin is stronger in the hippocampus of female animals than in that of male animals. Phosphorylation of aromatase, using letrozole, however, down-regulates synaptopodin immunohistochemistry in the hippocampus of both male and females. Similarly, in aromatase knock-out mice synaptopodin expression in the hippocampus is reduced sex independently. Using primary-dissociated hippocampal neurons, we found that evoked release of Ca^{2+} from internal stores down-regulates aromatase activity, which is paralleled by reduced expression of synaptopodin. Opposite effects were achieved after inhibition of the release. Calcium-dependent regulation of synaptopodin expression was abolished when the control of aromatase activity by the Ca^{2+} transients was disrupted. Our data suggest that the regulation of aromatase activity by Ca^{2+} transients in neurons contributes to synaptic plasticity in the hippocampus of male and female animals as an on-site regulatory mechanism.

4.1664 The upregulation of annexin A2 after spinal cord injury in rats may have implication for astrocyte proliferation

Chen, J., Cui, Z., yang, S., Wu, C., Li, W., Bao, G., Xu, G., Sun, Y., Wang, L. and Zhang, J.
Neuropeptides, ***61***, *67-76 (2017)*

Annexin A2 (ANXA2), is a member of the annexin family of proteins that exhibit Ca^{2+}-dependent binding to phospholipids. One attractive biological function of ANXA2 is participating in DNA synthesis and cell proliferation. Previous studies have shown that ANXA2 play a role in the development of the central nervous system. However, the biological function of ANXA2 after spinal cord injury (SCI) is still with limited acquaintance. In the present study, we performed a SCI model in adult rats and investigated the dynamic changes of ANXA2 expression in the spinal cord. Western blot analysis indicated a striking expression upregulation of ANXA2 after SCI. Immunohistochemistry further confirmed that ANXA2 immunoactivity was expressed at low levels in normal condition and increased at 5 day after SCI. Double immunofluorescence staining prompted that ANXA2 immunoreactivity was found in astrocytes and neurons. Interestingly, ANXA2 expression was increased predominantly in astrocytes. We also examined the expression profiles of proliferating cell nuclear antigen (PCNA), Cyclin D1 and active caspase-3 in the injured spinal cords by western blot. Co-expression of ANXA2/PCNA, ANXA2/Cyclin D1 was detected in glial fibrillary acidic protein. Importantly, double immunofluorescence staining revealed that cell proliferation evaluated by PCNA appeared in many ANXA2-expressing cells and rare caspase-3 was observed in ANXA2-expressing cells after SCI. In addition, ANXA2 knockdown in astrocytes resulted in the increase of PCNA expression after LPS stimulation, showing that ANXA2 inhibited astrocyte proliferation after inflammation. Our data suggested that ANXA2 might play important roles in CNS pathophysiology after SCI.

4.1665 Understanding quasi-apoptosis of the most numerous enucleated components of blood needs detailed molecular autopsy

Petrovich Gusev, G., Govekar, R., Gadewal, N. and Ivanovna Agalakova, N.
Ageing Res. Reviews, ***35***, *46-62 (2017)*

Erythrocytes are the most numerous cells in human body and their function of oxygen transport is pivotal to human physiology. However, being enucleated, they are often referred to as a sac of molecules and their cellularity is challenged. Interestingly, their programmed death stands a testimony to their cell-hood. They are capable of self-execution after a defined life span by both cell-specific mechanism and that resembling the cytoplasmic events in apoptosis of nucleated cells. Since the execution process lacks the nuclear and mitochondrial events in apoptosis, it has been referred to as quasi-apoptosis or eryptosis. Several studies on molecular mechanisms underlying death of erythrocytes have been reported. The data has generated a non-cohesive sketch of the process. The lacunae in the present knowledge need to be filled to gain deeper insight into the mechanism of physiological ageing and death of erythrocytes, as well as the effect of age of organism on RBCs survival. This would entail how the most numerous cells in the human body die and enable a better understanding of signaling mechanisms of their senescence and premature eryptosis observed in individuals of advanced age.

4.1666 Platelets Inhibit Migration of Canine Osteosarcoma Cells

Bulla, S.C., Badial, P.R., Silva, R.C., Lunsford, K. and Bulla, C.
J. Comp. Path., ***156***, *3-13 (2017)*

The interaction between platelets and tumour cells is important for tumour growth and metastasis. Thrombocytopenia or antiplatelet treatment negatively impact on cancer metastasis, demonstrating potentially important roles for platelets in tumour progression. To our knowledge, there is no information regarding the role of platelets in cancer progression in dogs. This study was designed to test whether canine platelets affected the migratory behaviour of three canine osteosarcoma cell lines and to give insights of molecular mechanisms. Intact platelets, platelet lysate and platelet releasate inhibited the migration of canine osteosarcoma cell lines. Addition of blood leucocytes to the platelet samples did not alter the inhibitory effect on migration. Platelet treatment also significantly downregulated the transcriptional levels of *SNAI2* and *TWIST1* genes. The interaction between canine platelets or molecules released during platelet activation and these tumour cell lines inhibits their migration, which suggests that canine platelets might antagonize metastasis of canine osteosarcoma. This effect is probably due to, at least in part, downregulation of genes related to epithelial–mesenchymal transition.

4.1667 Tumor progression locus 2 reduces severe allergic airway inflammation by inhibiting Ccl24 production in dendritic cells

Kannan, Y., Li, Y., Coomes, S.M., Okoye, I.S., Pelly, V.S., Sriskantharajah, S., Gückel, E., Webb, L., Czieso, S., Nikolov, N., MacDonald, A.S., Ley, S. and Wilson, M.S.
J. Allergy Clin. Immunol., ***139(2)***, *655-666e7 (2017)*

Background
The molecular and cellular pathways driving the pathogenesis of severe asthma are poorly defined. Tumor progression locus 2 (TPL-2) (COT, MAP3K8) kinase activates the MEK1/2-extracellular-signal regulated kinase 1/2 MAP kinase signaling pathway following Toll-like receptor, TNFR1, and IL-1R stimulation.
Objective
TPL-2 has been widely described as a critical regulator of inflammation, and we sought to investigate the role of TPL-2 in house dust mite (HDM)-mediated allergic airway inflammation.
Methods
A comparative analysis of wild-type and *Map3k8*$^{-/-}$ mice was conducted. Mixed bone marrow chimeras, conditional knockout mice, and adoptive transfer models were also used. Differential cell counts were performed on the bronchoalveolar lavage fluid, followed by histological analysis of lung sections. Flow cytometry and quantitative PCR was used to measure type 2 cytokines. ELISA was used to assess the production of IgE, type 2 cytokines, and Ccl24. RNA sequencing was used to characterize dendritic cell (DC) transcripts.
Results
TPL-2 deficiency led to exacerbated HDM-induced airway allergy, with increased airway and tissue eosinophilia, lung inflammation, and IL-4, IL-5, IL-13, and IgE production. Increased airway allergic responses in *Map3k8*$^{-/-}$ mice were not due to a cell-intrinsic role for TPL-2 in T cells, B cells, or LysM^{+} cells but due to a regulatory role for TPL-2 in DCs. TPL-2 inhibited *Ccl24* expression in lung DCs, and blockade of Ccl24 prevented the exaggerated airway eosinophilia and lung inflammation in mice given HDM-pulsed *Map3k8*$^{-/-}$ DCs.
Conclusions
TPL-2 regulates DC-derived Ccl24 production to prevent severe type 2 airway allergy in mice.

4.1668 489 – Modulation of platelet levels by an anti-IL-1α antibody (MABp1) in advanced colorectal cancer patients

Hickish, T., Mohanty, P., Shivaswamy, M.S., Sunley, K., Varshney, A., Martin, R. and Simard, J.
*Eur. J. Cancer, **72**, Suppl. 1, S70 (2017)*

Background: In a Phase III study, treatment with MABp1, an anti-IL-1a antibody, has demonstrated 76% relative increase in clinical response rate versus placebo in end-stage colorectal cancer patients. In addition to the primary end point (improved health status as measured by lean body mass and pain, fatigue and appetite), secondary measures included monitoring of pharmacodynamic parameters such as serum IL-6 levels and platelet counts. With respect to these secondary endpoints, patient receiving MABp1 treatment showed decreased serum IL6 levels as well as platelet counts. IL-1a is known to upregulate IL-6, a known inducer of megakaryocytopoesis. The reduction in IL-6 and platelet counts in patients treated with IL-1a suggests that platelet-derived IL-1a may be both a target of antibody therapy and play an important role in regulation of megakaryocytopoiesis. While the role of platelet-derived IL-1a has been established in animal models for vascular endothelial cell activation and the pathogenesis of cerebrovascular inflammation, few studies have even confirmed the expression of IL-1a on human platelets. Here we present findings to confirm theexpression of platelet IL-1a on platelets within a healthy human population.
Material and Methods: Platelets from human blood were isolated on a discontinuous iodixanol density gradient. Platelets were stained with MABp1 or isotype control before and after activation with thrombin and lipopolysaccharide, and observed by confocal microscopy and flow cytometry. In addition, the isolated platelets were lysed and the membranes were isolated by ultracentrifugation. The IL-1a on the membrane was immunoprecipitated using a pro-IL-1a antibody. The membrane proteins were resolved on a SDS-PAGE and human IL-1a was detected using various antibodies. The protein was digested with trypsin, and the isolatedpeptides were subjected to peptide mass fingerprinting.
Results: Our work has confirmed the presence of IL-1a on the surface of platelets. Confocal microscopy and Flow Cytometry has shown an activation dependent increase in membrane IL-1a levels. Western blotting and immunoprecipitation confirmed that the IL-1a is present on the membrane in its propeptide form. In addition, the propeptide is sensitive to cleavage into its mature form by calpain-like protease also present on the surface of platelets. Peptide mass fingerprinting of the immunoprecipitant using a QTof Micro Mass Spectrometer identified five unique peptides specific to human IL-1a at high confidence levels.
Conclusions: Based on our findings and results from previous studies, platelets appear to play an important role in the development of important conditions, including cancer. We confirm that IL-1a is present on human platelets and that this may represent an important factor in regulatingplatelet thrombocytosis and consequent pathology in cancer.

4.1669 Doxycycline attenuates breast cancer related inflammation by decreasing plasma lysophosphatidate concentrations and inhibiting NF-κB activation

Tang, X., Wang, X., Zhao, Y.Y., Curtis, J.M. and Brindley, D.N.
*Mol. Cancer, **16**:36 (2017)*

Background
We previously discovered that tetracyclines increase the expression of lipid phosphate phosphatases at the surface of cells. These enzymes degrade circulating lysophosphatidate and therefore doxycycline increases the turnover of plasma lysophosphatidate and decreases its concentration. Extracellular lysophosphatidate signals through six G protein-coupled receptors and it is a potent promoter of tumor growth, metastasis and chemo-resistance. These effects depend partly on the stimulation of inflammation that lysophosphatidate produces.
Methods
In this work, we used a syngeneic orthotopic mouse model of breast cancer to determine the impact of doxycycline on circulating lysophosphatidate concentrations and tumor growth. Cytokine/chemokine concentrations in tumor tissue and plasma were measured by multiplexing laser bead technology. Leukocyte infiltration in tumors was analyzed by immunohistochemistry. The expression of IL-6 in breast cancer cell lines was determined by RT-PCR. Cell growth was measured in Matrigel™ 3D culture. The effects of doxycycline on NF-κB-dependent signaling were analyzed by Western blotting.
Results
Doxycycline decreased plasma lysophosphatidate concentrations, delayed tumor growth and decreased the concentrations of several cytokines/chemokines (IL-1β, IL-6, IL-9, CCL2, CCL11, CXCL1, CXCL2, CXCL9, G-CSF, LIF, VEGF) in the tumor. These results were compatible with the effects of doxycycline in decreasing the numbers of $F4/80^+$ macrophages and $CD31^+$ blood vessel endothelial cells in the tumor.

Doxycycline also decreased the lysophosphatidate-induced growth of breast cancer cells in three-dimensional culture. Lysophosphatidate-induced Ki-67 expression was inhibited by doxycycline. NF-κB activity in HEK293 cells transiently expressing a NF-κB-luciferase reporter vectors was also inhibited by doxycycline. Treatment of breast cancer cells with doxycycline also decreased the translocation of NF-κB to the nucleus and the mRNA levels for IL-6 in the presence or absence of lysophosphatidate.
Conclusion
These results contribute a new dimension for understanding the anti-inflammatory effects of tetracyclines, which make them potential candidates for adjuvant therapy of cancers and other inflammatory diseases.

4.1670 Selective Osmotic Shock (SOS)-Based Islet Isolation for Microencapsulation
Enck, K., McQuilling, J.P., Orlando, G., Tamburrini, R., Sivanandane, S. and Opara, E.C.
Methods in Mol. Biol., ***1479***, *191-198 (2017)*

Islet transplantation (IT) has recently been shown to be a promising alternative to pancreas transplantation for reversing diabetes. IT requires the isolation of the islets from the pancreas, and these islets can be used to fabricate a bio-artificial pancreas. Enzymatic digestion is the current gold standard procedure for islet isolation but has lingering concerns. One such concern is that it has been shown to damage the islets due to nonselective tissue digestion. This chapter provides a detailed description of a nonenzymatic method that we are exploring in our lab as an alternative to current enzymatic digestion procedures for islet isolation from human and nonhuman pancreatic tissues. This method is based on selective destruction and protection of specific cell types and has been shown to leave the extracellular matrix (ECM) of islets intact, which may thus enhance islet viability and functionality. We also show that these SOS-isolated islets can be microencapsulated for transplantation.

4.1671 Neutrophil adhesion and crawling dynamics on liver sinusoidal endothelial cells under shear flow
Yang, H., Li, N., Du, Y., Tong, C., Lü, S., Hu, J., Zhang, Y. and Long, M.
Exp. Cell Res., ***351***, *91-00 (2017)*

Neutrophil (polymorphonuclear leukocyte, PMN) recruitment in the liver sinusoid takes place in almost all liver diseases and contributes to pathogen clearance or tissue damage. While PMN rolling unlikely appears in liver sinusoids and Mac-1 or CD44 is assumed to play respective roles during *in vivo* local or systematic inflammatory stimulation, the regulating mechanisms of PMN adhesion and crawling dynamics are still unclear from those *in vivo* studies. Here we developed a two-dimensional *in vitro* sinusoidal model with primary liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) to investigate TNF-α-induced PMN recruitment under shear flow. Our data demonstrated that LFA-1 dominates the static or shear resistant adhesion of PMNs while Mac-1 decelerates PMN crawling on LSEC monolayer. Any one of LFA-1, Mac-1, and CD44 molecules is not able to work effectively for mediating PMN transmigration across LSEC monolayer. The presence of KCs only affects the randomness of PMN crawling. These findings further the understandings of PMN recruitment under shear flow in liver sinusoids.

4.1672 Zinc finger protein ZPR9 functions as an activator of AMPK-related serine/threonine kinase MPK38/MELK involved in ASK1/TGF-β/p53 signaling pathways
Seong, H-A., Manoharan, R. and Ha, H.
Scientific Reports, ***7***:*42502 (2017)*

Murine protein serine-threonine kinase 38 (MPK38), an AMP-activated protein kinase (AMPK)-related kinase, has been implicated in the induction of apoptosis signal-regulating kinase 1 (ASK1)-, transforming growth factor-β (TGF-β)-, and p53-mediated activity involved in metabolic homeostasis. Here, zinc finger protein ZPR9 was found to be an activator of MPK38. The association of MPK38 and ZPR9 was mediated by cysteine residues present in each of these two proteins, Cys269 and Cys286 of MPK38 and Cys305 and Cys308 of ZPR9. MPK38 phosphorylated ZPR9 at Thr252. Wild-type ZPR9, but not the ZPR9 mutant T252A, enhanced ASK1, TGF-β, and p53 function by stabilizing MPK38. The requirement of ZPR9 Thr252 phosphorylation was validated using CRISPR/Cas9-mediated ZPR9 (T252A) knockin cell lines. The knockdown of endogenous ZPR9 showed an opposite trend, resulting in the inhibition of MPK38-dependent ASK1, TGF-β, and p53 function. This effect was also demonstrated in mouse embryonic fibroblast (MEF) cells that were haploinsufficient (+/−) for ZPR9, NIH 3T3 cells with inducible knockdown of ZPR9, and CRISPR/Cas9-mediated ZPR9 knockout cells. Furthermore, high-fat diet (HFD)-fed mice displayed reduced MPK38 kinase activity and ZPR9 expression compared to that in mice on control chow, suggesting that ZPR9 acts as a physiological activator of MPK38 that may participate in obesity.

4.1673 PARP inhibition protects against alcoholic and non-alcoholic steatohepatitis

Mukhopadhyay, P. et al

J. Hepatol., ***66***, *589-600 (2017)*

Background & Aims

Mitochondrial dysfunction, oxidative stress, inflammation, and metabolic reprograming are crucial contributors to hepatic injury and subsequent liver fibrosis. Poly(ADP-ribose) polymerases (PARP) and their interactions with sirtuins play an important role in regulating intermediary metabolism in this process. However, there is little research into whether PARP inhibition affects alcoholic and non-alcoholic steatohepatitis (ASH/NASH).

Methods

We investigated the effects of genetic deletion of PARP1 and pharmacological inhibition of PARP in models of early alcoholic steatohepatitis, as well as on Kupffer cell activation *in vitro* using biochemical assays, real-time PCR, and histological analyses. The effects of PARP inhibition were also evaluated in high fat or methionine and choline deficient diet-induced steatohepatitis models in mice.

Results

PARP activity was increased in livers due to excessive alcohol intake, which was associated with decreased NAD^+ content and SIRT1 activity. Pharmacological inhibition of PARP restored the hepatic NAD^+ content, attenuated the decrease in SIRT1 activation and beneficially affected the metabolic-, inflammatory-, and oxidative stress-related alterations due to alcohol feeding in the liver. *PARP1*$^{-/-}$ animals were protected against alcoholic steatohepatitis and pharmacological inhibition of PARP or genetic deletion of PARP1 also attenuated Kupffer cell activation *in vitro*. Furthermore, PARP inhibition decreased hepatic triglyceride accumulation, metabolic dysregulation, or inflammation and/or fibrosis in models of NASH.

Conclusion

Our results suggests that PARP inhibition is a promising therapeutic strategy in steatohepatitis with high translational potential, considering the availability of PARP inhibitors for clinical treatment of cancer.

4.1674 One step fabrication of hydrogel microcapsules with hollow core for assembly and cultivation of hepatocyte spheroids

Siltanen, C., Diakataou, M., Lowen, J., Haque, A., Rahimian, A., Stybayeva, G. and Revzin, A.

Acta Biomaterialia, ***50***, *428-436 (2017)*

3D hepatic microtissues can serve as valuable liver analogues for cell-based therapies and for hepatotoxicity screening during preclinical drug development. However, hepatocytes rapidly dedifferentiate *in vitro*, and typically require 3D culture systems or co-cultures for phenotype rescue. In this work we present a novel microencapsulation strategy, utilizing coaxial flow-focusing droplet microfluidics to fabricate microcapsules with liquid core and poly(ethylene glycol) (PEG) gel shell. When entrapped inside these capsules, primary hepatocytes rapidly formed cell-cell contacts and assembled into compact spheroids. High levels of hepatic function were maintained inside the capsules for over ten days. The microencapsulation approach described here is compatible with difficult-to-culture primary epithelial cells, allows for tuning gel mechanical properties and diffusivity, and may be used in the future for high density suspension cell cultures.

4.1675 Laquinimod enhances central nervous system barrier functions

Lühder, F., Kebir, H., Odoardi, F., Litke, T., Sonneck, M., Alvarez, J.I., Winchenbrch, J., Eckert, N., Hayardeny, L., Sorani, E., Lodygin, D., Flügel, A. and Prat, A.

Neurobiology of Disease, ***102***, *60-69 (2017)*

Laquinimod is currently being tested as a therapeutic drug in multiple sclerosis. However, its exact mechanism of action is still under investigation. Tracking of fluorescently-tagged encephalitogenic T cells during experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, revealed that laquinimod significantly reduces the invasion of pathogenic effector T cells into the CNS tissue. T-cell activation, differentiation and amplification within secondary lymphoid organs after immunization with myelin antigen, their migratory capacity and re-activation within the nervous tissue were either only mildly affected or remained unchanged. Instead, laquinimod directly impacted the functionality of the CNS vasculature. The expression of tight junction proteins p120 and ZO-1 in human brain endothelial cells was up-regulated upon laquinimod treatment, resulting in a significant increase in

the transendothelial electrical resistance of confluent monolayers of brain endothelial cells. Similarly, expression of the adhesion molecule activated leukocyte cell adhesion molecule (ALCAM) and inflammatory chemokines CCL2 and IP-10 was suppressed, leading to a significant reduction in the migration of memory T_H1 and T_H17 lymphocytes across the blood brain barrier (BBB). Our data indicate that laquinimod exerts its therapeutic effects by tightening the BBB and limiting parenchymal invasion of effector T cells, thereby reducing CNS damage.

4.1676 The cryoprotective effect of iodixanol in buffalo semen cryopreservation
Singh Swami, D., Kumar, P., Malik, R.K., Saini, M., Kumar, d. and Jan, M.H.
*Animal Reprod. Sci., **179**, 20-26 (2017)*

This is the first report to examine the effect of iodixanol (OptiPrep™) on cryosurvival of buffalo spermatozoa. A total of thirty ejaculates (five ejaculates from each bull) from six buffalo bulls were used for this experiment. Each ejaculate was divided into four aliquots and diluted in freezing extender supplemented with different concentrations of OptiPrep™ (0, 1.25, 2.5 and 5%) and then cryopreserved. The semen quality variables were evaluated before and after freezing of the semen. There were no effects of OptiPrep™ ($P > 0.05$) on sperm kinetics, motility, abnormality and membrane integrity of fresh extended spermatozoa. However, after freeze-thaw, sperm motility, plasma membrane integrity and distance travelled in cervical mucus of 2.5% OptiPrep™ treated samples showed significantly higher ($P < 0.05$) compared to other treated and control samples. No significant differences ($P > 0.05$) were seen in sperm abnormality and acrosomal integrity of treated and control frozen-thawed samples. The total antioxidant capacity of 2.5 and 5% OptiPrep™ treated frozen-thawed sperm were found to be higher ($P < 0.05$) as compared to other groups; whereas the MDA level in OptiPrep™ treated sperm was significantly lower than the control ($P < 0.05$). In incubation test, 2.5% OptiPrep™ proved to be better in preservation of sperm motility as compared to other treated and control samples. In conclusion, the present study has shown that iodixanol has the ability protect spermatozoa against oxidative stress and resulting overall improvement in the post-thaw semen quality.

4.1677 Identification of novel autoantigens via mass spectroscopy-based antibody-mediated identification of autoantigens (MS-AMIDA) using immune thrombocytopenic purpura (ITP) as a model disease
Kamhieh-Milz, J., Sterzer, V., Celik, H., Khorramshahi, O., Hassan Noftah, R.F. and Salama, A.
*J. Proteomics, **157**, 59-70 (2017)*

Immune thrombocytopenic purpura (ITP) is one of the best characterized autoimmune diseases. Autoantibodies (AABs) against platelet antigens are considered as the diagnostic hallmark of ITP, but are detectable in only 50% of patients. We designed and applied a novel proteomic approach termed Mass Spectroscopy-based Antibody-Mediated Identification of Autoantigens (MS-AMIDA) for platelet antigens. Patients were separated into patients with classical AABs [ITP(+)] and patients without AABs [ITP(−)]. Altogether, 181 potential AAGs were found in ITP(+) and 135 AAGs in ITP(−), with 34 and 23 AAGs reproducibly found in two runs of MS-AMIDA. After subtracting identifiers from the controls, 57 AAGs in ITP(+) and 29 AAGs in ITP(+) remained, with 16 AAGs commonly found in ITP(+) and ITP(−) patients. Label-free quantification (LFQ) revealed 15 potential AAGs that are quantitatively stronger in ITP. Dot blot validation was performed on hexokinase 1 (HK1), E1 pyruvate dehydrogenase (E1-PDH), coagulation factor XIII, filamin A (FLNA), non-muscle myosin 9. Eleven patients were found to have anti-HK1 AABs, one patient had anti-E1-PDH AABs, and two patients had anti-FLNA AABs. Most antigens were of intracellular origin with significant association with actin-cytoskeleton and regulation of programmed cell death. In conclusion, novel AAGs for ITP were identified using MS-AMIDA.

4.1678 The redox environment triggers conformational changes and aggregation of hIAPP in Type II Diabetes
Rodriguez Camargo, D.C. et al
Scientific Reports, 7:44041 (2017)

Type II diabetes (T2D) is characterized by diminished insulin production and resistance of cells to insulin. Among others, endoplasmic reticulum (ER) stress is a principal factor contributing to T2D and induces a shift towards a more reducing cellular environment. At the same time, peripheral insulin resistance triggers the over-production of regulatory hormones such as insulin and human islet amyloid polypeptide (hIAPP). We show that the differential aggregation of reduced and oxidized hIAPP assists to maintain the redox equilibrium by restoring redox equivalents. Aggregation thus induces redox balancing which can assist initially to counteract ER stress. Failure of the protein degradation machinery might finally result in β-cell

disruption and cell death. We further present a structural characterization of hIAPP in solution, demonstrating that the N-terminus of the oxidized peptide has a high propensity to form an α-helical structure which is lacking in the reduced state of hIAPP. In healthy cells, this residual structure prevents the conversion into amyloidogenic aggregates.

4.1679 Abseq: Ultrahigh-throughput single cell protein profiling with droplet microfluidic barcoding
Shahi, P., Kim, S.C., Haliburton, J.R., Gartner, Z.J. and Abate, A.R.
*Scientific Reports, **7**:44447 (2017)*

Proteins are the primary effectors of cellular function, including cellular metabolism, structural dynamics, and information processing. However, quantitative characterization of proteins at the single-cell level is challenging due to the tiny amount of protein available. Here, we present Abseq, a method to detect and quantitate proteins in single cells at ultrahigh throughput. Like flow and mass cytometry, Abseq uses specific antibodies to detect epitopes of interest; however, unlike these methods, antibodies are labeled with sequence tags that can be read out with microfluidic barcoding and DNA sequencing. We demonstrate this novel approach by characterizing surface proteins of different cell types at the single-cell level and distinguishing between the cells by their protein expression profiles. DNA-tagged antibodies provide multiple advantages for profiling proteins in single cells, including the ability to amplify low-abundance tags to make them detectable with sequencing, to use molecular indices for quantitative results, and essentially limitless multiplexing.

4.1680 An efficient method for isolation of representative and contamination-free population of blood platelets for proteomic studies
Wrzyszcz, A., Urbaniak, J., Sapa, A. and Wozniak, M.
*Platelets, **28(1)**, 43-53 (2017)*

To date, there has been no ideal method for blood platelet isolation which allows one to obtain a preparation devoid of contaminations, reflecting the activation status and morphological features of circulating platelets. To address these requirements, we have developed a method which combines the continuous density gradient centrifugation with washing from PGI_2-supplemented platelet-rich plasma (PRP). We have assessed the degree of erythrocyte and leukocyte contamination, recovery of platelets, morphological features, activation status, and reactivity of isolated platelets. Using our protocol, we were able to get a preparation free from contaminations, representing well the platelet population prior to the isolation in terms of size and activity. Besides this, we have obtained approximately 2 times more platelets from the same volume of blood compared to the most widely used method. From 10 ml of whole citrated blood we were able to get on average 2.7 mg of platelet-derived protein. The method of platelet isolation presented in this paper can be successfully applied to tests requiring very pure platelets, reflecting the circulating platelet state, from a small volume of blood.

4.1681 Deterministic encapsulation of single cells in thin tunable microgels for niche modelling and therapeutic delivery
Mao, A.S., Shin, J-W., utech, S., Wang, H., Uzun, O., Li, W., Cooper, M., Hu, Y., Zhang, L., Weitz, D.A. and Mooney, D.J.
*Nature Materials, **16(2)**, 236-243 (2017)*

Existing techniques to encapsulate cells into microscale hydrogels generally yield high polymer-to-cell ratios and lack control over the hydrogel's mechanical properties1. Here, we report a microfluidic-based method for encapsulating single cells in an approximately six-micrometre layer of alginate that increases the proportion of cell-containing microgels by a factor of ten, with encapsulation efficiencies over 90%. We show that in vitro cell viability was maintained over a three-day period, that the microgels are mechanically tractable, and that, for microscale cell assemblages of encapsulated marrow stromal cells cultured in microwells, osteogenic differentiation of encapsulated cells depends on gel stiffness and cell density. We also show that intravenous injection of singly encapsulated marrow stromal cells into mice delays clearance kinetics and sustains donor-derived soluble factors in vivo. The encapsulation of single cells in tunable hydrogels should find use in a variety of tissue engineering and regenerative medicine applications.

4.1682 Age-Related Expression of a Repeat-Rich Intergenic Long Noncoding RNA in the Rat Brain
Kour, S. and Rath, P.C.
*Mol. Neurobiol., **54**, 639-660 (2017)*

Genome-wide transcriptome analysis has shown that ~90 % of the mammalian genome undergoes pervasive transcription into various small and long noncoding RNAs with diverse biological functions and only ~1.5 % is protein coding. Recent literature suggests that various structurally diverse sense and antisense long noncoding RNAs (lncRNAs) (>200 nt) are expressed from the intronic, intergenic and repeat-rich regions in the mammalian central nervous system (CNS). Till date, many of them have been found to be regulated in developmental, spatio-temporal and cell type-specific manners and are involved in various neurological processes. However, still much is left to be understood regarding their functional relevance in mammalian brain development, maturation and ageing. Furthermore, various signalling factors and metabolites such as all-*trans* retinoic acid (atRA) have been known to regulate brain functions during development, though their role in adult brain function is much less known. Here, we report differential and age-related expression of a novel repeat sequence-rich, long intergenic nonprotein coding RNA (lincRNA), named as *LINC-RSAS* (repeat-rich sense-antisense transcript) in different neuroanatomical regions of the rat brain. The *LINC-RSAS* was found to be moderately conserved and contained regulatory elements of various cell growth- and development-specific transcriptional factors in its up/downstream flanking sequences in the genome. Through RNA expression by reverse transcription polymerase chain reaction (RT-PCR) and localization by in situ RNA hybridization, we found that both sense and antisense transcripts of *LINC-RSAS* were expressed in the cortex, hippocampus and cerebellum regions of the rat brain in cell type-specific and age-related manner. Furthermore, both the expression level and subcellular localization of the antisense *LINC-RSAS* transcript were significantly induced in the cultured primary hippocampal neurons after treatment with atRA. Overall, our study provides insights into the possible involvement of an atRA-inducible, intergenic lncRNA in different functional regions of mammalian brain and its association with brain maturation and ageing.

4.1683 Microfluidic fluorescence-activated cell sorting (μFACS) chip with integrated piezoelectric actuators for low-cost mammalian cell enrichment
Cheng, Z., Wu, X., Cheng, J. and Liu, P.
Microfluid. Nanofluid., ***21****:9 (2017)*

A low-cost, microfluidic fluorescence-activated cell sorting (μFACS) microchip integrated with two piezoelectric lead–zirconate–titanate actuators was demonstrated for automated, high-performance mammalian cell analysis and enrichment. In this PDMS–glass device, cells were hydrodynamically focused into a single file line in the lateral direction by two sheath flows, and then interrogated with a forward scattering and confocal fluorescent detection system. The selected cells were displaced transversely into a collection channel by two piezoelectric actuators that worked in a pull–push relay manner with a minimal switching time of ~0.8 ms. High detection throughput (~2500 cells/s), high sorting rate (~1250 cells/s), and high sorting efficiency (~98%) were successfully achieved on the μFACS system. Six cell mixture samples containing 22.87% of GFP-expressing HeLa cells were consecutively analyzed and sorted on the chip, revealing a stable sorting efficiency of 97.7 ± 0.93%. In addition, cell mixtures containing 37.65 and 3.36% GFP HeLa cells were effectively enriched up to 83.82 and 78.51%, respectively, on the microchip, and an enrichment factor of 105 for the low-purity (3.36%) sample was successfully obtained. This fully enclosed, disposable microfluidic chip provides an automated platform for low-cost fluorescence-based cell detection and enrichment, and is attractive to applications where cross-contamination between runs and aerosol hazard are the primary concerns.

4.1684 Rapid Encapsulation of Cell and Polymer Solutions with Bubble-Triggered Droplet Generation
Yan, Z., Clark, I.C. and Abate, A.R.
Macromolecul. Chem. Physics, ***218(2)****, 1600297 (2017)*

The generation of monodisperse droplets with microfluidics is valuable for applications ranging from material science to single cell analysis. However, conventional methods for forming droplets are limited in throughput, particularly when the fluids have low interfacial tension or high viscosity, like biological or polymer fluids. Rapid emulsification of biological and polymer fluids using bubble-triggered droplet generation is demonstrated. In addition to making droplets over tenfold faster than conventional drop makers with equivalent monodispersity, bubble-triggering can form droplets smaller than the nozzle, allowing droplets of the desired size to be generated in large channels that are robust against clogging.

4.1685 Single cell-laden protease-sensitive microniches for long-term culture in 3D
Lienemann, P.S., Rossow, T., Mao, A.S., Vallmajo-Martin, Q., Ehrbar, M. and Mooney, D.J.
Lab on a Chip, ***17****, 727-737 (2017)*

Single cell-laden three-dimensional (3D) microgels that can serve to mimic stem cell niches in vitro, and are therefore termed microniches, can be efficiently fabricated by droplet-based microfluidics. In this technique an aqueous polymer solution along with a highly diluted cell solution is injected into a microfluidic device to create monodisperse pre-microgel droplets that are then solidified by a polymer crosslinking reaction to obtain monodisperse single cell-laden microniches. However, problems limiting this approach studying the fate of single cells include Poisson encapsulation statistics that result in mostly empty microniches, and cells egressing from the microniches during subsequent cell culture. Here, we present a strategy to bypass Poisson encapsulation statistics in synthetic microniches by selective crosslinking of only cell-laden pre-microgel droplets. Furthermore, we show that we can position cells in the center of the microniches, and that even in protease-sensitive microniches this greatly reduces cell egress. Collectively, we present the development of a versatile protocol that allows for unprecedented efficiency in creation of synthetic protease-sensitive microniches for probing single stem cell fate in 3D.

4.1686 Thymic Dendritic Cell Subsets Display Distinct Efficiencies and Mechanisms of Intercellular MHC Transfer

Kroger, C.J., Spidale, N.A., Wang, B. and Tisch, R.
J. Immunol., ***198(1)***, *249-256 (2017)*

Thymic dendritic cells (DC) delete self-antigen–specific thymocytes, and drive development of Foxp3-expressing immunoregulatory T cells. Unlike medullary thymic epithelial cells, which express and present peripheral self-antigen, DC must acquire self-antigen to mediate thymic negative selection. One such mechanism entails the transfer of surface MHC–self peptide complexes from medullary thymic epithelial cells to thymic DC. Despite the importance of thymic DC cross-dressing in negative selection, the factors that regulate the process and the capacity of different thymic DC subsets to acquire MHC and stimulate thymocytes are poorly understood. In this study intercellular MHC transfer by thymic DC subsets was investigated using an MHC-mismatch–based in vitro system. Thymic conventional DC (cDC) subsets signal regulatory protein α ($SIRP\alpha^+$) and $CD8\alpha^+$ readily acquired MHC class I and II from thymic epithelial cells but plasmacytoid DC were less efficient. Intercellular MHC transfer was donor-cell specific; thymic DC readily acquired MHC from TEC plus thymic or splenic DC, whereas thymic or splenic B cells were poor donors. Furthermore DC origin influenced cross-dressing; thymic versus splenic DC exhibited an increased capacity to capture TEC-derived MHC, which correlated with direct expression of EpCAM by DC. Despite similar capacities to acquire MHC–peptide complexes, thymic $CD8\alpha^+$ cDC elicited increased T cell stimulation relative to $SIRP\alpha^+$ cDC. DC cross-dressing was cell-contact dependent and unaffected by lipid raft disruption of donor TEC. Furthermore, blocking PI3K signaling reduced MHC acquisition by thymic $CD8\alpha^+$ cDC and plasmacytoid DC but not $SIRP\alpha^+$ cDC. These findings demonstrate that multiple parameters influence the efficiency of and distinct mechanisms drive intercellular MHC transfer by thymic DC subsets.

4.1687 Extracellular vesicles released by hepatocytes from gastric infusion model of alcoholic liver disease contain a MicroRNA barcode that can be detected in blood

Eguchi, A., Lazaro, R.G., Wang, J., Kim, J., Povero, D., Williams, B., Ho, S.B., Stärkel, P., Schnabl, B., Ohno-Machado, L., Tsukamoto, H. and Feldstein, A.E.
Hepatology, ***65(2)***, *475-490 (2017)*

Extracellular vesicles (EVs) released during cell stress, or demise, can contain a barcode of the cell origin, including specific microRNAs (miRNAs). Here, we tested the hypothesis that during early alcoholic steatohepatitis (ASH) development, hepatocytes (HCs) release EVs with an miRNA signature that can be measured in circulation. A time-course experiment showed that after 2 weeks of intragastric infusion, a time point that results in isolated steatosis, there was no increase of blood EVs. After 4 weeks of infusion, mice developed features of early ASH accompanied by a marked increase in the level of EVs in blood ($P < 0.05$), as well as in culture media of isolated HCs ($P < 0.001$) and hepatic macrophages ($P < 0.001$), with HCs being the predominant source of EVs. The transcriptome analysis of HC-EVs from ASH mice detected differentially expressed miRNAs, including nine significantly up-regulated and four significantly down-regulated miRNAs. Target prediction and pathway analyses of the up-regulated miRNAs identified 121 potential target genes involved in inflammatory and cancer pathways, such as nuclear factor kappa B, EGF, Wnt, and B-cell lymphoma 2. Three miRNAs, let7f, miR-29a, and miR-340, were increased in blood EVs from ASH mice ($P < 0.05$), but not in blood EVs from three other models of chronic liver injury, including bile duct ligation, nonalcoholic steatohepatitis, and obese mice, as well as EVs released from hepatocytes exposed to ethanol. Blood EV level ($P < 0.01$) and three miRNAs ($P < 0.05$) were

significantly increased in patients with ambulatory mild ALD as compared to nonalcoholics. *Conclusion*: Damaged hepatocytes from ASH mice are a key EV source with a specific miRNA cargo, which are specific for ASH-related liver injury. These findings uncover EVs as a potentially novel diagnostic for ASH.

4.1688 Wildtype motoneurons, ALS-Linked SOD1 mutation and glutamate profoundly modify astrocyte metabolism and lactate shuttling
Hounoum, B.M., mavel, S., Coque, E., patin, F., Vourc'h, P., marouillat, S., nadaal-Desbarats, L., Emond, P., Corcia, P., Andres, C.R., Raoul, C. and Blasco, H.
*Glia, **65(4)**, 592-605 (2017)*

The selective degeneration of motoneuron that typifies amyotrophic lateral sclerosis (ALS) implicates non-cell-autonomous effects of astrocytes. However, mechanisms underlying astrocyte-mediated neurotoxicity remain largely unknown. According to the determinant role of astrocyte metabolism in supporting neuronal function, we propose to explore the metabolic status of astrocytes exposed to ALS-associated conditions. We found a significant metabolic dysregulation including purine, pyrimidine, lysine, and glycerophospholipid metabolism pathways in astrocytes expressing an ALS-causing mutated superoxide dismutase-1 (SOD1) when co-cultured with motoneurons. SOD1 astrocytes exposed to glutamate revealed a significant modification of the astrocyte metabolic fingerprint. More importantly, we observed that SOD1 mutation and glutamate impact the cellular shuttling of lactate between astrocytes and motoneurons with a decreased in extra- and intra-cellular lactate levels in astrocytes. Based on the emergent strategy of metabolomics, this work provides novel insight for understanding metabolic dysfunction of astrocytes in ALS conditions and opens the perspective of therapeutics targets through focusing on these metabolic pathways.

4.1689 Droplet size influences division of mammalian cell factories in droplet microfluidic cultivation
Rajaeswari, P.K.P., Joensson, H.N. and Andersson-Svahn, H.
*Electrophoresis, **38(2)**, 305-310 (2017)*

The potential of using droplet microfluidics for screening mammalian cell factories has been limited by the difficulty in achieving continuous cell division during cultivation in droplets. Here, we report the influence of droplet size on mammalian cell division and viability during cultivation in droplets. Chinese Hamster Ovary (CHO) cells, the most widely used mammalian host cells for biopharmaceuticals production were encapsulated and cultivated in 33, 180 and 320 pL droplets for 3 days. Periodic monitoring of the droplets during incubation showed that the cell divisions in 33 pL droplets stopped after 24 h, whereas continuous cell division was observed in 180 and 320 pL droplets for 72 h. The viability of the cells cultivated in the 33 pL droplets also dropped to about 50% in 72 h. In contrast, the viability of the cells in the larger droplets was above 90% even after 72 h of cultivation, making them a more suitable droplet size for 72-h cultivation. This study shows a direct correlation of microfluidic droplet size to the division and viability of mammalian cells. This highlights the importance of selecting suitable droplet size for mammalian cell factory screening assays.

4.1690 Upregulation of hydroxysteroid sulfotransferase 2B1b promotes hepatic oval cell proliferation by modulating oxysterol-induced LXR activation in a mouse model of liver injury
Wang, Z., yang, X., Chen, L., Zhi, X., Lu, H., Ning, Y., Yeong, J., Chen, S., Yin, L., Wang, X. and Li, X.
*Arch. Toxicol., **91**, 271-287 (2017)*

Hydroxysteroid sulfotransferase 2B1b (SULT2B1b) sulfates cholesterol and oxysterols. Hepatic oval cells (HOCs), thought to be progenitor cells, can be triggered in chemically injured livers. The present study focused on the role of SULT2B1b in HOC proliferation after liver injury. Our experiments revealed that the expression of SULT2B1b was increased dramatically in a chemical-induced liver injury model, mainly in HOCs. Upon challenge with a hepatotoxic diet containing 0.1 % 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), SULT2B1$^{-/-}$ mice presented alleviated liver injury and less HOC proliferation compared with wild-type (WT) mice, and these findings were verified by serum analysis, histopathology, immunofluorescence staining, RNA-seq, and Western blotting. HOCs derived from SULT2B1$^{-/-}$ mice showed lower proliferative capability than those from WT mice. SULT2B1b overexpression promoted growth of the WB-F344 hepatic oval cell line, whereas SULT2B1b knockdown inhibited growth of these cells. The IL-6/STAT3 signaling pathway also was promoted by SULT2B1b. Liquid chromatography and mass spectrometry indicated that the levels of 22-hydroxycholesterol, 25-hydroxycholesterol, and 24,25-

epoxycholesterol were higher in the DDC-injured livers of SULT2B1$^{-/-}$ mice than in livers of WT mice. The above oxysterols are physiological ligands of liver X receptors (LXRs), and SULT2B1b suppressed oxysterol-induced LXR activation. Additional in vivo and in vitro experiments demonstrated that LXR activation could inhibit HOC proliferation and the IL-6/STAT3 signaling pathway, and these effects could be reversed by SULT2B1b. Our data indicate that upregulation of SULT2B1b might promote HOC proliferation and aggravate liver injury via the suppression of oxysterol-induced LXR activation in chemically induced mouse liver injury.

4.1691 Droplet Microfluidic Flow Cytometer For Sorting On Transient Cellular Responses Of Genetically-Encoded Sensors

Fiedler, B.L., Van Buskirk, S., Carter, K.P., Qin, Y., Carpenter, M.C., Palmer, A.E. and Jimenez, R.
Anal. Chem., ***89(1)****, 711-719 (2017)*

Fluorescent biosensors are important measurement tools for in vivo quantification of pH, concentrations of metal ions and other analytes, and physical parameters such as membrane potential. Both the development of these sensors and their implementation in examining cellular heterogeneity requires technology for measuring and sorting cells based on the fluorescence levels before and after chemical or physical perturbations. We developed a droplet microfluidic platform for the screening and separation of cell populations on the basis of the in vivo response of expressed fluorescence-based biosensors after addition of an exogenous analyte. We demonstrate the capability to resolve the responses of two genetically encoded Zn^{2+} sensors at a range of time points spanning several seconds and subsequently sort a mixed-cell population of varying ratios with high accuracy.

4.1692 Anakinra Protects Against Serum Deprivation-Induced Inflammation and Functional Derangement in Islets Isolated From Nonhuman Primates

Jin, S-M., Shim, W., Oh, B.J., Oh, S-H., Yu, S.J., Choi, J.M., Park, H.J., Park, J.B. and Kim, J.H.
Am. J. Transplant., ***17(2)****, 365-376 (2017)*

We investigated whether serum deprivation induces islet amyloid polypeptide (IAPP) oligomer accumulation and/or a proinflammatory response and, if so, whether the addition of interleukin (IL)-1 receptor antagonist to the culture medium can relieve the proinflammatory response during serum-deprived culture of nonhuman primate (NHP) islets. After culture in medium with and without Ana under serum-deprived culture conditions, IAPP oligomer/amyloid accumulation, in vitro viability, islet function, cytokine secretion, and posttransplantation outcome in streptozotocin-induced diabetic nude mice were determined in islets isolated from heterozygote human IAPP transgenic (hIAPP$^{+/-}$) mice and/or NHP islets. Serum deprivation induced accumulation of IAPP oligomer, but not amyloid, in NHP islets. Anakinra (Ana) protected islets from the serum deprivation–induced impairment of in vitro viability and glucose-stimulated insulin secretion and attenuated serum deprivation–induced caspase-1 activation, transcription, and secretion of IL-1β, IL-6, and tumor necrosis factor-α in hIAPP$^{+/-}$ mice and NHP islets. Supplementation of medium with Ana during serum-deprived culture also improved posttransplantation in vivo outcomes of NHP islets. In conclusion, serum deprivation induced accumulation of IAPP oligomers and proinflammatory responses in cultured isolated islets. Supplementation of the culture medium with Ana attenuated the functional impairment and proinflammatory responses induced by serum deprivation in ex vivo culture of NHP islets.

4.1693 Transient Monocytosis Subjugates Low Platelet Count in Adult Dengue Patients

Tsai, J-J., Chang, J-S., Chang, K., Chen, P-C., Liu, L-T., Ho, T-C., Tan, S-S., Chien, Y-W., Lo, Y-C. and Perng, G.C.
Biomedicine Hub, ***2****:457785 (2017)*

Background: Dengue is one of the most important vector-borne human viral diseases globally. The kinetic changes of hematological parameters of dengue in adult Taiwanese patients have seldomly been systematically investigated and characterized. ***Methodology/Principal Findings:*** Serial laboratory data of 1,015 adult patients who were diagnosed with dengue virus serotype 2 (DENV2) and 3 (DENV3) infections in southern Taiwan were retrospectively examined. Prominent parameters were verified with specimens from a 2015 dengue outbreak. Higher absolute monocyte counts on day 5 in severe patients than mild fever subjects after the onset of fever was seen. The absolute number of monocytes was significantly greater in those with DENV2 than DENV3 infections in spite of subtle differences in laboratory tests. Platelet counts were lowest and activated partial thromboplastin time was highest on day 5 in patients with severe conditions. In addition, sudden downward platelet counts corresponding to a transient surge of

monocytes on day 4 onward was observed. Fluorescence-activated cell sorting analysis of peripheral blood mononuclear cells obtained from acute dengue patients and experimental investigations revealed that phagocytic effects of innate immune cells contribute to thrombocytopenia in dengue patients. ***Conclusion:*** Innate phagocytic cells play an essential role in low platelet counts in adult patients with dengue virus infections.

4.1694 ALS Along the Axons – Expression of Coding and Noncoding RNA Differs in Axons of ALS models
Rotem, N., magen, I., Ionescu, A., Gershoni-Emek, N., Altman, T., Costa, C.J., Gradus, T., Pasmanik-Chor, M., Willis, D.E., Ben-Dov, I.Z., Hornstein, E. and Perlson, E.
*Scientific Reports, **7**:44500 (2017)*

Amyotrophic lateral sclerosis (ALS) is a multifactorial lethal motor neuron disease with no known treatment. Although the basic mechanism of its degenerative pathogenesis remains poorly understood, a subcellular spatial alteration in RNA metabolism is thought to play a key role. The nature of these RNAs remains elusive, and a comprehensive characterization of the axonal RNAs involved in maintaining neuronal health has yet to be described. Here, using cultured spinal cord (SC) neurons grown using a compartmented platform followed by next-generation sequencing (NGS) technology, we find that RNA expression differs between the somatic and axonal compartments of the neuron, for both mRNA and microRNA (miRNA). Further, the introduction of SOD1G93A and TDP43A315T, established ALS-related mutations, changed the subcellular expression and localization of RNAs within the neurons, showing a spatial specificity to either the soma or the axon. Altogether, we provide here the first combined inclusive profile of mRNA and miRNA expression in two ALS models at the subcellular level. These data provide an important resource for studies on the roles of local protein synthesis and axon degeneration in ALS and can serve as a possible target pool for ALS treatment.

4.1695 Genome-wide mapping of histone H3K9me2 in acute myeloid leukemia reveals large chromosomal domains associated with massive gene silencing and sites of genome instability
Salzberg, A.C., Harris-Becker, A., Popova, E.Y., Keasey, N., Loughran, T.P., Claxton, D.F. and Grigoryev, S.A.
*PloS One, **12(3)**, e0173723 (2017)*

A facultative heterochromatin mark, histone H3 lysine 9 dimethylation (H3K9me2), which is mediated by histone methyltransferases G9a/GLP (EHMT2/1), undergoes dramatic rearrangements during myeloid cell differentiation as observed by chromatin imaging. To determine whether these structural transitions also involve genomic repositioning of H3K9me2, we used ChIP-sequencing to map genome-wide topography of H3K9me2 in normal human granulocytes, normal CD34+ hematopoietic progenitors, primary myeloblasts from acute myeloid leukemia (AML) patients, and a model leukemia cell line K562. We observe that H3K9me2 naturally repositions from the previously designated “repressed” chromatin state in hematopoietic progenitors to predominant association with heterochromatin regions in granulocytes. In contrast, AML cells accumulate H3K9me2 on previously undefined large (> 100 Kb) genomic blocks that are enriched with AML-specific single nucleotide variants, sites of chromosomal translocations, and genes downregulated in AML. Specifically, the AML-specific H3K9me2 blocks are enriched with genes regulated by the proto-oncogene *ERG* that promotes stem cell characteristics. The AML-enriched H3K9me2 blocks (in contrast to the heterochromatin-associated H3K9me2 blocks enriched in granulocytes) are reduced by pharmacological inhibition of the histone methyltransferase G9a/GLP in K562 cells concomitantly with transcriptional activation of *ERG* and *ETS1* oncogenes. Our data suggest that G9a/GLP mediate formation of transient H3K9me2 blocks that are preserved in AML myeloblasts and may lead to an increased rate of AML-specific mutagenesis and chromosomal translocations.

4.1696 PEGylated insulin-like growth factor-I affords protection and facilitates recovery of lost functions post-focal ischemia
Parker, K., Berretta, A., Saenger, S., Sivaramakrishnan, M., Shirley, S.A., Metzger, F. and Clarkson, A.N.
*Scientific Reports, **7**:241 (2017)*

Insulin-like growth factor-I (IGF-I) is involved in the maturation and maintenance of neurons, and impaired IGF-I signaling has been shown to play a role in various neurological diseases including stroke. The aim of the present study was to investigate the efficacy of an optimized IGF-I variant by adding a 40 kDa polyethylene glycol (PEG) chain to IGF-I to form PEG-IGF-I. We show that PEG-IGF-I has a slower clearance which allows for twice-weekly dosing to maintain steady-state serum levels in mice. Using a photothrombotic model of focal stroke, dosing from 3 hrs post-stroke dose-dependently (0.3–

1 mg/kg) decreases the volume of infarction and improves motor behavioural function in both young 3-month and aged 22–24 month old mice. Further, PEG-IGF-I treatment increases GFAP expression when given early (3 hrs post-stroke), increases Synaptophysin expression and increases neurogenesis in young and aged. Finally, neurons (P5–6) cultured *in vitro* on reactive astrocytes in the presence of PEG-IGF-I showed an increase in neurite length, indicating that PEG-IGF-I can aid in sprouting of new connections. This data suggests a modulatory role of IGF-I in both protective and regenerative processes, and indicates that therapeutic approaches using PEG-IGF-I should be given early and where the endogenous regenerative potential is still high.

4.1697 Tricyclic Antidepressants Promote Ceramide Accumulation to Regulate Collagen Production in Human Hepatic Stellate Cells
Chen, J.Y. et al
*Scientific Reports, **7**:44867 (2017)*

Activation of hepatic stellate cells (HSCs) in response to injury is a key step in hepatic fibrosis, and is characterized by trans-differentiation of quiescent HSCs to HSC myofibroblasts, which secrete extracellular matrix proteins responsible for the fibrotic scar. There are currently no therapies to directly inhibit hepatic fibrosis. We developed a small molecule screen to identify compounds that inactivate human HSC myofibroblasts through the quantification of lipid droplets. We screened 1600 compounds and identified 21 small molecules that induce HSC inactivation. Four hits were tricyclic antidepressants (TCAs), and they repressed expression of pro-fibrotic factors Alpha-Actin-2 (ACTA2) and Alpha-1 Type I Collagen (COL1A1) in HSCs. RNA sequencing implicated the sphingolipid pathway as a target of the TCAs. Indeed, TCA treatment of HSCs promoted accumulation of ceramide through inhibition of acid ceramidase (aCDase). Depletion of aCDase also promoted accumulation of ceramide and was associated with reduced COL1A1 expression. Treatment with B13, an inhibitor of aCDase, reproduced the antifibrotic phenotype as did the addition of exogenous ceramide. Our results show that detection of lipid droplets provides a robust readout to screen for regulators of hepatic fibrosis and have identified a novel antifibrotic role for ceramide.

4.1698 Petri Net computational modelling of Langerhans cell Interferon Regulatory Factor Network predicts their role in T cell activation
Polak, M.E., Ung, C.Y., Masapust, J., Freeman, T.C. and Ardern-Jones, M.R.
*Scientific Reports, **7**:668 (2017)*

Langerhans cells (LCs) are able to orchestrate adaptive immune responses in the skin by interpreting the microenvironmental context in which they encounter foreign substances, but the regulatory basis for this has not been established. Utilising systems immunology approaches combining *in silico* modelling of a reconstructed gene regulatory network (GRN) with *in vitro* validation of the predictions, we sought to determine the mechanisms of regulation of immune responses in human primary LCs. The key role of Interferon regulatory factors (IRFs) as controllers of the human Langerhans cell response to epidermal cytokines was revealed by whole transcriptome analysis. Applying Boolean logic we assembled a Petri net-based model of the IRF-GRN which provides molecular pathway predictions for the induction of different transcriptional programmes in LCs. *In silico* simulations performed after model parameterisation with transcription factor expression values predicted that human LC activation of antigen-specific CD8 T cells would be differentially regulated by epidermal cytokine induction of specific IRF-controlled pathways. This was confirmed by *in vitro* measurement of IFN-γ production by activated T cells. As a proof of concept, this approach shows that stochastic modelling of a specific immune networks renders transcriptome data valuable for the prediction of functional outcomes of immune responses.

4.1699 Papilloma-pseudovirus eradicates intestinal tumours and triples the lifespan of ApcMin/+ mice
Zhong, Z., Zhai, Y., Bu, P., Shah, S. and Qiao, L.
*Nature Communications, **8**:15004 (2017)*

Inducing tumour-specific adaptive immunity, such as cytotoxic T lymphocyte (CTL) response, can result in promising antitumour effect against several human malignancies, especially in combination with immune checkpoint blockade strategies. However, little is known whether activation of innate immunity can lead to direct tumoricidal effect. Here, we develop a papilloma pseudovirus-based oral immunotherapeutic approach that shows strong tumoricidal effects in the gut, resulting in an almost tripled lifespan of ApcMin/+ mice (an animal model of human intestinal tumorigenesis). Mechanistically, these pseudoviruses activate the NLRP3 and AIM2 inflammasomes, leading to caspase-1-mediated tumour

regression that is dependent on neither cytotoxic T lymphocytes nor humoral immune response. Blocking caspase-1 activation abrogated the therapeutic effects of the pseudoviruses. Thus, targeting innate immune sensors in tumours by the pseudoviruses might represent a strategy to treat intestinal tumours.

4.1700 Mimicking liver sinusoidal structures and functions using a 3D-configured microfluidic chip
Long, M. et al
Lab. Chip, ***17(5)****, 782-794 (2017)*

Physiologically, four major types of hepatic cells – the liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and hepatocytes – reside inside liver sinusoids and interact with flowing peripheral cells under blood flow. It is hard to mimic an *in vivo* liver sinusoid due to its complex multiple cell–cell interactions, spatiotemporal construction, and mechanical microenvironment. Here we developed an *in vitro* liver sinusoid chip by integrating the four types of primary murine hepatic cells into two adjacent fluid channels separated by a porous permeable membrane, replicating liver's key structures and configurations. Each type of cells was identified with its respective markers, and the assembled chip presented the liver-specific unique morphology of fenestration. The flow field in the liver chip was quantitatively analyzed by computational fluid dynamics simulations and particle tracking visualization tests. Intriguingly, co-culture and shear flow enhance albumin secretion independently or cooperatively, while shear flow alone enhances HGF production and CYP450 metabolism. Under lipopolysaccharide (LPS) stimulations, the hepatic cell co-culture facilitated neutrophil recruitment in the liver chip. Thus, this 3D-configured *in vitro* liver chip integrates the two key factors of shear flow and the four types of primary hepatic cells to replicate key structures, hepatic functions, and primary immune responses and provides a new *in vitro* model to investigate the short-duration hepatic cellular interactions under a microenvironment mimicking the physiology of a liver.

4.1701 CCR2 mediates Helicobacter pylori-induced immune tolerance and contributes to mucosal homeostasis
Sun, X., Zhang, M., El-Zaatari, M., Huffnagle, G.B. and Kao, J.Y.
Helicobacter, ***22(2)****, e12366 (2017)*

Background
We previously demonstrated that *H. pylori* infection leads to increased induction of regulatory T cells in local and systemic immune compartments. Here, we investigate the role of CCR2 in the tolerogenic programing of dendritic cells in a mouse model of *H. pylori* infection.
Materials and Methods
CCR2 deficient (CCR2KO) mice and wild-type (Wt) mice infected with *H. pylori* SS1 strain were analyzed by qPCR and FACS analysis. In vitro, bone marrow-derived DC on day 6 from CCR2KO and Wt mice cocultured with or without *H. pylori* were examined to determine the impact of CCR2 signaling on dendritic cells function by qPCR, ELISA, and FACS analyses.
Results
Acute *H. pylori* infection was associated with a threefold increase in CCR2 mRNA expression in the gastric mucosa. *H. pylori*-infected CCR2KO mice exhibited a higher degree of mucosal inflammation, that is, increased gastritis scores and pro-inflammatory cytokine mRNA levels, but lower degree of *H. pylori* gastric colonization compared to infected Wt mice. Peripheral *H. pylori*-specific immune response measured in the CCR2KO spleen was characterized by a higher Th17 response and a lower Treg response. In vitro, CCR2KO bone marrow-derived DC was less mature and shown a lower Treg/Th17 ratio. Moreover, blockade of CCR2 signaling by MCP-1 neutralizing antibody inhibited *H. pylori*-stimulated bone marrow-derived DC maturation.
Conclusions
Our results indicate that CCR2 plays an essential role in *H. pylori*-induced immune tolerance and shed light on a novel mechanism of CCR2-dependent DC Treg induction, which appears to be important in maintaining mucosal homeostasis during *H. pylori* infection.

4.1702 Adenovirus vector-mediated macrophage erythroblast attacher (MAEA) overexpression in primary mouse hepatocytes attenuates hepatic gluconeogenesis
Shimizu, K., Okamoto, M., Terada, T., Sakurai, F., Mizuguchi, H., Tomita, K. and Nishinaka, T.
Biochem. Biophys. Reports, ***10****, 192-197 (2017)*

Japanese patients with type 2 diabetes mellitus present a different responsiveness in terms of insulin

secretion to glucose and body mass index (BMI) from other populations. The genetic background that predisposes Japanese individuals to type 2 diabetes mellitus is under study. Recent genetic studies demonstrated that the locus mapped in macrophage erythroblast attacher (MAEA) increases the susceptibility to type 2 diabetes mellitus in East Asians, including Japanese individuals. MAEA encodes a protein that plays a role in erythroblast enucleation and in the normal differentiation of erythroid cells and macrophages. However, the contribution of MAEA to type 2 diabetes mellitus remains unknown. In this study, to overexpress MAEA in the mouse liver and primary mouse hepatocytes, we generated a MAEA-expressing adenovirus (Ad) vector using a novel Ad vector exhibiting significantly lower hepatotoxicity (Ad-MAEA). Blood glucose and insulin levels in Ad-MAEA-treated mice were comparable to those in control Ad-treated mice. Primary mouse hepatocytes transduced with Ad-MAEA showed lower levels of expression of gluconeogenesis genes than those transduced with the control Ad vector. Hepatocyte nuclear factor-4α (HNF-4α) mRNA expression in primary mouse hepatocytes was also suppressed by MAEA overexpression. These results suggest that MAEA overexpression attenuates hepatic gluconeogenesis, which could potentially lead to improvement of type 2 diabetes mellitus.

4.1703 Mu-opioid receptor and delta-opioid receptor differentially regulate microglial inflammatory response to control proopiomelanocortin neuronal apoptosis in the hypothalamus: effects of neonatal alcohol

Ahrivastava, P., Cabrera, M.A., Chastain, L.G., Boyadjieva, N.I., Jabbar, S., Franklin, T. and Sarkar, D.K.
*J. Neuroinflammatioan, **14**:83, (2017)*

Background
Opioid receptors are known to control neurotransmission of various peptidergic neurons, but their potential role in regulation of microglia and neuronal cell communications is unknown. We investigated the role of mu-opioid receptors (MOR) and delta-opioid receptors (DOR) on microglia in the regulation of apoptosis in proopiomelanocortin (POMC) neurons induced by neonatal ethanol in the hypothalamus.
Methods
Neonatal rat pups were fed a milk formula containing ethanol or control diets between postnatal days 2–6. Some of the alcohol-fed rats additionally received pretreatment of a microglia activation blocker minocycline. Two hours after the last feeding, some of the pups were sacrificed and processed for histochemical detection of microglial cell functions or confocal microscopy for detection of cellular physical interaction or used for gene and protein expression analysis. The rest of the pups were dissected for microglia separation by differential gradient centrifugation and characterization by measuring production of various activation markers and cytokines. In addition, primary cultures of microglial cells were prepared using hypothalamic tissues of neonatal rats and used for determination of cytokine production/secretion and apoptotic activity of neurons.
Results
In the hypothalamus, neonatal alcohol feeding elevated cytokine receptor levels, increased the number of microglial cells with amoeboid-type circularity, enhanced POMC and microglial cell physical interaction, and decreased POMC cell numbers. Minocycline reversed these cellular effects of alcohol. Alcohol feeding also increased levels of microglia MOR protein and pro-inflammatory signaling molecules in the hypothalamus, and MOR receptor antagonist naltrexone prevented these effects of alcohol. In primary cultures of hypothalamic microglia, both MOR agonist [D-Ala 2, N-MePhe 4, Gly-ol]-enkephalin (DAMGO) and ethanol increased microglial cellular levels and secretion of pro-inflammatory cell signaling proteins. However, a DOR agonist [D-Pen2,5]enkephalin (DPDPE) increased microglial secretion of anti-inflammatory cytokines and suppressed ethanol's ability to increase microglial production of inflammatory signaling proteins and secretion of pro-inflammatory cytokines. In addition, MOR-activated inflammation promoted while DOR-suppressed inflammation inhibited the apoptotic effect of ethanol on POMC neurons.
Conclusions
These results suggest that ethanol's neurotoxic action on POMC neurons results from MOR-activated neuroinflammatory signaling. Additionally, these results identify a protective effect of a DOR agonist against the pro-inflammatory and neurotoxic action of ethanol.

4.1704 Development of Improved HDAC6 Inhibitors as Pharmacological Therapy for Axonal Charcot–Marie–Tooth Disease

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*Neurotherapeutics, **14**, 417-428 (2017)*

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy, with an

estimated prevalence of 1 in 2500. The degeneration of motor and sensory nerve axons leads to motor and sensory symptoms that progress over time and have an important impact on the daily life of these patients. Currently, there is no curative treatment available. Recently, we identified histone deacetylase 6 (HDAC6), which deacetylates α-tubulin, as a potential therapeutic target in axonal CMT (CMT2). Pharmacological inhibition of the deacetylating function of HDAC6 reversed the motor and sensory deficits in a mouse model for mutant "small heat shock protein B1" (HSPB1)-induced CMT2 at the behavioral and electrophysiological level. In order to translate this potential therapeutic strategy into a clinical application, small drug-like molecules that are potent and selective HDAC6 inhibitors are essential. To screen for these, we developed a method that consisted of 3 distinct phases and that was based on the pathological findings in the mutant HSPB1-induced CMT2 mouse model. Three different inhibitors (ACY-738, ACY-775, and ACY-1215) were tested and demonstrated to be both potent and selective HDAC6 inhibitors. Moreover, these inhibitors increased the innervation of the neuromuscular junctions in the gastrocnemius muscle and improved the motor and sensory nerve conduction, confirming that HDAC6 inhibition is a potential therapeutic strategy in CMT2. Furthermore, ACY-1215 is an interesting lead molecule as it is currently tested in clinical trials for cancer. Taken together, these results may speed up the translation of pharmacological inhibition of HDAC6 into a therapy against CMT2.

4.1705 Hesperetin derivative-14 alleviates inflammation by activating PPAR-γ in mice with CCl4-induced acute liver injury and LPS-treated RAW264.7 cells

Chen, X., Ding, H-W., Li, H-D., Huang, H-M., Li, X-F., Yang, Y., Zhang, Y-L., Pan, X-Y., Huang, C. and Meng, X-M.
Toxicol. Lett., ***274***, *51-63 (2017)*

Hesperetin is a flavanone glycoside compound naturally occurring in the fruit peel of *Citrusaurantium L.* (*Rutaceae*). Previous studies revealed that hesperetin possesses various pharmacological effects, including anti-inflammation, anti-tumor, anti-oxidant and neuroprotective properties. Hesperetin derivative-14 (HD-14) is a derivative of hesperetin improved in water solubility and bioavailability. In this study, we indicated that HD-14 (2 μM) significantly attenuated inflammation in LPS-treated RAW264.7 cells, besides, HD-14 (100 mg/kg) exhibited hepato-protective effects and anti-inflammatory effects on C57BL/6J mice with CCl_4-induced acute liver injury. In addition, it was demonstrated that HD-14 dramatically up-regulated the expression of PPAR-γ in vivo and in vitro. Interestingly, over-expression of PPAR-γ had anti-inflammatory effects on the expressions of TNF-α, IL-6, and IL-1β, whereas, knockdown of PPAR-γ with small interfering RNA had pro-inflammatory effects in LPS-treated RAW264.7 cells. Thus, our findings demonstrated that HD-14 alleviated inflammation by activating PPAR-γ expression at least in part. Further studies founded that HD-14 remarkably inhibited the expression of p-JAK1 and p-STAT1 through up-regulating PPAR-γ. Together, these results suggested that HD-14 served as an activator of PPAR-γ and the JAK1/STAT1 signaling pathway may be involved in the progress of inflammation. Collectively, HD-14 may be utilized as a potential anti-inflammation monomeric compound in the treatment of acute liver injury.

4.1706 Both MAPK and STAT3 signal transduction pathways are necessary for IL-6-dependent hepatic stellate cells activation

Kagan, P., Sultan, M., Tachlytski, I., Safran, M. and Ben-Ari, Z.
PloS One, ***12(5)***, *e0176173 (2017)*

Background
During liver injury, hepatic stellate cells (HSCs) can undergo activation and transform into alpha-smooth muscle actin (αSMA)-expressing contractile myofibroblast-like cells, leading to deposition of excessive scar matrix. We have recently demonstrated that depletion of adenosine deaminase acting on double-stranded RNA (ADAR1) from mouse hepatocytes leads to HSC activation and induction of inflammation and hepatic fibrosis that is mediated by interleukin 6 (IL-6). Our aim was to identify and characterize the molecular pathways involved in the direct, inflammation-independent activation of HSCs by IL-6.
Methods
Primary HSCs were isolated from mouse livers. mRNA levels of αSMA and Col1a were analyzed using qRT-PCR. Protein levels of αSMA, MAPK, p-MAPK, p38, p-p38, STAT3 and p-STAT3 were assessed by Western Blot analysis. The effect of specific signal transduction pathway inhibitors (i.e., SB203580 (P-38 inhibitor), U0126 (MAPK inhibitor), S3I-201 (STAT3 inhibitor) and Ruxolitinib (Jak1/2 inhibitor)) was also studied.
Results
Primary HSCs treated with IL-6 demonstrated upregulation of αSMA and Col1a mRNA levels as well as

increased αSMA protein levels. Moreover, the phenotypic transition of quiescent HSCs toward myofibroblast-like cells was noted upon administration of IL-6 and not in untreated samples. In addition, the phosphorylation levels of p38, MAPK and STAT3 increased 30 minutes after treatment, and was followed by a decline in the phosphorylation levels 2–4 hours post-treatment. However, addition of specific signal transduction pathway inhibitors curbed this effect, and resulted in αSMA and Col1a expression levels similar to those measured in untreated control samples.

Conclusion

IL-6 can directly induce the transition of HSCs toward myofibroblast-like cells. The effect is mediated by the activation of both MAPK and JAK/STAT signaling pathways. Elimination of either MAPK or JAK/STAT signaling pathways inhibits HSC stimulation. These results might pave the road toward the development of potential therapeutic interventions for hepatic fibrosis.

4.1707 Xenotransplantation of layer-by-layer encapsulated non-human primate islets with a specified immunosuppressive drug protocol

Haque, M.R., Kim, J., Park, H., Lee, H.S., Lee, K.W., Al-Hilal, T.A., Jeong, J-H., Ahn, C-H., Lee, D.S., Kim, S.J. and Byun, Y.

*J. Controlled Release, **258**, 10-21 (2017)*

Islet transplantation is as effective as but also less immunogenic than pancreas transplantation for the treatment of type 1 diabetes mellitus. However, as the complete elimination of immunogenicity still remains a major obstacle in islet transplantation, layer-by-layer encapsulation (LbL) of pancreatic islets using biocompatible polymers offers a rational approach to reducing host immune response towards transplanted islets. We investigated the effect of LbL of non-human primate (NHP) islets on reducing immunogenicity as a preclinical model since NHPs have close phylogenetic and immunological relationship with humans. LbL with three-layers of polyethylene glycol (PEG) molecules (SH-6-arm-PEG-NHS, 6-arm-PEG-catechol and linear PEG-SH) showed a uniform nano-shielding on islets without the loss of viability or function of islets. An immunosuppressive drug protocol was also combined to improve the survival rate of the transplanted islets *in vivo*. A xenorecipient (C57BL/6 mice) of LbL islet transplanted along with our immunosuppressive drug protocol showed 100% survival rate for 150 days after transplantation. On the other hand, naked islet recipients showed poor survival time of 5.5 ± 1.4 days without drugs and 77.5 ± 42 days with the drug protocol. Immunohistochemistry of the transplanted grafts and serum cytokine concentration demonstrated less immunogenicity in the LbL islet transplanted recipients compared with the naked islet ones.

4.1708 Follicular size predicts success in artificial insemination with frozen-thawed sperm in donkeys

Saragusty, J., Lemma, A., Hildebrandt, T.B. and Göritz, F.

*PloS One, **12(5)**, e0175637 (2017)*

In asses, semen collection, cryopreservation, and artificial insemination (AI) with frozen-thawed semen have been scarcely described and success rate, particularly following AI, is reportedly low. In the absence of reliable protocols, assisted reproductive technologies cannot support the conservation efforts aimed at endangered wild ass species and domestic donkey breeds. Two experiments were conducted in this study. In experiment 1 we evaluated freezing Abyssinian donkey (N = 5, 4 ejaculates each) spermatozoa using three freezing extenders (Berliner Cryomedium + glycerol, BC+G; BotuCrio, BOTU; INRAFreeze, INRA) and two cryopreservation techniques (liquid nitrogen vapour, LNV; directional freezing, DF). Post-thaw evaluation indicated that BOTU and INRA were similar and both superior to BC+G ($P \leq 0.004$ for all motility tests), and that DF was superior to LNV ($P < 0.002$ for all evaluation parameters). In experiment 2, relying on these results, we used Abyssinian donkey sperm frozen in BOTU and INRA by DF for AI (N = 20). Prior to AI, thawed samples were diluted in corresponding centrifugation media or autologous seminal fluids at 1:1 ratio. No difference was found between BOTU and INRA or between the addition of seminal fluids or media, all resulting in ~50% pregnancy, and no differences were noted between males (N = 4). The size of pre-ovulatory follicle was a significant (P = 0.001) predictor for AI success with 9/10 pregnancies occurring when follicular size ranged between 33.1–37.4 mm, no pregnancy when it was smaller, and only one when larger. A number of ass species face the risk of extinction. Knowledge gained in this study on the Abyssinian donkey can be customised and transferred to its closely related endangered species and breeds.

4.1709 Clinical effectiveness of a pylorus-preserving procedure on total pancreatectomy with islet autotransplantation

Shahbazov, R., Yoshimatsu, G., Haque, W.Z., Khan, O.S., Saracino, G., Lawrence, M.C., Kim, P.T.,

Onaca, N., Naziruddin, B. and Levy, M.F.
Am. J. Surg., ***213***, *1065-1071 (2017)*

Background
The impact of pylorus preserving procedures (PP) on total pancreatectomy with islet autotransplantation (TPIAT) has not been examined. This study aimed to investigate the clinical impact of the PP on TPIAT.
Methods
The Baylor Simmons Transplant Institute database was queried to identify seventy-three patients who underwent TPIAT from 2006 to 2014. All patients were investigated in postoperative complications, long-term nutritional status, and graft function.
Results
Patients with PP did not face worse outcomes in terms of delayed gastric emptying and length of hospital stay. Also, nutritional status and metabolic outcome, such as body weight, serum albumin level, serum vitamin level, HbA1c level, graft survival rate and insulin independent rate, were similar between both groups.
Conclusions
Clinical results including the graft function indicated that patients undergoing TPIAT with PP did not amplify surgical complications such as delayed gastric emptying and showed no significant advantage of nutrition and metabolic outcome.

4.1710 **High-throughput generation of hyaluronic acid microgels via microfluidics-assisted enzymatic crosslinking and/or Diels–Alder click chemistry for cell encapsulation and delivery**
Ma, T., Gao, X., Dong, H., He, H. and Cao, X.
Applied Materials Today, ***9***, *49-59 (2017)*

Cell encapsulation in 3D microgels offers unique advantages to meet the complicated requirements in tissue engineering, regenerative medicine and cell therapy. Herein we report high-throughput microfluidic generation of cell-laden microgels based on a new hyaluronic acid (HA) derivative, i.e. furylamine and tyramine grafted HA molecules, which can be crosslinked via either enzymatic crosslinking, or Diels–Alder click chemistry, or both. Compared with traditional photoinitiated free radical polymerization and Michael conjugate addition reaction, enzymatic crosslinking and click chemistry crosslinking show higher chemical selectivity and milder reaction conditions. More importantly, the switching of crosslinking strategy in the same molecule makes it possible to fabricate microgels with almost the same composition but variable gelation time and elasticity. Using ATDC-5 cells as model cells, three types of cell-laden microgels were generated in high-throughput manner and their potentials as cell carriers were evaluated in detail by comparing their mechanical elasticity, gelation time, microgel size, swelling ratio, cell viability, enzymatic degradation and bioactivity of released cells. Our results reveal that the new HA-derived microgels crosslinked by enzymatic crosslinking and Diels–Alder click chemistry are very promising candidate for cell encapsulation and delivery.

4.1711 **GalR3 mediates galanin proliferative effects on postnatal hippocampal precursors**
Khan, D., Khan, M., Runesson, J., Zaben, M. and Gray, W.P.
Neuropeptides, ***63***, *14-17 (2017)*

Galanin, a neuropeptide co-released from noradrenergic and serotonergic projection neurons to the dentate gyrus, has recently emerged as an important mediator for signaling neuronal activity to the subgranular neurogenic stem cell niche supporting adult hippocampal neurogenesis. Galanin and its receptors appear to play key roles in depression-like behavior, and effects on hippocampal neurogenesis are relevant to pharmacological strategies for treating depression, which in part appear to rely on restoring altered neurogenesis. We previously demonstrated that the GalR2/3 receptor agonist Gal 2–11 is proliferative and proneurogenic for postnatal hippocampal progenitor cells; however, the specific receptor mediation remained to be identified.
With the recent availability of M1145 (a specific GalR2 agonist), and SNAP 37889 (GalR3 specific antagonist), we extend our previous studies and show that while M1145 has no proliferative effect, the co-treatment of postnatal rat hippocampal progenitors with Gal 2–11 and SNAP 37889 completely abolished the Gal 2–11 proliferative effects. Taken together, these results clearly demonstrate that GalR3 and not GalR2 is the specific receptor subtype that mediates the proliferative effects of galanin on hippocampal progenitor cells. These results implicate GALR3 in the mediation of galanin neurogenic effects and, potentially, its neurogenic anti-depressant effects.

4.1712 Foxa2 and Pdx1 cooperatively regulate postnatal maturation of pancreatic β-cells

Bastidas-Ponce, A., Roscioni, S.S., Burtscher, I., Bader, E., Sterr, M., Bakhti, M. and Lickert, H.
*Mol. Metabolism, **6**, 524-534 (2017)*

Objective
The transcription factors (TF) Foxa2 and Pdx1 are key regulators of beta-cell (β-cell) development and function. Mutations of these TFs or their respective cis-regulatory consensus binding sites have been linked to maturity diabetes of the young (MODY), pancreas agenesis, or diabetes susceptibility in human. Although Foxa2 has been shown to directly regulate Pdx1 expression during mouse embryonic development, the impact of this gene regulatory interaction on postnatal β-cell maturation remains obscure.
Methods
In order to easily monitor the expression domains of Foxa2 and Pdx1 and analyze their functional interconnection, we generated a novel double knock-in homozygous ($FVFPBF^{DHom}$) fluorescent reporter mouse model by crossing the previously described Foxa2-Venus fusion (FVF) with the newly generated Pdx1-BFP (blue fluorescent protein) fusion (PBF) mice.
Results
Although adult PBF homozygous animals exhibited a reduction in expression levels of Pdx1, they are normoglycemic. On the contrary, despite normal pancreas and endocrine development, the $FVFPBF^{DHom}$ reporter male animals developed hyperglycemia at weaning age and displayed a reduction in Pdx1 levels in islets, which coincided with alterations in β-cell number and islet architecture. The failure to establish mature β-cells resulted in loss of β-cell identity and trans-differentiation towards other endocrine cell fates. Further analysis suggested that Foxa2 and Pdx1 genetically and functionally cooperate to regulate maturation of adult β-cells.
Conclusions
Our data show that the maturation of pancreatic β-cells requires the cooperative function of Foxa2 and Pdx1. Understanding the postnatal gene regulatory network of β-cell maturation will help to decipher pathomechanisms of diabetes and identify triggers to regenerate dedifferentiated β-cell mass.

4.1713 IGF-II promotes neuroprotection and neuroplasticity recovery in a long-lasting model of oxidative damage induced by glucocorticoids

Martin-Montanez, E., Millon, C., Boraldi, F., Garcia-Guirades, F., Pedroza, C., Lara, e., Santin, L.J., Pavia, J. and Garcia-Fernandez, M.
*Redex Biol., **13**, 69-81 (2017)*

Insulin-like growth factor-II (IGF-II) is a naturally occurring hormone that exerts neurotrophic and neuroprotective properties in a wide range of neurodegenerative diseases and ageing. Accumulating evidence suggests that the effects of IGF-II in the brain may be explained by its binding to the specific transmembrane receptor, IGFII/M6P receptor (IGF-IIR). However, relatively little is known regarding the role of IGF-II through IGF-IIR in neuroprotection. Here, using adult cortical neuronal cultures, we investigated whether IGF-II exhibits long-term antioxidant effects and neuroprotection at the synaptic level after oxidative damage induced by high and transient levels of corticosterone (CORT). Furthermore, the involvement of the IGF-IIR was also studied to elucidate its role in the neuroprotective actions of IGF-II. We found that neurons treated with IGF-II after CORT incubation showed reduced oxidative stress damage and recovered antioxidant status (normalized total antioxidant status, lipid hydroperoxides and NAD(P) H:quinone oxidoreductase activity). Similar results were obtained when mitochondria function was analysed (cytochrome c oxidase activity, mitochondrial membrane potential and subcellular mitochondrial distribution). Furthermore, neuronal impairment and degeneration were also assessed (synaptophysin and PSD-95 expression, presynaptic function and FluoroJade B® stain). IGF-II was also able to recover the long-lasting neuronal cell damage. Finally, the effects of IGF-II were not blocked by an IGF-IR antagonist, suggesting the involvement of IGF-IIR. Altogether these results suggest that, in or model, IGF-II through IGF-IIR is able to revert the oxidative damage induced by CORT. In accordance with the neuroprotective role of the IGF-II/IGF-IIR reported in our study, pharmacotherapy approaches targeting this pathway may be useful for the treatment of diseases associated with cognitive deficits (*i.e.*, neurodegenerative disorders, depression, *etc.*).

4.1714 Simultaneous Subtotal Pancreatectomy and Streptozotocin Injection for Diabetes Modeling in Cynomolgus Monkeys

Park, H., Park, J.B., Kim, J.H., Lee, K.W., Lee, H.S., Kim, G.S., Shin, D-Y., Oh, S.H., Jin, S-M. and Kim, S.J.
*Transplant. Proceedings, **49**, 1142-1149 (2017)*

Background
In an experimental animal model of islet transplantation, stable induction of insulin-dependent diabetes mellitus (IDDM) and islet isolation from donor pancreas are essential. Total pancreatectomy for IDDM induction and islet procurement in nonhuman primates leads to unwanted loss of exocrine function and may lead to morbidities associated with IDDM.
Methods
IDDM induction with streptozotocin (STZ) is associated with drug toxicity of STZ and necessitates the killing of another animal for islet procurement. In this study, we performed a subtotal pancreatectomy combined with reduced STZ injection to induce IDDM and procure islets in a nonhuman primate model.
Results
Twelve cynomolgus monkeys received low-dose STZ injections (60 mg/kg) simultaneously with subtotal pancreatectomy. All monkeys recovered from the procedure without complications. IDDM was induced in the animals. 57,691 ± 16,050 islets were isolated from the resected pancreas and transplanted into other monkeys.
Conclusions
Simultaneous subtotal pancreatectomy and low-dose STZ injection represent an effective and safe method to create an animal model of insulin dependence diabetes, while at the same time providing sufficient amounts of fresh islet cells for allotransplantation without requiring killing of additional animals.
Nonhuman primate (NHP) models of insulin-dependent diabetes mellitus (IDDM) have been widely used to study islet allotransplantation and xenotransplantation [1]; [2]; [3] ; [4]. IDDM in NHPs by injection of the β-cell toxic drug streptozotocin (STZ) or total pancreatectomy is well-documented in the literature [5]; [6] ; [7].
Induction of IDDM in NHPs with the use of STZ is a relatively simple procedure involving intravenous injection of the drug. It does not require open abdominal surgery, and, accordingly, morbidities associated with surgery and anesthesia can be avoided. Furthermore, the peritoneal cavity remains naive, allowing for safer access during laparotomy for islet transplantation [8]. However, STZ has hepatotoxic and nephrotoxic side effects, and it is not clear what the optimal dose is of STZ for diabetes induction without invoking obvious adverse effects, because a wide range of values has been reported [9]; [10]; [11] ; [12].
Induction of IDDM with total pancreatectomy ensures a permanent state of diabetes with the added advantage of acquiring islet cells in the process. However, this is an invasive and complicated surgical procedure that does not ensure preservation of the pancreaticoduodenal arcade. Loss of pancreatic exocrine function is another disadvantage of this procedure [7] ; [13].

4.1715 Lipidomic characterization and localization of phospholipids in the human lung

Zemski Berry, K.A., Murphy, R.C., Kosmider, B. and Mason, R.J.
J. Lipid Res., ***58(5)***, *926-933 (2017)*

Lipids play a central role in lung physiology and pathology; however, a comprehensive lipidomic characterization of human pulmonary cells relevant to disease has not been performed. The cells involved in lung host defense, including alveolar macrophages (AMs), bronchial epithelial cells (BECs), and alveolar type II cells (ATIIs), were isolated from human subjects and lipidomic analysis by LC-MS and LC-MS/MS was performed. Additionally, pieces of lung tissue from the same donors were analyzed by MALDI imaging MS in order to determine lipid localization in the tissue. The unique distribution of phospholipids in ATIIs, BECs, and AMs from human subjects was accomplished by subjecting the large number of identified phospholipid molecular species to univariant statistical analysis. Specific MALDI images were generated based on the univariant statistical analysis data to reveal the location of specific cell types within the human lung slice. While the complex composition and function of the lipidome in various disease states is currently poorly understood, this method could be useful for the characterization of lipid alterations in pulmonary disease and may aid in a better understanding of disease pathogenesis.

4.1716 Liraglutide improves liver microvascular dysfunction in cirrhosis: Evidence from translational studies

De mesquite, F.C., Guixe-Muntet, s., Fernandez-Iglesias, A., Maeso-Diaz, R., Vila, S., Hide, D., Ortega-Ribera, M., Rosa, J.L., Garcia-Pagan, J.C., Bosch, J., de Oliveira, J.R. and Gracia-Sancho, J.
Scientific Reports, ***7***:*3255 (2017)*

Hepatic stellate cells (HSC) play a key role in the development of chronic liver disease (CLD). Liraglutide, well-established in type 2 diabetes, showed anti-inflammatory and anti-oxidant properties. We evaluated

the effects of liraglutide on HSC phenotype and hepatic microvascular function using diverse pre-clinical models of CLD. Human and rat HSC were *in vitro* treated with liraglutide, or vehicle, and their phenotype, viability and proliferation were evaluated. In addition, liraglutide or vehicle was administered to rats with CLD. Liver microvascular function, fibrosis, HSC phenotype and sinusoidal endothelial phenotype were determined. Additionally, the effects of liraglutide on HSC phenotype were analysed in human precision-cut liver slices. Liraglutide markedly improved HSC phenotype and diminished cell proliferation. Cirrhotic rats receiving liraglutide exhibited significantly improved liver microvascular function, as evidenced by lower portal pressure, improved intrahepatic vascular resistance, and marked ameliorations in fibrosis, HSC phenotype and endothelial function. The anti-fibrotic effects of liraglutide were confirmed in human liver tissue and, although requiring further investigation, its underlying molecular mechanisms suggested a GLP1-R-independent and NF-κB-Sox9-dependent one. This study demonstrates for the first time that liraglutide improves the liver sinusoidal milieu in pre-clinical models of cirrhosis, encouraging its clinical evaluation in the treatment of chronic liver disease.

4.1717 Stress Hormones Epinephrine and Corticosterone Selectively Modulate Herpes Simplex Virus 1 (HSV-1) and HSV-2 Productive Infections in Adult Sympathetic, but Not Sensory, Neurons
Ives, A.M. and Bertke, A.S.
*J. Virol., **91(13)**, e00582-17 (2017)*

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) infect and establish latency in peripheral neurons, from which they can reactivate to cause recurrent disease throughout the life of the host. Stress is associated with the exacerbation of clinical symptoms and the induction of recurrences in humans and animal models. The viruses preferentially replicate and establish latency in different subtypes of sensory neurons, as well as in neurons of the autonomic nervous system that are highly responsive to stress hormones. To determine if stress-related hormones modulate productive HSV-1 and HSV-2 infections within sensory and autonomic neurons, we analyzed viral DNA and the production of viral progeny after treatment of primary adult murine neuronal cultures with the stress hormones epinephrine and corticosterone. Both sensory trigeminal ganglion (TG) and sympathetic superior cervical ganglion (SCG) neurons expressed adrenergic receptors (activated by epinephrine) and the glucocorticoid receptor (activated by corticosterone). Productive HSV infection colocalized with these receptors in SCG but not in TG neurons. In productively infected neuronal cultures, epinephrine treatment significantly increased the levels of HSV-1 DNA replication and production of viral progeny in SCG neurons, but no significant differences were found in TG neurons. In contrast, corticosterone significantly decreased the levels of HSV-2 DNA replication and production of viral progeny in SCG neurons but not in TG neurons. Thus, the stress-related hormones epinephrine and corticosterone selectively modulate acute HSV-1 and HSV-2 infections in autonomic, but not sensory, neurons.

4.1718 MHC II−, but not MHC II+, hepatic Stellate cells contribute to liver fibrosis of mice in infection with Schistosoma japonicum
Zhou, C-L., Kong, D-L., Liu, J-F., Lu, Z-K., Guo, H-F., Wang, W., Qiu, J-F., Liu, X-J. and Wang, Y.
*BBA – Molecular Basis of Disase, **1863**, 1848-1857 (2017)*

Hepatic stellate cells (HSCs) are considered as the main effector cells in vitamin A metabolism and liver fibrosis, as well as in hepatic immune regulation. Recently, researches have revealed that HSCs have plasticity and heterogeneity, which depend on their lobular location and whether liver is normal or injured. This research aimed to explore the biological characteristics and heterogeneity of HSCs in mice with Schistosoma japonicum (S. japonicum) infection, and determine the subpopulation of HSCs in pathogenesis of hepatic fibrosis caused by S. japonicum infection. Results revealed that HSCs significantly increased the expressions of MHC II and fibrogenic genes after S. japonicum infection, and could be classified into MHC II^+ HSCs and MHC II^- HSCs subsets. Both two HSCs populations suppressed the proliferation of activated $CD4^+$ T cells, whereas only MHC II^- HSCs displayed a myofibroblast-like phenotype. In response to IFN-γ, HSCs up-regulated the expressions of MHC II and CIITA, while down-regulated the expression of fibrogenic gene Col1. In addition, praziquantel treatment decreased the expressions of fibrogenic genes in MHC II^- HSCs. These results confirmed that HSCs from S. japonicum-infected mice have heterogeneity. The MHC II^- α-SMA^+ HSCs were major subsets of HSCs contributing to liver fibrosis and could be considered as a potential target of praziquantel anti-fibrosis treatment.

4.1719 The hemagglutinin-neuramidinase protein of Newcastle disease virus upregulates expression of the TRAIL gene in murine natural killer cells through the activation of Syk and NF-κB
Liang, Y., Song, D-Z., Liang, S., Zhang, Z-F., Gao, L-X. And Fan, X-H.

*PloS One, **12(6)**, e0178746 (2017)*

Newcastle disease virus (NDV) is responsible for tumoricidal activity *in vitro* and *in vivo*. However, the mechanisms that lead to this activity are unclear. Natural killer cells are able to induce apoptosis of tumor cells through multiple pathways, including the tumor necrosis factor-related apoptosis-inducing ligand-death receptor pathway. We previously showed that exposure of NK and T cells to NDV resulted in enhanced tumoricidal activity that was mediated by upregulated expression of the TRAIL gene, *via* an interferon gamma -dependent pathway. Other pathways involved in the upregulated expression of TRAIL are yet to be identified. In the current study, we used mice in which the IFN-γ receptor one gene was inactivated functionally. We identified an IFN-γ-independent TRAIL pathway in the NDV-stimulated NK cells. Hemagglutinin-neuramidinase induced expression of the TRAIL gene in IFN-R1$^{-/-}$ NK cells by binding to the NKp46 receptor. This upregulation was inhibited by pretreatment of NDV with a neutralizing monoclonal antibody against HN, or desialylation of NK cells. Phosphorylation of spleen tryosine kinases and IκBα was increased in HN-induced IFN-R1$^{-/-}$ NK cells. Treatment with the HN neutralizing monoclonal antibody, pharmacological disialylation, or a Syk inhibitor decreased Syk and IκBα phosphorylation levels. We concluded that killer activation receptors pathway is involved in the IFN-γ-independent TRAIL expression of NDV-stimulated NK cells, and these are activated by Syk and NF-κB.

4.1720 **EV-3, an endogenous human erythropoietin isoform with distinct functional relevance**
Bonnas, C., Wüsterfeld, L., Winkler, D., Kronstein-Wiedemann, R., Dere, E., Specht, K., Boxberg, M., Tonn, T., Ehrenreich, H., Stadler, H. and Sillaber, I.
*Scientific Reports, **7**:3864 (2017)*

Generation of multiple mRNAs by alternative splicing is well known in the group of cytokines and has recently been reported for the human erythropoietin (EPO) gene. Here, we focus on the alternatively spliced *EPO* transcript characterized by deletion of exon 3 (hEPOΔ3). We show co-regulation of *EPO* and hEPOΔ3 in human diseased tissue. The expression of hEPOΔ3 in various human samples was low under normal conditions, and distinctly increased in pathological states. Concomitant up-regulation of hEPOΔ3 and *EPO* in response to hypoxic conditions was also observed in HepG2 cell cultures. Using LC-ESI-MS/MS, we provide first evidence for the existence of hEPOΔ3 derived protein EV-3 in human serum from healthy donors. Contrary to EPO, recombinant EV-3 did not promote early erythroid progenitors in cultures of human CD34+ haematopoietic stem cells. Repeated intraperitoneal administration of EV-3 in mice did not affect the haematocrit. Similar to EPO, EV-3 acted anti-apoptotic in rat hippocampal neurons exposed to oxygen-glucose deprivation. Employing the touch-screen paradigm of long-term visual discrimination learning, we obtained first *in vivo* evidence of beneficial effects of EV-3 on cognition. This is the first report on the presence of a naturally occurring EPO protein isoform in human serum sharing non-erythropoietic functions with EPO.

4.1721 **Thymosin beta-4 regulates activation of hepatic stellate cells via hedgehog signaling**
Kim, J., Hyun, J., Wang, S., Lee, C., Lee, J-W., Moon, E-Y., Cha, H., Diehl, A.M. and Jung, Y.
*Scientific Reports, **7**:3815 (2017)*

The molecular mechanisms of thymosin beta-4 (TB4) involved in regulating hepatic stellate cell (HSC) functions remain unclear. Therefore, we hypothesize that TB4 influences HSC activation through hedgehog (Hh) pathway. HSC functions declined in a TB4 siRNA-treated LX-2. *TB4* suppression down-regulated both integrin linked kinase (*ILK*), an activator of smoothened, and phosphorylated glycogen synthase kinase 3 beta (*pGSK-3B*), an inactive form of GSK-3B degrading glioblastoma 2 (GLI2), followed by the decreased expression of both smoothened and *GLI2*. A TB4 CRISPR also blocked the activation of primary HSCs, with decreased expression of smoothened, *GLI2* and *ILK* compared with cells transfected with nontargeting control CRISPR. Double immunostaining and an immunoprecipitation assay revealed that TB4 interacted with either smoothened at the cytoplasm or GLI2 at the nucleus in LX-2. Smoothened suppression in primary HSCs using a Hh antagonist or adenovirus transduction decreased *TB4* expression with the reduced activation of HSCs. Tb4-overexpressing transgenic mice treated with CCl_4 were susceptible to the development hepatic fibrosis with higher levels of ILK, pGSK3b, and Hh activity, as compared with wild-type mice. These findings demonstrate that TB4 regulates HSC activation by influencing the activity of Smoothened and GLI2, suggesting TB4 as a novel therapeutic target in liver disease.

4.1722 Sphingosine-1-Phosphate Prevents Egress of Hematopoietic Stem Cells From Liver to Reduce Fibrosis

King, A. ete al

*Gastroenterol., **153**, 233-248 (2017)*

Background & Aims
There is growing interest in the use of bone marrow cells to treat liver fibrosis, however, little is known about their antifibrotic efficacy or the identity of their effector cell(s). Sphingosine-1-phosphate (S1P) mediates egress of immune cells from the lymphoid organs into the lymphatic vessels; we investigated its role in the response of hematopoietic stem cells (HSCs) to liver fibrosis in mice.
Methods
Purified (c-kit+/sca1+/lin-) HSCs were infused repeatedly into mice undergoing fibrotic liver injury. Chronic liver injury was induced in BoyJ mice by injection of carbon tetrachloride (CCl_4) or placement on a methionine-choline–deficient diet. Some mice were irradiated and given transplants of bone marrow cells from C57BL6 mice, with or without the S1P antagonist FTY720; we then studied HSC mobilization and localization. Migration of HSC lines was quantified in Transwell assays. Levels of S1P in liver, bone marrow, and lymph fluid were measured using an enzyme-linked immunosorbent assay. Liver tissues were collected and analyzed by immunohistochemical quantitative polymerase chain reaction and sphingosine kinase activity assays. We performed quantitative polymerase chain reaction analyses of the expression of sphingosine kinase 1 and 2, sphingosine-1-phosphate lyase 1, and sphingosine-1-phosphate phosphatase 1 in normal human liver and cirrhotic liver from patients with alcohol-related liver disease (n = 6).
Results
Infusions of HSCs into mice with liver injury reduced liver scarring based on picrosirius red staining (49.7% reduction in mice given HSCs vs control mice; $P < .001$), and hepatic hydroxyproline content (328 mg/g in mice given HSCs vs 428 mg/g in control mice; $P < .01$). HSC infusion also reduced hepatic expression of α-smooth muscle actin (0.19 ± 0.007-fold compared with controls; $P < .0001$) and collagen type I α 1 chain (0.29 ± 0.17-fold compared with controls; $P < .0001$). These antifibrotic effects were maintained with infusion of lymphoid progenitors that lack myeloid potential and were associated with increased numbers of recipient neutrophils and macrophages in liver. In studies of HSC cell lines, we found HSCs to recruit monocytes, and this process to require C-C motif chemokine receptor 2. In fibrotic liver tissue from mice and patients, hepatic S1P levels increased owing to increased hepatic sphingosine kinase-1 expression, which contributed to a reduced liver:lymph S1P gradient and limited HSC egress from the liver. Mice given the S1P antagonist (FTY720) with HSCs had increased hepatic retention of HSCs (1697 ± 247 cells in mice given FTY720 vs 982 ± 110 cells in controls; $P < .05$), and further reductions in fibrosis.
Conclusions
In studies of mice with chronic liver injury, we showed the antifibrotic effects of repeated infusions of purified HSCs. We found that HSCs promote recruitment of endogenous macrophages and neutrophils. Strategies to reduce SIP signaling and increase retention of HSCs in the liver could increase their antifibrotic activities and be developed for treatment of patients with liver fibrosis.

4.1723 Augmented liver targeting of exosomes by surface modification with cationized pullulan

Tamura, R., Uemoto, S. and Tabata, Y.

*Acta Biomaterialia, **57**, 274-284 (2017)*

Exosomes are membrane nanoparticles containing biological substances that are employed as therapeutics in experimental inflammatory models. Surface modification of exosomes for better tissue targetability and enhancement of their therapeutic ability was recently attempted mainly using gene transfection techniques. Here, we show for the first time that the surface modification of exosomes with cationized pullulan, which has the ability to target hepatocyte asialoglycoprotein receptors, can target injured liver and enhance the therapeutic effect of exosomes. Surface modification can be achieved by a simple mixing of original exosomes and cationized pullulan and through an electrostatic interaction of both substances. The exosomes modified with cationized pullulan were internalized into HepG2 cells *in vitro* to a significantly greater extent than unmodified ones and this internalization was induced through the asialoglycoprotein receptor that was specifically expressed on HepG2 cells and hepatocytes. When injected intravenously into mice with concanavalin A-induced liver injury, the modified exosomes accumulated in the liver tissue, resulting in an enhanced anti-inflammatory effect *in vivo*. It is concluded that the surface modification with cationized pullulan promoted accumulation of the exosomes in the liver and the subsequent biological function, resulting in a greater therapeutic effect on liver injury.

4.1724 **Focal Adhesion Kinase Regulates Hepatic Stellate Cell Activation and Liver Fibrosis**
Zhao, X-Ke, et al
*Scientific Reports, **7**:4032 (2017)*

Understanding the underlying molecular mechanisms of liver fibrosis is important to develop effective therapy. Herein, we show that focal-adhesion-kinse (FAK) plays a key role in promoting hepatic stellate cells (HSCs) activation *in vitro* and liver fibrosis progression *in vivo*. FAK activation is associated with increased expression of α-smooth muscle actin (α-SMA) and collagen in fibrotic live tissues. Transforming growth factor beta-1 (TGF-β1) induces FAK activation in a time and dose dependent manner. FAK activation precedes the α-SMA expression in HSCs. Inhibition of FAK activation blocks the α-SMA and collagen expression, and inhibits the formation of stress fibers in TGF-β1 treated HSCs. Furthermore, inhibition of FAK activation significantly reduces HSC migration and small GTPase activation, and induces apoptotic signaling in TGF-β1 treated HSCs. Importantly, FAK inhibitor attenuates liver fibrosis *in vivo* and significantly reduces collagen and α-SMA expression in an animal model of liver fibrosis. These data demonstrate that FAK plays an essential role in HSC activation and liver fibrosis progression, and FAK signaling pathway could be a potential target for liver fibrosis.

4.1725 **KCC3 loss-of-function contributes to Andermann syndrome by inducing activity-dependent neuromuscular junction defects**
Bowermann, M. et al
*Neurobiol. Dis., **106**, 35-48 (2017)*

Loss-of-function mutations in the potassium-chloride cotransporter KCC3 lead to Andermann syndrome, a severe sensorimotor neuropathy characterized by areflexia, amyotrophy and locomotor abnormalities. The molecular events responsible for axonal loss remain poorly understood. Here, we establish that global or neuron-specific KCC3 loss-of-function in mice leads to early neuromuscular junction (NMJ) abnormalities and muscular atrophy that are consistent with the pre-synaptic neurotransmission defects observed in patients. KCC3 depletion does not modify chloride handling, but promotes an abnormal electrical activity among primary motoneurons and mislocalization of Na^+/K^+-ATPase α1 in spinal cord motoneurons. Moreover, the activity-targeting drug carbamazepine restores Na^+/K^+-ATPase α1 localization and reduces NMJ denervation in *Slc12a6*$^{-/-}$ mice. We here propose that abnormal motoneuron electrical activity contributes to the peripheral neuropathy observed in Andermann syndrome.

4.1726 **Bioenergetic status modulates motor neuron vulnerability and pathogenesis in a zebrafish model of spinal muscular atrophy**
Boyd, P.J. et al
*PloS Genetics, **13(4)**, e1006744 (2017)*

Degeneration and loss of lower motor neurons is the major pathological hallmark of spinal muscular atrophy (SMA), resulting from low levels of ubiquitously-expressed survival motor neuron (SMN) protein. One remarkable, yet unresolved, feature of SMA is that not all motor neurons are equally affected, with some populations displaying a robust resistance to the disease. Here, we demonstrate that selective vulnerability of distinct motor neuron pools arises from fundamental modifications to their basal molecular profiles. Comparative gene expression profiling of motor neurons innervating the extensor digitorum longus (disease-resistant), gastrocnemius (intermediate vulnerability), and tibialis anterior (vulnerable) muscles in mice revealed that disease susceptibility correlates strongly with a modified bioenergetic profile. Targeting of identified bioenergetic pathways by enhancing mitochondrial biogenesis rescued motor axon defects in SMA zebrafish. Moreover, targeting of a single bioenergetic protein, phosphoglycerate kinase 1 (Pgk1), was found to modulate motor neuron vulnerability in vivo. Knockdown of pgk1 alone was sufficient to partially mimic the SMA phenotype in wild-type zebrafish. Conversely, Pgk1 overexpression, or treatment with terazosin (an FDA-approved small molecule that binds and activates Pgk1), rescued motor axon phenotypes in SMA zebrafish. We conclude that global bioenergetics pathways can be therapeutically manipulated to ameliorate SMA motor neuron phenotypes in vivo.

4.1727 **Targeting the mitochondrial pyruvate carrier attenuates fibrosis in a mouse model of nonalcoholic steatohepatitis**
McCommis, K.S., Hodges, W.T., Brunt, E.M., Nalbantoglu, I., McDonald, W.G., Holley, C., Fujiwara, H., Schaffer, J.E., Colca, J.R. and Finck, B.N.
*Hepatology, **65(5)**, 1543-1556 (2017)*

Diseases of the liver related to metabolic syndrome have emerged as the most common and undertreated hepatic ailments. The cause of nonalcoholic fatty liver disease is the aberrant accumulation of lipid in hepatocytes, though the mechanisms whereby this leads to hepatocyte dysfunction, death, and hepatic fibrosis are still unclear. Insulin-sensitizing thiazolidinediones have shown efficacy in treating nonalcoholic steatohepatitis (NASH), but their widespread use is constrained by dose-limiting side effects thought to be due to activation of the peroxisome proliferator–activated receptor γ. We sought to determine whether a next-generation thiazolidinedione with markedly diminished ability to activate peroxisome proliferator–activated receptor γ (MSDC-0602) would retain its efficacy for treating NASH in a rodent model. We also determined whether some or all of these beneficial effects would be mediated through an inhibitory interaction with the mitochondrial pyruvate carrier 2 (MPC2), which was recently identified as a mitochondrial binding site for thiazolidinediones, including MSDC-0602. We found that MSDC-0602 prevented and reversed liver fibrosis and suppressed expression of markers of stellate cell activation in livers of mice fed a diet rich in trans-fatty acids, fructose, and cholesterol. Moreover, mice with liver-specific deletion of MPC2 were protected from development of NASH on this diet. Finally, MSDC-0602 directly reduced hepatic stellate cell activation *in vitro*, and MSDC-0602 treatment or hepatocyte MPC2 deletion also limited stellate cell activation indirectly by affecting secretion of exosomes from hepatocytes. *Conclusion*: Collectively, these data demonstrate the effectiveness of MSDC-0602 for attenuating NASH in a rodent model and suggest that targeting hepatic MPC2 may be an effective strategy for pharmacologic development.

4.1728 Retinoic Acid Regulates Immune Responses by Promoting IL-22 and Modulating S100 Proteins in Viral Hepatitis

Jie, Z., Liang, Y., Yi, P., Tang, H., Soong, L., Cong, Y., Zhang, K. and Sun, J.
J. Immunol., ***198(9)***, *3448-3460 (2017)*

Although large amounts of vitamin A and its metabolite all-*trans* retinoic acid (RA) are stored in the liver, how RA regulates liver immune responses during viral infection remains unclear. In this study, we demonstrated that IL-22, mainly produced by hepatic γδ T cells, attenuated liver injury in adenovirus-infected mice. RA can promote γδ T cells to produce mTORC1-dependent IL-22 in the liver, but inhibits IFN-γ and IL-17. RA also affected the aptitude of T cell responses by modulating dendritic cell (DC) migration and costimulatory molecule expression. These results suggested that RA plays an immunomodulatory role in viral infection. Proteomics data revealed that RA downregulated S100 family protein expression in DCs, as well as NF-κB/ERK pathway activation in these cells. Furthermore, adoptive transfer of S100A4-repressed, virus-pulsed DCs into the hind foot of naive mice failed to prime T cell responses in draining lymph nodes. Our study has demonstrated a crucial role for RA in promoting IL-22 production and tempering DC function through downregulating S100 family proteins during viral hepatitis.

4.1729 MicroRNA-649 promotes HSV-1 replication by directly targeting MALT1

Zhang, Y., Dai, J., Tang, J., Zhou, L. and Zhou, M.
J. Med. Virol., ***89***, *1069-1079 (82017)*

Herpes simplex virus type 1 (HSV-1), a member of the Herpes viridae, is associated with a wide variety of nervous system diseases including meningitis and encephalitis. The data presented here demonstrate that miR-649 promotes the replication of HSV-1 without affecting cell viability. Further mechanistic studies revealed that MALT1 (mucosa associated lymphoid tissue lymphoma translocation gene 1) is directly targeted by miR-649. We then found that MALT1 and the downstream NF-κB signaling pathway, are involved in miR-649-induced HSV-1 replication. Interestingly, miR-649 levels were downregulated after HSV-1 infection, and miR-649 expression was negatively associated with MALT1 expression in HSV-1-infected HeLa cells. Taken together, this present study indicates that miR-649 promotes HSV-1 replication through regulation of the MALT1-mediated antiviral signaling pathway and suggests a promising target for antiviral therapies.

4.1730 IRAP+ endosomes restrict TLR9 activation and signaling

Babdor, J. et al
Natuer Immunol., ***18(5)***, *509-518 (2017)*

The retention of intracellular Toll-like receptors (TLRs) in the endoplasmic reticulum prevents their activation under basal conditions. TLR9 is activated by sensing ligands in specific endosomal-lysosomal compartments. Here we identified $IRAP^{+}$ endosomes as major cellular compartments for the early steps of

TLR9 activation in dendritic cells (DCs). Both TLR9 and its ligand, the dinucleotide CpG, were present as cargo in $IRAP^+$ endosomes. In the absence of the aminopeptidase IRAP, the trafficking of CpG and TLR9 to lysosomes and signaling via TLR9 were enhanced in DCs and in mice following bacterial infection. IRAP stabilized CpG-containing endosomes by interacting with the actin-nucleation factor FHOD4, which slowed the trafficking of TLR9 toward lysosomes. Thus, endosomal retention of TLR9 via the interaction of IRAP with the actin cytoskeleton is a mechanism that prevents hyper-activation of TLR9 in DCs.

4.1731 **Social network architecture of human immune cells unveiled by quantitative proteomics**
Rickmann, J.C., Geiger, R., Hornburg, D., Wolf, T., Kveler, K., Jarrossay, D., Sallusto, F., Shen-Orr, S.S., Lanzavecchia, A., Mann, M. and Meissner, F.
Nature Immunol., ***18(5)***, *583-593 (2017)*

The immune system is unique in its dynamic interplay between numerous cell types. However, a system-wide view of how immune cells communicate to protect against disease has not yet been established. We applied high-resolution mass-spectrometry-based proteomics to characterize 28 primary human hematopoietic cell populations in steady and activated states at a depth of >10,000 proteins in total. Protein copy numbers revealed a specialization of immune cells for ligand and receptor expression, thereby connecting distinct immune functions. By integrating total and secreted proteomes, we discovered fundamental intercellular communication structures and previously unknown connections between cell types. Our publicly accessible (**http://www.immprot.org/**) proteomic resource provides a framework for the orchestration of cellular interplay and a reference for altered communication associated with pathology.

4.1732 **Targeting Extracellular Cyclophilin A Reduces Neuroinflammation and Extends Survival in a Mouse Model of Amyotrophic Lateral Sclerosis**
Pasetto, L. et al
J. Neurosci., ***37(6)***, *1413-1427 (2017)*

Neuroinflammation is a major hallmark of amyotrophic lateral sclerosis (ALS), which is currently untreatable. Several anti-inflammatory compounds have been evaluated in patients and in animal models of ALS, but have been proven disappointing in part because effective targets have not yet been identified. Cyclophilin A, also known as peptidylprolyl cis-/trans-isomerase A (PPIA), as a foldase is beneficial intracellularly, but extracellularly has detrimental functions. We found that extracellular PPIA is a mediator of neuroinflammation in ALS. It is a major inducer of matrix metalloproteinase 9 and is selectively toxic for motor neurons. High levels of PPIA were found in the CSF of $SOD1^{G93A}$ mice and rats and sporadic ALS patients, suggesting that our findings may be relevant for familial and sporadic cases. A specific inhibitor of extracellular PPIA, MM218, given at symptom onset, rescued motor neurons and extended survival in the $SOD1^{G93A}$ mouse model of familial ALS by 11 d. The treatment resulted in the polarization of glia toward a prohealing phenotype associated with reduced NF-κB activation, proinflammatory markers, endoplasmic reticulum stress, and insoluble phosphorylated TDP-43. Our results indicates that extracellular PPIA is a promising druggable target for ALS and support further studies to develop a therapy to arrest or slow the progression of the disease in patients.

4.1733 **Decreased Motor Neuron Support by SMA Astrocytes due to Diminished MCP1 Secretion**
Martin, J.E., Nguyen, T.T., Grunseich, C., Nofziger, J.H., Lee, P.R., Fields, D., Fishbeck, K.H. and Foran, E.
J. Neurosci., ***37(21)***, *5309-5318 (2017)*

Spinal muscular atrophy (SMA) is an autosomal-recessive disorder characterized by severe, often fatal muscle weakness due to loss of motor neurons. SMA patients have deletions and other mutations of the *survival of motor neuron 1* (*SMN1*) gene, resulting in decreased SMN protein. Astrocytes are the primary support cells of the CNS and are responsible for glutamate clearance, metabolic support, response to injury, and regulation of signal transmission. Astrocytes have been implicated in SMA as in in other neurodegenerative disorders. Astrocyte-specific rescue of SMN protein levels has been shown to mitigate disease manifestations in mice. However, the mechanism by which SMN deficiency in astrocytes may contribute to SMA is unclear and what aspect of astrocyte activity is lacking is unknown. Therefore, it is worthwhile to identify defects in SMN-deficient astrocytes that compromise normal function. We show here that SMA astrocyte cultures derived from mouse spinal cord of both sexes are deficient in supporting both WT and SMN-deficient motor neurons derived from male, female, and mixed-sex sources and that this deficiency may be mitigated with secreted factors. In particular, SMN-deficient astrocytes have decreased levels of monocyte chemoactive protein 1 (MCP1) secretion compared with controls and MCP1

restoration stimulates outgrowth of neurites from cultured motor neurons. Correction of MCP1 deficiency may thus be a new therapeutic approach to SMA.

4.1734 MicroRNA Profiling Reveals Marker of Motor Neuron Disease in ALS Models
Hoye, M.L. et al
J. Neurosci., ***37(22)***, *5574-5586 (2017)*

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder marked by the loss of motor neurons (MNs) in the brain and spinal cord, leading to fatally debilitating weakness. Because this disease predominantly affects MNs, we aimed to characterize the distinct expression profile of that cell type to elucidate underlying disease mechanisms and to identify novel targets that inform on MN health during ALS disease time course. microRNAs (miRNAs) are short, noncoding RNAs that can shape the expression profile of a cell and thus often exhibit cell-type-enriched expression. To determine MN-enriched miRNA expression, we used Cre recombinase-dependent miRNA tagging and affinity purification in mice. By defining the *in vivo* miRNA expression of MNs, all neurons, astrocytes, and microglia, we then focused on MN-enriched miRNAs via a comparative analysis and found that they may functionally distinguish MNs postnatally from other spinal neurons. Characterizing the levels of the MN-enriched miRNAs in CSF harvested from ALS models of MN disease demonstrated that one miRNA (miR-218) tracked with MN loss and was responsive to an ALS therapy in rodent models. Therefore, we have used cellular expression profiling tools to define the distinct miRNA expression of MNs, which is likely to enrich future studies of MN disease. This approach enabled the development of a novel, drug-responsive marker of MN disease in ALS rodents.

4.1735 Presence of diabetes autoantigens in extracellular vesicles derived from human islets
Hasilo, C.P., negli, S., Allaeys, I., Cloutier, N., Rutman, A.K., Gasparrini, M., Bonneil, E., Thibault, P., Boilard, E. and Paraskevas, S.
*Scientific Reports, **7**:500 (2017)*

Beta-cell (β-cell) injury is the hallmark of autoimmune diabetes. However, the mechanisms by which autoreactive responses are generated in susceptible individuals are not well understood. Extracellular vesicles (EV) are produced by mammalian cells under normal and stressed physiological states. They are an important part of cellular communication, and may serve a role in antigen processing and presentation. We hypothesized that isolated human islets in culture produce EV that contain diabetes autoantigens (DAA) from these otherwise normal, non-diabetic donors. Here we report the caspase-independent production of EV by human islets in culture, and the characterization of DAA glutamic acid decarboxylase 65 (GAD65) and zinc transporter 8 (ZnT8), as well as the β-cell resident glucose transporter 2 (Glut2), present within the EV.

4.1736 TRAIL regulatory receptors constrain human hepatic stellate cell apoptosis
Singh, H., Otano, I., Rombouts, K., Singh, K.P., Peppa, D., Gill, U., Böttcher, K., Kennedy, P.T.F., Oben, J., Pinzani, M., Walczak, H., Fusai, G., Rosenberg, W.C. and Maini, M.K.
*Scientific Reports, **7**:5514 (2017)*

The TRAIL pathway can mediate apoptosis of hepatic stellate cells to promote the resolution of liver fibrosis. However, TRAIL has the capacity to bind to regulatory receptors in addition to death-inducing receptors; their differential roles in liver fibrosis have not been investigated. Here we have dissected the contribution of regulatory TRAIL receptors to apoptosis resistance in primary human hepatic stellate cells (hHSC). hHSC isolated from healthy margins of liver resections from different donors expressed variable levels of TRAIL-R2/3/4 (but negligible TRAIL-R1) *ex vivo* and after activation. The apoptotic potential of TRAIL-R2 on hHSC was confirmed by lentiviral-mediated knockdown. A functional inhibitory role for TRAIL-R3/4 was revealed by shRNA knockdown and mAb blockade, showing that these regulatory receptors limit apoptosis of hHSC in response to both oligomerised TRAIL and NK cells. A close inverse *ex vivo* correlation between hHSC TRAIL-R4 expression and susceptibility to apoptosis underscored its central regulatory role. Our data provide the first demonstration of non-redundant functional roles for the regulatory TRAIL receptors (TRAIL-R3/4) in a physiological setting. The potential for these inhibitory TRAIL receptors to protect hHSC from apoptosis opens new avenues for prognostic and therapeutic approaches to the management of liver fibrosis.

4.1737 Cryptic amyloidogenic elements in mutant NEFH causing Charcot-Marie-Tooth 2 trigger aggresome formation and neuronal death
Jackuier, A. et al
*Acta Neuropathol. Comm., **5**:55 (2017)*

Neurofilament heavy chain (*NEFH*) gene was recently identified to cause autosomal dominant axonal Charcot-Marie-Tooth disease (CMT2cc). However, the clinical spectrum of this condition and the physio-pathological pathway remain to be delineated. We report 12 patients from two French families with axonal dominantly inherited form of CMT caused by two new mutations in the *NEFH* gene. A remarkable feature was the early involvement of proximal muscles of the lower limbs associated with pyramidal signs in some patients. Nerve conduction velocity studies indicated a predominantly motor axonal neuropathy. Unique deletions of two nucleotides causing frameshifts near the end of the *NEFH* coding sequence were identified: in family 1, c.3008_3009del (p.Lys1003Argfs*59), and in family 2 c.3043_3044del (p.Lys1015Glyfs*47). Both frameshifts lead to 40 additional amino acids translation encoding a cryptic amyloidogenic element. Consistently, we show that these mutations cause protein aggregation which are recognised by the autophagic pathway in motoneurons and triggered caspase 3 activation leading to apoptosis in neuroblastoma cells. Using electroporation of chick embryo spinal cord, we confirm that NEFH mutants form aggregates in vivo and trigger apoptosis of spinal cord neurons. Thus, our results provide a physiological explanation for the overlap between CMT and amyotrophic lateral sclerosis (ALS) clinical features in affected patients.

4.1738 A CXCL ortholog from Hippocampus abdominalis: Molecular features and functional delineation as a pro-inflammatory chemokine
Oh, M., bathige, S.D.N.K., Kim, Y., Lee, S., yang, H., Kim, M-J. and Lee, J.
*Fish & Shellfish Immunol., **67**, 218-227 (2017)*

Chemokines are a family of chemotactic cytokines that regulate leukocyte migration. They are classified into four groups namely, CXC, CC, C and CX_3C, based on the formation of a disulfide bridge. Among these, CXC chemokines have been identified as the largest group of chemokines in humans. In this study, we identified and functionally characterized a homolog of CXC chemokine from the big-belly seahorse, *Hippocampus abdominalis*, and designated it as *ShCXCL*. The cDNA of *ShCXCL* composed of a 342-bp open reading frame encoding 113 amino acids (aa). The CXC family-specific small cytokine domain (SCY) was identified from the mature peptide region, which comprised of a conserved CXC motif. As ShCXCL lacks an ELR (Glutamic acid-Leucine-Arginine) motif, it belongs to ELR^- subfamily. The recombinant ShCXCL protein strongly induced the nitric oxide (NO) production in macrophage cells (RAW 264.7 cell line) and showed the chemotactic effect on flounder peripheral blood leukocytes. Tissue profiling showed a ubiquitous expression pattern in all examined tissues, with a high abundance in spleen. The up-regulated mRNA expression pattern of *ShCXCL* was observed in blood and kidney tissues after immune stimulation by live bacteria, such as *Streptococcus iniae* and *Edwardsiella tarda*, and mitogens, such as lipopolysaccharides (LPS) and polyinosinic:polycytidylic acid (poly I:C), suggesting its important role in host immune defense against microbial infection.

4.1739 Chemoselective functionalization of nanogels for microglia treatment
Mauri, E., Veglianese, P., Papa, S., Mariani, A., De paola, M., Rigamonti, R., Chincarini, G.M.F., Rimondo, S., Sacchetti, A. and Rossi, F.
*Eur. Polymer J., **94**, 143-151 (2017)*

The development of nanogels as nanoscale multifunctional polymer-based matrices for controlled drug and gene delivery purposes has been the subject of intense research during the last decades. Indeed, polymeric nanoparticles are capable to interact with cells to different extents, depending on their size, shape, surface properties and ligands tagged to the surface. Moreover, coating these devices using appropriate functionalization strategies can greatly improve or not their adhesion and uptake by cells. In this work, we proposed different coatings and then we studied their ability to improve or reduce microglia internalization. Nanogels (NGs) were composed by polyethylene glycol (PEG) and polyethyleneimine (PEI) conjugated with rhodamine through click chemistry reaction. Coatings were prepared using PEG monomethyl ether (mPEG), modifying its terminal hydroxyl groups with different linkers to evaluate the amount of mPEG layer chemically bond to the nanogel and its effect over microglia internalization. Nanogels were also investigated to identify which procedure is able to form networks with adequate dimensions and stability, according to the physicochemical parameters for the microglia applications *in vitro*. The biological experimental results showed that NGs were efficiently internalized by cells and the coating-microglia

interactions allowed different cellular uptake. This outcome could be considered a promising perspective of nanogels use as carriers for drugs or genes delivery within microglia environment, improving their therapeutic effect through polymer surface modifications.

4.1740 A new method of isolating spinal motor neurons from fetal mouse
Wang, W., Qi, B., Lv, H., Wu, F., Liu, L., Wang, W., Wang, Q., Hu, L., Hao, Y. and Wang, Y.
*J. Neurosci. Methods, **288**, 57-61 (2017)*

Background
Isolating of primary motor neurons from animal embryos is critical for the study of neurological disease including mechanistic discovery and therapeutic development. Density gradient centrifuge taking advantage of the buoyant of motor neuron permits the enrichment of motor neurons. Despite the metrizamide, an OptiPrep medium has been introduced to separate the motor neurons by gradient centrifuge.
New method
We hereby used single density gradient of OptiPrep medium to isolate the spinal motor neurons from the fetal mouse.
Results
Single density gradient of OptiPrep medium is effective to isolate spinal motor neurons from the fetal mouse. The immunofluorescence staining analysis showed that the purity of cultured motor neurons at 72 h was between 90% and 95%.
Comparison with existing method
Four gradients of OptiPrep medium have been previously used to isolate the motor neurons from spinal cord of mouse. In this study, the single gradient of OptiPrep medium was demonstrated to effectively isolate spinal motor neurons from the fetal mouse.
Conclusions
The single gradient of OptiPrep medium is enough to produce high purity of spinal motor neurons from the fetal mouse.

4.1741 Sigma 1 receptor activation modifies intracellular calcium exchange in the G93AhSOD1 ALS model
Tadic, V., Malci, A., Goldhammer, N., Stubendorff, B., Sengupta, S., Prell, T., Keiner, S., Liu, J., Guenther, M., Frahm, C., Witte, O.W. and Grosskreutz, J.
*Neuroscience, **359**, 105-118 (2017)*

Aberrations in intracellular calcium (Ca^{2+}) have been well established within amyotrophic lateral sclerosis (ALS), a severe motor neuron disease. Intracellular Ca^{2+} concentration is controlled in part through the endoplasmic reticulum (ER) mitochondria Ca^{2+} cycle (ERMCC). The ER supplies Ca^{2+} to the mitochondria at close contacts between the two organelles, i.e. the mitochondria-associated ER membranes (MAMs). The Sigma 1 receptor (Sig1R) is enriched at MAMs, where it acts as an inter-organelle signaling modulator. However, its impact on intracellular Ca^{2+} at the cellular level remains to be thoroughly investigated.
Here, we used cultured embryonic mice spinal neurons to investigate the influence of Sig1R activation on intracellular Ca^{2+} homeostasis in the presence of $G93A^{hSOD1}$ (G93A), an established ALS-causing mutation. Sig1R expression was increased in G93A motor neurons relative to non-transgenic (nontg) controls. Furthermore, we demonstrated significantly reduced bradykinin-sensitive intracellular Ca^{2+} stores in G93A spinal neurons, which were normalized by the Sig1R agonist SA4503. Moreover, SA4503 accelerated cytosolic Ca^{2+} clearance following a) AMPAR activation by kainate and b) IP_3R–mediated ER Ca^{2+} release following bradykinin stimulation in both genotypes. PRE-084 (another Sig1R agonist) did not exert any significant effects on cytosolic Ca^{2+}. Both Sig1R expression and functionality were altered by the G93A mutation, indicating the centrality of Sig1R in ALS pathology. Here, we showed that intracellular Ca^{2+} shuttling can be manipulated by Sig1R activation, thus demonstrating the value of using the pharmacological manipulation of Sig1R to understand Ca^{2+} homeostasis.

4.1742 The anti-influenza M2e antibody response is promoted by XCR1 targeting in pig skin
Deloizy, C. et al
*Scientific Reports, **7**:7639 (2017)*

XCR1 is selectively expressed on a conventional dendritic cell subset, the cDC1 subset, through phylogenetically distant species. The outcome of antigen-targeting to XCR1 may therefore be similar

across species, permitting the translation of results from experimental models to human and veterinary applications. Here we evaluated in pigs the immunogenicity of bivalent protein structures made of XCL1 fused to the external portion of the influenza virus M2 proton pump, which is conserved through strains and a candidate for universal influenza vaccines. Pigs represent a relevant target of such universal vaccines as pigs can be infected by swine, human and avian strains. We found that cDC1 were the only cell type labeled by XCR1-targeted mCherry upon intradermal injection in pig skin. XCR1-targeted M2e induced higher IgG responses in seronegative and seropositive pigs as compared to non-targeted M2e. The IgG response was less significantly enhanced by CpG than by XCR1 targeting, and CpG did not further increase the response elicited by XCR1 targeting. Monophosphoryl lipid A with neutral liposomes did not have significant effect. Thus altogether M2e-targeting to XCR1 shows promises for a trans-species universal influenza vaccine strategy, possibly avoiding the use of classical adjuvants.

4.1743 Swarm Intelligence-Enhanced Detection of Non-Small-Cell Lung Cancer Using Tumor-Educated Platelets

Best, M.G., Sol, N., In t'Veld, S.G.J.G., Vancura, A., Muller, M., Niemeijer, A-L. N., Fejes, A.V.,Tjon Kon Fat, L-A. and In t'Veld, A.E.H.
*Cancer Cell, **32**, 238-252 (2017)*

Blood-based liquid biopsies, including tumor-educated blood platelets (TEPs), have emerged as promising biomarker sources for non-invasive detection of cancer. Here we demonstrate that particle-swarm optimization (PSO)-enhanced algorithms enable efficient selection of RNA biomarker panels from platelet RNA-sequencing libraries (n = 779). This resulted in accurate TEP-based detection of early- and late-stage non-small-cell lung cancer (n = 518 late-stage validation cohort, accuracy, 88%; AUC, 0.94; 95% CI, 0.92–0.96; $p < 0.001$; n = 106 early-stage validation cohort, accuracy, 81%; AUC, 0.89; 95% CI, 0.83–0.95; $p < 0.001$), independent of age of the individuals, smoking habits, whole-blood storage time, and various inflammatory conditions. PSO enabled selection of gene panels to diagnose cancer from TEPs, suggesting that swarm intelligence may also benefit the optimization of diagnostics readout of other liquid biopsy biosources.

4.1744 The Evaluation of Islet Purification Methods That Use Large Bottles to Create a Continuous Density Gradient

Miyagi-Shiohira, C., Kobayashi, N., Saitoh, I., Watanabe, M., Noguchi, Y., Matsushita, M. and Noguchi, H.
*Cell Med., **9**, 45-51 (2017)*

Islet purification is one of the most important steps of islet isolation for pancreatic islet transplantation. The most common method of islet purification is density gradient centrifugation using a COBE 2991 cell processor. However, this method can damage islets mechanically through its high shearing force. We recently reported that a new purification method using large plastic bottles effectively achieves a high yield of islets from the porcine pancreas. In the present study, we evaluated the methods of making a continuous density gradient. The gradient was produced with a gradient maker and two types of candy cane-shaped stainless steel pipes. One method was to use a "bent-tipped" stainless steel pipe and to load from a high-density solution to a low-density solution, uploading the stainless steel pipe. The other method was to use a regular stainless steel pipe and to load from a low-density solution to a high-density solution, leaving the stainless steel pipe in place. There were no significant differences between the two solutions in terms of the islet yield, rate of viability or purity, score, or the stimulation index after purification. Furthermore, there were no differences in the attainability or suitability of posttransplantation normoglycemia. Our study shows the equivalency of these two methods of islet purification.

4.1745 Comparison of Purification Solutions with Different Osmolality for Porcine Islet Purification

Miyagi-Shiora, C., Kobayashi, N., Saitoh, I., Watanabe, M., Noguchi, Y., Matsushita, M. and Noguchi, H.
*Cell Med., **9**, 53-59 (2017)*

The osmolality of the purification solution is one of the most critical variables in human islet purification during islet isolation. We previously reported the effectiveness of a combined continuous density/osmolality gradient for the supplemental purification of human islets. We herein applied a combined continuous density/osmolality gradient for regular purification. The islets were purified with a continuous density gradient without osmolality preparation [continuous density/normal osmolality (CD/NO)] or continuous density/osmolality solution with osmolality preparation by 10× Hank's balanced salt solution (HBSS) [continuous density/continuous osmolality (CD/CO)]. The osmolality of the low-

density solution was 400 mOsm/kg in both groups and that of the high-density solution was 410 mOsm/kg in the CD/NO group and 500 mOsm/kg in the CD/CO group. Unexpectedly, we noted no significant differences between the two solutions in terms of the islet yield, rate of viability and purity, score, stimulation index, or the attainability and suitability of posttransplantation normoglycemia. Despite reports that the endocrine and exocrine tissues of pancreata have distinct osmotic sensitivities and that high-osmolality solutions result in greater purification efficiency, the isolation and transplant outcomes did not markedly differ between the two purification solutions with different osmolalities in this study.

4.1746 Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding
Lan, F., Demaree, B., Ahmed, N. and Abate, A.R.
Nature Biotech., ***35(7)***, *640-646 (2017)*

The application of single-cell genome sequencing to large cell populations has been hindered by technical challenges in isolating single cells during genome preparation. Here we present single-cell genomic sequencing (SiC-seq), which uses droplet microfluidics to isolate, fragment, and barcode the genomes of single cells, followed by Illumina sequencing of pooled DNA. We demonstrate ultra-high-throughput sequencing of >50,000 cells per run in a synthetic community of Gram-negative and Gram-positive bacteria and fungi. The sequenced genomes can be sorted *in silico* based on characteristic sequences. We use this approach to analyze the distributions of antibiotic-resistance genes, virulence factors, and phage sequences in microbial communities from an environmental sample. The ability to routinely sequence large populations of single cells will enable the de-convolution of genetic heterogeneity in diverse cell populations.

4.1747 Cutting Edge: Eosinophils Undergo Caspase-1–Mediated Pyroptosis in Response to Necrotic Liver Cells
Palacios-Macapagal, D., Connor, J., Mustelin, T., Ramalingam, T.R., Wynn, T.A. and Davidson, T.S.
J. Immunol., ***199(3)***, *847-853 (2017)*

Many chronic liver disorders are characterized by dysregulated immune responses and hepatocyte death. We used an in vivo model to study the immune response to necrotic liver injury and found that necrotic liver cells induced eosinophil recruitment. Necrotic liver induced eosinophil IL-1β and IL-18 secretion, degranulation, and cell death. Caspase-1 inhibitors blocked all of these responses. Caspase-1–mediated cell death with accompanying cytokine release is the hallmark of a novel form of cell death termed pyroptosis. To confirm this response in a disease model, we isolated eosinophils from the livers of *Schistosoma mansoni*–infected mice. *S. mansoni* eggs lodge in the hepatic sinusoids of infected mice, resulting in hepatocyte death, inflammation, and progressive liver fibrosis. This response is typified by massive eosinophilia, and we were able to confirm pyroptosis in the infiltrating eosinophils. This demonstrated that pyroptosis is a cellular pathway used by eosinophils in response to large-scale hepatic cell death.

4.1748 A novel whole-bacterial enzyme linked-immunosorbant assay to quantify Chlamydia trachomatis specific antibodies reveals distinct differences between systemic and genital compartments
Albritton, H.L., Kozlowski, P.A:, Lillis, R.A., McGowin, C.L., Siren, J.D., Taylor, S.N., Ibana, J.A., Buckner, L.R., Shen, L and Quayle, J.
PloS One, ***12(8)***, *e0183101 (2017)*

Chlamydia trachomatis (CT) is the leading sexually transmitted bacterial infection. The continued global burden of CT infection strongly predicates the need for a vaccine to supplement current chlamydial control programs. The correlates of protection against CT are currently unknown, but they must be carefully defined to guide vaccine design. The localized nature of chlamydial infection in columnar epithelial cells of the genital tract necessitates investigation of immunity at the site of infection. The purpose of this study was to develop a sensitive whole bacterial enzyme-linked immunosorbent assay (ELISA) to quantify and compare CT-specific IgG and IgA in sera and genital secretions from CT-infected women. To achieve this, elementary bodies (EBs) from two of the most common genital serovars (D and E) were attached to poly-L-lysine-coated microtiter plates with glutaraldehyde. EB attachment and integrity were verified by the presence of outer membrane antigens and the absence of bacterial cytoplasmic antigens. EB-specific IgG and IgA standards were developed by pooling sera with high titers of CT-specific antibodies from infected women. Serum, endocervical and vaginal secretions, and endocervical cytobrush specimens from CT-infected women were used to quantify CT-specific IgG and IgA which were then normalized to total IgG and IgA, respectively. Analyses of paired serum and genital samples revealed significantly higher proportions of EB-specific antibodies in genital secretions compared to sera. Cervical and vaginal

secretions and cytobrush specimens had similar proportions of EB-specific antibodies, suggesting any one of these genital sampling techniques could be used to quantify CT-specific antibodies when appropriate normalization methodologies are implemented. Overall, these results illustrate the need to investigate genital tract CT antibody responses, and our assay provides a useful quantitative tool to assess natural immunity in defined clinical groups and CT vaccine trials.

4.1749 Improved yield of canine islet isolation from deceased donors

Harrington, S., Williams, S.J., Otte, V., Barchman, S., Jones, C., Ramachandran, K. and Stehno-Bittel, L.
*BMC Vet. Res., **13**:264 (2017)*

Background
Canine diabetes is a strikingly prevalent and growing disease, and yet the standard treatment of a twice-daily insulin injection is both cumbersome to pet owners and only moderately effective. Islet transplantation has been performed with repeated success in canine research models, but has unfortunately not been made available to companion animals. Standard protocols for islet isolation, developed primarily for human islet transplantation, include beating-heart organ donation, vascular perfusion of preservation solutions, specialized equipment. Unfortunately, these processes are prohibitively complex and expensive for veterinary use. The aim of the study was to develop a simplified approach for isolating canine islets that is compatible with the financial and logistical restrictions inherent to veterinary medicine for the purpose of translating islet transplantation to a clinical treatment for canine diabetes.
Results
Here, we describe simplified strategies for isolating quality islets from deceased canine donors without vascular preservation and with up to 90 min of cold ischemia time. An average of more than 1500 islet equivalents per kg of donor bodyweight was obtained with a purity of 70% (N = 6 animals). Islets were 95% viable and responsive to glucose stimulation for a week. We found that processing only the body and tail of the pancreas increased isolation efficiency without sacrificing islet total yield. Islet yield per gram of tissue increased from 773 to 1868 islet equivalents when the head of the pancreas was discarded (N = 3/group).
Conclusions
In summary, this study resulted in the development of an efficient and readily accessible method for obtaining viable and functional canine islets from deceased donors. These strategies provide an ethical means for obtaining donor islets.

4.1750 DNA strand breaks and TDP-43 mislocation are absent in the murine hSOD1G93A model of amyotrophic lateral sclerosis in vivo and in vitro

Pennendorf, D., Tadic, V., Witte, O., grosskreutz, J. and Kretz, A.
*PloS One, **12(8)**, e0183684 (2017)*

Mutations in the human *Cu/Zn superoxide dismutase type-1* (*hSOD1*) gene are common in familial amyotrophic lateral sclerosis (fALS). The pathophysiology has been linked to, e.g., organelle dysfunction, RNA metabolism and oxidative DNA damage conferred by SOD1 malfunction. However, apart from metabolically evoked DNA oxidation, it is unclear whether severe genotoxicity including DNA single-strand breaks (SSBs) and double-strand breaks (DSBs), originates from loss of function of nuclear SOD1 enzyme. Factors that endogenously interfere with DNA integrity and repair complexes in *hSOD1*-mediated fALS remain similarly unexplored. In this regard, uncontrolled activation of transposable elements (TEs) might contribute to DNA disintegration and neurodegeneration. The aim of this study was to elucidate the role of the fALS-causing $hSOD1^{G93A}$ mutation in the generation of severe DNA damage beyond well-characterized DNA base oxidation. Therefore, DNA damage was assessed in spinal tissue of $hSOD1^{G93A}$-overexpressing mice and in corresponding motor neuron-enriched cell cultures *in vitro*. Overexpression of the $hSOD1^{G93A}$ locus did not change the threshold for severe DNA damage *per se*. We found that levels of SSBs and DSBs were unaltered between $hSOD1^{G93A}$ and control conditions, as demonstrated in post-mitotic motor neurons and in astrocytes susceptible to replication-dependent DNA breakage. Analogously, parameters indicative of DNA damage response processes were not activated *in vivo* or *in vitro*. Evidence for a mutation-related elevation in TE activation was not detected, in accordance with the absence of TAR DNA binding protein 43 (TDP-43) proteinopathy in terms of cytoplasmic mislocation or nuclear loss, as nuclear TDP-43 is supposed to silence TEs physiologically. Conclusively, the superoxide dismutase function of SOD1 might not be required to preserve DNA integrity in motor neurons, at least when the function of TDP-43 is unaltered. Our data establish a foundation for further investigations addressing functional TDP-43 interaction with ALS-relevant genetic mutations.

4.1751 A versatile microfluidic device for high throughput production of microparticles and cell microencapsulation
Akbari, S., Pirbodaghi, T., Kamm, R.D. and Hammond, P.T.
*Lab on a Chip, **17**, 2067-2075 (2017)*

Biocompatible microparticles are valuable tools in biomedical research for applications such as drug delivery, cell transplantation therapy, and analytical assays. However, their translation into clinical research and the pharmaceutical industry has been slow due to the lack of techniques that can produce microparticles with controlled physicochemical properties at high throughput. We introduce a robust microfluidic platform for the production of relatively homogeneous microdroplets at a generation frequency of up to 3.1 MHz, which is about three orders of magnitude higher than the production rate of a conventional microfluidic drop maker. We demonstrated the successful implementation of our device for production of biocompatible microparticles with various crosslinking mechanisms and cell microencapsulation with high cell viability.

4.1752 Controlled rotation and translation of spherical particles or living cells by surface acoustic waves
Bernard, I., Donikov, A.A., Marmottant, P., Rabaud, D., Poulain, C. and Thibault, P.
*Lab on a Chip, **17**, 2470-2480 (2017)*

We show experimental evidence of the acoustically-assisted micromanipulation of small objects like solid particles or blood cells, combining rotation and translation, using high frequency surface acoustic waves. This was obtained from the leakage in a microfluidic channel of two standing waves arranged perpendicularly in a $LiNbO_3$ piezoelectric substrate working at 36.3 MHz. By controlling the phase lag between the emitters, we could, in addition to translation, generate a swirling motion of the emitting surface which, in turn, led to the rapid rotation of spherical polystyrene Janus beads suspended in the channel and of human red and white blood cells up to several rounds per second. We show that these revolution velocities are compatible with a torque caused by the acoustic streaming that develops at the particles surface, like that first described by [F. Busse *et al.*, *J. Acoust. Soc. Am.*, 1981, **69**(6), 1634–1638]. This device, based on standard interdigitated transducers (IDTs) adjusted to emit at equal frequencies, opens a way to a large range of applications since it allows the simultaneous control of the translation and rotation of hard objects, as well as the investigation of the response of cells to shear stress.

4.1753 Toward a Droplet-Based Single-Cell Radiometric Assay
Gallina, M.E., Kim, T.J., Shelor, M., Vasquez, J., Mogersum, A., Kim, M., Tang, S.K.Y., Abbyad, P. and Pratx, G.
*Anal. Chem., **89(12)**, 6472-6481 (2017)*

Radiotracers are widely used to track molecular processes, both *in vitro* and *in vivo*, with high sensitivity and specificity. However, most radionuclide detection methods have spatial resolution inadequate for single-cell analysis. A few existing methods can extract single-cell information from radioactive decays, but the stochastic nature of the process precludes high-throughput measurement (and sorting) of single cells. In this work, we introduce a new concept for translating radioactive decays occurring stochastically within radiolabeled single-cells into an integrated, long-lasting fluorescence signal. Single cells are encapsulated in radiofluorogenic droplets containing molecular probes sensitive to byproducts of ionizing radiation (primarily reactive oxygen species, or ROS). Different probes were examined in bulk solutions, and dihydrorhodamine 123 (DHRh 123) was selected as the lead candidate due to its sensitivity and reproducibility. Fluorescence intensity of DHRh 123 in bulk increased at a rate of 54% per Gy of X-ray radiation and 15% per MBq/ml of 2-deoxy-2-[^{18}F]-fluoro-d-glucose ([^{18}F]FDG). Fluorescence imaging of microfluidic droplets showed the same linear response, but droplets were less sensitive overall than the bulk ROS sensor (detection limit of 3 Gy per droplet). Finally, droplets encapsulating radiolabeled cancer cells allowed, for the first time, the detection of [^{18}F]FDG radiotracer uptake in single cells through fluorescence activation. With further improvements, we expect this technology to enable quantitative measurement and selective sorting of single cells based on the uptake of radiolabeled small molecules.

4.1754 Cyanidin-3-O-β-glucoside combined with its metabolite protocatechuic acid attenuated the activation of mice hepatic stellate cells
Jiang, X., Shen, T., tang, X., Yang, W., Guo, H. and Ling, w.
*Food Funct., **8**, 2945-2957 (2017)*

Previous studies indicated that cyanidin-3-*O*-β-glucoside (C3G) as a classical anthocyanin exerted an anti-

fibrotic effect in the liver, but its bioavailability was quite low. This study was undertaken to explore the restraining effect of C3G and its metabolite protocatechuic acid (PCA) on the activation of hepatic stellate cells (HSCs). Our data demonstrated that the treatment of a carbon tetrachloride-treated mice model with C3G inhibited liver fibrosis and HSC activation. *In vitro*, both C3G and PCA preserved the lipid droplets and retinol in primary HSCs, and additionally inhibited the mRNA expression of α-smooth muscle actin and collagen I, but elevated the level of matrix metalloproteinase-2 and liver X receptors. Only PCA suppressed the levels of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) secreted from HSCs significantly. In addition, C3G and PCA inhibited the proliferation and migration of HSCs. In conclusion, PCA mainly explained the *in vivo* inhibiting effect of C3G on HSC activation and liver fibrosis.

4.1755 Nanoemulsion-induced enzymatic crosslinking of tyramine-functionalized polymer droplets
Tom Kamperman, Sieger Henke, Bram Zoetebier, Niels Ruiterkamp, Rong Wang, Behdad Pouran, Harrie Weinans, Marcel Karperien and Jeroen Leijten
*J. Mater. Chem. B, **5**, 4835-4844 (2017)*

In situ gelation of water-in-oil polymer emulsions is a key method to produce hydrogel particles. Although this approach is in principle ideal for encapsulating bioactive components such as cells, the oil phase can interfere with straightforward presentation of crosslinker molecules. Several approaches have been developed to induce in-emulsion gelation by exploiting the triggered generation or release of crosslinker molecules. However, these methods typically rely on photo- or acid-based reactions that are detrimental to cell survival and functioning. In this work, we demonstrate the diffusion-based supplementation of small molecules for the in-emulsion gelation of multiple tyramine-functionalized polymers *via* enzymatic crosslinking using a H_2O_2/oil nanoemulsion. This strategy is compatible with various emulsification techniques, thereby readily supporting the formation of monodisperse hydrogel particles spanning multiple length scales ranging from the nano- to the millimeter. As proof of principle, we leveraged droplet microfluidics in combination with the cytocompatible nature of enzymatic crosslinking to engineer hollow cell-laden hydrogel microcapsules that support the formation of viable and functional 3D microtissues. The straightforward, universal, and cytocompatible nature of nanoemulsion-induced enzymatic crosslinking facilitates its rapid and widespread use in numerous food, pharma, and life science applications.

4.1756 From single-molecule detection to next-generation sequencing: microfluidic droplets for high-throughput nucleic acid analysis
Ding, Y., Choo, J. and deMello, A.J.
*Microfluid. Nanofluid., **21**:58 (2017)*

Droplet-based microfluidic technologies have proved themselves to be of significant utility in the performance of high-throughput chemical and biological experiments. By encapsulating and isolating reagents within femtoliter–nanoliter droplet, millions of (bio) chemical reactions can be processed in a parallel fashion and on ultra-short timescales. Recent applications of such technologies to genetic analysis have suggested significant utility in low-cost, efficient and rapid workflows for DNA amplification, rare mutation detection, antibody screening and next-generation sequencing. To this end, we describe and highlight some of the most interesting recent developments and applications of droplet-based microfluidics in the broad area of nucleic acid analysis. In addition, we also present a cursory description of some of the most essential functional components, which allow the creation of integrated and complex workflows based on flowing streams of droplets.

4.1757 Colonization with Helicobacter is concomitant with modified gut microbiota and drastic failure of the immune control of Mycobacterium tuberculosis
Majlessi, L., Sayes, F., Bureau, J-F., Pawlik, A., Michel, V., Jouvion, G., Huerre, M., Severgnini, M., Consolandi, C., Peano, C., Brosch, R., Touati, E. and Leclerc, C.
*Mucosal Immunol., **10**, 1178-1189 (2017)*

Epidemiological and experimental observations suggest that chronic microbial colonization can impact the immune control of other unrelated pathogens contracted in a concomitant or sequential manner. Possible interactions between Mycobacterium tuberculosis infection and persistence of other bacteria have scarcely been investigated. Here we demonstrated that natural colonization of the digestive tract with Helicobacter hepaticus in mice is concomitant with modification of the gut microbiota, subclinical inflammation, and drastic impairment of immune control of the growth of subsequently administered M. tuberculosis, which results in severe lung tissue injury. Our results provided insights upon the fact that this prior H. hepaticus colonization leads to failures in the mechanisms that could prevent the otherwise balanced cross-talk

between M. tuberculosis and the immune system. Such disequilibrium ultimately leads to the inhibition of control of mycobacterial growth, outbreak of inflammation, and lung pathology. Among the dysregulated immune signatures, we noticed a correlation between the detrimental lung injury and the accumulation of activated T-lymphocytes. Our findings suggest that the impact of prior Helicobacter spp. colonization and subsequent M. tuberculosis parasitism might be greater than previously thought, which is a key point given that both species are among the most frequent invasive bacteria in human populations.

4.1758 ADAR1 deletion induces NFκB and interferon signaling dependent liver inflammation and fibrosis
Be-Shoshan, S., Kagan, P., Sultan, M., Barabash, Z., Dor, C., Jacob-Hirsch, J., Harmelin, A., Pappo, O., Marcu-Malina, V., Ben-Ari, Z., Amariglio, N., Rechavi, G., Goldstein, I. and Safran, M.
RNA Biol., ***14(5)***, *587-602 (2017)*

Adenosine deaminase acting on RNA (ADAR) 1 binds and edits double-stranded (ds) RNA secondary structures found mainly within untranslated regions of many transcripts. In the current research, our aim was to study the role of ADAR1 in liver homeostasis. As previous studies show a conserved immunoregulatory function for ADAR1 in mammalians, we focused on its role in preventing chronic hepatic inflammation and the associated activation of hepatic stellate cells to produce extracellular matrix and promote fibrosis. We show that hepatocytes specific ADAR1 knock out (KO) mice display massive liver damage with multifocal inflammation and fibrogenesis. The bioinformatics analysis of the microarray gene-expression datasets of ADAR1 KO livers reveled a type-I interferons signature and an enrichment for immune response genes compared to control littermate livers. Furthermore, we found that *in vitro* silencing of ADAR1 expression in HepG2 cells leads to enhanced transcription of NFκB target genes, foremost of the pro-inflammatory cytokines IL6 and IL8. We also discovered immune cell-independent paracrine signaling among ADAR1-depleted HepG2 cells and hepatic stellate cells, leading to the activation of the latter cell type to adopt a profibrogenic phenotype. This paracrine communication dependent mainly on the production and secretion of the cytokine IL6 induced by ADAR1 silencing in hepatocytes. Thus, our findings shed a new light on the vital regulatory role of ADAR1 in hepatic immune homeostasis, chiefly its inhibitory function on the crosstalk between the NFκB and type-I interferons signaling cascades, restraining the development of liver inflammation and fibrosis.

4.1759 Thrombin-Induced Inflammation in Human Decidual Cells Is Not Affected By Heparin
Smrtka, M.P., Feng, L., Murtha, A.P. and Grotegut, C.A.
Reproductive Sciences, ***24(8)***, *1154-1163 (2017)*

Objective:
Thrombin (Thr) generation at the uteroplacental interface induces inflammation and weakens fetal membranes. Tissue factor (TF) is a powerful procoagulant that is increased by Thr in decidual cells (DCs). The TF expression may play an important role in modulating Thr-induced inflammation. The purpose of this study was to assess the effect of heparin, including nonanticoagulant (desulfated) heparins, on basal and Thr-induced expression of TF and inflammatory cytokines in DCs.
Methods:
Fetal membranes were collected from term pregnancies undergoing unlabored cesarean delivery and then DCs were isolated and cultured. Third passage DCs were conditioned in defined media for 1 week and then treated with 1 of the 4 heparins (enoxaparin, unfractionated heparin, and 2 desulfated heparins) with and without Thr (2.5 U/mL) for 24 hours. Supernatant levels of interleukin (IL) 6, IL-8, IL-10, tumor necrosis factor α, and interferon γ (IFN-γ) were determined by enzyme-linked immunosorbent assay. Western blots were performed on cell lysates to determine TF expression. A Kruskal-Wallis test was used to compare cytokine concentrations and normalized TF expression among treatments.
Results:
Treatment of DCs with Thr alone increased the expression of TF, IL-6, IL8, IL-10, and IFN-γ compared to basal levels ($P < .05$ for each). Cotreatment of DCs with Thr and any of the tested heparins did not decrease the expression of TF or inflammatory cytokines compared to treatment with Thr alone.
Discussion:
Heparins do not appear to affect basal or Thr-induced expression of TF or inflammatory cytokines in human term DCs. Additional work is needed to determine whether nonanticoagulant heparins can reduce inflammation and membrane weakening due to bleeding in pregnancy.

4.1760 Double conjugated nanogels for selective intracellular drug delivery
Mauri, E., Veglianese, P., Papa, S., Mariani, A., De Paola, M., Rigamonti, R., Chincarini, G.M.F., Vismara, I., Rimondo, S., Sacchetti, A. and Rossi, F.

*RSC Adv., **7**, 30345-30356 (2017)*

One of the most important drawbacks of nanomedicine is related to the unwanted rapid diffusion of drugs loaded within nanocarriers towards the external biological environment, according to the high clearance of body fluids. Therefore, colloids can carry only a small amount of their initial content in the target district, limiting their pharmacological activity and then the therapy. To overcome this limitation, we synthesized double conjugated nanogels: the first click strategy (1,3 Huisgen cycloaddition) guarantees the traceability of nanogels while the second one (disulfide bond) links drug molecules to polymeric chains. In this study, we proposed the above-mentioned double strategy and we validated the synthesized colloids and the selective release kinetics in microglia cells, dramatically involved in several diseases of the central nervous system. Cleavable linked drugs prove to be a promising tool for the selective administration of pharmacological compounds in microglia cells and potentially in many others counteracting some relevant events.

4.1761 **EphA receptors and ephrin-A ligands are upregulated by monocytic differentiation/maturation and promote cell adhesion and protrusion formation in HL60 monocytes**
Mukai, M., Suruga, N., Saeki, N. and ogawa, K.
*BMC Cell Biol., **18**:28 (2017)*

Background
Eph signaling is known to induce contrasting cell behaviors such as promoting and inhibiting cell adhesion/spreading by altering F-actin organization and influencing integrin activities. We have previously demonstrated that EphA2 stimulation by ephrin-A1 promotes cell adhesion through interaction with integrins and integrin ligands in two monocyte/macrophage cell lines. Although mature mononuclear leukocytes express several members of the EphA/ephrin-A subclass, their expression has not been examined in monocytes undergoing during differentiation and maturation.
Results
Using RT-PCR, we have shown that EphA2, ephrin-A1, and ephrin-A2 expression was upregulated in murine bone marrow mononuclear cells during monocyte maturation. Moreover, EphA2 and EphA4 expression was induced, and ephrin-A4 expression was upregulated, in a human promyelocytic leukemia cell line, HL60, along with monocyte differentiation toward the classical $CD14^{++}CD16^{-}$ monocyte subset. Using RT-PCR and flow cytometry, we have also shown that expression levels of αL, αM, αX, and β2 integrin subunits were upregulated in HL60 cells along with monocyte differentiation while those of α4, α5, α6, and β1 subunits were unchanged. Using a cell attachment stripe assay, we have shown that stimulation by EphA as well as ephrin-A, likely promoted adhesion to an integrin ligand-coated surface in HL60 monocytes. Moreover, EphA and ephrin-A stimulation likely promoted the formation of protrusions in HL60 monocytes.
Conclusions
Notably, this study is the first analysis of EphA/ephrin-A expression during monocytic differentiation/maturation and of ephrin-A stimulation affecting monocyte adhesion to an integrin ligand-coated surface. Thus, we propose that monocyte adhesion via integrin activation and the formation of protrusions is likely promoted by stimulation of EphA as well as of ephrin-A.

4.1762 **Non-equilibrium Inertial Separation Array for High-throughput, Large-volume Blood Fractionation**
Mutlu, B.R., Smith, K.C., Edd, J.F., Nadaar, P., Dlamini, M., Kapur, R. and Toner, M.
*Scientific Reports, **7**:9915 (2017)*

Microfluidic blood processing is used in a range of applications from cancer therapeutics to infectious disease diagnostics. As these applications are being translated to clinical use, processing larger volumes of blood in shorter timescales with high-reliability and robustness is becoming a pressing need. In this work, we report a scaled, label-free cell separation mechanism called non-equilibrium inertial separation array (NISA). The NISA mechanism consists of an array of islands that exert a passive inertial lift force on proximate cells, thus enabling gentler manipulation of the cells without the need of physical contact. As the cells follow their size-based, deterministic path to their equilibrium positions, a preset fraction of the flow is siphoned to separate the smaller cells from the main flow. The NISA device was used to fractionate 400 mL of whole blood in less than 3 hours, and produce an ultrapure buffy coat (96.6% white blood cell yield, 0.0059% red blood cell carryover) by processing whole blood at 3 mL/min, or ~300 million cells/second. This device presents a feasible alternative for fractionating blood for transfusion, cellular therapy and blood-based diagnostics, and could significantly improve the sensitivity of rare cell isolation devices by increasing the processed whole blood volume.

4.1763 **Image-based closed-loop feedback for highly mono-dispersed microdroplet production**
Crawford, D.F., Smith, C.A. and Whyte, G.
*Scientific Reports, **7**:10545 (2017)*

Micron-scale droplets isolated by an immiscible liquid can provide miniaturised reaction vessels which can be manipulated in microfluidic networks, and has seen a rapid growth in development. In many experiments, the precise volume of these microdroplets is a critical parameter which can be influenced by many external factors. In this work, we demonstrate the combination of imaging-based feedback and pressure driven pumping to accurately control the size of microdroplets produced in a microfluidic device. The use of fast-response, pressure-driving pumps allows the microfluidic flow to be quickly and accurately changed, while directly measuring the droplet size allows the user to define the more meaningful parameters of droplet size and generation frequency rather than flow rates or pressures. The feedback loop enables the drift correction of pressure based pumps, and leads to a large increase in the mono-dispersity of the droplets produced over long periods. We also show how this can be extended to control multiple liquid flows, allowing the frequency of droplet formation or the average concentration of living cells per droplet to be controlled and kept constant.

4.1764 **MicroRNAs contribute to postnatal development of laminar differences and neuronal subtypes in the rat medial entorhinal cortex**
Olsen, L.C., O'Reilly, K.C., Liabakk, N.B., Witter, M.P. and Sætrum, Å.
*Brain Struct. Funct., **222**, 3107-3126 (2017)*

The medial entorhinal cortex (MEC) is important in spatial navigation and memory formation and its layers have distinct neuronal subtypes, connectivity, spatial properties, and disease susceptibility. As little is known about the molecular basis for the development of these laminar differences, we analyzed microRNA (miRNA) and messenger RNA (mRNA) expression differences between rat MEC layer II and layers III–VI during postnatal development. We identified layer and age-specific regulation of gene expression by miRNAs, which included processes related to neuron specialization and locomotor behavior. Further analyses by retrograde labeling and expression profiling of layer II stellate neurons and in situ hybridization revealed that the miRNA most up-regulated in layer II, miR-143, was enriched in stellate neurons, whereas the miRNA most up-regulated in deep layers, miR-219-5p, was expressed in ependymal cells, oligodendrocytes and glia. Bioinformatics analyses of predicted mRNA targets with negatively correlated expression patterns to miR-143 found that miR-143 likely regulates the Lmo4 gene, which is known to influence hippocampal-based spatial learning.

4.1765 **Pancreatic stellate cell activation is regulated by fatty acids and ER stress**
Ben-Harorosh, Y., Anosov, M., Salem, H., yatchenko, Y. and Birk, R.
*Exp. Cell Res., **359**, 76-85 (2017)*

Introduction
Pancreatic pathologies are characterized by a progressive fibrosis process. Pancreatic stellate cells (PSC) play a crucial role in pancreatic fibrogenesis. Endoplasmic reticulum (ER) stress emerges as an important determinant of fibrotic remodeling. Overload of fatty acids (FA), typical to obesity, may lead to lipotoxic state and cellular stress.
Aim
To study the effect of different lipolytic challenges on pancreatic ER stress and PSC activation.
Methods
Primary PSCs were exposed to different FAs, palmitate (pal) and oleate (ole), at pathophysiological concentrations typical to obese state, and in acute caerulein-induced stress (cer). PSC activation and differentiation were analyzed by measuring fat accumulation (oil-red staining and quantitation), proliferation (cells count) and migration (wound- healing assay). PSC differentiation markers (α-sma, fibronectin, tgf-β and collagen secretion), ER stress unfolded protein response and immune indicators (Xbp1, CHOP, TNF-α, IL-6) were analyzed at the transcript and protein expression levels (quantitative RT-PCR and western blotting).
Results
PSC exposure to pal and ole FAs (500 μM) increased significantly fat accumulation. Proliferation and migration analysis demonstrated that ole FA retained PSC activation, while exposure to pal FA significantly halted proliferation rate and delayed migration. Cer significantly augmented PSC differentiation markers α- sma, fibronectin and collagen, and ER stress and inflammation markers

including Xbp1, CHOP, TNF-α and IL-6. The ole FA treatment significantly elevated PSC differentiation markers α-sma, fibronectin and collagen secretion. PSC ER stress was demonstrated following pal treatment with significant elevation of Xbp1 splicing and CHOP levels.
Conclusion
Exposure to pal FA halted PSC activation and differentiation and elevated ER stress markers, while cer and ole exposure significantly induced activation, differentiation and fibrosis. Thus, dietary FA composition should be considered and optimized to regulate PSC activation and differentiation in pancreatic pathologies.

4.1766 Protocol for High-Content Screening for the Impact of Overexpressed MicroRNAs on Primary Motor Neurons
Yardeni, T. and Hornstein, E.
*Neuromethods, **128**, 11-19 (2017)*

In this chapter we provide a protocol for the design and usage of automated, high-content microscopy screening that enables the investigation of microRNA (miRNA) impact on primary motor neuron. High-content screening (HCS) platforms facilitate superior precision in research and are scalable to study in parallel multiple genetic, molecular, or cellular conditions. miRNAs are critical for neuronal function and for brain integrity and are considered attractive candidate targets for therapy in many neuropathologies. Therefore, HCS platforms provide a novel paradigm for exploring the impact of miRNA expression, applicable for functional pathways discovery in an academic setting, or towards development of therapeutics in the pharma industry.

4.1767 Analysis of Pathological Activities of CCN Proteins in Fibrotic Diseases: Liver Fibrosis
Chen, L. and Brigstock, D.R.
*Methods in Mol. Biol., **1489**, 445-463 (2017)*

Hepatic fibrosis is a complex pathology arising from chronic injury. Pathological features are dominated by the excessive production of extracellular matrix proteins, particularly collagens which are deposited as insoluble scar material that can compromise tissue function. Fibrosis in the liver can often be assessed by staining for collagen in tissue sections and this is an approach that is widely used for grading of fibrosis in human biopsies. However, the recognition of the molecular components that drive fibrosis, including CCN proteins, and the involvement of hepatic stellate cells (HSC) as the principal collagen-producing cells in fibrosing liver, has resulted in a wide variety of molecular and cellular approaches to study the pathogenesis of fibrosis both in vivo and in vitro.

4.1768 Using Cloning to Amplify Neuronal Genomes for Whole-Genome Sequencing and Comprehensive Mutation Detection and Validation
Hazen, J.L., Duran, M.A., Smith, R.P., Rodriguez, A.R., Martin, G.S., Kupriyanov, S., Hall, I.M. and Baldwin, K.K.
*Neuromethods, **131**, 163-185 (2017)*

Recent studies of somatic mutation in neurons and other cell types suggest that somatic cells can acquire hundreds to thousands of new mutations over their lifetimes. Each individual mutation can have extremely low prevalence, with many mutations restricted to a single cell. Because of their rarity, somatic mutations can be challenging to detect and reliably distinguish from false-positive calls arising from amplification, sequencing, or bioinformatic methods. In these scenarios, a variety of methods are required to compensate for the limited applicability and technical artifacts inherent in any single approach. In the method we describe, somatic cell nuclear transfer (SCNT, also known as cloning) is used to reprogram single neurons to blastocysts from which we derive embryonic stem cells. Division of these cells faithfully amplifies the neuronal genome for next-generation sequencing and genome-wide mutation detection. This approach allows the detection of false positives due to amplification artifacts and is applicable to all classes of mutations. While it is both sensitive and reliable, our method is lower throughput than single-cell sequencing-based approaches and may also fail to amplify the most severely compromised neuronal genomes. In this chapter, we outline current methods for generating neuron-derived SCNT embryonic cell lines, discuss best practices for genome-wide mutation detection, and address the advantages and limitations of this approach.

4.1769 Cellular or Exosomal microRNAs Associated with CCN Gene Expression in Liver Fibrosis
Chen, L. and Brigstock, D.R.

Methods in Mol. Biol., ***1489***, *465-480 (2017)*

Liver fibrosis occurs during chronic injury and represents, in large part, an exaggerated matrigenic output by hepatic stellate cells (HSCs) which become activated as a result of injury-induced signaling pathways in parenchymal and infl ammatory cells (hepatocytes, macrophages, etc.). The molecular components in these pathways (e.g., CCN proteins) are modulated by transcription factors as well as by factors such as microRNAs (miRs) that act posttranscriptionally. MiRs are small (~23 nt) noncoding RNAs that regulate gene expression by specifi cally interacting with the 3′ untranslated region (UTR) of target gene mRNA to repress translation or enhance mRNA cleavage. As well as acting in their cells of production, miRs (and other cellular constituents such as mRNAs and proteins) can be liberated from their cells of origin in nanovesicular membrane exosomes, which traverse the intercellular spaces, and can be delivered to neighboring cells into which they release their molecular payload, causing alterations in gene expression in the target cells. Here we summarize some of the experimental approaches for studying miR action and exosomal traff cking between hepatic cells. Insights into the mechanisms involved will yield new information about how hepatic fi brosis is regulated and, further, may identify new points of therapeutic intervention.

4.1770 Printed droplet microfluidics for on demand dispensing of picoliter droplets and cells
Cole, R.H., Tang, S-Y., Siltanen, C.A., Shahi, P., Zhang, J.Q., Poust, S., Gartner, Z.J. and Abate, A.R.
PNAS, ***114(33)***, *8728-8733 (2017)*

Although the elementary unit of biology is the cell, high-throughput methods for the microscale manipulation of cells and reagents are limited. The existing options either are slow, lack single-cell specificity, or use fluid volumes out of scale with those of cells. Here we present printed droplet microfluidics, a technology to dispense picoliter droplets and cells with deterministic control. The core technology is a fluorescence-activated droplet sorter coupled to a specialized substrate that together act as a picoliter droplet and single-cell printer, enabling high-throughput generation of intricate arrays of droplets, cells, and microparticles. Printed droplet microfluidics provides a programmable and robust technology to construct arrays of defined cell and reagent combinations and to integrate multiple measurement modalities together in a single assay.

4.1771 Paired related homeobox protein 1 regulates PDGF-induced chemotaxis of hepatic stellate cells in liver fibrosis
Gong, J., Han, J., He, J., Liu, J., Han, P., Wang, Y., Li, M., Li, D., Ding, X., Du, Z., Liao, J. and Tian, D.
Lab. Invest., ***97(9)***, *1020-1032 (2017)*

Activation of the platelet-derived growth factor (PDGF)/PDGF beta receptor (PDGFβR) axis has a critical role in liver fibrosis. However, the mechanisms that regulate the PDGF signaling are yet to be elucidated. The present study demonstrates that paired related homeobox protein 1 (Prrx1) is involved in PDGF-dependent hepatic stellate cell (HSCs) migration via modulation of the expression of metalloproteinases MMP2 and MMP9. PDGF elevated the level of Prrx1 through the activation of ERK/Sp1 and PI3K/Akt/Ets1 pathways. In vivo, an adenoviral-mediated Prrx1 shRNA administration attenuated liver fibrosis in thioacetamide-induced fibrotic models. These studies reveal a role of Prrx1 as a modulator of PDGF-dependent signaling in HSCs, and inhibiting its expression may offer a therapeutic approach for hepatic fibrosis.

4.1772 Warmer night-time temperature promotes microbial heterotrophic activity and modifies stream sediment community
Freixa, A., Acuna, V., Casellas, M., Percheva, S. and Romani, A.M.
Global Change Biol., ***23(9)***, *3825-3837 (2017)*

Diel temperature patterns are changing because of global warming, with higher temperatures being predicted to be more pronounced at night. Biological reactions are temperature dependent, with some occurring only during the daylight hours (e.g., light photosynthesis) and other during the entire day (e.g., respiration). Consequently, we expect the modification of daily temperature cycles to alter microbial biological reactions in stream sediments. Here, we aimed to study the effect of warming and changes of the diel temperature patterns on stream sediment biofilm functions tied to organic carbon decomposition, as well as on biofilm meiofaunal community structure. We performed an eight-week experiment with 12 artificial streams subjected to three different diel temperature patterns: warming, warmer nights and control. Significant effects of warming on biofilm function and structure were mainly detected in the long

term. Our results showed that warming altered biofilm function, especially in the warmer nights' treatment, which enhanced β-glucosidase enzyme activity. Interestingly, clear opposite diel patterns were observed for dissolved organic carbon and β-glucosidase activity, suggesting that, at night, sediment bacteria quickly consume the input of photosynthetic dissolved organic carbon labile compounds created during light-time. The biofilm structure was also altered by warming, as both warming and warmer night treatments enhanced copepod abundance and diminished abundances of turbellaria and nematodes, which, in turn, controlled bacterial, algal and ciliate communities. Overall, we conclude that warming has strong effect on sediment biofilm structure and enhanced microbial organic matter degradation which might, consequently, affect higher trophic levels and river carbon cycling.

4.1773 Effects of supplementation of iodixanol to semen extender on quality and fertilization ability of frozen–thawed Thai native bull sperm
Chuawongboon, P., Sirisathien, S., Pongpeng, J., Sakhong, D., Nagai, T. and Vongpralub, T.
Animal Sci. J., ***88(9)****, 1311-1320 (2017)*

This study investigates the effects of iodixanol supplementation in varied concentrations to Tris egg yolk (TEY) extender on the quality and fertilization ability of frozen–thawed sperm of Thai native bulls. Each ejaculate was divided into four different groups, as follows: sperm were treated with TEY extender (control group) and TEY extender supplemented with three different concentrations of iodixanol (1.25%, 2.50% and 5.00%). Semen straws were frozen in liquid nitrogen vapor. After thawing, sperm motility characteristics, viability, plasma membrane integrity and acrosome integrity were determined. Also, frozen–thawed spermatozoa from all groups were used for *in vitro* fertilization and artificial insemination (AI) in natural estrus Thai native cows. The results showed that the post-thaw quality of the 2.50% iodixanol group was superior to the other iodixanol groups ($P < 0.05$). However, iodixanol had no beneficial effect on post-thaw sperm *in vitro* fertilization ability and pregnancy rate after AI ($P > 0.05$). It can be concluded that the supplementation of 2.50% iodixanol extender significantly improves the progressive motility, viability, plasma membrane integrity and acrosome integrity of cryopreserved semen from Thai native bulls, but it has no beneficial effect on *in vitro* fertilization ability and pregnancy rate after AI.

4.1774 Scaffold-Free Liver-On-A-Chip with Multiscale Organotypic Cultures
Weng, Y-S., Chang, S-F., Shih, M-C., Tseng, S-H. and Lai, C-H.
Advanced Materials, ***29****, 1701545 (2017)*

The considerable advances that have been made in the development of organotypic cultures have failed to overcome the challenges of expressing tissue-specific functions and complexities, especially for organs that require multitasking and complex biological processes, such as the liver. Primary liver cells are ideal biological building blocks for functional organotypic reconstruction, but are limited by their rapid loss of physiological integrity in vitro. Here the concept of lattice growth used in material science is applied to develop a tissue incubator, which provides physiological cues and controls the 3D assembly of primary cells. The cues include a biological growing template, spatial coculture, biomimetic radial flow, and circulation in a scaffold-free condition. The feasibility of recapitulating a multiscale physiological structural hierarchy, complex drug clearance, and zonal physiology from the cell to tissue level in long-term cultured liver-on-a-chip is demonstrated. These methods are promising for future applications in pharmacodynamics and personal medicine.

4.1775 Complement Protein C3 Suppresses Axon Growth and Promotes Neuron Loss
Peterson, S.L., Nguyen, H.X., Mendez, O.A. and Anderson, A.J.
Scientific Reports, ***7****:12904 (2017)*

The inflammatory response to spinal cord injury (SCI) involves localization and activation of innate and adaptive immune cells and proteins, including the complement cascade. Complement C3 is important for the classical, alternative, and lectin pathways of complement activation, and its cleavage products C3a and C3b mediate several functions in the context of inflammation, but little is known about the potential functions of C3 on regeneration and survival of injured neurons after SCI. We report that 6 weeks after dorsal hemisection with peripheral conditioning lesion, $C3^{-/-}$ mice demonstrated a 2-fold increase in sensory axon regeneration in the spinal cord in comparison to wildtype $C3^{+/+}$ mice. *In vitro*, addition of C3 tripled both myelin-mediated neurite outgrowth inhibition and neuron loss versus myelin alone, and ELISA experiments revealed that myelin serine proteases cleave C3 to generate active fragments. Addition of purified C3 cleavage products to cultured neurons suggested that C3b is responsible for the growth

inhibitory and neurotoxic or anti-adhesion activities of C3. These data indicate that C3 reduces neurite outgrowth and neuronal viability *in vitro* and restricts axon regeneration *in vivo*, and demonstrate a novel, non-traditional role for this inflammatory protein in the central nervous system.

4.1776 Antidepressants promote formation of heterocomplexes of dopamine D2 and somatostatin subtype 5 receptors in the mouse striatum

Szafran-Pilch, K., Faron-Gorecka, A., Kolasa, M., Zurawek, D., Szlachta, M., Solich, J., Kusmider, M. and Dziedzicka-Wasylewska, M.
*Brain Res. Bull.,**135**, 92-97 (2017)*

The interaction between the dopaminergic and somatostatinergic systems is considered to play a potential role in mood regulation. Chronic administration of antidepressants influences release of both neurotransmitters. The molecular basis of the functional cooperation may stem from the physical interaction of somatostatin receptor subtypes and dopamine D2 receptors since they colocalize in striatal interneurons and were shown to undergo ligand-dependent heterodimerization in heterologous expression systems. In present study we adapted in situ proximity ligation assay to investigate the occurrence of D2-Sst5 receptor heterocomplexes, and their possible alterations in the striatum of mice treated acutely and repeatedly (21 days) with antidepressant drugs of different pharmacological profiles (escitalopram and desipramine). Additionally we analysed number of heterocomplexes in primary striatal neuronal cultures incubated with both antidepressant drugs for 1 h and 6 days. The studies revealed that antidepressants increase formation of D2-Sst5 receptors heterodimers. These findings provide interesting evidence that dopamine D2 and somatostatin Sst5 heterodimers may be considered as potential mediators of antidepressant effects, since the heterodimerization of these receptors occurs in native brain tissue as well as in primary striatal neuronal cultures where receptors are expressed at physiological levels.

4.1777 Genome Editing of Plants

Songstad, D.D., Petolino, J.F., Voytas, D.F. and Reichert, N.A.
*Critical Reviews in Plant Sci., **36(1)**, 1-23 (2017)*

Genome editing in organisms via random mutagenesis is a naturally occurring phenomenon. As a technology, genome editing has evolved from the use of chemical and physical mutagenic agents capable of altering DNA sequences to biological tools such as designed sequence-specific nucleases (SSN) to produce knock-out (KO) or knock-in (KI) edits and Oligonucleotide Directed Mutagenesis (ODM) where specific nucleotide changes are made in a directed manner resulting in custom single nucleotide polymorphisms (SNPs). Cibus' SU Canola™, which the US Department of Agriculture (USDA) views as non-genetically modified (non-GM), is Cibus' first commercial product arising from plant genome editing and had its test launch in 2014. Regulatory aspects of the various genome editing tools will be discussed.

4.1778 Molecular and expression analysis of the Allograft inflammatory factor 1 (AIF-1) in the coelomocytes of the common sea urchin Paracentrotus lividus

Barca, A., Vacca, F., Viziolo, J., Grago, F., Vertrugna, c., Verri, T. and Pagliara, P.
*Fish & Shellfish Immunol., **71**, 136-143 (2017)*

Allograft inflammatory factor 1 (AIF-1) is a highly conserved gene involved in inflammation, cloned and characterized in several evolutionary distant animal species. Here, we report the molecular identification, characterization and expression of AIF-1 from the common sea urchin *Paracentrotus lividus*. In this species, AIF-1 encodes a predicted 151 amino acid protein with high similarity to vertebrate AIF-1 proteins. Immunocytochemical analyses on coelomocytes reveal localization of the AIF-1 protein in amoebocytes (perinuclear cytoplasmic zone) and red sphaerulocytes (inside granules), but not in vibratile cells and colorless sphaerula cells. The significant increase of AIF-1 expression (mRNA and protein) found in the coelomocytes of the sea urchin after Gram + bacterial challenge suggests the involvement of AIF-1 in the inflammatory response. Our analysis on *P. lividus* AIF-1 contributes to elucidate AIF-1 function along the evolutionary scale and consolidate the key evolutionary position of echinoderms throughout metazoans with respect to the common immune paths.

4.1779 Proteomic Analysis Reveals Dab2 Mediated Receptor Endocytosis Promotes Liver Sinusoidal Endothelial Cell Dedifferentiation

Lao, Y., Li, Y., Hou, Y., Chen, H., Qiu, B., Lin, W., Sun, A., Wei, H., Jiang, Y. and He, F.
*Scientific Reports, **7**, 13456 (2017)*

Sinusoidal dedifferentiation is a complicated process induced by several factors, and exists in early stage

of diverse liver diseases. The mechanism of sinusoidal dedifferentiation is poorly unknown. In this study, we established a NaAsO2-induced sinusoidal dedifferentiation mice model. Liver sinusoidal endothelial cells were isolated and isobaric tag for relative and absolute quantitation (iTRAQ) based proteomic approach was adopted to globally examine the effects of arsenic on liver sinusoidal endothelial cells (LSECs) during the progression of sinusoidal dedifferentiation. In all, 4205 proteins were identified and quantified by iTRAQ combined with LC-MS/MS analysis, of which 310 proteins were significantly changed in NaAsO2 group, compared with the normal control. Validation by western blot showed increased level of clathrin-associated sorting protein Disabled 2 (Dab2) in NaAsO2 group, indicating that it may regulate receptor endocytosis, which served as a mechanism to augment intracellular VEGF signaling. Moreover, we found that knockdown of Dab2 reduced the uptake of VEGF in LSECs, furthermore blocking VEGF-mediated LSEC dedifferentiation and angiogenesis.

4.1780 The impact of allogenic blood transfusion on the outcomes of total pancreatectomy with islet autotransplantation

Yoshimatsu, G., Shahbazov, R., Saracino, G., Lawrence, M.C., Kim, P.T., Onaca, N., Beecherl, E.E., Naziruddin, B. and Levy, M.F.
Am. J. Surg., ***214***, *849-855 (2017)*

Background
Allogenic blood transfusion (ABT) may be needed for severe bleeding during total pancreatectomy with autotransplantation (TPIAT), but may induce inflammation. This study investigated the impact of ABT.
Methods
With a population of 83 patients who underwent TPIAT from 2006 to 2014, this study compared cytokine levels, patient characteristics, islet characteristics, metabolic outcomes, insulin requirements, and hemoglobin A1c for those who received a blood transfusion (BT) versus no blood transfusion (NBT).
Results
Initially, proinflammatory cytokines were moderately higher in the BT group than the NBT group. Despite longer procedures and more severe bleeding, the BT group had similar values to the NBT group for insulin requirements, serum C-peptide, hemoglobin A1c, and insulin independence rate. The probability of insulin independence was slightly higher in patients receiving ≥3 units of blood.
Conclusion
ABT induced elevation of proinflammatory cytokines during the perioperative period in TPIAT, but these changes did not significantly change posttransplant islet function.

4.1781 Bio-enriched Pleurotus mushrooms for deficiency control and improved antioxidative protection of human platelets?

Poniedzialek, B., Mleczek, M., Niedzielski, P., Siwulski, M., Gasecka, m., Kozak, L. and Komosa, P.
Eur. Food Res. Technol., ***243***, *2187-2198 (2017)*

The study investigated effect of substrate supplementation with Se alone or in combination with Cu or/and Zn Se on (1) the growth of *Pleurotus ostreatus* and *Pleurotus eryngii*; (2) elements accumulation in mushrooms; (3) the antioxidant activities of bio-enriched mushroom extracts in human platelets. The accumulation of elements generally increased over concentration gradient reaching its maximum at 1.2 mM for *P. ostreatus* and *P. eryngii*: (1) over 100 and 80 mg kg^{-1} of Se, respectively (Se supplementation); (2) over 15 and 30 mg kg^{-1} of Cu, respectively (Se+Cu); (3) over 30 and 85 mg kg^{-1} of Zn, respectively. Se was predominantly accumulated as an organic fraction. Contrary to *P. eryngii*, the *P. ostreatus* biomass decreased with substrate elements concentration but was satisfactory up to 0.9 mM of Se, Se+Cu and Se+Zn. The Se+Cu+Zn model yielded low biomass and elements accumulation. Extracts from mushrooms bio-enriched with Se and Se+Zn (0.6–1.2 mM) revealed significant antioxidant activities in human platelets by ameliorating reactive oxygen species (ROS) and preventing lipid peroxidation. The study demonstrated the potential application of *Pleurotus* mushrooms as functional food products bio-enriched with essential elements. ROS inhibition by extracts of these mushrooms may be useful in control of platelets activation cascade.

4.1782 Bacillus anthracis lethal toxin negatively modulates ILC3 function through perturbation of IL-23-mediated MAPK signaling

Seshadri, S., Allan, D.S., Carlyle, J.R. and Zenewicz, L.A.
PloS Pathogens, ***13(10)***, *e1006690 (2017)*

Bacillus anthracis, the causative agent of anthrax, secretes lethal toxin that down-regulates immune

functions. Translocation of *B. anthracis* across mucosal epithelia is key for its dissemination and pathogenesis. Group 3 innate lymphocytes (ILC3s) are important in mucosal barrier maintenance due to their expression of the cytokine IL-22, a critical regulator of tissue responses and repair during homeostasis and inflammation. We found that *B. anthracis* lethal toxin perturbed ILC3 function *in vitro* and *in vivo*, revealing an unknown IL-23-mediated MAPK signaling pathway. Lethal toxin had no effects on the canonical STAT3-mediated IL-23 signaling pathway. Rather lethal toxin triggered the loss of several MAP2K kinases, which correlated with reduced activation of downstream ERK1/2 and p38, respectively. Inhibition studies showed the importance of MAPK signaling in IL-23-mediated production of IL-22. Our finding that MAPK signaling is required for optimal IL-22 production in ILC3s may lead to new approaches for targeting IL-22 biology.

4.1783 Isolation and Culture of Primary Murine Hepatic Stellate Cells
Weiskirchen, S., Tag, C.G., Sauer-Lehnen, S., Tacke, F. and Weiskirchen, R.
Methods in Mol. Biol., ***1627****, 165-191 (2017)*

Hepatic stellate cells (HSCs) are found in the perisinusoidal space of the liver (i.e., the space of Dissé). They represent 5–8% of the total number of liver cells. In normal liver, these cells have a quiescent phenotype and are characterized by numerous fat vacuoles that store vitamin A in a form of retinyl ester. In injured liver, these cells transdifferentiate into a myofibroblast phenotype, become highly proliferative and are responsible for excess collagen synthesis and deposition during fibrosis. Due to their exceptional pathophysiological relevance, several isolation and purification protocols of primary HSCs have been established that provide the basis for studying HSC biology in vitro. We here describe a method for high-purity isolation of HSCs from mice. This protocol includes the enzymatic digestion of the liver tissue by pronase and collagenase, cellular enrichment by centrifugation of the crude cell suspension through a Nycodenz density gradient, and a final (optional) flow cytometric enrichment that allows generating ultrapure HSC fractions.

4.1784 Isolation, Purification, and Culture of Primary Murine Sensory Neurons
Katzenell, S., Cabrera, J.R., North, B.J. and Leib, D.A.
Methods in Mol. Biol., ***1656****, 229-251 (2017)*

Cultured primary neurons have been of extraordinary value for the study of neuronal anatomy, cell biology, and physiology. While use of neuronal cell lines has ease and utility, there are often caveats that arise due to their mitotic nature. This methods article presents detailed methodology for the preparation, purification, and culture of adult murine sensory neurons for the study of herpes simplex virus lytic and latent infections. While virology is the application for our laboratory, these cultures also have broad utility for neurobiologists and cell biologists. While these primary cultures have been highly informative, the methodology is challenging to many investigators. Through publication of this highly detailed protocol, it is our hope that the use of this culture system can spread in the field to allow more rapid progress in furthering our understanding of neurotropic virus infection.

4.1785 Lectins identify distinct populations of coelomocytes in Strongylocentrotus purpuratus
Liao, W-Y., Fugmann, S.D.
PloS One, ***12(11)****, e0187987 (2017)*

Coelomocytes represent the immune cells of echinoderms, but detailed knowledge about their roles during immune responses is very limited. One major challenge for studying coelomocyte biology is the lack of reagents to identify and purify distinct populations defined by objective molecular markers rather than by morphology-based classifications that are subjective at times. Glycosylation patterns are known to differ significantly between cell types in vertebrates, and furthermore they can vary depending on the developmental stage and activation states within a given lineage. Thus fluorescently labeled lectins that recognize distinct glycan structures on cell surface proteins are routinely used to identify discrete cell populations in the vertebrate immune system. Here we now employed a panel of fifteen fluorescently-labeled lectins to determine differences in the glycosylation features on the surface of *Strongylocentrotus purpuratus* coelomocytes by fluorescence microscopy and flow cytometry. Eight of the lectins (succinylated wheat germ agglutinin, *Len culinaris* lectin, *Pisum sativum* agglutinin, *Saphora japonica* agglutinin, *Solanum tuberosum* lectin, *Lycopersicon esculentum* lectin, *Datura stramonium* lectin, *Vicia villosa* lectin) showed distinct binding patterns to fixed and live cells of three major coelomocyte classes: phagocytic cells, red spherule cells, and vibratile cells. Importantly, almost all lectins bound only to a subgroup of cells within each cell type. Lastly, we established fluorescently-labeled lectin-based

fluorescence activated cell sorting as a strategy to purify distinct *S. purpuratus* coelomocyte (sub-)populations based on molecular markers. We anticipate that this will become a routine approach in future studies focused on dissecting the roles of different coelomocytes in echinoderm immunity.

4.1786 Dissecting hematopoietic and renal cell heterogeneity in adult zebrafish at single-cell resolution using RNA sequencing
Tang, Q. et al
J. Exp. Med., ***214(10)***, *2875-2887 (2017)*

Recent advances in single-cell, transcriptomic profiling have provided unprecedented access to investigate cell heterogeneity during tissue and organ development. In this study, we used massively parallel, single-cell RNA sequencing to define cell heterogeneity within the zebrafish kidney marrow, constructing a comprehensive molecular atlas of definitive hematopoiesis and functionally distinct renal cells found in adult zebrafish. Because our method analyzed blood and kidney cells in an unbiased manner, our approach was useful in characterizing immune-cell deficiencies within DNA–protein kinase catalytic subunit (*prkdc*), interleukin-2 receptor γ a (*il2rga*), and double-homozygous–mutant fish, identifying blood cell losses in T, B, and natural killer cells within specific genetic mutants. Our analysis also uncovered novel cell types, including two classes of natural killer immune cells, classically defined and erythroid-primed hematopoietic stem and progenitor cells, mucin-secreting kidney cells, and kidney stem/progenitor cells. In total, our work provides the first, comprehensive, single-cell, transcriptomic analysis of kidney and marrow cells in the adult zebrafish.

4.1787 A novel Cre-inducible knock-in ARL13B-tRFP fusion cilium reporter
Schmitz, F., Burtscher, I., Stauber, M., Gossler, A. and Lickert, H.
Genesis, ***55***, *e23073 (2017)*

Cilia play a major role in the regulation of numerous signaling pathways and are essential for embryonic development. Mutations in genes affecting ciliary function can cause a variety of diseases in humans summarized as ciliopathies. To facilitate the detection and visualization of cilia in a temporal and spatial manner in mouse tissues, we generated a Cre-inducible cilium-specific reporter mouse line expressing an ARL13B-tRFP fusion protein driven by a CMV enhancer/chicken β actin promotor (pCAG) from the *Hprt* locus. We detected bright and specific ciliary signals by immunostainings of various mono- and multiciliated tissues and by time-lapse live-cell analysis of cultured embryos and organ explant cultures. Additionally, we monitored cilium assembly and disassembly in embryonic fibroblast cells using live-cell imaging. Thus, the ARL13B-tRFP reporter mouse strain is a valuable tool for the investigation of ciliary structure and function in a tissue-specific manner to understand processes, such as ciliary protein trafficking or cilium-dependent signaling *in vitro* and *in vivo*.

4.1788 Metabolic, Reproductive, and Neurologic Abnormalities in Agpat1-Null Mice
Agarwal, A.K., Tunison, K., Dalal, J.S., Nagamma, S.S., Hamra, F.K., Sankella, S., Shao, X., Auchus, R.J., and Garg, A.
Endocrinology, ***158(11)***, *3954-3973 (2017)*

Defects in the biosynthesis of phospholipids and neutral lipids are associated with cell membrane dysfunction, disrupted energy metabolism, and diseases including lipodystrophy. In these pathways, the 1-acylglycerol-3-phosphate *O*-acyltransferase (AGPAT) enzymes transfer a fatty acid to the *sn-2* carbon of *sn*-1-acylglycerol-3-phosphate (lysophosphatidic acid) to form *sn*-1, 2-acylglycerol-3-phosphate [phosphatidic acid (PA)]. PA is a precursor for key phospholipids and diacylglycerol. AGPAT1 and AGPAT2 are highly homologous isoenzymes that are both expressed in adipocytes. Genetic defects in *AGPAT2* cause congenital generalized lipodystrophy, indicating that AGPAT1 cannot compensate for loss of AGPAT2 in adipocytes. To further explore the physiology of AGPAT1, we characterized a loss-of-function mouse model (*Agpat1*$^{-/-}$). The majority of *Agpat1*$^{-/-}$ mice died before weaning and had low body weight and low plasma glucose levels, independent of plasma insulin and glucagon levels, with reduced percentage of body fat but not generalized lipodystrophy. These mice also had decreased hepatic messenger RNA expression of *Igf-1* and *Foxo1*, suggesting a decrease in gluconeogenesis. In male mice, sperm development was impaired, with a late meiotic arrest near the onset of round spermatid production, and gonadotropins were elevated. Female mice showed oligoanovulation yet retained responsiveness to gonadotropins. *Agpat1*$^{-/-}$ mice also demonstrated abnormal hippocampal neuron development and developed audiogenic seizures. In summary, *Agpat1*$^{-/-}$ mice developed widespread disturbances of metabolism, sperm development, and neurologic function resulting from disrupted phospholipid

homeostasis. AGPAT1 appears to serve important functions in the physiology of multiple organ systems. The *Agpat1*-deficient mouse provides an important model in which to study the contribution of phospholipid and triacylglycerol synthesis to physiology and diseases.

4.1789 Applications of particulate oxygen-generating substances (POGS) in the bioartificial pancreas
McQuilling, J.P., Sittadjody, S., Pendergraft, S., farney, A.C. and Opara, E.C.
*Biomater. Sci., **5**, 2437-2447 (2017)*

Type-1 Diabetes (T1D) is a devastating autoimmune disorder which results in the destruction of beta cells within the pancreas. A promising treatment strategy for T1D is the replacement of the lost beta cell mass through implantation of immune-isolated microencapsulated islets referred to as the bioartificial pancreas. The goal of this approach is to restore blood glucose regulation and prevent the long-term comorbidities of T1D without the need for immunosuppressants. A major requirement in the quest to achieve this goal is to address the oxygen needs of islet cells. Islets are highly metabolically active and require a significant amount of oxygen for normal function. During the process of isolation, microencapsulation, and processing prior to transplantation, the islets' oxygen supply is disrupted, and a large amount of islet cells are therefore lost due to extended hypoxia, thus creating a major barrier to clinical success with this treatment. In this work, we have investigated the oxygen generating compounds, sodium percarbonate (SPO) and calcium peroxide (CPO) as potential supplemental oxygen sources for islets during isolation and encapsulation before and immediately after transplantation. First, SPO particles were used as an oxygen source for islets during isolation. Secondly, silicone films containing SPO were used to provide supplemental oxygen to islets for up to 4 days in culture. Finally, CPO was used as an oxygen source for encapsulated cells by co-encapsulating CPO particles with islets in permselective alginate microspheres. These studies provide an important proof of concept for the utilization of these oxygen generating materials to prevent beta cell death caused by hypoxia.

4.1790 Erythropoietin's Beta Common Receptor Mediates Neuroprotection in Spinal Cord Neurons
Foley, L.S., Fullerton, D.A., mares, J., Sungelo, M., Weyant, M.J., Cleveland, J.C. and Reece, T.B.
*Ann. Thorac. Surg., **104**, 1909-1914 (2017)*

Background
Paraplegia from spinal cord ischemia-reperfusion (SCIR) remains an elusive and devastating complication of complex aortic operations. Erythropoietin (EPO) attenuates this injury in models of SCIR. Upregulation of the EPO beta common receptor (βcR) is associated with reduced damage in models of neural injury. The purpose of this study was to examine whether EPO-mediated neuroprotection was dependent on βcR expression. We hypothesized that spinal cord neurons subjected to oxygen-glucose deprivation would mimic SCIR injury in aortic surgery and EPO treatment attenuates this injury in a βcR-dependent fashion.
Methods
Lentiviral vectors with βcR knockdown sequences were tested on neuron cell cultures. The virus with greatest βcR knockdown was selected. Spinal cord neurons from perinatal wild-type mice were harvested and cultured to maturity. They were treated with knockdown or nonsense virus and transduced cells were selected. Three groups (βcR knockdown virus, nonsense control virus, no virus control; n = 8 each) were subjected to 1 hour of oxygen-glucose deprivation. Viability was assessed. βcR expression was quantified by immunoblot.
Results
EPO preserved neuronal viability after oxygen-glucose deprivation (0.82 ± 0.04 versus 0.61 ± 0.01; $p < 0.01$). Additionally, EPO-mediated neuron preservation was similar in the nonsense virus and control mice (0.82 ± 0.04 versus 0.80 ± 0.05; $p = 0.77$). EPO neuron preservation was lost in βcR knockdown mice compared with nonsense control mice (0.46 ± 0.03 versus 0.80 ± 0.05; $p < 0.01$).
Conclusions
EPO attenuates neuronal loss after oxygen-glucose deprivation in a βcR-dependent fashion. This receptor holds immense clinical promise as a target for pharmacotherapies treating spinal cord ischemic injury.

4.1791 Hyaluronic Acid/Collagen Hydrogel as an Alternative to Alginate for Long-Term Immunoprotected Islet Transplantation No Access
Harrington, S., Williams, J., Rawal, s., Ramachandran, K and Stehno-Bittel, L.
*Tissue Engineering: Part A, **23(19-20)**, 1088-1099 (2017)*

Alginate has long been the material of choice for immunoprotection of islets due to its low cost and ability to easily form microspheres. Unfortunately, this seaweed-derived material is notoriously prone to fibrotic

overgrowth *in vivo,* resulting in premature graft failure. The purpose of this study was to test an alternative, hyaluronic acid (HA-COL), for *in vitro* function, viability, and allogeneic islet transplant outcomes in diabetic rats. *In vitro* studies indicated that the HA-COL gel had diffusion characteristics that would allow small molecules such as glucose and insulin to enter and exit the gel, whereas larger molecules (70 and 500 kDa dextrans) were impeded from diffusing past the gel edge in 24 h. Islets encapsulated in HA-COL hydrogel showed significantly improved *in vitro* viability over unencapsulated islets and retained their morphology and glucose sensitivity for 28 days. When unencapsulated allogeneic islet transplants were administered to the omentum of outbred rats, they initially were normoglycemic, but by 11 days returned to hyperglycemia. Immunohistological examination of the grafts and surrounding tissue indicated strong graft rejection. By comparison, when using the same outbred strain of rats, allogeneic transplantation of islets within the HA-COL gel reversed long-term diabetes and prevented graft rejection in all animals. Animals were sacrificed at 40, 52, 64, and 80 weeks for evaluation, and all were non-diabetic at sacrifice. Explanted grafts revealed viable islets in the transplant site as well as intact hydrogel, with little or no evidence of fibrotic overgrowth or cellular rejection. The results of these studies demonstrate great potential for HA-COL hydrogel as an alternative to sodium alginate for long-term immunoprotected islet transplantation.

4.1792 Differential detection photothermal spectroscopy: towards ultra-fast and sensitive label-free detection in picoliter & femtoliter droplets
Maceiczyk, R.M., Hess, D., Chiu, F.W., Stavrakis, s. and deMello, A.J.
*Lab on a Chip, **17**, 3654-3663 (2017)*

Despite the growing importance of droplet-based microfluidics in high-throughput experimentation, few current methods allow the sensitive measurement of absorbance within rapidly moving droplets. To address this significant limitation, we herein present the application of differential detection photothermal interferometry (DDPI) for single-point absorbance quantification in pL- and fL-volume droplets. To assess the efficacy of our approach, we initially measure absorbance in 100 pL droplets at frequencies in excess of 1 kHz and determine a detection limit of 1.4 μmol L^{-1} for Erythrosin B ($A = 3.8 \times 10^{-4}$). Subsequently, we apply the method to the analysis of fL-volume droplets and droplets generated at frequencies in excess of 10 kHz. Finally, we demonstrate the utility of DDPI as a detection scheme for colorimetric assays. Specifically, we extract the Michaelis–Menten constant for the reaction of β-galactosidase and chlorophenol-red-β-D-galactopyranoside and monitor the metabolomic activity of a population of HL-60 cells at the single cell level. Results establish single-point absorbance detection as a powerful, sensitive and rapid alternative to fluorescence for a wide range of assays within segmented flows.

4.1793 Potent analogues of etiprednol dicloacetate, a second generation of soft corticosteroids†
Bodor, N., Zubovics, Z., Kurucz, I., Solyom, S. and Bodor, E.
*J. Pharmacy Pharmacol., **69(12)**, 1745-1753 82017)*

Objectives
Loteprednol etabonate (LE) is the first, highly successful soft corticosteroid (SC) designed using the 'inactive metabolite' approach, starting with Δ^1-cortienic acid (d-CA). The next generation of SCs based on d-CA was etiprednol dicloacetate (ED). The 17α-dichloroacetyl function serves both as a unique pharmacophore and as the source of the molecule's softness. Highly potent SCs were designed based on a combination of ED and LE, introducing 6, 9 and 16 substituents in the molecule.
Methods
The new 6α, 9α, 16α and β 17α-dichloroacetyl 17β-esters were synthesized from the correspondingly substituted Δ^1-cortienic acids. The anti-inflammatory activity was assessed using LPS-induced TNF α-release under various conditions to determine intrinsic activity vs. systemic biological stability. *In vivo* anti-inflammatory activity was studied in the widely used ovalbumin-sensitized and ovalbumin-challenged Brown Norway rat model.
Key findings
The 6α or 9α-fluoro substitution produced highly potent corticosteroids, but the 17α-dichloroacetyl substituent provided 'softness' in all cases. Local application of these steroids will significantly reduce systemic activity, due to the facile hydrolytic deactivation of these molecules.
Conclusions
A 17α-dichloroacetyl derivative of fluticasone (FLU) is highly potent but much safer than the currently used propionate or furoate ester.

4.1794 Up-regulation of Interleukin-21 Contributes to Liver Pathology of Schistosomiasis by Driving GC

Immune Responses and Activating HSCs in Mice
*Scientific Reports, **7**:16682 (2017)*

The pathology of schistosome egg-induced liver granuloma, fibrosis and eventually liver scarring is complicated. CD4+ helper T (Th) cells play critical roles in both host humoral immunity and cellular immunity against parasitic infection and immunopathology in schistosomiasis. Follicular helper T (Tfh) cells are another specialized subset of Th cells and involved in infectious diseases. However, the immune regulatory mechanism of Tfh cells in severe liver pathology of schistosomiasis is still poorly understood. In this study, using a S. japonicum-infected mouse model, we studied the dynamics and effects of Tfh cells in vivo and demonstrated that Tfh phenotype molecules ICOS, PD-1 and functional factor IL-21 were positively correlated with disease development by flow cytometry. Meanwhile, our results also showed that Tfh cells enriched in splenic germinal center (GC) and promoted B cells producing IgM with the progress of hepatic immunopathology by B-T co-culture experiments. More importantly, our data indicated that IL-21 contributed to the formation and development of hepatic egg granuloma and subsequent fibrosis by driving GC responses and activating HSCs by immunohistochemical detection and blocking assay in vitro. Our findings contribute to the better understanding of the immunopathogenesis of schistosomiasis and have implications for therapeutic intervention of hepatic fibrotic diseases.

4.1795 Functional analysis of human intrafusal fiber innervation by human γ-motoneurons
Colon, A., Guo, X., Akanda, N., Cai, Y. and Hickman, J.J.
*Scientific Reports, **7**:17202 (2017)*

Investigation of neuromuscular deficits and diseases such as SMA, as well as for next generation prosthetics, utilizing *in vitro* phenotypic models would benefit from the development of a functional neuromuscular reflex arc. The neuromuscular reflex arc is the system that integrates the proprioceptive information for muscle length and activity (sensory afferent), to modify motoneuron output to achieve graded muscle contraction (actuation efferent). The sensory portion of the arc is composed of proprioceptive sensory neurons and the muscle spindle, which is embedded in the muscle tissue and composed of intrafusal fibers. The gamma motoneurons (γ-MNs) that innervate these fibers regulate the intrafusal fiber's stretch so that they retain proper tension and sensitivity during muscle contraction or relaxation. This mechanism is in place to maintain the sensitivity of proprioception during dynamic muscle activity and to prevent muscular damage. In this study, a co-culture system was developed for innervation of intrafusal fibers by human γ-MNs and demonstrated by morphological and immunocytochemical analysis, then validated by functional electrophysiological evaluation. This human-based fusimotor model and its incorporation into the reflex arc allows for a more accurate recapitulation of neuromuscular function for applications in disease investigations, drug discovery, prosthetic design and neuropathic pain investigations.

4.1796 GDF15 deficiency exacerbates chronic alcohol- and carbon tetrachloride-induced liver injury
Chung, H., Kim, J.T., Kim, H-W., Kwon, M., Kim, S.Y., Shong, M., Kim, K.S. and Yi, H-s.
*Scientific Reports, **7**:17238 (2017)*

Growth differentiation factor 15 (GDF15) has recently been shown to have an important role in the regulation of mitochondrial function and in the pathogenesis of complex human diseases. Nevertheless, the role of GDF15 in alcohol-induced or fibrotic liver diseases has yet to be determined. In this study, we demonstrate that alcohol- or carbon tetrachloride (CCl_4)-mediated hepatic GDF15 production ameliorates liver inflammation and fibrosis. Alcohol directly enhanced GDF15 expression in primary hepatocytes, which led to increased oxygen consumption. Moreover, GDF15 reduced the expression of pro-inflammatory cytokines in liver-resident macrophages, leading to an improvement in inflammation and fibrosis in the liver. GDF15 knockout (KO) mice had more TNF-α-producing T cells and more activated $CD4^+$ and $CD8^+$ T cells in the liver than wild-type mice. Liver-infiltrating monocytes and neutrophils were also increased in the GDF15 KO mice during liver fibrogenesis. These changes in hepatic immune cells were associated with increased tissue inflammation and fibrosis. Finally, recombinant GDF15 decreased the expression of pro-inflammatory cytokines and fibrotic mediators and prevented the activation of T cells in the livers of mice with CCl_4-induced liver fibrosis. These results suggest that GDF15 could be a potential therapeutic target for the treatment of alcohol-induced and fibrotic liver diseases.

4.1797 Effectiveness of different molecular forms of C. histolyticum class I collagenase to recover islets
Green, M.L., Breite, A.G., Beechler, C.A., Dwuler, F.E. and McCarthy, R.C.
*Islets, **9(6)**, 177-181 (2017)*

One factor that may contribute to variability between different lots of purified collagenase to recover islets is the molecular form of *C. histolyticum* class I (C1) collagenase used in the isolation procedure. Two different enzyme mixtures containing C1, class II (C2) collagenase and BP Protease were compared for their effectiveness to recover islets from split adult porcine pancreas. The same enzyme activities per g trimmed tissue were used for all isolations with the only difference being the mass of C1 required to achieve 25,000 collagen degradation activity U/g tissue. The results show no differences in performance of the two enzyme mixtures. The only significant difference is 19 fold more truncated C1 was required to achieve the same result as intact C1.

4.1798 Analysis of Argonaute 2–microRNA complexes in ex vivo stored red blood cells

Vu, L., Ragupathy, V., Kulkarni, S. and Atreya, C.
Transfusion, ***57(12)***, *2995-3000 (2017)*

BACKGROUND
Human enucleated mature red blood cells (RBCs) contain both mature microRNAs (miRNAs) and mRNAs, and we have previously correlated RBC storage lesion processes such as eryptosis, adenosine 5′-triphosphate loss, and RBC indices with differentially expressed miRNAs. Here we have characterized Argonaute 2 (AGO2)–miRNA complexes in stored mature RBCs as a first step toward understanding their role, if any.
STUDY DESIGN AND METHODS
In this report AGO2-bound miRNAs in mature RBCs isolated from RBCs collected from three different healthy donors and stored for 24 hours at 4 to 6°C were identified by anti-AGO2 immunoprecipitation (IP) followed by next-generation sequencing of the RNA isolated from the IP. The data were analyzed by various bioinformatics tools.
RESULTS
The analysis highlighted 28 mature AGO2-bound miRNAs that are common to all three donors, representing 95.6% of the identified miRNAs. Among these, miR-16-5p (20.6%), miR-451a-5p (16.7%), miR-486-5p (12.6%), and miR-92a-3p (12.6%) are the most abundant miRNAs. Functional enrichment analysis for mRNA targets of the 28 common miRNAs identified molecules related to various diseases, biofunctions, and toxicity functions such as cardio-, hepato-, and nephrotoxicity.
CONCLUSION
Overall, these results demonstrate the existence of multiple intracellular AGO2-bound miRNAs in 24-hour-stored RBCs and warrant further experiments to determine whether AGO2-miRNAs are functional in RBCs.

4.1799 Compound heterozygous CASQ2 mutations and long-term course of catecholaminergic polymorphic ventricular tachycardia

Josephs, K., Patel, K., Janson, C.M., Montagna, C. and McDonald, T.V.
Mol. Genet. Genom. Med., ***5(6)***, *788-794 (2017)*

Background
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a potentially lethal inherited cardiac disorder characterized by episodic ventricular tachycardia during adrenergic stimulation. It is associated with significant morbidity and mortality. Knowledge of the underlying genetic cause, pathogenesis, and the natural history of the disease remains incomplete. Approximately 50% of CPVT cases are caused by dominant mutations in the cardiac ryanodine receptor (*RYR2*) gene, <5% of cases are accounted for by recessive mutations in cardiac calsequestrin (*CASQ2*) or Triadin (*TRDN*).
Methods
We report a family with two *CASQ2* gene mutations. A research-based next-generation sequencing (NGS) initiative was used in a patient with a severe CPVT phenotype and her clinically unaffected son. Reverse transcription polymerase chain reaction (RT-PCR) from platelet RNA was used to assess the consequences of predicted splice variants.
Results
NGS revealed that the proband carried a novel c.199C>T (p.Gln67*) mutation and a previously reported splice site mutation c.532+1G>A in *CASQ2*. Her son is a heterozygous carrier of the c.199C>T (p.Gln67*) mutation alone and the proband was compound heterozygous at *CASQ2*. RNA analysis demonstrated that the splice site mutation results in the retention of intron 3 with no full-length *CASQ2* mRNA.
Conclusion
This study describes a novel CPVT genotype and further characterizes the effect of a previously reported

CASQ2 splice site mutation. The long-term follow-up of 23 years since first symptom provides additional insight into the natural history of *CASQ2*-associated CPVT.

4.1800 Thirst-associated preoptic neurons encode an aversive motivational drive
Allen, W.E., Denardo, L.A., Chen, M.Z., Liu, C.D., Loh, K.M., Fenno, L.E., Ramakrisshnan, C., Deisseroth, K. and Luo, L.
*Science, **357(6356)**, 1149-1155 (2017)*

Water deprivation produces a drive to seek and consume water. How neural activity creates this motivation remains poorly understood. We used activity-dependent genetic labeling to characterize neurons activated by water deprivation in the hypothalamic median preoptic nucleus (MnPO). Single-cell transcriptional profiling revealed that dehydration-activated MnPO neurons consist of a single excitatory cell type. After optogenetic activation of these neurons, mice drank water and performed an operant lever-pressing task for water reward with rates that scaled with stimulation frequency. This stimulation was aversive, and instrumentally pausing stimulation could reinforce lever-pressing. Activity of these neurons gradually decreased over the course of an operant session. Thus, the activity of dehydration-activated MnPO neurons establishes a scalable, persistent, and aversive internal state that dynamically controls thirst-motivated behavior.

4.1801 Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4–MD2 complex
Kim, S.Y. et al
*Nature Communications, **8**:2247 (2017)*

Reactive oxygen species (ROS) contribute to the development of non-alcoholic fatty liver disease. ROS generation by infiltrating macrophages involves multiple mechanisms, including Toll-like receptor 4 (TLR4)-mediated NADPH oxidase (NOX) activation. Here, we show that palmitate-stimulated $CD11b^{+}F4/80^{low}$ hepatic infiltrating macrophages, but not $CD11b^{+}F4/80^{high}$ Kupffer cells, generate ROS via dynamin-mediated endocytosis of TLR4 and NOX2, independently from MyD88 and TRIF. We demonstrate that differently from LPS-mediated dimerization of the TLR4–MD2 complex, palmitate binds a monomeric TLR4–MD2 complex that triggers endocytosis, ROS generation and increases pro-interleukin-1β expression in macrophages. Palmitate-induced ROS generation in human $CD68^{low}CD14^{high}$ macrophages is strongly suppressed by inhibition of dynamin. Furthermore, *Nox2*-deficient mice are protected against high-fat diet-induced hepatic steatosis and insulin resistance. Therefore, endocytosis of TLR4 and NOX2 into macrophages might be a novel therapeutic target for non-alcoholic fatty liver disease.

4.1802 H3.3K27M Cooperates with Trp53 Loss and PDGFRA Gain in Mouse Embryonic Neural Progenitor Cells to Induce Invasive High-Grade Gliomas
Pathania, m., De Jay, N., Maestro, N. et al
*Cancer Cell, **32(5)**, 684-700 (2017)*

Gain-of-function mutations in histone 3 (H3) variants are found in a substantial proportion of pediatric high-grade gliomas (pHGG), often in association with *TP53* loss and platelet-derived growth factor receptor alpha (*PDGFRA*) amplification. Here, we describe a somatic mouse model wherein $H3.3^{K27M}$ and *Trp53* loss alone are sufficient for neoplastic transformation if introduced *in utero*. $H3.3^{K27M}$-driven lesions are clonal, H3K27me3 depleted, Olig2 positive, highly proliferative, and diffusely spreading, thus recapitulating hallmark molecular and histopathological features of pHGG. Addition of wild-type PDGFRA decreases latency and increases tumor invasion, while ATRX knockdown is associated with more circumscribed tumors. $H3.3^{K27M}$-tumor cells serially engraft in recipient mice, and preliminary drug screening reveals mutation-specific vulnerabilities. Overall, we provide a faithful $H3.3^{K27M}$-pHGG model which enables insights into oncohistone pathogenesis and investigation of future therapies.

4.1803 Antifibrotic effect of rapamycin containing polyethylene glycol-coated alginate microcapsule in islet xenotransplantation
Park, H-S., Kim, J-W., Lee, S-H., Yang, H.K., Ham, D-S., Sun, C-L., Hong, T.H., Khang, G., Park, C-G. and Yoon, K-H.
*J. Tissue Eng. Regen. Med., **11(4)**, 1274-1284 (2017)*

Islet microencapsulation is an attractive strategy for the minimization or avoidance of life-long immunosuppression after transplantation. However, the clinical implementation of this technique is

currently limited by incomplete biocompatibility. Thus, the aim of the present study was to demonstrate the improved biocompatibility of rapamycin-containing polyethylene glycol (Rapa–PEG)-coating on alginate microcapsules containing xenogeneic islets. The Rapa–PEG-coating on the alginate layer was observed using scanning electron microscopy (SEM) and the molecular cut-off weight of the microcapsules was approximately 70 kDa. The viabilities of the alginate-encapsulated and Rapa–PEG-coated alginate-encapsulated islets were lower than the viability of the naked islets just after encapsulation, but these the differences diminished over time in culture dishes. Rapa–PEG-coating on the alginate capsules effectively decreased the proliferation of macrophage cells compared to the non-coating and alginate coating of xenogeneic pancreas tissues. Glucose-stimulated insulin secretion did not significantly differ among the groups prior to transplantation. The random blood glucose levels of diabetic mice significantly improved following the transplantation of alginate-encapsulated and Rapa–PEG-coated alginate-encapsulated islets, but there were no significant differences between these two groups. However, there was a significant decrease in the number of microcapsules with fibrotic cell infiltration in the Rapa–PEG-coated alginate microcapsule group compared to the alginate microcapsule group. In conclusion, Rapa–PEG-coating might be an effective technique with which to improve the biocompatibility of microcapsules containing xenogeneic islets.

4.1804 A microfluidic-based cell encapsulation platform to achieve high long-term cell viability in photopolymerized PEGNB hydrogel microspheres
Jiang, Z., Xia, B., McBride, R.M. and Oakey, J.
J. Mater. Chem. B., **5**, *173-180 (2017)*

Cell encapsulation within photopolymerized polyethylene glycol (PEG)-based hydrogel scaffolds has been demonstrated as a robust strategy for cell delivery, tissue engineering, regenerative medicine, and developing *in vitro* platforms to study cellular behavior and fate. Strategies to achieve spatial and temporal control over PEG hydrogel mechanical properties, chemical functionalization, and cytocompatibility have advanced considerably in recent years. Recent microfluidic technologies have enabled the miniaturization of PEG hydrogels, thus enabling the fabrication of miniaturized cell-laden vehicles. However, rapid oxygen diffusive transport times on the microscale dramatically inhibit chain growth photopolymerization of polyethylene glycol diacrylate (PEGDA), thus decreasing the viability of cells encapsulated within these microstructures. Another promising PEG-based scaffold material, PEG norbornene (PEGNB), is formed by a step-growth photopolymerization and is not inhibited by oxygen. PEGNB has also been shown to be more cytocompatible than PEGDA and allows for orthogonal addition reactions. The step-growth kinetics, however, are slow and therefore challenging to fully polymerize within droplets flowing through microfluidic devices. Here, we describe a microfluidic-based droplet fabrication platform that generates consistently monodisperse cell-laden water-in-oil emulsions. Microfluidically generated PEGNB droplets are collected and photopolymerized under UV exposure in bulk emulsions. In this work, we compare this microfluidic-based cell encapsulation platform with a vortex-based method on the basis of microgel size, uniformity, post-encapsulation cell viability and long-term cell viability. Several factors that influence post-encapsulation cell viability were identified. Finally, long-term cell viability achieved by this platform was compared to a similar cell encapsulation platform using PEGDA. We show that this PEGNB microencapsulation platform is capable of generating cell-laden hydrogel microspheres at high rates with well-controlled size distributions and high long-term cell viability.

4.1805 Lipopolysaccharide mediates hepatic stellate cell activation by regulating autophagy and retinoic acid signaling
Chen, M., Liu, J., Yang, W. and Ling, W.
Autophagy, ***13(11)***, *1813-1827 (2017)*

Bacterial translocation and lipopolysaccharide (LPS) leakage occur at a very early stage of liver fibrosis in animal models. We studied the role of LPS in hepatic stellate cell (HSC) activation and the underlying mechanisms in vitro and in vivo. Herein, we demonstrated that LPS treatment led to a dramatic increase in autophagosome formation and autophagic flux in LX-2 cells and HSCs, which was mediated through the AKT-MTOR and AMPK-ULK1 pathway. LPS significantly decreased the lipid content, including the lipid droplet (LD) number and lipid staining area in HSCs; pretreatment with macroautophagy/autophagy inhibitors or silencing ATG5 attenuated this decrease. Furthermore, lipophagy was induced by LPS through the autophagy-lysosomal pathway in LX-2 cells and HSCs. Additionally, LPS-induced autophagy further reduced retinoic acid (RA) signaling, as demonstrated by a decrease in the intracellular RA level and *Rar* target genes, resulting in the downregulation of *Bambi* and promoting the sensitization of the

HSC's fibrosis response to TGFB. Compared with CCl_4 injection alone, CCl_4 plus LPS injection exaggerated liver fibrosis in mice, as demonstrated by increased *Col1a1* (collagen, type I, α 1), *Acta2, Tgfb* and *Timp1* mRNA expression, ACTA2/α-SMA and COL1A1 protein expression, and Sirius Red staining area, which could be attenuated by injection of an autophagy inhibitor. LPS also reduced lipid content in HSCs in vivo, with this change being attenuated by chloroquine (CQ) administration. In conclusion, LPS-induced autophagy resulted in LD loss, RA signaling dysfunction, and downregulation of the TGFB pseudoreceptor *Bambi*, thus sensitizing HSCs to TGFB signaling.

4.1806 Elasto-Inertial Focusing of Mammalian Cells and Bacteria Using Low Molecular, Low Viscosity PEO Solutions

Holzner, G., Stavrakis, S. and Demello, A.
Anal. Chem., ***89(21)***, *11653-11663 (2017)*

The ability to manipulate biological cells is critical in a diversity of biomedical and industrial applications. Microfluidic-based cell manipulations provide unique opportunities for sophisticated and high-throughput biological assays such as cell sorting, rare cell detection, and imaging flow cytometry. In this respect, cell focusing is an extremely useful functional operation preceding downstream biological analysis, since it allows the accurate lateral and axial positioning of cells moving through microfluidic channels, and thus enables sophisticated cell manipulations in a passive manner. Herein, we explore the utility of viscoelastic carrier fluids for enhanced elasto-inertial focusing of biological species within straight, rectangular cross section microfluidic channels. Since the investigated polymer solutions possess viscosities close to that of water and exhibit negligible shear thinning, focusing occurs over a wide range of elasticity numbers and a large range of Reynolds numbers. With a view to applications in the robust focusing of cells and bacteria, we assess and characterize the influence of accessible focusing parameters, including blockage ratio, volumetric flow rate, cell concentration, and polymer chain length.

4.1807 Extremely low frequency magnetic field protects injured spinal cord from the microglia- and iron-induced tissue damage

Dey, S., Bose, S., Kumar, S., Rathmore, R., Mathur, R. and Jain, S.
Electromagnetic Biol. Med., ***36(4)***, *330-340 (2017)*

Spinal cord injury (SCI) is insult to the spinal cord, which results in loss of sensory and motor function below the level of injury. SCI results in both immediate mechanical damage and secondary tissue degeneration. Following traumatic insult, activated microglia release proinflammatory cytokines and excess iron due to hemorrhage, initiating oxidative stress that contributes to secondary degeneration. Literature suggests that benefits are visible with the reduction in concentration of iron and activated microglia in SCI. Magnetic field attenuates oxidative stress and promotes axonal regeneration in vitro and in vivo. The present study demonstrates the potential of extremely low frequency magnetic field to attenuate microglia- and iron-induced secondary injury in SCI rats. Complete transection of the spinal cord (T13 level) was performed in male Wistar rats and subsequently exposed to magnetic field (50 Hz,17.96 μT) for 2 h daily for 8 weeks. At the end of the study period, spinal cords were dissected to quantify microglia, macrophage, iron content and study the architecture of lesion site. A significant improvement in locomotion was observed in rats of the SCI + MF group as compared to those in the SCI group. Histology, immunohistochemistry and flow cytometry revealed significant reduction in lesion volume, microglia, macrophage, collagen tissue and iron content, whereas, a significantly higher vascular endothelial growth factor expression around the epicenter of the lesion in SCI + MF group as compared to SCI group. These novel findings suggest that exposure to ELF-MF reduces lesion volume, inflammation and iron content in addition to facilitation of angiogenesis following SCI.

4.1808 A proteome analysis of pig pancreatic islets and exocrine tissue by liquid chromatography with tandem mass spectrometry

Nakashima, Y., Miyagi-Shiohira, C., Kobayashi, N., Saitoh, I., Watanabe, M. and Noguchi, H.
Islets, ***9(6)***, *159-176 (2017)*

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is a proteome analysis method, and the shotgun analysis by LC-MS/MS comprehensively identifies proteins from tissues and cells with high resolving power. In this study, we analyzed the protein expression in pancreatic tissue by LC-MS/MS. Islets isolated from porcine pancreata (purity ≥95%) and exocrine tissue (purity ≥99%) were used in this study. LC-MS/MS showed that 13 proteins were expressed in pancreatic islets only (Group I), 43 proteins were expressed in both islets and exocrine tissue (Group I&E), and 102 proteins were expressed in

exocrine tissue only (Group E). Proteins involved in islet differentiation and cell proliferation were identified in Group I (e.g. CLUS, CMGA, MIF). In addition, various functional proteins (e.g. SCG2, TBA1A) were identified in islet by using the new method of 'principal component analysis (PCA)'. However, the function of such proteins on islets remains unclear. EPCAM was identified in Group E. Group E was found to include proteins involved in clinical inflammatory diseases such as pancreatitis (e.g. CBPA1, CGL, CYTB, ISK1 and PA21B). Many of these identified proteins were reported less frequently in previous studies, and HS71B, NEC2, PRAF3 and SCG1 were newly detected in Group I while CPNS1, DPEP1, GANAB, GDIB, GGT1, HSPB1, ICTL, VILI, MUTA, NDKB, PTGR1, UCHL3, VAPB and VINC were newly detected in Group E. These results show that comprehensive expression analysis of proteins by LC-MS/MS is useful as a method to investigate new factors constructing cellular component, biological process, and molecular function.

4.1809 **Extending antigen release from particulate vaccines results in enhanced antitumor immune response**
Kapadia, C.H., Tian, S., Perry, J.L., Sailer, D., Luft, J.C. and DeSimone, J.M.
J. Controlled Release, ***269***, *393-404 (2018)*

Tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) play a critical role in an anti-tumor immune response. However, vaccination intended to elicit a potent $CD8^+$ T cell responses employing tumor-associated peptide antigens, are typically ineffective due to poor immunogenicity. Previously, we engineered a polyethylene glycol (PEG) hydrogel-based subunit vaccine for the delivery of an antigenic peptide and CpG (adjuvant) to elicit potent CTLs. In this study, we further examined the effect of antigen release kinetics on their induced immune responses. A $CD8^+$ T cell epitope peptide from OVA (CSIINFEKL) and CpG were co-conjugated to nanoparticles utilizing either a disulfide or a thioether linkage. Subsequent studies comparing peptide release rates as a function of linker, determined that the thioether linkage provided sustained release of peptide over 72 h. Ability to control the release of peptide resulted in both higher and prolonged antigen presentation when compared to disulfide-linked peptide. Both NP vaccine formulations resulted in activation and maturation of bone marrow derived dendritic cells (BMDCs) and induced potent $CD8^+$ T cell responses when compared to soluble antigen and soluble CpG. Immunization with either disulfide or thioether linked vaccine constructs effectively inhibited EG7-OVA tumor growth in mice, however only treatment with the thioether linked vaccine construct resulted in enhanced survival.

4.1810 **Increased number of tissue factor protein expressing thrombocytes in canine idiopathic immune mediated hemolytic anemia**
Hennink, I., van Leeuwen, M.W., Penning, L.C. and Piek, C.J.
Vet. Immunol. Immunopathol., ***196***, *22-29 (2018)*

Dogs suffering from canine idiopathic immune mediated hemolytic anemia (cIIMHA) are at great risk of dying particularly in the first two weeks after the diagnosis is made. This high mortality risk may be associated with the development of thromboembolism (TE) and/or disseminated intravascular coagulation (DIC) resulting in organ failure. The exact mechanism of the development of TE and/or DIC in cIIMHA is still undetermined. Therefore, this study investigates the presence of tissue factor (TF) in thrombocytes of dogs suffering from cIIMHA, using *OptiPrep*™ for the isolation of blood cells and immunocytochemistry (ICC) to visualize TF on thrombocytes. The normalised TF quantity, acquired with 'colour deconvolution' (*ImageJ* plug in), revealed that in cIIMHA dogs the fraction TF positive thrombocytes was statistically significant higher ($P < 0.001$; mean 0.79; $n = 7$) compared to the fraction TF positive thrombocytes of the healthy dogs (mean 0.43; $n = 9$). We further have indications that the fraction of TF positive thrombocytes decreases with time and therapy, but that the progression rate differs individually. Since cIIMHA dogs have more thrombocytes that are TF-positive compared to healthy dogs, this may explain the increased risk to develop TE and DIC. Furthermore, it seems that the number of TF-positive thrombocytes in cIIMHA dogs remains high during the first two weeks of the disease, the time when the animals are at greatest health risk.

4.1811 **Decreased macrophage phagocytic function due to xenobiotic exposures in vitro, difference in sensitivity between various macrophage models**
Berntsen, H.F., Bølling, A.K., Bjørklund, C.G., Zimmer, K., Ropstad, E., Zienolddiny, S., Becher, R., Holme, J.A., Dirve, H., Nygaard, U.C. and Bodin, J.
Food Chem. Toxicol., ***112***, *86-90 (2018)*

Both autoimmune disease prevalence and exposure to immunotoxic chemicals have increased the last

decades. As a first screening of immunotoxic chemicals possibly affecting development of autoimmunity through attenuated macrophage function, we demonstrate a promising model measuring macrophage function in isolated peritoneal macrophages (PCM) from Wistar rats and C57Bl/6 mice. Immunotoxic effects of bisphenol A (BPA) and a selection of perfluoroalkyl acids (PFAAs) were analysed *in vitro* assessing phagocytic function of macrophages from different sources. Phagocytosis was reduced in PCM of C57Bl/6 mice and Wistar rats after BPA and perfluoroundecanoic acid (PFUnDA) exposure, but not in macrophages derived from human and rat monocyte derived macrophages (MDM). On the other hand, *in vitro* exposure to mixtures of persistent organic pollutants (POPs) showed similar reductions in rat PCM and rat and human MDM phagocytosis. Reduced phagocytosis was partly due to cytotoxicity. PCM isolated from non-obese diabetic (NOD) mice, interleukin 1α/β knockout (IL-1KO) mice and new-born rats were less sensitive to the xenobiotics than PCM from adult wild type rodents. Finally, *in vivo* studies with NOD mice verified that POP exposure also decreased the number of pancreatic macrophages in pancreatic islets, reflecting early signs of autoimmunity development, similarly as previously described for BPA.

4.1812 Inhibition of histone deacetylase 6 (HDAC6) protects against vincristine-induced peripheral neuropathies and inhibits tumor growth

Van Helleputte, L. et al

Neurobiology of Disease, ***111***, *59-69 (2018)*

As cancer is becoming more and more a chronic disease, a large proportion of patients is confronted with devastating side effects of certain anti-cancer drugs. The most common neurological complications are painful peripheral neuropathies. Chemotherapeutics that interfere with microtubules, including plant-derived vinca-alkaloids such as vincristine, can cause these chemotherapy-induced peripheral neuropathies (CIPN). Available treatments focus on symptom alleviation and pain reduction rather than prevention of the neuropathy. The aim of this study was to investigate the potential of specific histone deacetylase 6 (HDAC6) inhibitors as a preventive therapy for CIPN using multiple rodent models for vincristine-induced peripheral neuropathies (VIPN). HDAC6 inhibition increased the levels of acetylated α-tubulin in tissues of rodents undergoing vincristine-based chemotherapy, which correlates to a reduced severity of the neurological symptoms, both at the electrophysiological and the behavioral level. Mechanistically, disturbances in axonal transport of mitochondria is considered as an important contributing factor in the pathophysiology of VIPN. As vincristine interferes with the polymerization of microtubules, we investigated whether disturbances in axonal transport could contribute to VIPN. We observed that increasing α-tubulin acetylation through HDAC6 inhibition restores vincristine-induced defects of axonal transport in cultured dorsal root ganglion neurons. Finally, we assured that HDAC6-inhibition offers neuroprotection without interfering with the anti-cancer efficacy of vincristine using a mouse model for acute lymphoblastic leukemia. Taken together, our results emphasize the therapeutic potential of HDAC6 inhibitors with beneficial effects both on vincristine-induced neurotoxicity, as well as on tumor proliferation.

4.1813 Effect of silicon-rich water intake on the systemic and peritoneal inflammation of rats with chronic low levels of aluminum ingestion

Radvanovic, Z., Djindjic, B., Dzopalic, T., Veljkovic, A., Dunjic, M., Krstic, D., Djindjic, N., Bozic, B. and Nedeljkovic, B.

J. Trace Elements in Med. Biol., ***46***, *96-102 (2018)*

Background and Objectives
Study evaluated effect of silicon-rich water intake on systemic inflammation and functional characteristics of peritoneal macrophages (PMs) of rats that were chronically exposed to dietary aluminum.
Methods
One month-old female Wistar Albino rats were administered aluminum chloride dissolved in distilled water (1.6 mg/kg body weight in 0.5 mL) by gavage for 90 days. The rats were then given standard (6 mg/L) or silicon-rich water (19 mg/L silicon) (n = 7/group). Control rats underwent sham gavage and received standard or silicon-rich water (n = 7/group). Blood was assessed for cytokine levels. Unstimulated and lipopolysaccharide (LPS)-stimulated PMs were assessed in terms of phagocytic activity and cytokine secretion in vitro.
Results
Chronic exposition to dietary aluminum and silicon-rich drinking water did not change serum TNF-α levels. Aluminum increased serum IL-2 and this was reversed by silicon-rich water. The aluminum-exposed rats had higher serum sICAM-1 than sham-gavaged, unrelated to type of water. LPS-stimulated

PMs from aluminum-intoxicated animals exhibited low phagocytic activity and release of TNF-α, this was significantly improved by silicon-rich water intake. In the presence of silicon-rich water, LPS-stimulated and unstimulated PMs from aluminum-exposed rats produced significantly more IL-10.
Conclusions
Chronic ingestion of aluminum, increases systemic and peritoneal inflammation and PM dysfunction. The presence of high levels of the natural aluminum antagonist silicon in the drinking water restored IL-10 and TNF-α PM secretion, preventing prolonged inflammation. Thus, silicon intake can decrease the immunotoxicity of aluminum.

4.1814 Targeting secreted cytokine BMP9 gates the attenuation of hepatic fibrosis
Li, P., Li, Y., Zhu, L., yang, Z., He, J., Wang, L., Shang, Q., pan, H., Wang, H., Ma, X., Li, B., Fan, X., Ge, S., Jia, R. and Zhang, H.
BBA – Mol. Basis of Disease, ***1864***, *709-720 (2018)*

Liver fibrosis is overly exuberant wound healing that leads to portal hypertension or liver cirrhosis. Recent studies have demonstrated the functions of bone morphogenetic protein 9 (BMP9) in liver fibrosis, and thus, targeting liver-specific BMP9 abnormalities will become an attractive approach for developing therapeutics to treat liver fibrosis. Here, we reveal that BMP9 serves as a valuable serum diagnostic indicator and efficient therapeutic target to attenuate liver fibrogenesis. Our analysis of biopsies from liver fibrotic patients revealed that higher BMP9 levels accompanied advanced stages of liver fibrosis. In mouse models, recombinant Bmp9 overexpression accelerated liver fibrosis, and adenovirus-mediated Bmp9 knockdown attenuated liver fibrogenesis. Intriguingly, BMP9 directly stimulated hepatic stellate cell activation via the SMAD signaling pathway to enhance hepatic fibrosis. Moreover, an inhibitory monoclonal antibody targeting Bmp9 was efficacious in treatment of mice with liver fibrosis. These observations delineate a novel model in which BMP9 directly drives SMAD/ID1 signaling in hepatic stellate cells, which modulates liver fibrogenesis development. Moreover, the findings unveil a promising surrogate biomarker for the diagnosis of hepatic fibrosis, thereby representing an efficient “BMP9 neutralization” approach in alleviating hepatic fibrosis.

4.1815 Dairy Heifers Naturally Exposed to Fasciola hepatica Develop a Type 2 Immune Response and Concomitant Suppression of Leukocyte Proliferation
Graham-Brown, J., Hartley, C., Clough, H., Kadioglu, A., Baylis, M. and Williams, D.J.L.
Infect.f Immun., ***86(1)***, *e00607-17 (2018)*

Fasciola hepatica is a parasitic trematode of global importance in livestock. Control strategies reliant on anthelmintics are unsustainable due to the emergence of drug resistance. Vaccines are under development, but efficacies are variable. Evidence from experimental infection suggests that vaccine efficacy may be affected by parasite-induced immunomodulation. Little is known about the immune response to F. hepatica following natural exposure. Hence, we analyzed the immune responses over time in calves naturally exposed to F. hepatica infection. Cohorts of replacement dairy heifer calves (n = 42) with no prior exposure to F. hepatica, on three commercial dairy farms, were sampled over the course of a grazing season. Exposure was determined through an F. hepatica-specific serum antibody enzyme-linked immunosorbent assay (ELISA) and fluke egg counts. Concurrent changes in peripheral blood leukocyte subpopulations, lymphocyte proliferation, and cytokine responses were measured. Relationships between fluke infection and immune responses were analyzed by using multivariable linear mixed-effect models. All calves from one farm showed evidence of exposure, while cohorts from the remaining two farms remained negative over the grazing season. A type 2 immune response was associated with exposure, with increased interleukin-4 (IL-4) production, IL-5 transcription, and eosinophilia. Suppression of parasite-specific peripheral blood mononuclear cell (PBMC) proliferation was evident, while decreased mitogen-stimulated gamma interferon (IFN-γ) production suggested immunomodulation, which was not restricted to parasite-specific responses. Our findings show that the global immune response is modulated toward a nonproliferative type 2 state following natural challenge with F. hepatica. This has implications in terms of the timing of the administration of vaccination programs and for host susceptibility to coinfecting pathogens.

4.1816 Recombinant human B cell repertoires enable screening for rare, specific, and natively paired antibodies
Rajan, S., Kierny, M.R., Mercer, A., Wu, J., Tovchigrechko, A., Wu, H., Dall’Acqua, W.F., Xiao, X. and Chowdhury, P.S.
Communications Biol., ***1***:*5 (2018)*

The human antibody repertoire is increasingly being recognized as a valuable source of therapeutic grade antibodies. However, methods for mining primary antibody-expressing B cells are limited in their ability to rapidly isolate rare and antigen-specific binders. Here we show the encapsulation of two million primary B cells into picoliter-sized droplets, where their cognate V genes are fused in-frame to form a library of scFv cassettes. We used this approach to construct natively paired phage-display libraries from healthy donors and drove selection towards cross-reactive antibodies targeting influenza hemagglutinin. Within 4 weeks we progressed from B cell isolation to a panel of unique monoclonal antibodies, including seven that displayed broad reactivity to different clinically relevant influenza hemagglutinin subtypes. Most isolated antibody sequences were not detected by next-generation sequencing of the paired repertoire, illustrating how this method can isolate extremely rare leads not likely found by existing technologies.

4.1817 High content image analysis reveals function of miR-124 upstream of Vimentin in regulating motor neuron mitochondria

Yardani, T. et al

*Scientific Reports, **8**:59 (2018)*

microRNAs (miRNAs) are critical for neuronal function and their dysregulation is repeatedly observed in neurodegenerative diseases. Here, we implemented high content image analysis for investigating the impact of several miRNAs in mouse primary motor neurons. This survey directed our attention to the neuron-specific miR-124, which controls axonal morphology. By performing next generation sequencing analysis and molecular studies, we characterized novel roles for miR-124 in control of mitochondria localization and function. We further demonstrated that the intermediate filament Vimentin is a key target of miR-124 in this system. Our data establishes a new pathway for control of mitochondria function in motor neurons, revealing the value of a neuron-specific miRNA gene as a mechanism for the re-shaping of otherwise ubiquitously-expressed intermediate filament network, upstream of mitochondria activity and cellular metabolism.

4.1818 Lack of Fgf18 causes abnormal clustering of motor nerve terminals at the neuromuscular junction with reduced acetylcholine receptor clusters

Ito, K., Ohkawara, B., yagi, H., Nakashima, H., Tsushima, M., Ota, K., Konishi, H., Masuda, A., Imagama, S., Kiyama, H., Ishiguro, N. and Ohno, K.

*Scientific Reports, **8**:434 (2018)*

FGF receptor 2 is involved in the formation of the neuromuscular junction (NMJ), but its *in vivo* ligand remains to be determined. Laser capture microdissection of the mouse spinal motor neurons (SMNs) revealed that *Fgf18* mRNA is highly expressed in SMNs in adults. Expression of *Fgf18* mRNA was the highest in the spinal cord at embryonic day (E) 15.5, which gradually decreased to postnatal day 7. FGF18 protein was localized at the NMJs of the tibialis anterior muscle at E18.5 and in adults. *Fgf18*−/− mice at E18.5 showed decreased expressions of the NMJ-specific *Chrne* and *Colq* genes in the diaphragm. In *Fgf18*−/− diaphragms, the synaptophysin-positive areas at the nerve terminals and the acetylcholine receptor (AChR)-positive areas at the motor endplates were both approximately one-third of those in wild-type embryos. *Fgf18*−/− diaphragms ultrastructurally showed abnormal aggregation of multiple nerve terminals making a gigantic presynapse with sparse synaptic vesicles, and simplified motor endplates. In *Fgf18*−/− diaphragms, miniature endplate potentials were low in amplitude with markedly reduced frequency. In C2C12 myotubes, FGF18 enhanced AChR clustering, which was blocked by inhibiting FGFRs or MEK1. We propose that FGF18 plays a pivotal role in AChR clustering and NMJ formation in mouse embryogenesis.

4.1819 The alpha7-nicotinic receptor contributes to gp120-induced neurotoxicity: implications in HIV-associated neurocognitive disorders

Capo-Velez, C.M., Morales-Vargas, B., Garcia-Gonzalez, A., Grajales-Reyes, J.G., Delgado-Velez, M., Madera, B., Baez-pagan, C.A., Quesada, O. and Lasalde-Dominicci, J.A.

*Scientific Reports, **8**:1829 (2018)*

Currently, there are no specific therapies to treat HIV-1 associated neurocognitive disorders (HAND). The HIV-1 envelope, gp120, induces neuropathological changes similar to those in HAND patients; furthermore, it triggers an upregulation of the α7-nicotinic acetylcholine receptor (α7-nAChR), facilitating intracellular calcium overload and neuronal cell death. Using a $gp120_{IIIB}$-transgenic mouse (gp120-tgm) model, we demonstrate that α7-nAChRs are upregulated on striatal neurons. Activation of α7-nAChRs

leads to an increase in both intracellular calcium and percentage of apoptotic cells, which can be abrogated by antagonizing the receptor, suggesting a role for α7-nAChRs in gp120-induced neurotoxicity. Moreover, we demonstrate for the first time that gp120-tgm have learning deficiencies on a striatum-dependent behavioral task. They also show locomotor deficiencies, which improved with α7-nAChR antagonists, further supporting a role for this receptor in gp120-induced neurotoxicity. Together, these results uncover a new mechanism through which gp120-induced modulation of α7-nAChRs in the striatum can contribute to HAND development.

4.1820 Parallel droplet microfluidics for high throughput cell encapsulation and synthetic microgel generation

Headen, D.M., Garcia, J.R. and Garcia, A.J.
*Microsystems & Nanoengineering, **4**:17076 (2018)*

Cells can be microencapsulated in synthetic hydrogel microspheres (microgels) using droplet microfluidics, but microfluidic devices with a single droplet generating geometry have limited throughput, especially as microgel diameter decreases. Here we demonstrate microencapsulation of human mesenchymal stem cells (hMSCs) in small (<100 μm diameter) microgels utilizing parallel droplet generators on a two-layer elastomer device, which has 600% increased throughput vs. single-nozzle devices. Distribution of microgel diameters were compared between products of parallel vs. single-nozzle configurations for two square nozzle widths, 35 and 100 μm. Microgels produced on parallel nozzles were equivalent to those produced on single nozzles, with substantially the same polydispersity. Microencapsulation of hMSCs was compared for parallel nozzle devices of each width. Thirty five micrometer wide nozzle devices could be operated at twice the cell concentration of 100 μm wide nozzle devices but produced more empty microgels than predicted by a Poisson distribution. Hundred micrometer wide nozzle devices produced microgels as predicted by a Poisson distribution. Polydispersity of microgels did not increase with the addition of cells for either nozzle width. hMSCs encapsulated on 35 μm wide nozzle devices had reduced viability (~70%) and a corresponding decrease in vascular endothelial growth factor (VEGF) secretion compared to hMSCs cultured on tissue culture (TC) plastic. Encapsulating hMSCs using 100 μm wide nozzle devices mitigated loss of viability and function, as measured by VEGF secretion.

4.1821 Carnosol-mediated Sirtuin 1 activation inhibits Enhancer of Zeste Homolog 2 to attenuate liver fibrosis

Zhao, H., Wang, Z., Tang, F., Zhao, Y., Feng, D., Li, Y., Hu, Y., Wang, C., Zhou, J., Tian, X. and Yao, J.
*Pharmacol. Res., **128**, 327-337 (2018)*

Quiescent hepatic stellate cell (HSC) activation and subsequent conversion into myofibroblasts is the central event in hepatic fibrosis pathogenesis. Epithelial−mesenchymal transition (EMT), another vital participant in liver fibrosis, has the potential to initiate HSC activation, which promotes abundant myofibroblast production. Previous studies suggest that Enhancer of Zeste Homolog 2 (EZH2) plays a significant role in myofibroblast transdifferentiation; however, the underlying mechanisms remain largely unaddressed. Carnosol (CS), a compound extracted from rosemary, displays multiple pharmacological activities. This study aimed to investigate the signaling mechanisms underlying EZH2 inhibition and the anti-fibrotic effect of CS in liver fibrosis. We found that CS significantly inhibited CCl_4- and TGFβ1-induced liver fibrosis and reduced both HSC activation and EMT. EZH2 knockdown also prevented these processes induced by TGFβ1 in HSCs and AML-12 cells. Interestingly, the protective effect of CS was positively associated with Sirtuin 1 (SIRT1) activation and accompanied by EZH2 inhibition. SIRT1 knockdown attenuated the EZH2 inhibition induced by CS and increased EZH2 acetylation, which enhanced its stability. Conversely, upon TGFβ1 exposure, SIRT1 activation significantly reduced the level of EZH2 acetylation; however, EZH2 overexpression prevented the SIRT1 activation that primed myofibroblast inhibition, indicating that EZH2 is a target of SIRT1. Thus, SIRT1/EZH2 regulation could be used as a new therapeutic strategy for fibrogenesis. Together, this study provides evidence of activation of the SIRT1/EZH2 pathway by CS that inhibits myofibroblast generation, and thus, CS may represent an attractive candidate for anti-fibrotic clinical therapy.

4.1822 Quality of cryopreserved buffalo spermatozoa improved from poor quality ejaculates

Ali, A., Ahmad, E., Ijaz, N., Ahmad, W. and Ul-hassan, F.
*Cryobiology, **80**, 167 abstract S44 (2018)*

Use of assisted reproductive technology, especially density gradient centrifugation, is being used as a technique of semen preparation in animals and humans. The technique was efficient in improving semen

quality after cryopreservation. Single layer centrifugation provides a large amount of high quality sperm before conservation. Single layer centrifugation studied in stallions, boars and cow bulls, limited data available for buffalo bulls. Also bulls experience a transient reduction in semen quality, thus techniques that allow improvement in semen quality could be applied. The aim of this study was the evaluation of single layer and double layer centrifugation by the use of iodixanol, compared with conventional centrifugation and non-centrifuged semen, on the sperm characteristics during the cryopreservation process in buffalo bulls with normal and poor semen quality. Single layer centrifugation and double layer centrifugation both significantly increased the percentage of normal sperm and decreased the percentage of non-sperm cells in poor quality samples, while both were ineffective in those of normal quality. Sperm characteristics in poor quality samples increased after single and double layer centrifugation, reaching values similar to those recorded in normal samples, and this trend is maintained after equilibration and after cryopreservation. Single Layer and Double Layer Centrifugation resulted in a reduction of the sperm recovered, and this resulted in a reduction of the absolute number of sperm preserved in the normal samples, without a clear improvement in sperm characteristics in this type of sample. Data suggested that both techniques could be performed in practice, but their application should be limited to the cases in which the quality of the sperm recovered is more important than the total number of sperm.

4.1823 Cryosurvival of Mus musculus and Peromyscus spermatozoa in the presence of Iodixanol
Agca, C., Timonin, M., Kim, S., Epperson, K. and Agca, Y.
*Cryobiology, **80**, 185, abstract P22 (2018)*

Cryopreservation of spermatozoa provides a valuable means of maintaining transgenic mouse strains used in biomedical research. Addition of Iodixanol (OptiPrep™) had beneficial effects on post-thaw motility of bull, rat and ram sperm. In the current study, we compared effects of addition of OptiPrep™ in freezing solution on *Mus musculus* and *Peromyscus maniculatus* (PM) mouse sperm post-thaw survival. Four levels of OptiPrep™ (0, 5, 15 and 25%) in combination with three levels of raffinose (18, 15.5 and 14.5%) were compared: The freezing solutions contained 18% raffinose (18R), 18% raffinose and 5% OptiPrep™ (18R5O), 15.5% raffinose and 15% OptiPrep™ (15.5R15O), and 14.5% raffinose and 25% OptiPrep™ (14.5R25O). All the freezing solutions were supplemented with 3% skim milk. The freezing solutions were tested on *Mus musclus* species C57BL/6, 129S1/SvImJ, and FVB/NJ and *Peromyscus maniculatus*. Post-thaw sperm motility, progressive motility, velocity, Live-dead sperm, acosomal integrity, mitochondrial membrane potential were determined. *Mus musculus* sperm post-thaw motility, progressive motility, live sperm, and acosomal integrity improved when 15.5R15O was used as the cryoprotectant. Sperm velocity and mitochondrial membrane potential were not different among sperm freezing solutions. Addition of OptiPrep™ significantly improved (P=0.05) PM live sperm concentration compared to sperm frozen in 18R. Peromyscus maniculatus post-thaw motility was significantly greater (P=0.01) for 15.5R15O compared to 18R and 18R5O. These data suggest that in addition to raffinose and skim milk, iodixanol may be an effective cryoprotectant for mouse and Peromyscus spermatozoa.

4.1824 Liver 'organ on a chip'
Beckwitt, C.H., Clark, A.M., Wheeler, S., taylor, D.L., Stolz, D.B., Griffith, L. and Wells, A.
*Exp. Cell Res., **363**, 15-25 (2018)*

The liver plays critical roles in both homeostasis and pathology. It is the major site of drug metabolism in the body and, as such, a common target for drug-induced toxicity and is susceptible to a wide range of diseases. In contrast to other solid organs, the liver possesses the unique ability to regenerate. The physiological importance and plasticity of this organ make it a crucial system of study to better understand human physiology, disease, and response to exogenous compounds. These aspects have impelled many to develop liver tissue systems for study in isolation outside the body. Herein, we discuss these biologically engineered organoids and microphysiological systems. These aspects have impelled many to develop liver tissue systems for study in isolation outside the body. Herein, we discuss these biologically engineered organoids and microphysiological systems.

4.1825 EphB2 receptor tyrosine kinase promotes hepatic fibrogenesis in mice via activation of hepatic stellate cells
Mimche, P.N., Lee, C.M., Mimche, S.M., Thapa, M., Grakoui, A., Henkemeyer, M. and Lamb, T.J.
*Scientific Reports, **8**:2532 (2018)*

Hepatic fibrosis is the result of an excessive wound-healing response subsequent to chronic liver injury. A feature of liver fibrogenesis is the secretion and deposition of extracellular matrix proteins by activated

hepatic stellate cells (HSCs). Here we report that upregulation of EphB2 is a prominent feature of two mouse models of hepatic fibrosis and also observed in humans with liver cirrhosis. EphB2 is upregulated and activated in mouse HSCs following chronic carbon tetrachloride (CCl_4) exposure. Moreover, we show that EphB2 deficiency attenuates liver fibrosis and inflammation and this is correlated with an overall reduction in pro-fibrotic markers, inflammatory chemokines and cytokines. In an *in vitro* system of HSCs activation we observed an impaired proliferation and sub-optimal differentiation into fibrogenic myofibroblasts of HSCs isolated from *EphB2−/−* mice compared to HSCs isolated from wild type mice. This supports the hypothesis that EphB2 promotes liver fibrosis partly via activation of HSCs. Cellular apoptosis which is generally observed during the regression of liver fibrogenesis was increased in liver specimens of CCl_4-treated *EphB2−/−* mice compared to littermate controls. This data is suggestive of an active repair/regeneration system in the absence of EphB2. Altogether, our data validate this novel pro-fibrotic function of EphB2 receptor tyrosine kinase.

4.1826 Rho-inhibiting C2IN-C3 fusion toxin inhibits chemotactic recruitment of human monocytes ex vivo and in mice in vivo

Martin, T., Möglich, A., Felix, I., Förtsch, C., Rittlinger, A., Palmer, A., Denk, S., Schneider, J., Notbohm, L.,Vogel, M., Geiger, H., Pascheke, S., Huber-lang, M. and Barth, H.
Arch. Toxicol., ***92(1)***, *323-336 (2018)*

Bacterial protein toxins became valuable molecular tools for the targeted modulation of cell functions in experimental pharmacology and attractive therapeutics because of their potent and specific mode of action in human cells. C2IN-C3lim, a recombinant fusion toxin (~50 kDa) of the Rho-inhibiting C3lim from *Clostridium* (*C.*) *limosum* and a non-toxic portion of the *C. botulinum* C2 toxin (C2IN), is selectively internalized into the cytosol of monocytic cells where C3lim specifically ADP-ribosylates Rho A and -B, thereby inhibiting Rho-mediated signaling. Thus, we hypothesized that these unique features make C2IN-C3lim an attractive molecule for the targeted pharmacological down-regulation of Rho-mediated functions in monocytes. The analysis of the actin structure and the Rho ADP-ribosylation status implied that C2IN-C3lim entered the cytosol of primary human monocytes from healthy donors ex vivo within 1 h. Moreover, it inhibited the fMLP-induced chemotaxis of human monocytes in a Boyden chamber model ex vivo. Similarly, in a 3-dimensional ex vivo model of extravasation, single cell analysis revealed that C2IN-C3lim-treated cells were not able to move. In a clinically relevant mouse model of blunt chest trauma, the local application of C2IN-C3lim into the lungs after thorax trauma prevented the trauma-induced recruitment of monocytes into the lungs in vivo. Thus, C2IN-C3lim might be an attractive lead compound for novel pharmacological strategies to avoid the cellular damage response caused by monocytes in damaged tissue after trauma and during systemic inflammation. The results suggest that the pathophysiological role of clostridial C3 toxins might be a down-modulation of the innate immune system.

4.1827 Culture of somatic cells isolated from frozen-thawed equine semen using fluorescence-assisted cell sorting

Brom-de-Luna, J., Canesin, H.S., Wright, G. and Hinrichs, K.
Animal Reprod. Sci., ***190***, *10-17 (2017)*

Nuclear transfer using somatic cells from frozen semen (FzSC) would allow cloning of animals for which no other genetic material is available. Horses are one of the few species for which cloning is commercially feasible; despite this, there is no information available on the culture of equine FzSC. After preliminary trials on equine FzSC, recovered by density-gradient centrifugation, resulted in no growth, we hypothesized that sperm in the culture system negatively affected cell proliferation. Therefore, we evaluated culture of FzSC isolated using fluorescence-assisted cell sorting. In Exp. 1, sperm were labeled using antibodies to a sperm-specific antigen, SP17, and unlabeled cells were collected. This resulted in high sperm contamination. In Exp. 2, FzSC were labeled using an anti-MHC class I antibody. This resulted in an essentially pure population of FzSC, 13–25% of which were nucleated. Culture yielded no proliferation in any of nine replicates. In Exp. 3, 5×10^3 viable fresh, cultured horse fibroblasts were added to the frozen-thawed, washed semen, then this suspension was labeled and sorted as for Exp. 2. The enriched population had a mean of five sperm per recovered somatic cell; culture yielded formation of monolayers. In conclusion, an essentially pure population of equine FzSC could be obtained using sorting for presence of MHC class I antigens. No equine FzSC grew in culture; however, the proliferation of fibroblasts subjected to the same processing demonstrated that the labeling and sorting methods, and the presence of few sperm in culture, were compatible with cell viability.

4.1828 PD-L1 Prevents the Development of Autoimmune Heart Disease in Graft-versus-Host Disease
Juchem, K.W., Sacirbegovic, F., Zhang, C., Sharpe, A.H., Russell, K., McNiff, J.M., Demetris, A.J., Shlomchik, M.J. and Shlomchik, W.D.
*J. Immunol., **200(2)**, 834-846 (2018)*

Effector memory T cells (T_{EM}) are less capable of inducing graft-versus-host disease (GVHD) compared with naive T cells (T_N). Previously, in the TS1 TCR transgenic model of GVHD, wherein TS1 CD4 cells specific for a model minor histocompatibility Ag (miHA) induce GVHD in miHA-positive recipients, we found that cell-intrinsic properties of TS1 T_{EM} reduced their GVHD potency relative to TS1 T_N. Posttransplant, TS1 T_{EM} progeny expressed higher levels of PD-1 than did TS1 T_N progeny, leading us to test the hypothesis that T_{EM} induce less GVHD because of increased sensitivity to PD-ligands. In this study, we tested this hypothesis and found that indeed TS1 T_{EM} induced more severe skin and liver GVHD in the absence of PD-ligands. However, lack of PD-ligands did not result in early weight loss and colon GVHD comparable to that induced by TS1 T_N, indicating that additional pathways restrain alloreactive T_{EM}. TS1 T_N also caused more severe GVHD without PD-ligands. The absence of PD-ligands on donor bone marrow was sufficient to augment GVHD caused by either T_{EM} or T_N, indicating that donor PD-ligand–expressing APCs critically regulate GVHD. In the absence of PD-ligands, both TS1 T_{EM} and T_N induced late-onset myocarditis. Surprisingly, this was an autoimmune manifestation, because its development required non-TS1 polyclonal $CD8^+$ T cells. Myocarditis development also required donor bone marrow to be PD-ligand deficient, demonstrating the importance of donor APC regulatory function. In summary, PD-ligands suppress both miHA-directed GVHD and the development of alloimmunity-induced autoimmunity after allogeneic hematopoietic transplantation.

4.1829 Effector CD4+ T cells recognize intravascular antigen presented by patrolling monocytes
Westhorpe, C.L.V., Norman, M.U., Hall, P., Snelgrove, S.L., Finsterbusch, M., Li, A., Lo, C., Tan, Z.H., Li, S., Nilsson, S.K., Kitching, A.R. and Hiskey, M.J.
*Nature Communications, **9**:747 (2018)*

Although effector $CD4^+$ T cells readily respond to antigen outside the vasculature, how they respond to intravascular antigens is unknown. Here we show the process of intravascular antigen recognition using intravital multiphoton microscopy of glomeruli. $CD4^+$ T cells undergo intravascular migration within uninflamed glomeruli. Similarly, while MHCII is not expressed by intrinsic glomerular cells, intravascular MHCII-expressing immune cells patrol glomerular capillaries, interacting with $CD4^+$ T cells. Following intravascular deposition of antigen in glomeruli, effector $CD4^+$ T-cell responses, including NFAT1 nuclear translocation and decreased migration, are consistent with antigen recognition. Of the $MHCII^+$ immune cells adherent in glomerular capillaries, only monocytes are retained for prolonged durations. These cells can also induce T-cell proliferation in vitro. Moreover, monocyte depletion reduces $CD4^+$ T-cell-dependent glomerular inflammation. These findings indicate that $MHCII^+$ monocytes patrolling the glomerular microvasculature can present intravascular antigen to $CD4^+$ T cells within glomerular capillaries, leading to antigen-dependent inflammation.

4.1830 Neuregulin-1 elicits a regulatory immune response following traumatic spinal cord injury
Alizadeh, A., Santhosh, K.T., Kataria, H., Gounni, A.S. and Karimi-Abdolrezaee, S.K.
J. Neuroinflamm., 15:53 (2018)

Background
Spinal cord injury (SCI) triggers a robust neuroinflammatory response that governs secondary injury mechanisms with both degenerative and pro-regenerative effects. Identifying new immunomodulatory therapies to promote the supportive aspect of immune response is critically needed for the treatment of SCI. We previously demonstrated that SCI results in acute and permanent depletion of the neuronally derived Neuregulin-1 (Nrg-1) in the spinal cord. Increasing the dysregulated level of Nrg-1 through acute intrathecal Nrg-1 treatment enhanced endogenous cell replacement and promoted white matter preservation and functional recovery in rat SCI. Moreover, we identified a neuroprotective role for Nrg-1 in moderating the activity of resident astrocytes and microglia following injury. To date, the impact of Nrg-1 on immune response in SCI has not yet been investigated. In this study, we elucidated the effect of systemic Nrg-1 therapy on the recruitment and function of macrophages, T cells, and B cells, three major leukocyte populations involved in neuroinflammatory processes following SCI.
Methods
We utilized a clinically relevant model of moderately severe compressive SCI in female Sprague-Dawley rats. Nrg-1 (2 μg/day) or saline was delivered subcutaneously through osmotic mini-pumps starting 30 min

after SCI. We conducted flow cytometry, quantitative real-time PCR, and immunohistochemistry at acute, subacute, and chronic stages of SCI to investigate the effects of Nrg-1 treatment on systemic and spinal cord immune response as well as cytokine, chemokine, and antibody production.
Results
We provide novel evidence that Nrg-1 promotes a pro-regenerative immune response after SCI. Bioavailability of Nrg-1 stimulated a regulatory phenotype in T and B cells and augmented the population of M2 macrophages in the spinal cord and blood during the acute and chronic stages of SCI. Importantly, Nrg-1 fostered a more balanced microenvironment in the injured spinal cord by attenuating antibody deposition and expression of pro-inflammatory cytokines and chemokines while upregulating pro-regenerative mediators.
Conclusion
We provide the first evidence of a significant regulatory role for Nrg-1 in neuroinflammation after SCI. Importantly, the present study establishes the promise of systemic Nrg-1 treatment as a candidate immunotherapy for traumatic SCI and other CNS neuroinflammatory conditions.

4.1831 **Neutrophil–Hepatic Stellate Cell Interactions Promote Fibrosis in Experimental Steatohepatitis**
Zhou, Z., Xu, M-J., Cai, Y., Wang, W., Jiang, J.X., Varga, Z.V., Feng, D., Pacher, P., Kunos, G., Torok, N.J. and Gao, B.
Cell. Mol. Gastroenterol. Hepatol., ***5***, *399-413 (2018)*

Background & Aims
Hepatic infiltration of neutrophils is a hallmark of steatohepatitis; however, the role of neutrophils in the progression of steatohepatitis remains unknown.
Methods
A clinically relevant mouse model of steatohepatitis induced by high-fat diet (HFD) plus binge ethanol feeding was used. Liver fibrosis was examined. *In vitro* cell culture was used to analyze the interaction of hepatic stellate cells (HSCs) and neutrophils.
Results
HFD plus one binge ethanol (HFD+1B) feeding induced significant hepatic neutrophil infiltration, liver injury, and fibrosis. HFD plus multiple binges of ethanol (HFD+mB) caused more pronounced liver fibrosis. Microarray analyses showed that the most highly activated signaling pathway in this HFD+1B model was related to liver fibrosis and HSC activation. Blockade of chemokine (C-X-C motif) ligand 1 or intercellular adhesion molecule-1 expression reduced hepatic neutrophil infiltration and ameliorated liver injury and fibrosis. Disruption of the *p47^{phox}* gene (also called *neutrophil cytosolic factor 1*), a critical component of reactive oxygen species producing nicotinamide adenine dinucleotide phosphate-oxidase in neutrophils, diminished HFD+1B–induced liver injury and fibrosis. Co-culture of HSCs with neutrophils, but not with neutrophil apoptotic bodies, induced HSC activation and prolonged neutrophil survival. Mechanistic studies showed that activated HSCs produce granulocyte-macrophage colony-stimulating factor and interleukin-15 to prolong the survival of neutrophils, which may serve as a positive forward loop to promote liver damage and fibrosis.
Conclusions
The current data from a mouse model of HFD plus binge ethanol feeding suggest that obesity and binge drinking synergize to promote liver fibrosis, which is partially mediated via the interaction of neutrophils and HSCs. Microarray data in this article have been uploaded to NCBI's Gene Expression Omnibus (GEO accession number: GSE98153).

4.1832 **The cytoprotective role of DJ-1 and p45 NFE2 against human primary alveolar type II cell injury and emphysema**
Tan, L.H., Bahmed, K., Lin, C-R., marchatti, N., Bolla, S., Criner, G.J., kelsen, S., Madesh, M. and Kosmider, B.
Scientific Reports, ***8****:3555 (2018)*

Emphysema is characterized by irreversibly enlarged airspaces and destruction of alveolar walls. One of the factors contributing to this disease pathogenesis is an elevation in extracellular matrix (ECM) degradation in the lung. Alveolar type II (ATII) cells produce and secrete pulmonary surfactants and proliferate to restore the epithelium after damage. We isolated ATII cells from control non-smokers, smokers and patients with emphysema to determine the role of NFE2 (nuclear factor, erythroid-derived 2). NFE2 is a heterodimer composed of two subunits, a 45 kDa (p45 NFE2) and 18 kDa (p18 NFE2) polypeptides. Low expression of p45 NFE2 in patients with emphysema correlated with a high ECM degradation. Moreover, we found that NFE2 knockdown increased cell death induced by cigarette smoke

extract. We also studied the cross talk between p45 NFE2 and DJ-1. DJ-1 protein is a redox-sensitive chaperone that protects cells from oxidative stress. We detected that cigarette smoke significantly increased p45 NFE2 levels in DJ-1 KO mice compared to wild-type mice. Our results indicate that p45 NFE2 expression is induced by exposure to cigarette smoke, has a cytoprotective activity against cell injury, and its downregulation in human primary ATII cells may contribute to emphysema pathogenesis.

4.1833 Metabolic Reprogramming in Amyotrophic Lateral Sclerosis
Szelechowski, M., Amoedo, N., Obre, E., leger, C., Allard, l., Bonneu, M., Claverol, S., Lacombe, D., Oliet, S., Chevallier, S., La Masson, G. and Rossignol., R.
*Scientific Reports, **8**:3953 (2018)*

Mitochondrial dysfunction in the spinal cord is a hallmark of amyotrophic lateral sclerosis (ALS), but the neurometabolic alterations during early stages of the disease remain unknown. Here, we investigated the bioenergetic and proteomic changes in ALS mouse motor neurons and patients' skin fibroblasts. We first observed that SODG93A mice presymptomatic motor neurons display alterations in the coupling efficiency of oxidative phosphorylation, along with fragmentation of the mitochondrial network. The proteome of presymptomatic ALS mice motor neurons also revealed a peculiar metabolic signature with upregulation of most energy-transducing enzymes, including the fatty acid oxidation (FAO) and the ketogenic components HADHA and ACAT2, respectively. Accordingly, FAO inhibition altered cell viability specifically in ALS mice motor neurons, while uncoupling protein 2 (UCP2) inhibition recovered cellular ATP levels and mitochondrial network morphology. These findings suggest a novel hypothesis of ALS bioenergetics linking FAO and UCP2. Lastly, we provide a unique set of data comparing the molecular alterations found in human ALS patients' skin fibroblasts and SODG93A mouse motor neurons, revealing conserved changes in protein translation, folding and assembly, tRNA aminoacylation and cell adhesion processes.

4.1834 Phytosterols Synergize With Endotoxin to Augment Inflammation in Kupffer Cells but Alone Have Limited Direct Effect on Hepatocytes
Guthrie, G., Tackett, B., Stoll, B., Martin, C., Olutoye, O. and Burrin, D.G.
*J. Parenteral and Enteral Nutrition, **42(1)**, 37-48 (2018)*

Introduction: Phytosterols are implicated in the development of parenteral nutrition–associated liver disease. A newly proposed mechanism for phytosterol-mediated parenteral nutrition–associated liver disease is through phytosterol-facilitated hepatic proinflammatory cytokine release following exposure to intestinally derived bacteria. Whether the proinflammatory effects are liver cell specific is not known. *Aim*: To determine if phytosterols cause inflammation in hepatocytes or Kupffer cells independently or require costimulation by lipopolysaccharide (LPS). *Methods*: In an in vivo study, neonatal piglets on parenteral nutrition for 11 days received an 8-hour infusion of LPS. In the in vitro studies, neonatal piglet Kupffer cells and hepatocytes were treated with media, media + 1% soy oil, or media + 1% soy oil + 100μM phytosterols. After 24-hour incubation, cells were treated with farnesoid X receptor (FXR) agonist obeticholic acid or liver X receptor (LXR) agonist GW3965 and challenged with LPS or interleukin 1β. *Results*: LPS administration in piglets led to transient increases in proinflammatory cytokines and suppression of the transporters bile salt export pump and ATP-binding cassette transporter G5. In hepatocytes, phytosterols did not activate inflammation. Phytosterol treatment alone did not activate inflammation in Kupffer cells but, combined with LPS, synergistically increased interleukin 1β production. FXR and LXR agonists increased transporter expression in hepatocytes. GW3965 suppressed proinflammatory cytokine production in Kupffer cells, but obeticholic acid did not. *Conclusions*: LPS suppresses transporters that control bile acid and phytosterol clearance. Phytosterols alone do not cause inflammatory response. However, with costimulation by LPS, phytosterols synergistically maximize the inflammatory response in Kupffer cells.

4.1835 HDAC6 is a therapeutic target in mutant GARS-induced Charcot-Marie-Tooth disease
Benoy, V. eet al
*Brain, **141**, 673-687 (2018)*

Peripheral nerve axons require a well-organized axonal microtubule network for efficient transport to ensure the constant crosstalk between soma and synapse. Mutations in more than 80 different genes cause Charcot-Marie-Tooth disease, which is the most common inherited disorder affecting peripheral nerves. This genetic heterogeneity has hampered the development of therapeutics for Charcot-Marie-Tooth disease. The aim of this study was to explore whether histone deacetylase 6 (HDAC6) can serve as a therapeutic

target focusing on the mutant glycyl-tRNA synthetase (GlyRS/*GARS*)-induced peripheral neuropathy. Peripheral nerves and dorsal root ganglia from the C201R mutant *Gars* mouse model showed reduced acetylated α-tubulin levels. In primary dorsal root ganglion neurons, mutant GlyRS affected neurite length and disrupted normal mitochondrial transport. We demonstrated that GlyRS co-immunoprecipitated with HDAC6 and that this interaction was blocked by tubastatin A, a selective inhibitor of the deacetylating function of HDAC6. Moreover, HDAC6 inhibition restored mitochondrial axonal transport in mutant GlyRS-expressing neurons. Systemic delivery of a specific HDAC6 inhibitor increased α-tubulin acetylation in peripheral nerves and partially restored nerve conduction and motor behaviour in mutant *Gars* mice. Our study demonstrates that α-tubulin deacetylation and disrupted axonal transport may represent a common pathogenic mechanism underlying Charcot-Marie-Tooth disease and it broadens the therapeutic potential of selective HDAC6 inhibition to other genetic forms of axonal Charcot-Marie-Tooth disease.