Polymorphprep

1. **Quantitative analysis of membrane cofactor protein (MCP) of complement. High expression of MCP on human leukemia cell lines, which is down- regulated during cell differentiation**
Seya, T., Hara, T., Matsumoto, M. and Akedo, H.
J. Immunol., 145, 238 (1990)

Membrane cofactor protein (MCP) is a cell-associated regulatory molecule for C system with C3b/C4b binding and factor I-dependent cofactor activity. mAb were raised against MCP and amounts and distribution examined on normal human cells and cell lines. The mean quantity of MCP was 3000 to 7000 copies/cell in normal blood cells, except for E which have no MCP. Of note, PMN did not fully reveal all MCP sites until incubated for greater than 30 min at 37 degrees C. In most tumor cell lines, except for B cell lineages, expression of MCP increased by 2- to 8-fold in comparison with the normal cell counterparts. Strikingly, recombinant granulocyte CSF treatment of myeloid cell lines and hemin treatment of an erythroblastoid cell line, K562, led to a decrease of MCP to near normal levels. In contrast, C3b/C4b receptor (CR1) tended to increase with granulocyte-CSF treatment in several cell lines. We simultaneously determined levels of decay-accelerating factor (DAF) and CR1 in these tumor cells, and tested susceptibility to C3 deposition via activation of the alternative C pathway. Of 21 cell lines we examined, 14 lacked CR1 and two lacked DAF; none, however, lacked MCP. A slight amount of C3 deposition was observed in some myeloid cell lines and EBV-infected B cell lines. However, C3 deposition did not reflect a defect in the regulatory proteins. Tumor cells bearing MCP, lacking CR1 or DAF, and undergoing no C3 deposition, may escape C attack due to the compensatory effect of MCP in the absence of the other regulatory proteins. High expression of MCP provides a convenient means for tumor cells to block C attack and survive in blood stream. We favor the interpretation that MCP is up-regulated in association with certain malignant disorders, and that cell differentiation results in a switch from an MCP-dominant state to a CR1-dominant state.

2. **Molecular characterization and functional analysis of the leukocyte surface protein CD31**
Stockinger, H. et al
J. Immunol., 145, 3889 (1990)

The CD31 Ag is a surface glycoprotein of 130 kDa with a broad cellular distribution. We show that among peripheral human blood cells, it is expressed on monocytes, granulocytes, platelets, and a subpopulation of lymphocytes. Activation of granulocytes leads to down-regulation of CD31 molecule expression. Sequence analysis and quantitative measurements of the relatedness of the CD31 molecule to other known proteins demonstrate that it consists of six Ig constant domains and that each domain bears substantial similarity to Fc gamma R domains. We find, however, that the CD31 molecule does not bind Ig Fc domains. On human monocytes we demonstrate that CD31 mAb recognizing certain epitopes of the CD31 molecule induce the generation of reactive oxygen metabolites. No such effect was seen with human granulocytes. By using two CD31 mAb, termed 1B5 and 7E4, we analyzed the requirements for activation of the monocyte respiratory burst via CD31 Ag in more detail. We show that signal transduction occurs via formation of a CD31 Ag-mAb-Fc gamma R complex involving either Fc gamma RI (CD64) or Fc gamma RII (CDw32) molecules.

3. **Activation of human complement by IgG antisperm antibody and the demonstration of C3 and C5b-9-mediated immune injury to human sperm**
D'Cruz, O.J., Haas, G.G., Wang, B.L. and DeBault, L.E.
J. Immunol., 146, 611 (1991)

To investigate the role of C in the pathogenesis of antisperm antibody (ASA)-mediated infertility, we evaluated the binding and biologic effects of antisperm IgG and autologous C on human sperm. A flow cytometric assay using motile sperm as a target for IgG ASA+ (n = 30) and ASA- (n = 5) sera was developed for the concomitant detection of sperm-bound IgG and the initial (C3d) and terminal (C5b-9) C components on the surface of human sperm. Of the 30 IgG ASA+ sera evaluated by flow cytometry, 15 (50%) and 22 (73.3%) sera were also positive for sperm-bound C3d and C5b-9, respectively. Monomeric IgG purified from C- fixing ASA+ serum was able to bind to sperm and induced deposition of C3 on the sperm surface in the presence of human C. Incubation of motile sperm with C-fixing immune sera resulted in a significant loss (43 to 87%) of motility associated with characteristic C5b-9-induced alterations in sperm morphology leading ultimately to sperm lysis. When motile sperm were cocultured with purified polymorphonuclear leukocytes (PMN) in the presence of C-fixing immune sera, the binding of sperm heads to the PMN resulted in the formation of sperm rosettes, whereas non C-fixing or control sera had no such effect. Transmission electron microscopy of thin sections of the rosettes revealed ingestion of the sperm by the human PMN. These data suggested that 1) antibody bound to sperm is capable of activating autologous C by the classical pathway; 2) binding of both IgG and C proteins initiates C3-mediated sperm binding to PMN and sperm inactivation by deposition of membrane attack complex (MC5b-9) of C; and 3) concomitant detection of sperm-bound IgG, C3d, and C5b-9 may serve as an indicator of C-fixing cytotoxic ASA in the sera of infertile couples.

4. **Enhanced modulation of antibodies coating guinea pig leukemic cells in vitro and in vivo. The role of Fc gamma R expressing cells**
Lane, A.C., Foroozan, S., glennie, M.J., Kowalski-Saunders, P. and Stevenson, G.T.
J. Immunol., 146, 2461 (1991)

We have investigated the antigenic modulation induced by a number of antibody fragments and derivatives directed against the idiotype of the surface Ig of the L2C guinea pig B lymphoblastic leukemia, and studied the effects upon such modulation of the simultaneous presence of cells expressing Fc gamma receptors (FcR). In vitro studies confirmed previous work showing that antibody bivalency is required to induce modulation in vitro in simple systems. However, in the presence of isolated Kupffer cells, Fc-containing univalent antibodies were found to induce significant antigenic modulation, and the modulation induced by intact IgG was also found to be more rapid and extensive. Fragments that did not contain Fc regions behaved similarly in the presence or absence of Kupffer cells. Further investigations demonstrated that all three classes of human FcR can mediate modulation enhancement, and suggest that the mechanism involves indirect cross-linking of cell surface Ag via the antibody and effector cell FcR. In vivo studies showed that univalent antibody derivatives containing Fc regions did induce antigenic modulation, but that this was significantly reduced in comparison with bivalent antibodies, confirming their potential advantage for immunotherapy.

5. **Conversion of monocyte chemoattractant protein-1 into a neutrophil attractant by substitution of two amino acids**
Beall, C.J., Mahajan, S. and Kolattukudy, P.E.
*J. Biol. Chem., **267**, 3455-3459 (1992)*

The small cytokine monocyte chemoattractant protein-1 has structural similarity to the neutrophil chemoattractant interleukin-8, but each protein is specific in attracting its own target cell. To investigate the structural basis of this cell type specificity, we have developed an Escherichia coli expression system for the monocyte chemoattractant and mutagenized selected amino acid residues to ones found at the corresponding positions of interleukin-8. We find that a double mutation of tyrosine 28 and arginine 30 to leucine and valine, respectively, causes a drastic decrease in chemotactic activity toward monocytes with the appearance of a novel (interleukin-8-like) neutrophil chemotactic activity. Computer graphic analysis predicts that, with the double substitution, a putative receptor binding groove of the monocyte chemoattractant protein would become topographically similar to that of interleukin-8. We therefore postulate that one or both of these amino acid residues are part of the binding contact of these small cytokines and their receptors.

6. **Phagocytosis of immunoglobulin G and C3-bound human sperm by human polymorphonuclear leukocytes is not associated with the release of oxidative radicals**
D'Cruz, O.J., Wang, B.L. and Haas, G.G.
Biol. Reprod., 46, 721 (1992)

Antisperm antibody (ASA)- and complement (C)-mediated immune injury to human sperm is thought to be caused in part by phagocytic neutrophils. To investigate this process, we co-cultured purified human polymorphonuclear leukocytes (PMN) with swim-up sperm in the presence of ASA-positive and ASA-negative sera and assayed for PMN respiratory burst activity, monitored by the release of superoxide anion (O2-) and hydrogen peroxide (H2O2). Phorbol myristate acetate (PMA) and opsonized zymosan were used as positive controls. Phagocytosis of ASA-positive and C-bound sperm by PMN did not enhance O2- production when compared to incubation of sperm with ASA-negative sera. Phagocytosis of ASA- positive and C-bound sperm also resulted in minimal release of H2O2 when compared with ASA-positive and C-negative sperm that were not phagocytosed. In contrast, PMN were maximally stimulated to release O2- in response to either opsonized zymosan or PMA. The kinetics of PMA- induced O2- release was unaffected by the presence of ASA-positive and C-bound sperm. Cytocentrifuge preparations of PMN incubated with ASA- positive and C-bound sperm revealed limited O2- release at the site of PMN/sperm contact. These results indicated that 1) phagocytosis of motile sperm by PMN requires the binding of both ASA and C to the sperm surface; 2) phagocytosis of ASA-positive and C-positive sperm by PMN fails to release reactive oxygen species; and 3) metabolic processes associated with PMN respiratory burst activity may not be coupled to the ingestion of ASA-positive and C-bound sperm.

7. **Inhibition of neutrophil chemotaxis and activation following decentralization of the superior cervical ganglia**
Carter, L., Ferrari, J.K., Dawson, J.S. and Befus, D.
J. Leukoc. Biol., 51, 597 (1992)

Recent studies have shown that bilateral decentralization (sympathectomy) of the superior cervical ganglia (SCG) of rats sensitized to the parasite Nippostrongylus brasiliensis attenuated the development of pulmonary inflammation following allergen challenge. Sympathectomy inhibited total leukocyte infiltration into lung lavage fluids, particularly neutrophil infiltration. To define the effects of decentralization of the SCG on neutrophil responses, peripheral blood neutrophils of rats were isolated and tested in in vitro chemotaxis and phagocytosis assays. Neutrophils from rats that were sympathectomized 7 days previously displayed a marked reduction in chemotaxis to N-formyl-methionyl-leucyl-phenylalanine and leukotriene B4 compared to neutrophils from sham-operated or unoperated groups. Although the degree of chemotaxis was greater in blood neutrophils from parasite-infected rats than from uninfected rats, sympathectomy markedly reduced the chemotactic responses of both groups. In addition, neutrophils of sympathectomized rats were

unresponsive to lipopolysaccharide-induced metabolic activation as assessed by in vitro phagocytosis and oxidative reduction of nitroblue tetrazolium. Thus, decentralization of the SCG of rats affects the chemotactic responses and functions of neutrophils. Understanding the role of the sympathetic nervous system in modulating the behavior of neutrophils will shed light on the interactions between the nervous and immune systems.

8. Thymosin beta 4 sequesters the majority of G-actin in resting human polymorphonuclear leukocytes
Cassimeris, L., Safer, D., Nachmias, V.T. and Zigmond, S.H.
J. Cell Biol., 119(5), 1261-1270 (1992)

Thymosin beta 4 (T beta 4), a 5-kD peptide which binds G-actin and inhibits its polymerization (Safer, D., M. Elzinga, and V. T. Nachmias. 1991. J. Biol. Chem. 266:4029-4032), appears to be the major G-actin sequestering protein in human PMNs. In support of a previous study by Hannappel, E., and M. Van Kampen (1987. J. Chromatography. 397:279- 285), we find that T beta 4 is an abundant peptide in these cells. By reverse phase HPLC of perchloric acid supernatants, human PMNs contain approximately 169 fg/cell +/- 90 fg/cell (SD), corresponding to a cytoplasmic concentration of approximately 149 +/- 80.5 microM. On non- denaturing polyacrylamide gels, a large fraction of G-actin in supernatants prepared from resting PMNs has a mobility similar to the G- actin/T beta 4 complex. Chemoattractant stimulation of PMNs results in a decrease in this G-actin/T beta 4 complex. To determine whether chemoattractant induced actin polymerization results from an inactivation of T beta 4, the G-actin sequestering activity of supernatants prepared from resting and chemoattractant stimulated cells was measured by comparing the rates of pyrenyl-actin polymerization from filament pointed ends. Pyrenyl actin polymerization was inhibited to a greater extent in supernatants from stimulated cells and these results are qualitatively consistent with T beta 4 being released as G- actin polymerizes, with no chemoattractant-induced change in its affinity for G-actin. The kinetics of bovine spleen T beta 4 binding to muscle pyrenyl G-actin are sufficiently rapid to accommodate the rapid changes in actin polymerization and depolymerization observed in vivo in response to chemoattractant addition and removal.

9. Expression of 92-kDa Type IV Collagenase mRNA by Eosinophils Associated with Basal Cell Carcinoma
Stahle-Backdahl, M., Sudbeck, B.D., Eisen, A.Z., Welgus, H.G. and Parks, W.C.
*J. Invest. Dermatol., **99(4)**, 497-503 (1992)*

Metalloproteinases are thought to be important for tumor invasion and metastasis. We used in situ hybridization with 35S-labeled cRNA probes to localize sites of expression for 92-kDa type IV collagenase mRNA in sections of nodular basal cell carcinoma. Positive signal for 92-kDa type IV collagenase mRNA was detected in eosinophilic granulocytes within inflammatory infiltrates surrounding the tumor nodules. Eosinophils, however, were not adjacent to tumor cells, suggesting that metalloenzyme production by these granulocytes in this disease may be targeted more to stromal components than to remodeling or destruction of the basement lamina. The identity of the eosinophils was confirmed by cell morphology and specific histochemical staining. No resident or other migratory cells were positive for enzyme mRNA in these samples. Signal specificity for in situ hybridization was shown by a duplication of the results with complementary oligomeric probes and by a lack of signal in sections hybridized with a sense RNA probe or nonspecific oligomer. No signal for 92-kDa type IV collagenase mRNA was detected in circulating eosinophils or in eosinophils associated with Hodgkin's lymphoma. These data suggest that eosinophils migrate into the dermis and express type IV collagenase in response to basal cell carcinoma and that this process may have a role in tumor growth.

10. Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages
Heinecke, J.W., Li, W., Daehnke, H.L. and Goldstein, J.A.
*J. Biol. Chem., **268**, 4069-4077 (1993)*

Myeloperoxidase, secreted by activated phagocytes, produces the powerful cytotoxin hypochlorous acid from H2O2 and Cl-. We show that the enzyme can also employ H2O2 to oxidize L-tyrosine to tyrosyl radical, yielding the stable cross-linked product dityrosine. Dityrosine synthesis by the myeloperoxidase-H2O2 system did not require halide and was partially inhibited by Cl-. At physiological concentrations of Cl-, L-tyrosine, and other plasma amino acids, purified myeloperoxidase utilized 26% of the H2O2 in the reaction mixture to form dityrosine. Aminotriazole, cyanide, and azide inhibited the reaction. Phorbol ester-stimulated human neutrophils and monocyte- derived macrophages similarly generated dityrosine from L-tyrosine by a pathway inhibited by catalase, aminotriazole, and azide. The requirement for H2O2 and the inhibition by heme poisons suggest that activated phagocytes synthesize dityrosine by a peroxidative mechanism. These results indicate that L-tyrosine can compete effectively with Cl- as a substrate for myeloperoxidase and raise the possibility that formation of tyrosyl radical may play a role in the phagocyte inflammatory response. Because dityrosine is protease-resistant, stable to acid hydrolysis, and intensely fluorescent, its identification in tissues may pinpoint targets where phagocytes inflict oxidative damage in vivo.

11. Neutrophil CD14: biochemical properties and role in the secretion of tumor necrosis factor-alpha in response to lipopolysaccharide
Haziot, A., Tsuberi, B.Z. and Goyert, S.M.

*J. Immunol., **159**, 5556-5565 (1993)*

CD14 is a myeloid cell differentiation Ag expressed primarily by monocytes and macrophages. CD14 has recently been shown to function as a receptor for a complex of LPS and LPS binding protein (LBP), an acute phase serum protein also present in normal serum in trace amounts. In the presence of LBP, LPS strongly activates monocytes via CD14 as measured by TNF secretion. This pathway of monocyte activation is thought to be a major contributor to the symptoms of endotoxin shock. Another major cell type involved in the response to Gram-negative infection is the neutrophil. Recent studies have shown that neutrophils also express CD14 and suggest that they can respond to LPS through a similar pathway. However, the biochemical nature of neutrophil CD14 has not previously been described. In this report, we have analyzed several biochemical characteristics of neutrophil CD14. We show that CD14 is actively synthesized by neutrophils as a glycosylphosphatidyl-inositol- anchored protein, indistinguishable in size from monocyte CD14. Furthermore, neutrophils, like monocytes, shed a smaller soluble form of CD14 into culture supernatants. In addition, like monocytes, neutrophils respond to LPS/LBP complexes via CD14 by releasing TNF- alpha. The described properties and function of neutrophil CD14 suggest that it may directly participate in the acute inflammatory response and in endotoxin shock.

12. Response of blood leukocytes to thrombin receptor peptides
Hoffmann, M. and Church, F.C.
*J. Leukoc. Biol., **54**, 145 (1993)*

Thrombin has receptor-mediated effects on a variety of cell types. A recently cloned platelet thrombin receptor exerts its effects by a tethered-ligand mechanism. A similar receptor was shown in at least two nonplatelet cell types, fibroblasts and endothelial cells. Thrombin has biologically important effects on leukocytes, but the type of receptor mediating the effects is not known. Therefore, we examined the responses of monocytes, neutrophils, and lymphocytes to thrombin and to an agonist specific for the platelet-type thrombin receptor. We compared the effects of a peptide (SFLLRNPNDKYEPF) corresponding to residues 42-55 of the cloned platelet thrombin receptor on calcium flux in platelets and leukocytes. The thrombin receptor peptide induced increases in intracellular calcium in platelets and monocytes that reached a maximum at 5 microM peptide. The maximal increase was similar in magnitude to the response to thrombin. Lymphocytes showed a small and variable increase in intracellular calcium in response to thrombin or the thrombin receptor agonist. The thrombin receptor peptide had no effect on neutrophil calcium concentrations. When the amino acid corresponding to Arg 46 was replaced with Ala in the synthetic peptide, the ability to increase intracellular calcium was abolished for both platelets and monocytes. The peptide instead had thrombin antagonist activity. Thus, monocytes respond to thrombin receptor peptides similarly to platelets. We conclude that human monocytes possess a thrombin receptor similar to that present on platelets. Furthermore, the residue corresponding to Arg 46 of the thrombin receptor is critical for receptor agonist activity.

13. Granulocytes enhance LPS-induced tissue factor activity in monocytes via an interaction with platelets
Halvorsen, H., Olsen, J.O: and Osterud, B.
*J. Leukoc. Biol., **54**, 275 (1993)*

In the present study we have investigated the effect of platelets and granulocytes on bacterial lipopolysaccharide (LPS)-induced tissue factor (TF) activity in monocytes. Experiments were performed on freshly isolated cells resuspended in heparinized plasma and recombined with platelet-poor or platelet-rich plasma. In a platelet-dependent reaction the granulocytes enhanced LPS-induced TF activity by an average of 100%. The effect was dose dependent with regard to the number of both granulocytes and platelets, respectively. Granulocytes and/or platelets did not affect LPS-induced tumor necrosis factor (TNF) secretion from monocytes. Phorbol myristate acetate (PMA) per se was not able to induce TF activity in our system. In contrast, the agonist caused a substantial increase in TF activity induced by LPS. The effect was totally dependent on the presence of platelets and was shown to be due to stimulation of both granulocytes and monocytes (the activity rose from 30 +/- 7 to 83 +/- 12 mU/10(6) cells in the presence of platelets and from 69 +/- 8 to 143 +/- 22 mU/10(6) cells in the presence of platelets and granulocytes). Effects similar to those observed with PMA were obtained with physiological concentrations (10 ng/ml) of TNF. A combination of these two agonists gave no further amplification of LPS-induced TF activity compared with the effect of the agonists separately. Low concentrations of a monoclonal anti-CD15 antibody abolished the stimulatory effects of platelets and granulocytes. Furthermore, the anti-CD15 antibody neutralized the effect of TNF, whereas the PMA effect was reduced by almost 75%. These results were confirmed in a whole-blood system. The inhibitory effect of the antibody may be associated with CD15's role as a complementary ligand for PADGEM. Our study indicates that a close interaction between granulocytes, platelets, and monocytes is essential for optimal TF activity induced by LPS. It is hypothesized that the effect of granulocytes is related to their ability to activate platelets. We propose that upon activation granulocytes secrete a product that enhances the capacity of platelets to stimulate TF activity in monocytes.

14. Regulation of CD11b/CD18 expression in human neutrophils by phospholipase A2
Jacobson, P.B: and Schrier, D.J.
*J. Immunol., **151**, 5639 (1993)*

Recent evidence suggests that phospholipase A2 (PLA2)-derived lipid mediators may regulate a number of neutrophil responses including degranulation and adhesion. In view of the potential role of PLA2 in stimulus-secretion coupling, we examined the

relationship between PLA2 activation and the surface expression of CD11b/CD18 (MAC-1) in human polymorphonuclear leukocytes (hPMNL), including the functional consequences of PLA2 inactivation on MAC-1-dependent adhesion. The selective inhibition of PLA2 by the marine natural products manoalide (MLD) and scalaradial (SLD) blocks [3H]arachidonic acid (AA) release in calcium ionophore A23187-stimulated neutrophils, and also inhibits secretion of specific and azurophilic granule constituents. Additional studies demonstrate that MLD, SLD, and other less potent PLA2 inhibitors such as 4-bromophenacylbromide and nordihydroguiaretic acid inhibit the surface expression of MAC-1 (IC50: MLD, 0.33 microM; SLD, 0.23 microM; 4-bromophenacylbromide, 2.8 microM; NDGA, 3.5 microM) at concentrations similar to those at which they inhibit [3H]AA release. Inhibitors of cyclooxygenase, 5-lipoxygenase, protein kinase C, or calcium channel antagonists have no effect on MAC-1 expression. PLA2 inactivation also prevents MAC-1 up-regulation in hPMNL stimulated with FMLP, IL-8, TNF-alpha, PMA, or platelet activating factor. In FMLP- stimulated hPMNL, under conditions in which no secondary granule constituents are secreted, MAC-1 and alkaline phosphatase up-regulation from intracellular granules is inhibited by MLD and SLD. Functional assays also demonstrate that MLD and SLD block MAC-1-dependent adhesion of activated neutrophils to keyhole limpet hemocyanin at concentrations that block the surface expression of MAC-1. [3H]AA release and MAC-1 expression in MLD and SLD-treated hPMNL could be recovered in the presence of 1 mM hydroxylamine in a time-dependent fashion, consistent with reported data that MLD and SLD inactivate PLA2 through Schiff base formation. In summary, these data emphasize the role of PLA2 as a key regulator of MAC-1 expression in models of neutrophil adhesion.

15. Quartz selectively down-regulates CR1 on activated human granulocytes
Lundahl, J et al
J. Leukoc. Biol., 53, 99 (1993)

We have investigated the interaction between quartz and granulocytes with respect to complement receptor expression. When N-formylmethionyl-leucyl-phenylalanine (fMLP)-stimulated leukocytes were exposed to quartz at 37 degrees C, CR1 was down-regulated but CR3 was not affected. This was a direct effect on granulocytes because it occurred in a similar fashion when mixed leukocyte suspensions and isolated granulocyte populations were used as targets for quartz. The observed down-regulation by quartz was not affected by the microfilament-disrupting agent cytochalasin B and the total detectable pool of CR1 was reduced after quartz exposure. When protease inhibitors, such as aprotinin or phenylmethanesulfonyl fluoride, were present during quartz exposure, the down-regulation of CR1 was less pronounced, but this was not the case not when protease inhibitors such as EDTA-Na2 and pepstatin were present. Exposure to quartz was not accompanied by a pronounced release of beta-glucuronidase (marker for the primary granules) or vitamin B12 binding protein (marker for the secondary granules). In contrast to quartz, exposure to alumina did not affect the expression of CR1 and CR3. The spontaneous mobilization of CR1 at 37 degrees C was reduced when quartz was present but the CR3 mobilization was unaffected. Our results indicate that quartz induces a granule protease-dependent selective shedding of CR1 but not CR3 despite a low degree of degranulation.

16. Pili and the interaction of Aeromonas species with humanperipheral blood polymorphonuclear cells
Kamperman, L. and Kirov, S.M.
FEMS Immunol. Med. Microbiol., 7(2), 187-195 (1993)

The interaction of differentiallu piliated *Aeromonas* strains expressing pili of two broadly different morphologic types (short, rigid (S/R) and/or long, wavy (L/W)) with human peripheral blood mononuclear leukocytes (PMN) was investigated to determine whether host defense cells might exert a selective pressure on pili expression in vivo accounting for the different pili phenotypes of clinical and environmental strains. A majority of *Aeromonas veronii* biotype sobria strains from water (6/6) and faeces (8/11) readily associated with PMN (>60% PMN with adherent and/or internalised bacteria), irrespective of their degree, or predominant type, of piliation. Rigid pili of *Aeromonas* species did not promote interaction with PMN. However, the majority (55%) of strains which interacted well with PMN were adherent to HEp-2 cells. Interactio with PMN is unlikely to be the reason few S/R pili are seen on faecal strains, but it may be a selective pressure on L/W adhesive pili, or other OMP adhesins, resulting in the shedding of strains which have lost critical adhesins.

17. Sulfite stimulates NADPH oxidase of human neutrophils to produce active oxygen radicals via protein kinase C and Ca2+/calmodulin pathways
Beck-Speier, I., Liese, J.G., Belohradsky, B.H. and Godleski, J.J.
Free Rad. Biol. Med., 14(6), 661-668 (1993)

The effect of sulfite on the oxidative metabolism of human neutrophils was studied in vitro. Superoxide anion production of PMN was determined using superoxide dismutase-inhibitale lucigenin-dependent CL. The addition of sulfite in concentration of 0.01 mM–1 mM results in an up to 6-fold increase in CL of nonstimulated neutrophils at 37°C and pH 7. Neutrophils stimulated with zymosan or PMA have an additional 2-fold stimulation when sulfite is added. Higher sulfite concentrations (2 mM –10 mM) decrease the CL of both nonstimulated and stimulated cells. The activity of NADPH oxidase, responsible for $O_2^{\cdot-}$ production, is significantly increased in neutrophils incubated with 1 mM sulfite. Neutrophils from patients with chronic granulomatous disease, which are cytochrome b_{558} negative or have $p47^{phox}$ deficiency, exhibit no significant NADPH oxidase activity and show no increase in CL by sulfite. Inhibitors of protein kinase C, H7, and calphostin C, as well as inhibitors of CA^{2+}- and calmodulin-dependent processes, W7 and R 24 571, completely inhibited the increased CL of sulfite-treated neutrophils. These findings indicate that sulfite in low concentrations stimulates

neutrophils to produce superoxide anions by activation of NADPH oxidase through a signal transduction pathway involving protein kinase C and Ca^{2+}/calmodulin.

18. Distribution profile and properties of peripheral-type benzodiazepine receptors on human hemopoietic cells
Canat, X. et al
Life Sciences, 52(1), 107-118 (1993)

The cellular localization of peripheral-type benzodiazepine receptors (PBRs) was characterized in several human blood cell subpopulations including erythrocytes, platelets, monocytes and polymorphonuclear neutrophils (PMN), B, NK, T8 and T4-cells. Pharmacological properties of the PBR were established by binding studies and PBR mRNA expression was measured by quantitative polymerase chain reaction based method. These data clearly indicate 1) the PBR is pharmacologically homogeneous in the various types of blood cells, 2) the rank order of PBR cell density is monocytes = PMN > lymphocytes ≫ platelets > erythrocytes, 3) the PBR appears to be transcriptionally regulated since mRNA levels are roughly correlated with PBR density.

19. Benzodiazepines and their solvents influence neutrophil granulocyte function
Weiss, M., Mirow, N., Birkhahn, A., Schneider, M. and Wernet, P.
Br. J: Anaesth., 70(3), 317-321 (1993)

We have examined the effects of commercially available preparations and drug-free so/vents of diazepam (Valium, Diazepam-Lipuro) and midaz-olam (Dormicum) by N-formylmethionyl-leucyl-phenylalanine (FMLP)- and zymosan-induced polymorphonuclear cell (PMN) chemilumines-cence and in a cell-free chemiluminescence system. In the case of Valium, drug-free solvent and diazepam suppressed PMN chemiluminescence. With Diazepam-Lipuro, the so/vent stimulated and diazepam inhibited PMN chemiluminescence. With midazolam (Dormicum), only the active drug depressed PMN chemiluminescence. FMLP-induced PMN chemiluminescence was depressed 10–100 fold more by diazepam and midazolam than zymosan-induced chemiluminescence.

20. Regulation of interleukin-8 expression in porcine alveolar macrophages by bacterial lipopolysaccharide
Lin, G. et al
*J. Biol. Chem., **269**, 77-85 (1994)*

Interleukin (IL)-8 is a macrophage-derived neutrophil chemotactic factor that plays an important role in the recruitment of neutrophils to inflammatory loci. Hence, expression of IL-8 by alveolar macrophages may be a significant factor in host defense in the lung and in the pathogenesis of pneumonia in swine. To initiate molecular studies of IL- 8 regulation in pigs, we cloned IL-8 cDNA and examined the regulation of its mRNA in alveolar macrophages. The porcine IL-8 cDNA consists of 1491 base pairs including a coding region of 309 base pairs. The deduced amino acid sequence was 75 and 81% similar to human and rabbit IL-8, respectively. Resting macrophages contained low levels of IL-8 mRNA, which increased markedly after exposure to bacterial lipopolysaccharide (LPS). LPS induction of IL-8 was direct, not mediated through elevation of tumor necrosis factor or interleukin-1. The effect of LPS on IL-8 expression was dose dependent, and induction was observed at a concentration of 10 pg/ml. IL-8 mRNA expression was detectable within 0.5 h after stimulation with LPS, peaked at 3-6 h at about 30-fold higher levels than in resting cells, and was maintained for 24 h. Secreted IL-8, measured by neutrophil chemotaxis, was induced within 4 h by LPS, and accumulated in the media throughout the 24-h period. The mechanism of induction of IL-8 mRNA appeared to involve transcription and RNA processing. Nuclear run-on analysis showed that the IL-8 gene was actively transcribed in noninduced cells; upon stimulation with LPS, the rate of IL-8 transcription was increased about 4-fold. A single mature mRNA species was detected by primer extension analysis. The half-life of IL-8 mRNA transcripts in aveolar macrophages was approximately 2 h and did not change after LPS stimulation. The ability of LPS to induce IL-8 expression was suppressed by recombinant human IL-4 and dexamethasone in a concentration-dependent manner. These observations indicate that the expression of IL-8 is an early event in the sequelae to bacterial infection in the lung.

21. 2-aminofluorene metabolism and DNA adduct formation by mononuclear leukocytes from rapid and slow acetylator mouse strains
Levy, G.N., Chung, J-G. and Weber, W.W.
*Carcinogenesis, **15**, 353-357 (1994)*

Following exposure of mice to the arylamine carcinogen 2-aminofluorene, DNA-carcinogen adducts can be found in the target tissues liver and bladder, and also in circulating leukocytes. Evidence is presented here that mouse mononuclear leukocytes (MNL) are capable of metabolizing 2-aminofluorene to DNA-binding metabolites which give rise to the adducts found in the MNL. Both lymphocytes and monocytes were able to acetylate arylamines during 18 h of culture. The degree of acetytation was determined by the *N*-acetyltransferase genotype of the mice as shown through use of acetylator congenic strains which differ only in the *Nat*-2 gene. Cultured MNL from rapid acetylator mice (C57BL/6J and A.B6-*Nat*^{1}) produced about twice as much N-acetylaminofluorene from 2-aminofluorene and 6- to 8-

fold as much N-acetyl-p-amino-benzoic acid from *p*-aminobenzoic acid as cells from slow acetylator mice (B6.A-*Nat*^{5} and A/J). Other differences in arylamine metabolism by MNL in culture were observed and shown to be due to genetic factors, currently unidentified, other than *N*-acetyltransferase. DNA adduct formation following incubation of MNL with the arylamine carcinogen 2-aminofluorene was related to both acetylation capacity and to other genetic metabolic factors in the mouse genome. MNL from rapid acetylator mice with the C57BL/6J background (B6) had 3-fold the DNA adduct levels of cells from the corresponding slow acetylator congenic (B6.A-*Nat*^{s}). Similarly, MNL from rapid acetylator mice with the A/J background (A.B6-*Nat*^{r}) had twice the DNA adduct levels of those from their corresponding slow congenic (A). Adduct levels in MNL from C57BL/6J were nearly the same as those of MNL from A/J, again indicating the involvement of loci other than acetylation in DNA adduct formation. The finding of genetically dependent arylamine carcinogen metabolism and DNA adduct formation in cultured MNL suggests the possibility of using cultured MNL for assessing individual susceptibility to arylamine-induced DNA damage.

22. Targeting HIV-1 to Fc gamma R on human phagocytes via bispecific antibodies reduces infectivity of HIV-1 to T cells
Howell, A.L., Guyre, P.M. and Fanger, M.W.
*J. Leukoc. Biol., **55**, 385 (1994)*

In addition to CD4, the primary receptor to which the human immunodeficiency virus type 1 (HIV-1) binds, mononuclear phagocytes (monocytes) express three classes of Fc receptors for immunoglobulin G (Fc gamma R). We have previously shown that infection of monocytes by HIV-1 is inhibited when bispecific antibodies (BsAbs) are used to target the virus to either the type I, type II, or type III Fc gamma R on these cells. Infection of monocytes was not inhibited when HIV-1 was targeted to either human leukocyte antigen class I or CD33. We have extended these studies to examine the ability of BsAbs plus polymorphonuclear leukocytes (neutrophils, PMNs) and monocytes to reduce infectivity of HIV-1 to cells from the human T cell lymphoma line, H9. The production of HIV-1 following interaction of virus with BsAb and phagocytes was determined in an indicator cell assay by mixing BsAb, HIV-1, and phagocytes with uninfected H9 cells. Productive infection of H9 cells was quantitated on subsequent days by measuring p24 gag antigen levels in supernatants by enzyme-linked immunosorbent assay. Our findings show that the addition of interferon-gamma-activated PMNs or monocytes to cultures of HIV-1 plus H9 cells in the absence of BsAb results in a marked reduction in p24 levels equivalent to 85 to 90% of control levels. With the combination of BsAb (anti-Fc gamma RI x anti-gp120) plus IFN-gamma-activated phagocytes, levels of p24 in H9 cultures were below those at culture initiation. These findings demonstrate that IFN-gamma-activated phagocytes can affect the natural course of HIV-1 infection of T cells, a finding of potential clinical importance.

23. Inositol polyanions. Noncarbohydrate inhibitors of L- and P-selectin that block inflammation
Cecconi, O. et al
J. Biol. Chem., 269, 15060-15066 (1994)

Selectins are cell adhesion molecules known to support the initial attachment of leukocytes to inflamed vascular endothelium through their recognition of carbohydrate ligands such as the tetrasaccharide sialyl Lewisx (Neu5Ac alpha 2-3Gal beta 1-4(Fuc alpha 1-3)GlcNAc-). In the present study, we describe the inhibition of L- and P-selectin function by inositol polyanions, simple 6-carbon ring structures that have multiple ester-linked phosphate or sulfate groups. In a purified component competition assay, binding of L- and P-selectin-Ig fusion proteins to immobilized bovine serum albumin-sialyl Lewisx neoglycoprotein was inhibited by inositol hexakisphosphate (InsP6, IC50 = 2.1 +/- 1.4 microM and 160 +/- 40 microM), by inositol pentakisphosphate (InsP5, IC50 = 1.4 +/- 0.2 and 260 +/- 40 microM), and by inositol hexakissulfate (InsS6, IC50 = 210 +/- 80 microM and 2.8 +/- 0.9 mM); E-selectin-Ig binding was unaffected. Inositol polyanions diminished the adhesion of LS180 colon carcinoma cells to plates coated with L- and P-selectin-Ig but not with E-selectin-Ig. Inositol polyanions blocked polymorphonuclear leukocyte (PMN) adhesion to COS cells expressing recombinant transmembrane P-selectin but not to those expressing E-selectin. In addition, inositol polyanions diminished PMN adhesion to activated endothelial cells under rotation-induced shear stress, a process known to require L-selectin function. In vivo, the effects of inositol polyanions were studied in two murine models of acute inflammation. Intravenously administered InsP6 (two doses of 40 mumol/kg) inhibited PMN accumulation in thioglycolate-induced inflammation (55 +/- 10% inhibition) and in zymosan-induced inflammation (61 +/- 4% inhibition). InsP5 and InsS6 also inhibited inflammation in these models, although higher doses were required for InsS6. In conclusion, inositol polyanions are noncarbohydrate small molecules that inhibit L- and P-selectin function in vitro and inflammation in vivo.

24. Lipopolysaccharide enhances CD11b/CD18 function but inhibits neutrophil aggregation
Lynam, E.B., Simon, S.I., Rochon, Y.P. and Sklar, L.A.
*Blood, **83**, 3303-3311 (1994)*

Human neutrophils are primed in the presence of complexes of lipopolysaccharide (LPS) with its serum binding protein (LBP) in a manner dependent on CD14. Cellular consequences of priming include increased responsiveness, the upregulation of surface proteins including the adhesive integrin CD11b/CD18 (Mac-1), the increased binding of certain ligands to CD11b/CD18, and the concurrent shedding of the L-selectin homing receptor. Because expression of both CD11b/CD18 and L-selectin is obligatory for formyl peptide-stimulated neutrophil aggregation in vitro (Simon et al, Blood 82:1097, 1993), we have examined the consequences of bacterial endotoxin on the expression of neutrophil adhesive molecules. We observed that the exposure of neutrophils to LPS/LBP, while

enhancing the surface numbers and adhesive function of CD11b/CD18 for latex particles, did not induce aggregation. In contrast, as the LPS/LBP concentration increased (ED50 = 30 ng/mL LPS/LBP), the ability of neutrophils to aggregate decreased in parallel with the shedding of L-selectin. Moreover, when L-selectin adhesive activity was blocked by treatment with Fab fragments of Dreg- 200, aggregation was inhibited to an extent roughly proportional to the available L-selection. Blocking of LPS/LBP with CD14-specific monoclonal antibodies suppressed L-selectin shedding and preserved formyl peptide-stimulated aggregation. Taken together, the data suggest that inhibition of neutrophil aggregation by LPS/LBP is related to the expression of L-selectin via CD14 rather than LPS inhibition of CD11b/CD18 function during cellular stimulation.

25. The active monomeric form of macrophage inflammatory protein-1 alpha interacts with high- and low-affinity classes of receptors on human hematopoietic cells
Avalos, B.R. et al
*Blood, **84**, 1790-1801 (1994)*

Macrophage inflammatory protein-1 alpha (MIP-1 alpha) and its human homologue GOS19.1/LD78 are members of the C-C chemokine/intercrine family of secreted proteins. They have proinflammatory properties and also inhibit cell cycle progression of hematopoietic stem cells. Characterization of MIP-1 alpha receptor(s) has been confused because of its reported aggregation to inactive forms. Using a defined monomeric form of MIP-1 alpha that is biologically active for stem cell inhibition and induction of oxidative metabolism in polymorphonuclear cells, we report the detection of high- and low-affinity receptor classes on human leukemic CD34+ blast cells, promyelocytic cells, monocytes, peripheral blood neutrophils, and T cells. Both high- and low-affinity classes are expressed simultaneously in promyelocytes and neutrophils. The calculated kd for high-affinity receptors correlates with the concentrations of MIP-1 alpha required to induce a biologic effect on stem cells and neutrophils. Cross-linking studies show that MIP-1 alpha associates with two cell surface proteins with apparent molecular masses of 92 kD and 52 kD. Direct competition binding studies combined with studies on the inhibition of stem cells show that human and murine MIP-1 alpha have different receptor-binding and biologic properties.

26. Exercise-induced changes in immune function: effects of zinc supplementation
Singh, A., Failla, M.L. and Deuster, P.A.
J. Appl. Physiol., 76, 2298 (1994)

To examine the effect of zinc (Zn) supplementation on exercise-induced changes in immune function, five male runners were randomly assigned in a double-blind crossover design to take a supplement (S; 25 mg of Zn and 1.5 mg of copper) or placebo (P) twice daily for 6 days. On morning 4 of each phase, 1 h after taking S or P, subjects ran on a treadmill at 70-75% of maximal oxygen uptake until exhaustion (approximately 2 h). Blood samples were obtained before (Pre), immediately after (Post), and 1 (Rec1) and 2 (Rec2) days after the run. [3H]thymidine incorporation by mitogen-treated mononuclear cell cultures was significantly lower ($P < 0.05$) Post than Pre, Rec1, or Rec2 for both S and P. Respiratory burst activity of isolated neutrophils was enhanced after exercise with P but not with S (P: Pre 12.0 +/- 1.1 vs. Post 17.6 +/- 2.3 nmol O2-/10(6) cells; S: Pre 11.7 +/- 0.3 vs. Post 12.1 +/- 1.2 nmol O2-/10(6) cells). Thus supplemental Zn blocked the exercise-induced increase in reactive oxygen species. Whether this antioxidant effect of Zn will benefit individuals exposed to chronic physical stress remains to be determined.

27. Structural requirements of platelet chemokines for neutrophil activation
Yan, Z. et al
Blood, 84, 2329-2339 (1994)

Using recombinantly expressed proteins and synthetic peptides, we examined the structural/functional features of the platelet chemokines, neutrophil-activating peptide-2 (NAP-2) and platelet factor 4 (PF4); that were important in their activation of neutrophils. Previous studies with the chemokine interleukin-8 (IL-8) had shown that the N- terminal region preceding the first cysteine residue was critical in defining neutrophil-activating properties. We examined whether NAP-2 and PF4 had similar structural requirements. In the Ale-glu-leu-arg (AELR) N-terminus of NAP-2, substitution of E or R abolished Ca2+ mobilization and elastase secretion. Unlike the parent molecule PF4, AELR/PF4, the hybrid formed by replacing the N-terminal sequence of PF4 before the first cysteine residue with the homologous sequence of NAP- 2, stimulated Ca2+ mobilization and elastase secretion. Furthermore, the effect of amino acid substitutions in the ELR motif differed from those seen with NAP-2 in that conserved substitutions of E or R in NAP- 2 abolished activity, but only reduced neutrophil activation in the hybrid. These studies show that just as with IL-8, the N-termini of NAP- 2 and PF4 are critical for high-level neutrophil-activating function. Desensitization studies provided information on receptor binding. NAP- 2, which binds almost exclusively to the type 2 IL-8 receptor (IL-8R), did not desensitize neutrophils to activation by IL-8 because IL-8 could bind to and activate via both type 1 and 2 IL-8R. AELR/PF4 appears to bind to both types of receptors because it desensitized neutrophils to NAP-2 activation; but was not desensitized by NAP-2, and because it desensitized to and was desensitized by IL-8. Thus, although NAP-2 and AELR/PF4 share approximately 60% amino acid homology, they have different receptor affinities. Studies were performed to define the role of the C-termini of these platelet chemokines in receptor binding. Heparin and a monoclonal antibody specific for the heparin- binding domain of PF4 both inhibited Ca2+ mobilization and elastase release, further suggesting that the C-terminus of these chemokines is important in receptor binding. Synthetic NAP-2(51-70) failed to mobilize Ca2+, whereas PF4(47-70) and PF4(58-70) induced Ca2+ mobilization and secretion of elastase at high concentrations. Pertussis toxin inhibited neutrophil

activation by 40% to 50%, establishing a role for G-protein-coupled receptors such as the IL-8Rs in activation by the PF4 C-terminal peptides

28. The alpha 3 chain of type IV collagen prevents activation of human polymorphonuclear leukocytes
Monboisse, J.C. et al
J. Biol. Chem., 269, 25475-25482 (1994)

Our initial observation that type I collagen activates polymorphonuclear leukocytes (PMN) prompted the testing of the activating potential of type IV collagen. It was noted, however, that type IV collagen isolated from bovine lens capsule did not activate PMN but rather prevented their stimulation by N-formylmethionyl-leucyl- phenylalanine, phorbol myristate acetate, or type I collagen. This observation led to the present study, which demonstrates that the inhibitory effect of lens capsule type IV collagen resides in the noncollagenous (NC1) domain of the alpha 3 chain and specifically in the region comprising residues 185-203 of the NC1 domain of both the human and bovine molecules. Synthetic peptides from the same region of the NC1 domains of the alpha 1, alpha 2, alpha 4, and alpha 5 chains did not possess the inhibitory effect seen with the alpha 3 chain. The sequence S-N-S (residues 189-191) is unique to the peptide of the alpha 3 chain, and substitution of either serine with alanine abolishes the inhibition. Type IV collagen isolated from the mouse Engelbreth-Holm- Swarm (EHS) tumor, a molecule that lacks the alpha 3 chain, did not prevent PMN activation but instead stimulated the secretion of elastase and type IV collagenase. Incubation of PMN with intact lens capsule type IV collagen or a peptide comprising residues 185-203 of the alpha 3 (IV) chain resulted in a 3-fold increase of intracellular cAMP, whereas, Ca2+ levels remained unchanged. Incubating PMN with forskolin or with dibutyryl-cAMP resulted in the inhibition of O2- production and degranulation by PMN, thus mimicking the effects of type IV collagen and the alpha 3 (IV) 185-203 peptide. The data suggest that type IV collagen, through its alpha 3 chain, down-regulates PMN activation and thus decreases the potential for damage as these cells traverse the capillary wall. Our in vitro experiments suggest that the higher the content of the alpha 3 (IV) chain is in a basement membrane, the wider would be its capacity for self-protection.

29. Superoxide dismutase mimetics inhibit neutrophil-mediated human aortic endothelial cell injury in vitro
Hardy, M.M., Flickinger, A.G., Mueller, D.M. and Heinecke, J.W.
J. Biol. Chem., 269, 18535-18540 (1994)

In this study, we evaluated the ability of low molecular weight manganese-based superoxide dismutase mimetics to attenuate neutrophil-mediated oxygen radical damage to human aortic endothelial cells in vitro. Human neutrophils, when exposed to tumor necrosis factor-alpha and the complement compound C5a, induced endothelial damage assessed by the release of 51Cr into the medium. This damage correlated with the amount of superoxide generated by neutrophils. Three superoxide dismutase mimetics, with catalytic rate constants for superoxide dismutation ranging from 4 to 9 x 10(7) M-1 S-1, inhibited neutrophil- or xanthine oxidase-mediated endothelial cell injury in a concentration- dependent manner. A similar manganese-based compound with no detectable superoxide dismutase activity was ineffective in inhibiting injury. Fluorescent studies of the neutrophil respiratory burst showed that the superoxide dismutase mimetics were protective without interfering with the generation of superoxide by activated neutrophils. Catalase, elastase inhibitors, and desferrioxamine mesylate (an iron chelator and hydroxyl radical scavenger) were not protective against cell injury. This investigation demonstrates that neutrophil-mediated human aortic endothelial cell injury in vitro is mediated by the superoxide anion and that low molecular weight manganese-based superoxide dismutase mimetics are effective in abrogating this damage.

30. Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein
Savenkova, M.L., Mueller, D.M. and Heinecke, J.W.
J. Biol. Chem., 269, 20394-20400 (1994)

Myeloperoxidase, a heme protein secreted by activated phagocytes, is expressed in human atherosclerotic lesions. The enzyme uses H2O2 generated by the cells to oxidize L-tyrosine to tyrosyl radical, a catalyst for protein dityrosine synthesis. We have explored the possibility that tyrosyl radical initiates lipid peroxidation, which may be of pivotal importance in transforming low density lipoprotein (LDL) into atherogenic particles. Exposure of LDL to L-tyrosine and activated human neutrophils caused peroxidation of LDL lipids. LDL oxidation required L-tyrosine but was independent of free metal ions; catalase and heme poisons were inhibitory. Incubation of LDL with L- tyrosine, myeloperoxidase, and H2O2 likewise caused lipid peroxidation, and this reaction was inhibited by heme poisons and catalase. Replacement of L-tyrosine with O-methyltyrosine, which cannot form tyrosyl radical, inhibited LDL oxidation by both activated neutrophils and myeloperoxidase. The antioxidants ascorbate and probucol, but not vitamin E, inhibited LDL oxidation by myeloperoxidase, H2O2, and L- tyrosine. Ascorbate blocked dityrosine synthesis, while probucol scavenged chain-propagating peroxyl radicals in the lipid phase of LDL. These results indicate that tyrosyl radical stimulates LDL lipid peroxidation. In striking contrast to other cell-mediated mechanisms for LDL oxidation, the myeloperoxidase-catalyzed reaction is independent of free metal ions. This raises the possibility that tyrosyl radical generated by myeloperoxidase is of physiological importance in making LDL atherogenic.

31. Flow cytometric detection of anti-neutrophil cytoplasmic autoantibodies
Yang, Y.H., Hutchinson, P., Littlejohn, G.O. and Boyce, N.

J. Immunol. Methods, 172(1), 77-84 (1994)

An intracytoplasmic immunofluorescence staining technique which allows the detection and quantification of anti-neutrophil cytoplasmic autoantibodies (ANCA) by flow cytometry is described. A polymorph neutrophil population from human peripheral blood was used in this study as indicator cells. These were fixed and permeabilized by paraformaldehyde, Tween 20 and saponin, to allow ANCA in the patients sera to reach their intracellular antigen targets. The numbers of indicator cells remained unaltered by the permeabilization protocol and no cell aggregation or loss of intracellular antigenicity was observed. An excellent agreement (91% (207/228)) between ANCA detection by immunofluorescence microscopy (IF) and flow cytometry was noted. Compared with IF assay, the flow cytometric method has a sensitivity of 93% (42/45) and a specificity of 90% (165/183). Although not able to discriminate between P-ANCA or C-ANCA, this flow cytometric method has the adBantage of providing an objective, reproducible and quantitative measure of ANCA, which makes it an ideal technique for screening of patients sera for ANCA reactivities.

32. Do barbiturates impair zymosan-induced granulocyte function?
Weiss, M. et al
J. Crit. Care, 9(2), 83-89 (1994)

The dose-response relationship of commercially available preparations of methohexital, pentobarbital, phenobarbital, and thiopental and their respective drug-free solutions on granulocyte function was investigated to evaluate whether suppression of neutrophil chemiluminescence is mediated by the barbiturates themselves or by their drug-free solutions. Furthermore, it was assessed whether suppression of chemiluminescence is due to an interaction mainly with neutrophils or to free radical scavenging.
The dose-response effects of the four barbiturates on granulocyte function were tested by zymosan-induced neutrophil chemiluminescence and, in addition, in a cell-free chemiluminescence system.
Methohexital and pentobarbital did not influence zymosan-induced neutrophil chemiluminescence, whereas phenobarbital and thiopental decreased neutrophil chemiluminescence in a dose-dependent fashion. Nonphysiological osmolality (531 mosmol/kg) caused this impaired neutrophil chemiluminescence at the greatest concentration of phenobarbital. Thiopental solely suppressed neutrophil chemiluminescence drug specifically. Because thiopental also reduced chemiluminescence generated in a cell-free system, free radical scavenging might contribute to the impaired neutrophil chemiluminescence observed with thiopental.
With the exception of thiopental, barbiturates do not impair oxygen radical production during phagocytosis of neutrophils.

33. Application of PCR to multiple specimen types for diagnosis of cytomegalovirus infection: comparison with cell culture and shell vial assay
Miller, M.J., Bovey, S., Pado, K., Bruckner, D.A. and Wagar, E.A.
J. Clin. Microbiol., 32(1), 5-10 (1994)

Human cytomegalovirus (CMV) is a herpesvirus that is responsible for significant morbidity and mortality in congenitally infected infants and immunocompromised patients. Antiviral therapies are available, thus making timely diagnosis of significant importance to at-risk patients. A PCR system was devised. The newly devised system, unlike previously described systems, can be applied to a wide variety of specimen types in a clinical microbiology laboratory setting. Specimens from all sites routinely accepted for CMV culture were shown to be acceptable for CMV PCR. Sensitivity and specificity were established in comparison with those of both monolayer culture and shell vial assay (SVA). The sensitivity and specificity of PCR for detection of CMV in specimens exclusive of urine and blood were 97.5 (77 of 79 specimens) and 87.2% (41 of 47 specimens), respectively. The sensitivity and specificity of PCR for urine and blood specimens were 100 (10 of 10) and 95.7% (45 of 47) and 66.7 (4 of 6) and 78.8% (41 of 52), respectively. Discrepancies of positive PCR results with negative culture or SVA results occurred for specimens flanked chronologically by other culture- or SVA-positive specimens and were likely culture failures, increasing the specificity (100%) of PCR. Discrepancies of negative PCR results with positive culture or SVA results occurred in specimens with few cells or infectious foci by SVA or culture and may represent sampling variability associated with low virus titers.

34. Effect of centrifuging shell vials at 3,500 x g on detection of viruses in clinical specimens
Engler, H.D. and Selepak, S.T.
J. Clin. Microbiol., 32(6), 1580-1582 (1994)

An increase in shell vial centrifugation force to 3,500 x g and a concomitant reduction in spin time to 15 min did not decrease the sensitivity of detecting viruses in clinical specimens compared with the accepted practice of using 700 x g for 40 min. No damage to the cell monolayer (ML) at the higher g force was observed. Toxicity to the ML is decreased with the shorter spin, probably because of reduced time of contact between the specimen and the ML.

35. Differential effects of the anti-inflammatory compounds heparin, mannose-6-phosphate, and castanospermine on degradation of the vascular basement membrane by leukocytes, endothelial cells, and platelets
Bartlett, M.R., Cowden, W.B. and Parish, C.R.

*J. Leukoc. Biol., **57**, 207 (1995)*

Recent studies suggest that heparin, mannose-6-phosphate (M6P), and castanospermine (CS) may mediate their anti-inflammatory effects by inhibiting the passage of leukocytes through the subendothelial basement membrane (BM). In order to test this hypothesis, heparin, M6P, and CS were examined for their ability to prevent the in vitro degradation of a 35SO4-labeled extracellular matrix (ECM) by neutrophils, lymphocytes, endothelial cells (ECs), and platelets, the labeled ECM degradation products being analyzed by gel filtration chromatography. All three compounds inhibited 35SO4-labeled ECM degradation, but M6P and CS were cell-type specific in their effects. Heparin inhibited the heparanase activity of all cell types examined, confirming the results of previous studies using similar in vitro techniques. M6P selectively inhibited lymphocyte heparanase but not that of platelets, neutrophils, or ECs. CS selectively inhibited phorbol myristate acetate (PMA)-induced EC heparanase and sulfatase activity but did not affect the constitutive expression of degradative enzymes by non-stimulated ECs. These findings provide important clues to the mode of action of these compounds and the characteristic inflammatory pathology associated with the use of each anti-inflammatory agent. In particular, the data support the view that leukocytes markedly differ in the mechanisms they use to degrade BM/ECM to enable extravasation and that some degree of cooperation with EC is required in this process.

36. TNF induced superoxide anion production in adherent human neutrophils involves both the p55 and p75 TNF receptor
Richter, J., Gullberg, U. and Lantz, M.
*J. Immunol., **154**, 4142 (1995)*

TNF, a potent activator of neutrophil granulocytes, acts via two cell- surface receptors: the p55-TNF receptor (TNF-R55) and the p75-TNF receptor (TNF-R75), which can be cleaved from the cell surface and thus form soluble TNF-binding proteins (TNF-BP). The role of the two receptors in activation of the neutrophil respiratory burst was investigated. Two mAbs reacting with TNF-R55 (H398 and TBP2) induced O2 release in a similar manner but to a lesser extent than TNF. TBP2, however, required preincubation at 4 degrees C to exert its effect. Preincubation of neutrophils (both at 4 and 37 degrees C) with mAb to TNF-R75 decreased TNF-induced superoxide anion production by 67 and 64%, respectively, indicating the essential role also for TNF-R75 in neutrophil activation. This inhibitory effect could not be explained by cross-down-regulation of TNF-R55 because the TNF-R75 mAb had no effect on TNF binding to TNF-R55 as determined by binding of 125I-labeled TNF or release of TNF-R55-BP as measured by ELISA. Furthermore, the TNF-R75 mAb did not decrease superoxide anion generation induced by the TNF-R55 mAb H398, thus ruling out that the inhibitory effect of the TNF-R75 mAb is due to inhibition of the signaling pathway downstream of TNF-R55. In contrast to the TNF-R75 mAb, TNF-R55 mAbs induced down-regulation of TNF-R75 and shedding of both TNF-R55-BP and TNF-R75-BP. We conclude that both TNF-R55 and TNF-R75 are involved in TNF-induced activation of the neutrophil respiratory burst.

37. Evidence for participation of vicinal dithiols in the activation sequence of the respiratory burst of human neutrophils
Kutsumi, H., Kawai, K., Johnston, R.B. and Rokutan, K.
*Blood, **85**, 2559-2569 (1995)*

Phenylarsine oxide (PAO) specifically forms a stable ring complex with vicinal dithiols that can be reversed with 2,3-dimercaptopropanol (DMP). Pretreatment of human neutrophils with micromolar concentrations of PAO inhibited release of superoxide anion (O2-) stimulated by N- formyl-methionyl-leucyl-phenylalanine (FMLP) or phorbol 12-myristate 13- acetate (PMA); the inhibition was reversed with DMP, but not with 2- mercaptoethanol. PAO did not affect O2- release in previously stimulated cells. PAO did not affect the FMLP-induced Ca2+ response, suggesting that PAO affects a postreceptor event that does not modulate the Ca2+ transient. Treatment of isolated membrane or cytosolic fractions with PAO did not change the rates of arachidonate-stimulated O2- production in a cell-free system. Pretreatment of unstimulated neutrophils with PAO inactivated cytosolic protein kinase C (PKC); the inactivation was reversed with DMP. However, PAO did not affect PMA- induced translocation of beta-PKC protein or reduce the PKC activity translocated to the membrane. PAO had no effect on tyrosine kinase activity but inactivated phosphotyrosine phosphatase; stimulus-induced tyrosine phosphorylation of several proteins was markedly enhanced. These results suggest that vicinal dithiols play an essential role in activation of the respiratory burst oxidase. Possible sites for the activity of these essential vicinal dithiols include PKC and the regulatory balance of tyrosine phosphatase activity and tyrosine phosphorylation.

38. Evidence for a third component in neutrophil aggregation: potential roles of O-linked glycoproteins as L-selectin counter-structures
Bennett, T.A. et al
*J. Leukoc. Biol., **58**, 510 (1995)*

The homotypic aggregation of neutrophils requires the participation of L-selectin and the beta 2-integrins, but it has not been clear whether the two receptors recognize one another as counter-structures or whether other adhesion molecules are involved. We have examined aggregation of live neutrophils with target populations, manipulated to alter expression of adhesive epitopes, using flow cytometry. A target population depleted of both L-selectin and activatable beta 2-integrin displayed an ability to aggregate with live neutrophils, suggesting that these two molecules are not counter-structures. We also found that an O-sialoglycoprotease (GCP) from Pasteurella haemolytica is capable of inhibiting homotypic aggregation. Neutrophils treated with GCP lose O-glycosylated proteins but

retain L-selectin and activatable beta 2-integrin. One or more of the GCP substrates appears to function in L-selectin-dependent binding but not in beta 2-integrin-dependent binding. Together the data suggest a mechanism of aggregation that is analogous to leukocyte-endothelial cell adhesion in which a low-affinity carbohydrate-dependent interaction precedes a high-affinity integrin-dependent adhesion.

39. In vitro killing of neuroblastoma cells by neutrophils derived from granulocyte colony-stimulating factor-treated cancer patients using an anti-disialoganglioside/anti-Fc gamma RI bispecifc antibody
Michon, J. et al
Blood, 86, 1124-1130 (1995)

Neutrophils isolated from cancer patients treated with granulocyte colony-stimulating factor (G-CSF) express high levels of Fc gamma RI. They exhibited an efficient killing of GD2+ neuroblastoma cells in the presence of an antidisialoganglioside (GD2) mouse monoclonal antibody (MoAb; 7A4, IgG3 kappa). However, this cytotoxicity was totally blocked by human monomeric IgG. In contrast, a bispecific antibody (7A4 bis 22/MDX-260), prepared by chemically linking an F(ab') fragment of 7A4 with an F(ab') fragment of an anti-Fc gamma RI MoAb, 22, which binds outside the Fc binding domain, triggered antibody-dependent cell cytotoxicity, even when neutrophils were preincubated with human monomeric IgG. F(ab')2 22 MoAb abrogated the MDX-260 killing without affecting that of 7A4. The 3G8 MoAb, directed against the Fc gamma RIII binding site, did not inhibit the cytotoxicity induced by either antibody. Thus, these results indicate that G-CSF-activated neutrophils exert their cytotoxic effect against neuroblastoma cells through Fc gamma RI and not Fc gamma RIII, and that the saturation of the high affinity Fc gamma RI by monomeric IgG can be overcome by the use of bispecific antibodies binding epitopes outside the IgG Fc gamma RI binding site. A combined administration of such bispecific antibodies and G-CSF may be, therefore, an efficient therapeutic approach to trigger tumor lysis by cytotoxic neutrophils in vivo.

40. Paracetamol inhibits copper iron-induced, azo compound-initiated, and mononuclear cell-mediated oxidative modification of LDL
Nenseter, M.S., Halvorsen, B., Rosvold, Ø., Rustan, A.C. and Drevon, C.A.
*Arterioscler. Thromb. Vasc. Biol., **15**, 1338 (1995)*

Abstract The effects of paracetamol and sodium salicylate on the susceptibility of LDL to oxidative modification were studied. LDL was subjected to Cu^{2+}-, azo compound–, or peripheral blood mononuclear cell–initiated oxidation in the absence and presence of paracetamol and salicylate. Paracetamol (100 μmol/L; 25 μg LDL/mL) reduced the rate of formation of conjugated dienes and the amount of conjugated dienes formed during Cu^{2+}-induced oxidation by 67% and 58%, respectively. Paracetamol (400 μmol/L; 100 μg LDL/mL) reduced the generation of lipid peroxides during Cu^{2+}-induced oxidation by 43% (P<.05), the relative electrophoretic mobility in agarose gels by 16% (P<.05), and the amount of oxidized LDL taken up by J774 macrophages by 22% (P<.05). Paracetamol (100 μmol/L; 100 μg LDL/mL) reduced the 2,2'-azobis-(2-amidinopropane hydrochloride)–initiated lipid peroxidation by 70% (P<.05) and the relative electrophoretic mobility by 34% (P<.05). Paracetamol (100 μmol/L; 100 μg LDL/mL) reduced the amount of lipid peroxides generated in LDL during mononuclear cell–mediated oxidation by 69% (P<.01) and the relative electrophoretic mobility by 38% (P<.01). In comparison, 10 μmol/L α-tocopherol reduced the amount of lipid peroxides formed during cellular LDL oxidation and the relative electrophoretic mobility by 52% and 65%, respectively (P<.05). In the absence of paracetamol, SOD and catalase inhibited the modification of LDL (P<.05), suggesting that superoxide anions and hydrogen peroxide might be involved in the cell-mediated modification pathway. In the presence of paracetamol, SOD showed no additional inhibitory effect. The 1,1-diphenyl-2-pikrylhydracyl radical–scavenging test showed that paracetamol itself was a free-radical scavenger. In contrast, sodium salicylate (25 to 4000 μmol/L) showed no free radical–scavenging property and failed to protect LDL against mononuclear cell–mediated oxidation. In conclusion, the results indicate that paracetamol, but not salicylate, protects LDL against Cu^{2+}-induced, azo compound–initiated, and mononuclear cell–mediated oxidative modification in vitro and that this may be due to the radical scavenger capacity of paracetamol.

41. The effect of pregnancy on polymorphonuclear leukocyte function
Crouch, S.P., Crocker, J.P. and Fletcher, J.
J. Immunol., 155, 5436 (1995)

Pregnancy exerts suppressive effects on a number of chronic inflammatory conditions, particularly rheumatoid arthritis. We isolated peripheral blood polymorphonuclear leukocytes (PMN) from pregnant women at 30 to 34 wk (n = 34) and showed significant reductions in respiratory burst activity compared with nonpregnant controls (n = 34), as determined by lucigenin-enhanced chemiluminescence (LUCL). Responses to FMLP were reduced by 54% (p = 0.0046) and to zymosan-activated serum (ZAS) by 69% (p = 0.0043). Following LUCL responses to these agonists in women throughout the course of their pregnancy (n = 7) revealed significantly reduced responses by the second and third trimesters (p < 0.005). Intracellular H2O2 production in PMN at 30 to 34 wk gestation was significantly reduced (p = 0.0454) in response to FMLP, compared with the nonpregnant controls. Investigation of adhesion molecule expression revealed no differences in CD11b or CD18. However, loss of CD62L from the PMN surface in response to FMLP and ZAS was significantly reduced at 30 to 34 wk, as compared with controls (FMLP, p = 0.049; ZAS, p = 0.01; n = 34). There were no significant differences in cell surface formyl peptide receptor expression, although there were statistical differences in LUCL responses to all concentrations of FMLP used (p < 0.05). Incubating PMN with TNF, IL-8, and granulocyte-macrophage CSF increased formyl

peptide receptor expression but revealed no differences between the two groups. Priming of pregnancy PMN with the same cytokines gave significantly reduced LUCL when cells were subsequently stimulated with FMLP ($p < 0.05$; $n = 6$). Our results show a reduction in PMN NADPH-oxidase activity during pregnancy and may offer a partial explanation for the remission of symptoms observed in rheumatoid arthritis.

42. The Binding of Type I Collagen to Lymphocyte Function-associated Antigen (LFA) 1 Integrin Triggers the Respiratory Burst of Human Polymorphonuclear Neutrophils
Garnotel, R. et al
J. Biol. Chem., 270, 27495 (1995)

Monoclonal antibodies to the $\alpha_L\beta_2$ integrin inhibit the binding of type I collagen to PMN (polymorphonuclear neutrophil leukocytes) as well as the subsequent stimulation of superoxide production and enzyme secretion elicited by this collagen. Pepsinized collagen still binds PMN but no longer stimulates them. The I domain of the α chain of the integrin is involved in the binding. Two sequences of the α-1(I) polypeptide chain of collagen participate in the process. Experiments of competitive inhibition by synthetic peptides showed that the sequence RGD (915-917) is used for binding to the cells and DGGRYY (1034-1039) serves to stimulate PMN. Experiments of radioactive labeling of the cells and affinity chromatography on Sepharose-collagen confirmed the presence in PMN extracts of two proteins, 95 and 185 kDa, respectively, corresponding to the molecular weights of the β_2 and α_L chains of the integrin and recognized by their specific monoclonal antibodies.
The transduction pathways depending on the $\alpha_L\beta_2$ integrin do not involve a G protein (ruled out by the use of cholera and pertussis toxins), whereas the cytoskeleton was found to participate in the process, as evidenced by inhibition by cytochalasin B. After collagen stimulation, cytoplasmic inositol trisphosphate and calcium ion increased sharply for less than 2 min. The use of the inhibitors staurosporine and calphostin C demonstrated that protein kinase C was involved. Evaluation of the activity of this enzyme showed that, upon stimulation of PMN with collagen I, it was translocated to plasma membrane.
Acrylamide gel electrophoresis of the protein bands corresponding to the integrin $\alpha_L\beta_2$, followed by immunoblotting using monoclonal antibodies to phosphotyrosine, permitted us to demonstrate that, prior to stimulation by type I collagen, there was no phosphorylation, whereas after stimulation, both α_L and β_2 chains were stained by anti-phosphotyrosine antibodies. The adhesion of PMN to pepsinized type I collagen triggered tyrosine phosphorylation of the β_2 chain of the integrin, without stimulating O&cjs1138;$_2$ production by these cells, whereas their stimulation by complete type I collagen induced the tyrosine phosphorylation of both α_L and β_2 subunits. The tyrosine phosphorylation of both integrin subunits during transduction of stimuli is a heretofore undescribed phenomenon that may correspond to a new system of transmembrane communication.

43. An improved method for separation of neutrophils from human blood using methylcellulose
Zhou, D., Stewart, G.J., Kowalska, M.A. and Niewiarowski, S.
Thromb. Res., 8083), 271-275 (1995)

No abstract available

44. Quantitative multiwell myeloid differentiation assay using dichlorodihydrofluorescein diacetate (H2DCF-DA) or dihydrorhodamine 123 (H2R123)
Trayner, I.D., Rayner, A.P., Freeman, G.E. and Farzaneh, F.
J. Immunol. Methods, 186(2), 275-284 (1995)

It is well established that the fluorescent probes dihydrodichlorofluorescein diacetate (H_2DCF-DA) and dihydrorhodamine 123 (H_2R123) can be used to detect the respiratory burst response of mature myeloid cells. We describe a simple, fast and quantitative assay for myeloid differentiation based on the oxidation of these probes, which can be performed from start to finish in 96-well dishes. A bis(acetoxymethyl) ester of H_2DCF-DA, 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (CODCF-DA) is also capable of detecting the respiratory burst, but is less suitable than H_2DCF-DA or H_2R123 in our system. The amount of fluorescence produced can be quantified using a calibration curve, and values can be normalised to cell numbers using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. Results are expressed as ‘equivalents of soluble fluorescein’ (ESF) produced per cell under the defined reaction conditions. The extent to which HL60 cells reduce MTT is unaffected by differentiation induced by retinoic acid or 1α,25-dihydroxyvitamin D_3, and normalisation of fluorescence values using the MTT assay appears to be valid for a wide range of myeloid cell lines and differentiation inducers or cytokines.

45. Bimodal distribution of proteinase 3 (PR3) surface expression reflects a constitutive heterogeneity in the polymorphonuclear neutrophil pool
Halbwachs-Mecarelli, L., Bessou, G., Lesavre, P., Lopez, S. and Witko-Sarsat, V.
FEBS Lett., 374(1), 29-33 (1995)

Proteinase 3, which is known as an intracellular serine protease of neutrophils, was detected at the surface of a subpopulation of freshly isolated PMN. The proportion of PR3-positive and -negative PMN, observed by flow cytometry with anti-PR3 mAbs or ANCA autoantibodies, varies among individuals but is extremely stable for each individual over prolonged time periods. After PMN degranulation by FMLP with cyt. B, membrane PR3 expression increases but the proportion of low and high PR3-expressing cells remains stable. The existence of a subset of PMN which spontaneously expresses PR3 and varies among individuals, may be relevant to the pathogenesis of anti-PR3 ANCA autoantibody-related vasculitis.

46. Immunophenotyping of canine bronchoalveolar and peripheral blood lymphocytes
Dirscherl, P., Beisker, W., Kremmer, E., Mihalkov, A. and Ziesenis, A.
Vet. Immunol. Immunopathol., 48(1-2), 1-10 (1995)

The immunophenotype of canine lymphocytes obtained by bronchoalveolar lavage (BAL) was investigated and compared with that of peripheral blood leukocytes (PBL). Indirect immunofluorescence, generated by monoclonal antibodies (mAb) specific for canine CD5, CD4, CD8, CD45pan, CD45RA, MHCII and THY-1, was detected by flow cytometry. In comparison with PBL, in BAL there are fewer lymphocytes positive for CD45RA (75.4 ± 12.6% vs. 42.3 ± 9.4%; $P < 0.05$) and MHCI I (97.0 ± 2.9% vs. 74.0 ± 7.6%; $P < 0.01$), while there are more cells positive for CD8 (19.0 ± 3.6% vs. 29.5 ± 12.0%; $P < 0.05$). Thus there is a lower *CD*4/*CD*8 ratio in the cell compartment accessible by BAL (2.2 ± 0.3 vs. 1.3 ± 0.6; $P < 0.005$). The immunophenotype may be stable over time, as indicated by reexamination of cells obtained from one dog at four times over 1 year. Investigating the phenotype of lymphocytes from three different locations of the right lung, the cranial lobe lymphocytes show a lower *CD*4/*CD*8 ratio in comparison with PBL (1.81 ± 0.35 vs. 1.12 ± 0.31, $n = 5$; $P < 0.02$). In general, less MHCII positive lymphocytes ($P < 0.001$) and greater immunophenotype variability of results were found in these separate samples compared with pooled samples from these locations.

47. An immunological enrichment method for epithelial cells from peripheral blood
Griwatz, C., Brandt, B., Assmann, G. and Zänker, K.S.
J. Immunol. Methods, 183(2), 251-265 (1995)

The ability of primary tumours to metastasize accounts for the majority of cancer deaths. The emergence of circulating carcinoma cells in the peripheral blood is supposed to be an indicator for cancer cell spread. We have focused on this phenomenon in order to develop a sensitive technique for enriching epithelial derived cells on the basis of a two-layer density gradient and subsequent immune magnetic cell sorting.
Epithelial cells are possess a cytoskeleton containing an assembly of intermediate filaments. During carcinogenesis these filaments do not undergo modifications of antibody binding epitopes such as occur in the protein domains of surface markers.
We have developed a two-layer density gradient in which the epithelial cells form a single density band. This was demonstrated by recovery experiments using [^{3}H]thymidine-labelled epithelial cells which showed epithelial cells were enriched within this first step by a factor of 20. In a second step the MACS system was applied. Cells were stained with a preformed FITC-conjugated mouse anti-human cytokeratin antibody bound to a rat anti-mouse antibody coupled to superparamagnetic particles (immune paramagnetic separation complex; IPSC) and subjected to high gradient magnetic fields. The two-step procedure was confirmed by dispersing 50 epithelial cells in 5×10^5, 5×10^6, 5×10^7, 5×10^8, 5×10^9 peripheral blood leucocytes. Specific binding of the preformed IPSC was demonstrated by flow cytometry, confocal laser, fluorescent and electron microscopy. The specificity of the method was further proved by dual staining with IPSC and anti-human PSA antibody of epithelial prostatic cells separated from peripheral blood in vitro. By means of this double-step separation method it was possible to isolate up to 15–20 cells out of 50 epithelial cells originally suspended into 5×10^7 to 5×10^9 human peripheral blood leucocytes. This represented an enrichment factor between 20 000 and 200 000, depending on the initial cell number. The immunologically captured epithelial cells can be used for further cytogenetic investigation such as in situ dybridization (ISH) and/or polymerase chain reaction (PCR) to detect cancer cell specific gene aberrations. This sensitive combined buoyant density immune magnetic cell separation technique is capable of detecting free carcinoma cells in the peripheral blood.

48. In vitro activation of woodchuck lymphocytes measured by radiopurine incorporation and interleukin-2 production: Implications for modeling immunity and therapy in hepatitis B virus infection
Cote, P.J. and Gerin, J.L.
Hepatology, 22(3), 687-699 (1995)

Cellular immune responses to hepatitis B virus (HBV) play an important role in the resolution of acute infection. They also influence the course of chronic infection and disease but are inadequate to completely clear the infection. Woodchuck hepatitis virus (WHV) infection of the woodchuck can provide a model to study these processes. Lymphocyte responses of woodchucks were assessed by *in vitro* proliferation and/or interleukin (IL)-2 assays using mitogen (Concanavalin A [Cona]), cytokine (IL-2), superantigen (*Staphylococcus aureus* enterotoxin B [Seb]), major histocompatibility complex (MHC) alloantigen (mixed lymphocyte reaction [Mlr]), and viral antigens (woodchuck hepatitis virus core antigen [WHcag] and woodchuck hepatitis virus surface antigen [WHsag]). ConA-stimulated woodchuck lymphocytes underwent cell division based on cell counting experiments and produced IL-2 as detected using an IL-2-dependent murine cell line but failed to incorporate sufficient tritiated thymidine; however, they did incorporate sufficient tritiated adenosine and deoxyadenosine to permit development of a meaningful proliferation assay. The IL-2 assay was sensitive and specific for

detection of wood-chuck IL-2 induced by mitogen, superantigen, and MLR, as shown by quantitative titration analysis and anti-body neutralization of ConA-supernatant activity. Cyclosporin A and FK506 specifically inhibited ConAand SEB-induced IL-2 production by woodchuck lymphocytes. Positive two-way MLRs were detected by IL-2 production and proliferation assay between woodchucks from different geographic regions, thus indicating divergence among MHC molecules; however, occasional negative MLR reactions among indigenous pairs of woodchucks indicated that some woodchucks were mutually immunocompatible to some degree. The radioadenosine proliferation assay was sensitive for detecting peripheral blood lymphocyte responses to WHcAg and WHsAg in adult woodchucks with recently resolved acute infections. The above systems should facilitate the design of adoptive therapy and liver transplantation experiments in the woodchuck, and also enable modeling of immune responses that promote and maintain chronic hepadnavirus infection.

49. Immune deficiency following thermal trauma is associated with apoptotic cell death
Theodorczyk-Injeyan, J.A., Cembrzynska, M., Lalani, S., Peters, W.J. and Mills, P.G.B.
J. Clin. Immunol., 15(6), 318-328 (1995)

Thermal injury-associated specific immune deficiency occurs despite indicators of systemic activation of the lymphoid compartment. We investigated the possibility that postburn immune failure and T cell activation are causally related through activation-induced (apoptotic) cell death. The relationship between the cellular immune response and cell mortality was examined in cultures of peripheral blood mononuclear cells (PBMC) from 14 immunosuppressed patients with extensive burns (35–90% total body surface area). Impaired cellular immunity coincided with significantly reduced cell viability as ascertained by propidium iodide staining and dye reduction assays. Following stimulation with the mitogenic lectin, phytohemagglutinin (PHA), the majority of DNA in patient cultures was fragmented, suggesting the occurrence of apoptotic cell death. Even without stimulation a portion of patient cells was apoptotic as indicated by oligonucleosomal bands on agarose gel electrophoresis. Exogenous interleukin-2 or phorbol ester markedly reduced constitutive as well as PHAinduced DNA fragmentation.*In situ* demonstration of DNA strand breaks in freshly isolated patient PBMC, by a TdT-based labeling technique, confirmed that a larger fraction (up to 60%) of circulating lymphocytes was undergoing apoptosis on the periphery. These novel observations suggest that apoptosis may play a major role in thermal injury-related cellular immunodeficiency.

50. Antiinflammatory effects of second-generation leukotriene B4 receptor antagonist, SC-53228: Impact upon Leukotriene B4- and 12(R)-HETE- mediated events
Fretland, D.J. et al
Inflammation, 19(2), 193-205 (1995)

Leukotriene B_4 (LTB_4) and 12(*R*)-hydroxyeicosatetraenoic acid [12(*R*)-HETE] are proinflammatory products of arachidonic acid metabolism that have been implicated as mediators in a number of inflammatory diseases. When injected intradermally into the guinea pig, LTB_4 and 12(*R*)-HETE elicit a dose-dependent migration (chemotaxis) of neutrophils (PMNs) into the injection sites as assessed by the presence of a neutrophil marker enzyme myeloperoxidase. SC-41930 {7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxyl]-3,4-dihydro-8-propyl-2H-1-benzopyran-2-carboxylic acid}, a first-generation LTB_4 receptor antagonist, inhibited the chemotactic actions of LTB_4 when given orally with an ED_{50} value of 1.7 mg/kg. The second-generation LTB_4 receptor antagonist, SC-53228 [(+)-(*S*)-7-(3-{2-(cyclopropylmethyl)-3-methoxy-4-[(methylamino)carbonyl]phenoxy} propoxy)-3,4-dihydro-8-propyl-2H-1-benzopyran-2-propanoic acid], inhibited LTB_4-induced chemotaxis when given intragastrically with an ED_{50} value of 0.07 mg/kg. Furthermore, SC-53228 inhibited 12(*R*)-HETE-induced granulocyte chemotaxis with an oral ED_{50} value of 5.8 mg/kg. When dosed orally over a range of 0.03–100 mg/kg, SC-53228 gaveC_{max} plasma concentrations of 0.015–41.1 μg/ml. SC-53228 inhibited LTB_4-primed membrane depolarization of human neutrophils with an IC_{50} value of 34 nM. As a potent LTB_4 receptor antagonist, SC-53228 may well have application in the medical management of disease states such as asthma, rheumatoid arthritis, inflammatory bowel disease, contact dermatitis, and psoriasis, in which LTB_4 and/or 12(*R*)-HETE are implicated as inflammatory mediators.

51. Characterization of *Vibrio cholerae* El Tor cytolysin as an oligomerizing pore-forming toxin
Zitzer, A., Walev, I., Palmer, M. and Bhakdi, S.
Med. Microbiol. Immunol., 184(1), 37-44 (1995)

V. cholerae El Tor cytolysin is a secreted, water-soluble protein of M_r 60,000 that may be relevant to the pathogenesis of acute diarrhea. In this communication, we demonstrate that the toxin binds to and oligomerizes in target membranes to form SDS-stable aggregates of M_r 200000–250000 that generate small transmembrane pores. Pores formed in erythrocytes were approximately 0.7 nm in size, as demonstrated by osmotic protection experiments. Binding was shown to occur in a temperature-independent manner preceding the temperature-dependent oligomerization step. Pores were also shown to be formed in L929 and HEp-2 cells, human fibroblasts and keratinocytes, albeit with highly varying efficacy. At neutral pH and in the presence of serum, human fibroblasts were able to repair a limited number of lesions. The collective data identify *V. cholerae* El Tor cytolysin as an oligomerizing toxin that damages cells by creating small transmembrane pores.

52. Serum and cellular pharmacokinetics of clarithromycin 500 mg q.d. and 250 mg b.i.d. in volunteers
Kees, F., Wellenhofer, M. and Grobecker, H.
Infection, 23(3), 168-172 (1995)

In an open-label, randomized, crossover study 12 healthy volunteers were given clarithromycin orally 250 mg twice daily (b.i.d.) and 500 mg once a day (q.d.). Blood and saliva samples were collected on study days 1 and 5 to determine the pharmacokinetics of clarithromycin and its 14-hydroxy metabolite in plasma and saliva, and to measure concentrations of clarithromycin in mononuclear cells (MNCs) and polymorphonuclear leucocytes (PMNs). The mean peak levels of clarithromycin on day 5 of therapy in serum (2.3 vs. 1.2 mg/l), saliva (1.1 vs. 0.3 mg/l) and blood cells 60 vs. 26 mg/l in MNCs and 29 vs. 14 mg/l in PMNs) were at least doubled, the trough levels were lower with 500 mg q.d. vs. 250 mg b.i.d. (0.09 vs. 0.28 mg/l in serum; 0.06 vs. 0.13 mg/l in saliva; <1 vs. 6.8 mg/l in MNCs; 0.8 vs. 2.8 mg/l in PMNs). The mean relative peak serum concentrations of the 14-hydroxy metabolite were somewhat lower with the 500 mg dosage (0.78 vs. 0.46 mg/l). The peak concentrations of clarithromycin and its 14-hydroxy metabolite in saliva were 25–40% and 50–80% of the maximum serum concentrations with both dosage regimens. Clarithromycin exhibits good and rapid penetration into intracellular as well as into extravasal extracellular body compartments. Clarithromycin 500 mg q.d. compares favourably with 250 mg b.i.d., as far as peak serum levels and bioavailability are concerned, but trough levels are lower at the end of the 24-hour dosing interval.

53. Anti-neutrophil cytoplasmic antibodies and anti-endothelial cell antibodies are not increased in Kawasaki disease
Nash, M.C., Shar, V., Reader, J.A. and Dillon, M.J.
Br. J. Rheumatol., 34, 882-887 (1995)

We studied anti-neutrophil cytoplasmic antibodies (ANCA) and anti-endothelial cell antibodies (AECA) in 58 children with acute Kawasaki disease (KD) before i.v. gamma globulin treatment, 35 children with infection and fever > 38.5°C, and 48 healthy afebrile children. ANCA were studied by indirect immunofluorcscence (IIF) on ethanol-fixed neutrophils and by ELISA with crude neutrophil extract as antigen. AECA were studied using ELISA on resting and activated endothelial cells. ANCA IIF was weakly positive, cytoplasmic, diffuse and homogeneous in all three groups. ANCA IIF, ANCA ELISA and AECA ELISA were no higher in KD than in febrile children. There was no difference between KD with and KD without coronary artery aneurysms. AECA differences between the KD and afebrile group were not significant after correction for total IgM. In contrast with our previous findings, we conclude that ANCA and AECA are not raised in KD compared with febrile controls. It therefore seems unlikely that they are important in the pathogenesis of vasculitis in KD.

54. Rapid detection of HSV with an enzyme-linked virus inducible system™ (ELVIS™) employing a genetically modified cell line
Proffitt, M.R. and Schindler, S.A.
Clin. Diag. Virol., ***4(2),*** *175-182 (1995)*

Background: Infections with herpes simplex viruses (HSV) are common and may cause severe disease in immunocompromised hosts and in neonates. Isolation of infectious HSV in tissue culture is the most sensitive method of detection, but is not the most rapid. Recently, however, an Enzyme-Linked Virus Inducible System™ (ELVIS™) for rapid detection of HSV in culture has been developed. The system employs genetically engineered baby hamster kidney (BHK) cells (ELVIS™ cells) whose DNA bears and HSV inducible promoter gene chimerically linked to an *E. coli* LacZ "reporter" gene. Induction of the promoter by HSV leads to the production of LacZ product, β-galactosidase, which is readily detected histochemically.
Objective: To evaluate these ELVIS™ cells, as a test for HSV, in comparison with HSV detection in MRC-5 cells in shell vial cultures confirmed by staining with fluorescent antibodies.
Study design: Over a period of one month, 167 specimens submitted to the laboratory for detection of HSV were evaluated. Specimens were inoculated onto MRC-5 cells growing on glass coverslips in each of two shell vials and into two wells of a 24-well cluster plate containing ELVIS™ cells. MRC-5 shell vial cultures were observed daily for cpe for up to 7 days. With the appearance of cpe, the coverslips were fixed and the cells were typed for HSV-1 and HSV-2 with monoclonal antibodies. Specimens inoculated onto ELVIS™ cells were incubated for 16–24 h, then substrate was added to stain for β-galactosidase. ELVIS™ cells, induced by HSV infection to express β-galactosidase, stained blue upon reaction with substrate.
Results: Of 167 specimens inoculated onto MRC-5 cells, 13 were excluded because of contamination or toxicity. Among the remaining 154 specimens, 24 were positive for HSV in the MRC-5 shell vials. Of 166 specimens inoculated into the ELVIS™ cell, all were completed within 24 h. Twenty-three (23) of the 24 shell-vial-positive cultures also were positive on the ELVIS™ cells. All 23 specimens detected in the ELVIS™ cells were positive within 24 h, whereas only nine were positive within 24 hours in MRC-5 shell vial cultures. The remaining 15 became positive after 24 h. Specimens positive for viruses other than HSV-1 or HSV-2 were not positive on the ELVIS™ cells.
Conclusions: The ELVIS™ assay for HSV is simple to perform, is rapid, sensitive, and specific. The assay detects both HSV-1 and HSV-2. No antibodies are required unless typing, which can be done on the ELVIS™ cells, is necessary.

55. Postinjury neutrophil priming and activation: An early vulnerable window
Botha, A.J. et al
Surgery, 118(2), 358-365 (1995)

Background. Generation of extracellular, cytotoxic superoxide anion (O_2^-) by polymorphonuclear neutrophils (PMNs) contributes to an unbridled inflammatory response that can precipitate multiple organ failure (MOF). Release of O_2^- is markedly enhanced when activated PMNs have been previously "primed" by inflammatory mediators, such as those expressed after trauma. We therefore hypothesized that PMN priming occurs as an integral part of the early inflammatory response to trauma.
Methods. PMNs were obtained from 17 high-risk patients with torso trauma at 3, 6, 12, 24, 48, and 72 hours after injury, as well as from 10 healthy donors, and the in vitro release of O_2^- was quantitated with a kinetic, superoxide dismutase (SOD)-inhibitable cytochrome c reduction assay. PMN O_2^- release was measured in the presence and absence of 1 μmol/L N-formyl-methionyl-leucyl-phenylalanine (fMLP) and after priming and activation with 20 nmol/L platelet-activating factor (PAF) and 1 μmol/L fMLP, respectively.
Results. In vitro PMN O_2^- release was used to determine whether postinjury PMNs were (1) activated in vivo, (2) primed in vivo, or (3) primable in vitro. Unstimulated PMNs from trauma patients spontaneously expressed modest amounts of O_2^- in vitro from 6 to 48 hours after injury endogenous activation. Also, fMLP-activated PMNs collected between 3 and 24 hours after injury expressed more O_2^- than controls ($p \leq 0.02$), indicating in vivo, trauma-related priming. Furthermore, postinjury PMNs were maximally primed in vivo (i.e., in vitro exposure to PAF before fMLP activation failed to significantly enhance O_2^- release) as compared to PMNs treated with fMLP.
Conclusions. These data indicate that major torso trauma (first hit) primes and activates PMNs within 3 to 6 hours after injury. Consequently, we postulate that postinjury priming of PMNs may create an early vulnerable window during which a second hit (e.g., a secondary operation or delayed hemorrhage) activates exuberant PMN O_2^- release, rendering the injured patient at high risk for MOF.

56. Differences in oxidative response of subpopulations of neutrophils from healthy subjects and patients with rheumatoid arthritis
Eggleton, P., Wang, L., Penhallow, J., Crawford, N. and Brown, K.A.
*Annals of the Rheunatic Diseases, **54(11)**, 916-923 (1995)*

Objectives: To determine whether blood neutrophils from healthy individuals and blood and synovial fluid neutrophils from patients with rheumatoid arthritis (RA) responded differently to priming agonists and stimuli of the oxidative burst and, if so, whether this was a property of a subpopulation of neutrophils.
Methods: Continuous flow electrophoresis was used to separate neutrophils into subpopulations based upon quantitative differences in net negative surface charge. The generation of superoxide anion (O2-) was used as a measure of oxidative activity using 10-7 mol/l N-formyl-methionylleucyl-phenylalanine (FMLP) as the stimulating agonist and 10-8 mol/l platelet activating factor (PAF) as the priming agent.
Results: The production of O2- by blood and synovial fluid neutrophils from RA patients in response to FMLP was greater than that observed with control blood neutrophils (p less than 0.001). Priming of normal blood neutrophils with PAF increased their FMLP induced oxidative burst (p less than 0.001), but PAF treatment had no effect on rheumatoid neutrophils. Neutrophils from synovial fluid of RA patients were less electronegative than paired blood samples and exposure of blood neutrophils to FMLP but not PAF reduced their surface charge. Continuous flow electrophoresis isolated three neutrophil subpopulations: cells of least surface electronegativity were ascribed to pool P1 and cells of greatest surface electro-negativity to P3. Normal blood neutrophils from P3, but not P1, showed increased oxidative activity after PAF priming (twofold increase; p less than 0.01), whereas the responsiveness of rheumatoid blood and synovial fluid neutrophils from P1 and P3 was not modified by PAF treatment under the same conditions.
Conclusion: It is suggested that most of the circulating neutrophils in RA are already in a state of readiness to generate O2- upon activation by an inflammatory stimulus. This is in contrast to normal blood neutrophils, which have both responsive and non-responsive subpopulations with respect to priming agonists.

57. Type IV collagen-binding proteins of neutrophils: possible involvement of L-selectin in the neutrophil binding to type IV collagen
Iwabuchi, K., Nagaoka, I., Someya, A. and Yamashita, T.
Blood, 87, 365-372 (1996)

To isolate type IV collagen-binding proteins, 125I-labeled human- neutrophil extracts were chromatographed on a type IV collagen-Sepharose column. The affinity chromatography-separated fraction contained the four radioactive proteins with apparent molecular masses of 28, 49, 67, and 95 kD on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Western blot analysis indicated that the 95-kD proteins contained both L-selectin and nonspecific cross-reacting antigen 90 (NCA90), and that the 67-kD protein was the 67-kD elastin/laminin-binding protein (67BP). The data obtained with the type IV collagen-affinity chromatography and the immunoaffinity chromatographies using anti-L-selectin and anti-NCA90 monoclonal antibodies (MoAbs) have shown that L-selectin is closely associated with 67BP and the 49-kD protein, and that NCA90 is associated with 67BP, the 28-kD and 49-kD proteins. Among these binding proteins, sialic acid residues were contained in 67BP, L-selectin, and NCA90, but not in the 28-kD and 49-kD proteins. Sialidase treatment completely abolished both the binding affinity of the type IV collagen-binding proteins to type IV collagen and the

neutrophil adherence to type IV collagen-coated plastic. Thus, the sialic acid residues of 67BP, L- selectin, and NCA90 seem to be important for the binding of neutrophils to type IV collagen. Furthermore, L-selectin IgG chimeric protein directly bound to type IV collagen-Sepharose column, and anti-L- selectin MoAb DREG56 inhibited the neutrophil adherence to type IV collagen-coated plastic by 51%. These observations suggest that L- selectin likely plays a role in the neutrophil binding to type IV collagen, although neutrophils have several kinds of adhesion molecules for type IV collagen such as L-selectin, NCA90, 67BP, and the 28-kD and 49-kD proteins.

58. Comparison of two leukocyte extraction methods for cytomegalovirus antigenemia assay
Garcia, A., Niubo, J., Benitez, M.A., Viqueira, M. and Perez, J.L.
J. Clin. Microbiol., 34, 182-184 (1996)

We carried out a prospective, parallel, and blind study on 113 blood samples from immunocompromised patients in order to compare two leukocyte extraction methods (6% dextran sedimentation and **Polymorphprep** separation) for cytomegalovirus (CMV) antigenemia assay. CMV was detected in 38 samples by antigenemia assay (34 by dextran sedimentation and 35 by **Polymorphprep** separation). No differences either in the number of positive specimens (P = 1) or in the mean CMV- positive cell counts (P = 0.41) were observed between the two leukocyte extraction methods. In conclusion, the two methods performed equally well for this assay.

59. Metalloproteinase-mediated regulation of L-selectin levels on leucocytes
Preece, G., Murphy, G. and Ager, A.
J. Biol. Chem., 271, 11634 (1996)

Leucocyte (L)-selectin can be proteolytically cleaved in the membrane proximal extracellular region to yield a soluble fragment that contains the functional lectin and epidermal growth factor domains. A variety of stimuli are known to stimulate L-selectin shedding including chemoattractants, phorbol esters, and L-selectin cross-linking; however, the enzymes that regulate L-selectin expression are not characterized. In this study we have used phorbol ester to stimulate endoproteolytic release of L-selectin and identified a major role for a cell surface metalloproteinase (L-selectin sheddase) in this process.
The hydroxamic acid-based inhibitor of zinc-dependent matrix metalloproteinases Ro 31-9790 completely prevented shedding of cell surface L-selectin from leucocytes in mouse, rat, and man. L-selectin was susceptible to cleavage by known matrix metalloproteinases. Recombinant human fibroblast collagenase (MMP1) reduced the number of L-selectin-positive lymphocytes to a similar extent as phorbol ester activation, and stromelysin (MMP3) had a partial effect on L-selectin expression. Gelatinases A (MMP2) and B (MMP9) were without effect. Lymphocytes did not express fibroblast collagenase or stromelysin at the cell surface, and tissue inhibitor of metalloproteinases (TIMP) did not affect L-selectin levels. L-selectin sheddase was not detected in media harvested from phorbol ester-stimulated lymphocytes and was only able to cleave L-selectin in the *cis* but not the *trans* configuration.

60. Id2 expression increases with differentiation of human myeloid cells
Ishiguro, A. et al
Blood, 87, 5225-5231 (1996)

Id proteins are helix-loop-helix (HLH) transcriptional factors that lack the basic DNA binding domain. The Id proteins have been reported generally to function as inhibitors of cell differentiation, and their gene expression is often downregulated during cell differentiation. We examined the expression of human Id mRNAs by Northern hybridization in 11 human myeloid cell lines, several myeloid cell lines induced to differentiate, fresh myeloid leukemia samples, and normal human myeloid cells. Id2 mRNA was expressed in myelomonoblastic and monoblastic leukemic cells (PLB-985, THP-1, and U-937) but was weakly expressed in myeloblastic leukemic cells (KG-1 and HL-60). Id2 mRNA levels markedly increased with induction of differentiation of myeloid blasts (HL-60, PLB-985, THP-1, and U-937) toward either granulocytes or macrophages. Examination of fresh acute myeloid leukemic samples from 22 individuals also showed prominent Id2 mRNA expression in those samples having more differentiated blasts. Using the French-American-British classification, only 2 of 8 M0/M1 samples expressed Id2 mRNA; however, 10 of 13 M2/M3/M4 samples expressed it. In normal human myeloid cells, Id2 mRNA was expressed in cultured macrophages from bone marrow and in mature granulocytes and monocytes from peripheral blood. The half-life of Id2 mRNA was short (1 hour), and its expression was inducible by cessation of protein synthesis. Id3 mRNA was moderately expressed in monoblastic cell lines (THP-1 and U-937), and levels decreased with their differentiation. Almost no Id3 expression was detectable in either other myeloid leukemia lines, fresh leukemic samples, or normal human myeloid cells by Northern analyses. Id1 mRNA was not detected by polymerase chain reaction in either leukemic or normal myeloid cells except in K562 myeloid/erythroid cells. These results showed that Id2 mRNA was constitutively expressed in more mature myeloid blast cells and level markedly increased with terminal myeloid differentiation, suggesting that Id2 protein may inhibit an HLH transcriptional complex that normally represses myeloid differentiation.

61. Effect of homocysteine on copper ion-catalyzed, azo compound-initiated, and mononuclear cell-mediated oxidative modification of low density lipoprotein
Halvorsen, B. et al
J. Lipid Res., 37, 1591 (1996)

Homocysteine is an independent risk factor for cardiovascular diseases. The mechanisms by which elevated plasma concentrations of homocysteine are related to the pathogenesis of atherosclerosis are not fully understood. To examine whether homocysteine is implicated in atherogenesis through the modification of low density lipoprotein (LDL), the effect of homocysteine on the oxidation of LDL was studied by three different oxidation systems. Thus, LDL was subjected to Cu(2+)- catalyzed, azo compound-initiated, and peripheral blood mononuclear cell-mediated oxidative modification. The extent of modification was assessed by measuring the formation of conjugated dienes, lipid peroxides, thiobarbituric acid-reactive substances, and the relative electrophoretic mobility. Homocysteine at a normal plasma concentration (6 microM) showed no effect, whereas a concentration corresponding to moderate hyperhomocysteinemia (25 microM) or to concentrations seen in homocystinuria patients (100, 250, and 500 microM) protected LDL from modification of the lipid as well as of the protein moiety. One exception was observed: when the oxidation was initiated by copper ions, homocysteine at concentrations 6 and 25 microM stimulated the lipid peroxidation of LDL to a small, but statistically significant extent. High concentrations of homocysteine showed antioxidative properties as long as the thiol groups were intact, thereby delaying the onset of the oxidation. The 1,1-diphenyl-2-picrylhydracyl radical test demonstrated that homocysteine at concentrations > or = 50 microM possessed marked free radical scavenging capacity. Finally, LDL isolated from two patients with homozygous homocystinuria showed similar extent of Cu(2+)-catalyzed oxidation as LDL from a group of healthy control subjects. Taken together, our data suggest that low concentrations of homocysteine in the presence of copper ions may enhance the lipid peroxidation of LDL, whereas high concentrations of homocysteine may protect LDL against oxidative modification in the lipid as well as in the protein moiety. Thus, homocysteine-induced atherosclerosis may be explained by mechanisms other than oxidative modification of low density lipoprotein.

62. Neutrophil adhesion to 24-hour IL-1-stimulated endothelial cells under flow conditions
Jones, D.A., Smith, C.W., Picker, L.J. and McIntire, L.V.
J. Immunol., 157, 858 (1996)

In this study, we examine neutrophil adhesion under flow conditions to cultured HUVEC stimulated for 4 or 24 h with IL-1. Interactions are measured using videomicroscopy and a parallel-plate flow system which is capable of distinguishing primary adhesion and rolling from secondary (firm) adhesion. We find that after 24 h, E-selectin does not contribute to primary adhesion, in contrast to a significant contribution after 4 h. Endothelial cell P-selectin does not contribute at either time point. Blocking or removing L-selectin from the neutrophil surface decreases adhesion 50 to 70% at either time point, and neuraminidase treatment of neutrophils decreases adhesion by 85%. These results suggest that after a 24-h stimulation, primary adhesion depends on a distinct selectin-like interaction in which a HUVEC receptor binds carbohydrates on neutrophil glycoproteins, including L- selectin. We also find that secondary adhesion in this system can be inhibited 90% with Ab blockade of CD18/ICAM-1 interactions after a 24-h stimulation, and that a combination of IL-1 and IL-4 selectively down- regulates the pathway for primary adhesion at 24 h. These results demonstrate that neutrophils adhere using different receptor pathways following 4- and 24-h stimulations, and provide experimental data characterizing some properties of the receptors involved. One of the pathways that is evident at 24 h appears to be a novel selectin-like interaction.

63. Identification of Bruton's tyrosine kinase (Btk) gene mutations and characterization of the derived proteins in 35 X-linked agammaglobulinemia families: a nationwide study of Btk deficiency in Japan
Hashimoto, S. et al
Blood, 88, 561-573 (1996)

Deficiencies of Bruton's tyrosine kinase (Btk) have been implicated in the pathogenesis of human X-linked agammaglobulinemia (XLA). The distinctive phenotype observed in B-cell deficiency indicates the crucial role of Btk in B-cell development. This report describes a nationwide study of Btk deficiency in Japan, covering 51 XLA patients (35 independent families). Along with the identification of mutations, the resulting protein products were characterized by an in vitro kinase assay and a Western blot analysis. Thirty-one of the families were found to have mutations in the coding region of Btk. Although mutations were not found in the cDNA of 4 families, the Btk transcripts of these patients were greatly reduced. The identification of several novel missense mutations, in combination with the result of other studies, clarified the presence of two (missense) mutation hot spots, one in the SH1 and the other in the PH domain. The absence of kinase activity seen in 32 of the families underscored the importance of Btk protein analysis as a diagnostic indicator of XLA. The protein analysis also clarified the different effects of missense mutations on kinase activity and protein stability.

64. Soluble Fcgamma receptor type III (FcgammaRIII, CD16) triggers cell activation through interaction with complement receptors
Galon, J. et al
J. Immunol., 157, 1184 (1996)

The type III-B Fcgamma receptor (FcgammaRIII-B) is a glycosyl- phosphatidylinositol-linked receptor found on human neutrophils. A soluble form of FcgammaRIII-B (sCD16) corresponding to the extracellular region of the receptor circulates in plasma. In the present work, we have identified membrane receptors for sCD16. Soluble CD16 bound to CR3 (CDllb/CD18)- and CR4 (CDllc/CD18)- positive leukocytes and cell lines, the labeling was inhibited by anti-CD11b, CD11c or CD18 mAbs, and the up-regulation of CR3 and CR4 led

to an increased fixation of sCD16. Transfected eukaryotic cells expressing recombinant CD11b/CD18 or CD11c/CD18 heterodimers but not those expressing CD11a/CD18 bound sCD16. Moreover, the lectin-like binding site of CR3 is probably involved in the interaction with sCD16, as suggested by inhibition studies using mAbs against CR3 or sugars such as N-acetyl D-glucosamine, alpha- or beta-methyl D-glucoside, alpha- or beta-methyl D-mannoside, or zymosan. Thus, the complement receptors CR3 and CR4 are membrane receptors for sCD16. Through this interaction, sCD16 induces a CR3-dependent production of IL-6 and IL-8 by monocytes. These results suggest that sCD16 plays a regulatory role in inflammatory processes and provide a molecular basis for the interaction between FcgammaRIII-B and CR3 described on the cell membrane.

65. Interleukin-8 production by macrophages from atheromatous plaques
Apostoloulos, J., Davenport, P. and Tipping, P.G.
Arterioscler. Thromb. Vasc. Biol., 16, 1007 (1996)

Interleukin-8 (IL-8) is a chemotactic peptide produced by macrophages that may be involved in the recruitment of inflammatory cells into atherosclerotic plaques. In vitro, IL-8 production by macrophages isolated from carotid plaques (1240±510 pg·10^5 $cells^{-1}$·24 h^{-1}, mean±SEM, n=6) and noncarotid plaques (4312±1588 pg·10^5 $cells^{-1}$·24 h^{-1}, n=9) was significantly greater than IL-8 production by blood monocytes isolated from the same patients (526±278 pg·10^5 $cells^{-1}$·24 h^{-1}, n=6, $P<.05$ and 726±384 pg·10^5 $cells^{-1}$·24 h^{-1}, n=9, $P<.01$, respectively). IL-8 produced by atherosclerotic macrophages was demonstrated to be biologically active in a neutrophil chemotaxis assay. IL-8 mRNA was detectable in plaque macrophages and blood monocytes from these patients, but blood monocytes from normal donors did not exhibit detectable IL-8 mRNA. IL-8 mRNA was localized in macrophage-rich areas of atherosclerotic plaques by in situ hybridization. These studies demonstrate that macrophages from atherosclerotic plaques show an enhanced capacity to produce IL-8 compared with normal and patient blood monocytes and that macrophages are a major site of IL-8 mRNA production in atherosclerotic plaques. These results provide further evidence for a proinflammatory role for macrophages in atherosclerosis.

66. Fibronectin enhances the migration rate of human neutrophils in vitro
Everitt, E.A., Malik, A.B. and Hendey, B.
J. Leukoc. Biol., 60, 199 (1996)

Efficient polymorphonuclear neutrophil (PMN) migration depends on specific interactions between PMNs, endothelial cells, and extracellular matrix (ECM) proteins. We investigated the relationship between PMN migration and the ECM molecule fibronectin (FN). We used an in vitro migration assay system to show that human PMNs migrated across an FN-coated filter barrier toward a formyl-Met-Leu-Phe (fMLP) chemoattractant gradient in greater numbers than across (uncoated) bare fitters. In 1 h of fMLP stimulation, 69 +/- 6% of the PMNs had migrated across the FN-coated filters, whereas 46 +/- 5% of PMNs migrated across bare filters. This effect was specific to FN; coating the filters with the ECM protein vitronectin did not enhance migration. Monoclonal antibodies against FN or against the alpha5 or beta1 integrin subunits of the FN receptor inhibited the enhanced PMN migration response across FN-coated filters. These findings indicate that the extracellular matrix protein FN enhances PMN migration and that this response is mediated by the alpha5beta1 FN receptor.

67. CGM1a antigen of neutrophils, a receptor of gonococcal opacity protein
Chen, T. and Gotschlich, B.C.
PNAS, 93, 14851 (1996)

Neisseria gonorrhoeae (GC) or *Escherichia coli* expressing phase-variable opacity (Opa) protein (Opa^+ GC or Opa^+ *E. coli*) adhere to human neutrophils and stimulate phagocytosis, whereas their counterparts not expressing Opa protein (Opa^- GC or Opa^- *E. coli*) do not. Opa^+ GC or *E. coli* do not adhere to human lymphocytes and promyelocytic cell lines such as HL-60 cells. The adherence of Opa^+ GC to the neutrophils can be enhanced dramatically if the neutrophils are preactivated. These data suggest that the components binding the Opa^+ bacteria might exist in the granules. CGM1a antigen, a transmembrane protein of the carcinoembryonic antigen family, is exclusively expressed in the granulocytic lineage. The predicted molecular weight of CGM1a is ≈30 kDa. We observed specific binding of $OpaI^+$ *E. coli* to a 30-kDa band of polymorphonuclear leukocytes lysates. To prove the hypothesis that the 30-kDa CGM1a antigen from neutrophils was the receptor of Opa^+ bacteria, we showed that a HeLa cell line expressing human CGM1a antigen (HeLa-CGM1a) bound Opa^+ *E. coli* and subsequently engulfed the bacteria. Monoclonal antibodies (COL-1) against CGM1 blocked the interaction between Opa^+ *E. coli* and HeLa-CGM1a. These results demonstrate that HeLa cells when expressing the CGM1a antigens bind and internalize $OpaI^+$ bacteria.

68. p-Hydroxyphenylacetaldehyde Is the Major Product of L-Tyrosine Oxidation by Activated Human Phagocytes
Hazen, S.L., Hsu, F.F. and Heinecke, J.W.
J. Biol. Chem., 271, 1861 (1996)

Reactive aldehydes generated during lipid peroxidation have been implicated in the pathogenesis of atherosclerosis as well as other inflammatory diseases. A potential catalyst for such reactions is myeloperoxidase, a hemeprotein secreted by activated phagocytes. We now report that activated neutrophils utilize the myeloperoxidase-H_2O_2-chloride system to convert L-tyrosine to *p*-

hydroxyphenylacetaldehyde. Production of *p*-hydroxyphenylacetaldehyde was nearly quantitative at physiological concentrations of L-tyrosine and chloride. Aldehyde generation required myeloperoxidase, H_2O_2, L-tyrosine, and chloride ion; it was inhibited by the H_2O_2 scavenger catalase and by the heme poisons azide and cyanide. Phorbol ester- and calcium ionophore-stimulated human neutrophils likewise generated *p*-hydroxyphenylacetaldehyde from L-tyrosine by a pathway inhibited by azide, cyanide, and catalase. Aldehyde production accounted for 75% of H_2O_2 generated by optimally stimulated neutrophils at plasma concentrations of L-tyrosine and chloride. Collectively, these results indicate that activated phagocytes, under physiological conditions, utilize myeloperoxidase to execute the chloride-dependent conversion of L-tyrosine to the lipid-soluble aldehyde, *p*-hydroxyphenylacetaldehyde, in near quantitative yield. Moreover, like aldehydes derived from lipid peroxidation, amino acid-derived aldehydes may exert potent biological effects in vascular lesions and other sites of inflammation.

69. Human Phagocytes Employ the Myeloperoxidase-Hydrogen Peroxide System to Synthesize Dityrosine, Trityrosine, Pulcherosine, and Isodityrosine by a Tyrosyl Radical-dependent Pathway
Jacob, J.S. et al
J. Biol. Chem., 271, 19950 (1996)

Myeloperoxidase, a heme protein secreted by activated phagocytes, may be a catalyst for lipoprotein oxidation *in vivo*. Active myeloperoxidase is a component of human atherosclerotic lesions, and atherosclerotic tissue exhibits selective enrichment of protein dityrosine cross-links, a well characterized product of myeloperoxidase. Tyrosylation of lipoproteins with peroxidase-generated tyrosyl radical generates multiple protein-bound tyrosine oxidation products in addition to dityrosine. The structural characterization of these products would thus serve as an important step in determining the role of myeloperoxidase in lipoprotein oxidation in the artery wall. We now report the identification and characterization of four distinct tyrosyl radical addition products generated by human phagocytes. Activated neutrophils synthesized three major fluorescent products from L-tyrosine; on reverse phase HPLC, each compound coeluted with fluorescent oxidation products formed by myeloperoxidase. We purified the oxidation products to apparent homogeneity by cation and anion exchange chromatographies and identified the compounds as dityrosine (3,3′-dityrosine), trityrosine (3,3′,5′,3"-trityrosine) and pulcherosine (5-[4"-(2-carboxy-2-aminoethyl)phenoxy]3,3′-dityrosine) by high resolution NMR spectroscopy and mass spectrometry. Additionally, we have found that dityrosine is a precursor to trityrosine, but not pulcherosine. In a search for a precursor to pulcherosine, we identified isodityrosine (3-[4′-(2-carboxy-2-aminoethyl)phenoxy]tyrosine), a non-fluorescent product of L-tyrosine oxidation by human phagocytes. Our results represent the first identification of this family of tyrosyl radical addition products in a mammalian system. Moreover, these compounds may serve as markers specific for tyrosyl radical-mediated oxidative damage in atherosclerosis and other inflammatory conditions.

70. Antibodies to human major histocompatibility complex products inhibit Fcγ receptors types I and II
Neppert, J. and Jungi, T.W.
Transfusion Med., 6(2), 125-131 (1996)

We have analysed the effect of polyclonal and monoclonal antibodies of distinct IgG isotypes directed against products of the human major histocompatibility complex (MHC) on the function of Fcγ receptors types I (FcγRI) and II (FcγRII). Human anti-D sensitized red blood cells (RBC), selectively binding to FcγRI, and bovine or murine IgG1 (bIgG1 or mIgG1) sensitized RBC, selectively binding to FcγRII, were employed as targets for the effector cell in order to distinguish the inhibition of both types. Using these targets it could be shown that human antibodies to products of the human MHC or murine monoclonal antibodies with the same specificity and mIgG2a isotype inhibited both the FcγRI- and the FcγRII-mediated function. In contrast, murine monoclonal antibodies with the same specificity but of the mIgG1 isotype only inhibited the FcγRII-mediated function. In a control experiment, human and murine antibodies to products of the MHC did not impair the FcγR-independent phagocytosis of *Saccharomyces* by monocytes and neutrophils. The present study suggests that this mechanism involves at least two different Fcγ receptors.

71. Modulation of cytokine production in activated human monocytes by somatostatin
Peluso, G. et al
Neuropeptides, 30(5), 443-451 (1996)

The immunosuppressor effects of the widely distributed neuropeptide somatostatin were examined on purified peripheral blood human monocytes. Somatostatin, at concentrations thought to be physiologic (10^{-10}–10^{-7} M), regulated monocyte/macrophage responses to (LPS) stimulation, as reflected by interleukin production. In particular, somatostatin had direct inhibitory effects on TNF-α, IL-1β, and IL-6 secretion by LPS-activated monocytes, while the decrease on IL-8 synthesis was modulated mainly by the action of somatostatin on TNF-α and IL-1β. In fact, the addition of these two inflammatory cytokines to the monocyte culture medium was able to induce IL-8 expression, as demonstrated by mRNA analysis, also in presence of the neuropeptide. Although somatostatin affected IL-8 production in an indirect way, it suppressed directly the chemotactic response of neutrophils to IL-8. Finally, somatostatin downregulation of monocyte activation was confirmed by the decrease of HLA-DR expressionon cell plasmamembranes (52% versus 33%). Our results confirm that somatostatin exerts preferential effects on the suppression of immunoreactions by modulating cytokine production and activity.

72. Ca2+ entry induced by calcium influx factor and its regulation by protein kinase C in rabbit neutrophils
Shibata, K., Morita, K., Kitayama, S., Okamoto, H. and Dohi, T.
Biochem. Pharmacol., 5281), 167-171 (1996)

Extracellular application of acid extract from platelet-activating factor- or thapsigargin-treated rabbit neutrophils induced a rise of cytosolic free calcium concentration ($[Ca^{2+}]_i$) in neutrophils and adrenal chromaffin cells suspended in Ca^{2+}-containing, but not in Ca^{2+}-deficient, medium. The ability of the extract to selectively induce Ca^{2+} entry was also confirmed by the increase in $^{45}Ca^{2+}$ uptake and failure to stimulate Ca^{2+} release in digitonin-permeabilized neutrophils. 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibited the extract-induced $[Ca^{2+}]_i$ rise in a staurosporine (ST)-sensitive fashion, neither of which had any effect on its production. SK&F 96365 and econazole also reduced extract-induced Ca^{2+} entry. These results suggest that a Ca^{2+} entry-inducible substrate (calcium influx factor) is extracted from Ca^{2+} store-depleted neutrophils, and that its action may be regulated by protein kinase C and certain pharmacological agents.

73. Diagnosis of cytomegalovirus (CMV) infection in pediatric transplant patients by the antigenemia, shell vial, and conventional culture assays performed on blood: correlation with CMV disease
Pedneault, L., Anglow, M., Alfieri, C. and Rubin, E.
Clin. Diagn. Virol., 6(1), 51-61 (1996)

Background: Human cytomegalovirus (CMV) is a significant cause of morbidity and mortality in transplant recipients. Isolation of CMV from blood leukocytes (CMV viremia) is considered predictive of CMV disease in transplant recipients. Therefore, investigation of methods for the rapid detection of CMV in the blood is important for diagnosis and management of these patients.
Objective: To compare three techniques for the diagnosis and monitoring of CMV infection in a pediatric transplant population through the quantitative detection of CMV in peripheral blood leukocytes (PBL).
Methods: Serial blood specimens were obtained for most patients. After separation of the PBL from each specimen, aliquots of the PBL were used for direct detection of CMV antigenemia by immunoperoxidase staining of acetone-fixed cells (CMV-vue kit, INCSTAR), and by immunofluorescence staining of formaldehyde-fixed cells (Complete 1C3 kit, Biosoft Argene). PBL were also inoculated into conventional cell culture tubes and shell vials. Patients' medical records were reviewed to ascertain the clinical significance of the results.
Results: A total of 154 specimens obtained from 38 pediatric transplant recipients were evaluated. CMV was detected in 16 specimens obtained from eight patients: 11 specimens were found positive with the CMV-vue kit, 10 with the Complete 1C3 kit, four by conventional culture, and one by the shell vial assay. Seven of the eight patients with CMV-positive PBL had clinical signs and other laboratory evidence of active CMV infection. In general, a high-level antigenemia was demonstrated in the presence of clinical disease, but there were exceptions.
Conclusions: The two antigenemia kits were more sensitive than conventional culture and the shell vial assay for the detection of CMV in the blood of pediatric transplant patients. Our results suggest that CMV antigenemia is a sensitive and specific rapid method for the diagnosis and monitoring of CMV infection in our patient population.

74. Human oral neutrophils: Isolation and characterization
Sato, E.F., Utsumi, K. and Inoue, M.
Methods in Enzymol., 268, 503-509 (1996)

No abstract available

75. The neutrophil oxidative burst in diarrhoea?-?associated haemolytic uraemic syndrome
Hughes, D.A., Smith, G.C., Davidson, J.E., Murphy, A.V. and Beattie, M.T.J.
Pediatric Nephrol., 10(4), 445-447 (1996)

Neutrophil-mediated tissue damage has been implicated in the pathogenesis of diarrhoea-associated haemolytic uraemic syndrome (D+ HUS). This study evaluates priming and activation of the neutrophil oxidative burst in D+ HUS using chemiluminescent techniques. Peripheral blood neutrophils from 11 children with acute D+ HUS were examined. No difference was found in the oxidative burst of neutrophils from patients and controls. Serum elastase levels were measured in 8 patients and found to be significantly elevated. Although elastase results suggest neutrophil activation, chemiluminescence studies do not confirm this in the peripheral blood neutrophil. This does not support a significant role for circulating agents in priming and activating the peripheral blood neutrophil.

76. Human neutrophil interactions of a bispecific monoclonal antibody targeting tumor and human Fc??RIII
Weiner, L.M. et al
Cancer Immunol. Immunother., 42(3), 141-150 (1996)

2B1 is a bispecific murine monoclonal antibody (bsmAb) targeting the c-erbB-2 and CD16 (Fc%RIII) antigens. c-erbB-2 is over-expressed by a variety of adenocarcinomas, and CD16, the low-affinity Fc% receptor for aggregated immunoglobulins, is expressed by

polymorphonuclear leukocytes (PMN), natural killer (NK) cells and differentiated mononuclear phagocytes. 2B1 potentiates the in vitro lysis of c-erbB-2 over-expressing tumors by NK cells and macrophages. In this report, the interactions between 2B1 and PMN were investigated to assess the impact of these associations on in vitro 2B1-promoted tumor cytotoxicity by human NK cells. The peak binding of 2B1 to PMN was observed at a concentration of 10 7g/ml 2B1. However, 2B1 rapidly dissociated from PMN in vitro at 37°C in non-equilibrium conditions. This dissociation was not caused by CD16 shedding. When PMN were labeled with ^{125}I-2B1 and incubated at 37°C and the supernatants examined by HPLC analysis, the Fab regions of dissociated 2B1 were not complexed with shed CD16 extracellular domain. While most of the binding of 2B1 to PMN was solely attributable to Fab-directed binding to Fc%RIII, PMN-associated 2B1 also bound through Fc%-domain/Fc%RII interactions. 2B1 did not promote in vitro PMN cytotoxicity against c-erbB-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with peripheral blood lymphocytes, SK-OV-3 tumor and 2B1, the concentration of 2B1 required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered 2B1 in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

77. Detection of the metastatic potential of blood-borne and immunomagnetically enriched epithelial cells by quantitative erbB-2 RT-PCR

Brandt, B., Griwatz, C., Heidl, S., Assmann, G., Zänker, K.S.
Clin. Exp. Metastasis, 14(4), 399-408 (1996)

A quantitative competitive RT-PCR targeting the specific mRNA of c-erbB-2 is described. It is well known that in many cancers the c-erbB-2 gene dosage is elevated and/or the mRNA is overexpressed leading to a poor prognosis. The recently established method to enrich epithelial cells from peripheral blood made it reasonable and logical to develop a sophisticated competitive RT-PCR method to estimate the c-erbB-2 mRNA load of such cells. By this enrichment method it was discovered that cancer cells can be either isolated as single cells or clusters from patients' blood. The plausibility for their metastatic potential is derived from the measurement of the c-erbB-2 mRNA expression. A SKBR3 cell model suggested that the c-erbB-2 mRNA load is between 168 and 336 molecules per cell. Results of the quantitative competitive RT-PCR method can be used as a rational to determine the efficiency of anti-oncogenic therapies and as a criterion in adjuvant therapy decision.

78. Human Neutrophil Functions Are Inhibited In Vitro by Clinically Relevant Ethanol Concentrations

Patel, M. et al
Alcoholism Clin. Exp. Res., 20(2), 275-283 (1996)

Neutrophils [polymorphonuclear neutrophils (PMNs)] play a pivotal role in host defense in man. These defenses may be compromised, however, in alcohol users and abusers. We therefore evaluated the effect of ethanol levels (12.5 to 500 mg/dl), on key functions of human PMNs—chemotaxis and production of reactive oxygen species—and on changes in cytosolic-free calcium ($[Ca^{2+}]_i$), a pivotal intracellular mechanism of PMN activation. Ethanol significantly inhibited chemotaxis as evaluated by formyl-methionyl-leucyl-phenylalanine (fMLP)-induced upregulation of surface adhesion molecules (CD11b), fMLP-induced PMN elongation was only inhibited by a very high ethanol concentration of 500 mg/dl. Production of reactive oxygen species by normal PMNs was assessed by either chemiluminescence (CL) for hypochlorous acid or ferricytochrome c reduction (FCR) for superoxide anions. For PMN stimulated by fMLP, ethanol inhibited CL but not FCR. For PMNs activated by phorbol myristate acetate, ethanol inhibited both CL and FCR. Ethanol did not alter baseline $[Ca^{2+}]_i$, as assessed by videomicroscopy using the Ca^{2+}-sensing fluorescent dye Fura-2-AM, but did significantly potentiate the increase in peak $[Ca^{2+}]_i$, levels that occurs in response to stimulation by fMLP. Calcium channel blockers attenuated ethanol's inhibition of CL. Thus, acute in vitro ethanol, at clinically relevant concentrations, can inhibit several critical aspects of PMN functions. But, in PMNs, unlike neural cells, these inhibitory effects do not seem to be mediated by decreases in Ca^{2+} influx or in $[Ca^{2+}]_i$.

79. Ethanol Suppresses Endotoxin But Not Platelet Activating Factor-Induced Hypotension and Nitric Oxide

Greenberg, S.S., Xie, J., Powers, D.R. and Giles, T.D.
Alcoholism Clin. Exp. Res., 20(7), 1260-1268 (1996)

Ethanol (ETOH) inhibits the immune response to endotoxemia. The early stage of endotoxin (LPS)-induced shock is associated with an acute phase cardiovascular depression (APCD). Release of platelet activating factor (PAF) and tumor necrosis factor alpha (TNFa) with upregulation of nitric oxide (NO) production may initiate the APCD. Since ETOH inhibits induction of NO synthase (iNOS) mNRA by LPS, we postulate that ETOH may mask the APCD associated with endotoxemia. To test this, Sprague-Dawley rats (280–320 g, n = 5–6/group) were given LPS [0.75 mg/kg, intravenously (iv)] or PAF (10 to 150 pg/kg, iv) 30 min after administration of sterile saline (PBS), BN-5073 a mixed PAF antagonist (0.50 μg/kg, iv), or ETOH t2.2-5.5 g/kg, intra-peritoneally (ip)]. Cardiovascular parameters and plasma concentrations of nitrate and nitrite (RNI), ETOH, TNFα, and neutrophil (PMN) generation of RNI were measured. LPS and PAF both produced APCD. LPS-induced APCD was associated with tachycardia, elevated plasma TNFα and RNI, and ex wivo generation of RNI by PMNs. ETOH and EN-50730 prevented LPS-induced APCD and increases in RNI and TNFα. ETOH, however, increased the mortality associated with APCD. PAF produced only hypotension, bradycardia and elevated plasma levels of TNFα. ETOH and LNMMA did not affect PAF-induced APCD. EN-50730 inhibited PAF-induced APCD and plasma TNFα. We conclude that

1) ETOH inhibits the APCD and induction of NO characteristic of endotoxemia and 2) ETOH-induced suppression of LPS-mediated APCD may be mediated in part by suppression of release of intracellular PAF. Ethanol may increase the morbidity and mortality of endotoxemia by masking the hypotension and humoral changes characteristic of early endotoxemia thereby delaying appropriate therapy

80. Activation of human neutrophils after contact with cellulose-based haemodialysis membranes: intracellular calcium signalling in single cells
Hänsch, G.M. et al
Nephrol. Dial. Transplant., 11, 2453-2460 (1996)

PURPOSE OF STUDY.: *In vitro* contact of human leukocytes with cellulose-based dialysis membranes under complement-independent conditions results in activation of various leukocyte functions. To analyse signals involved in the mechanism of cell activation, we measured changes in cytosolic free calcium ($[Ca^{2+}]_i$) in individual human blood neutrophils (PMN) upon contact with flat sheet haemodialysis membranes.
RESULTS.: By confocal laser-scanning microscopy (CLSM), changes in $[Ca^{2+}]_i$ were monitored in Fluo-3-labelled cells up to 10 min after contact with a regenerated cellulose (RC) membrane. Multiple $[Ca^{2+}]_i$ transients were observed for cells in contact with RC; biostochastic analysis showed that up to 67% of the PMN responded with a high increase in $[Ca^{2+}]_i$ the rest were low- or non-responding cells. After contact with the new synthetic polycarbonate-polyether (PC-PE) membrane only non-responding cells were seen, indicating reduced cellular contact activation. The increase in $[Ca^{2+}]_i$ of cells on RC could be inhibited by 5 mM L-fucose. This monosaccharide was recently found to bepresent in cellulose-based polymers in picomolar concentrations.
CONCLUSIONS.: The data supports the hypothesis that dialysis-membrane-associated L-fucose residues participate in complement-independent leukocyte activation during haemodialysis therapy.

81. Quantitative RT-PCR combined with time-resolved fluorometry for determination of BCR-ABL mRNA
Bortolin, S. and Christopoulos, K.
Clin. Chem., 42(12), 1924-1929 (1996)

A microtiter well-based quantitative reverse transcriptase-PCR assay for determination of BCR-ABL mRNA, which relies on coamplification of the target with an RNA internal standard (IS), was developed. The hapten digoxigenin (Dig) is incorporated during PCR. Target RNA and IS contain identical primer recognition sites and generate same-sized amplification products distinguishable by hybridization with probes specific to the molecules' central part. The hybrids are determined with an anti-Dig-alkaline phosphatase conjugate with fluorosalicylphosphate as substrate. Fluorescent complexes of fluorosalicylate-Tb(III)-EDTA are measured by time-resolved fluorometry. The ratio of fluorescence values for target and IS is linearly related to initial target RNA in the range of 1000 to 200000 molecules. Samples containing K562 total RNA amidst 1 microgram of RNA from normal cells give fluorescence ratios that are linearly related to 30-10000 K562 cells. CVs for 30, 200, and 900 K562 cells are approximately 11%.

82. Is inhibition of oxygen radical production of neutrophils by sympathomimetics mediated via beta-2 adrenoceptors?
Weiss, M. et al
Pharmacol. Exp. Ther., 278(3), 1105-1113 (1996)

Despite their beneficial effects on cardiovascular derangements in patients with severe sepsis, high doses of sympathomimetics might contribute to an impaired neutrophil function. This study was conducted to examine whether various sympathomimetics [(-)-epinephrine (EPI), dopamine (DA) and dobutamine (DOB)] differ in their potency to suppress the formation of oxygen radicals by neutrophils and whether this potency correlates with their affinity to or intrinsic activity for beta-2 adrenoceptors (beta-2 AR). Oxygen radical production of human neutrophils was induced by N-formyl-methionyl-leucyl-phenyl-alanine and detected by chemiluminescence measurements. Dose-response curves for the inhibition of chemiluminescence by sympathomimetics were measured in the absence and presence of 0.1 microM CGP 20,712 A (1-[2(3- carbamoyl-4-hydroxy phenoxy)-ethylamino]-3-[4-(1-methyl-4- trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol methanesulfonate) and 0.1 microM ICI 118,551 (erythro-(+/-)-1-(7-methylindan-4-yloxy)-3 isopropylaminobutan-2-ol hydrochloride) to selectively antagonize beta- 1 AR and beta-2 AR, respectively. Inhibition of chemiluminescence of neutrophils by EPI was approximately 100-fold more potent than that by DA and DOB. Only the inhibition curve by EPI exhibited two components, one at nanomolar and one at micromolar concentrations. The nanomolar component was sensitive against beta-2 AR blockade, whereas the micromolar one was insensitive against both beta AR antagonists. Dose- response curves for DA and DOB exhibited a simple hyperbolic shape at micromolar concentrations and were insensitive against both beta AR antagonists. Maximum inhibition by DA and DOB was equipotent to that by EPI. However, the EC50 for DA was much lower than its dissociation constants, KD, assayed in membrane preparations by radioligand binding, whereas the EC50 of DOB matched KD. This difference could not be explained by a different efficiency of signal transduction, which was determined in receptor-coupled adenylate cyclase activity and which only showed a slightly higher efficiency of DA (51%) than of DOB (34%). Therefore, sympathomimetics were also investigated in a cell-free system, in which chemiluminescence was generated by horseradish peroxidase with hydrogen peroxide as substrate. Surprisingly, all of the sympathomimetics suppressed chemiluminescence with micromolar concentrations. We conclude that

sympathomimetics with high affinity and high intrinsic activity (EPI) inhibit neutrophil function via occupation of beta-2 AR, whereas sympathomimetics with low affinity (DA) or low intrinsic activity (DOB) may act by direct scavenging of oxygen radicals.

83. Expression of ceruloplasmin gene in human and rat lymphocytes
Pan, Y., Katula, K. and Failla, M.L.
Biochim. Biophys. Acta, ***1307(2)****, 233-238 (1996)*

The acute phase plasma protein ceruloplasmin (Cp) appears to play some role in host defense. The possibility that production of Cp in extrahepatic sites may also be essential for the activation, effector functions and cytoprotection of immune cells in localized environments has received minimal attention. Here, we have surveyed various types of human and rat immune cells for the presence of Cp mRNA using RT-PCR with primers that span exons 17–19 as an initial step in addressing this possibility. Validated Cp Rt-PCR bands were obtained from RNA samples isolated from resting and activated human lymphocytes, CD4 and CD8 T-cells and B-cells. Semiquantitative RT-PCR indicated that Cp mRNA in immune cells is present at about 0.2% the level of Cp mRNA in HepG-2 human liver cell line. Various human cell lines derived from the immune system, rat splenic MNC and purified rat T-lymphocytes also constitutively express Cp gene.

84. Site-directed mutagenesis of monocyte chemoattractant protein-1 identifies two regions of the polypeptide essential for biological activity
Beall, C.J., Mahajan, S., Kuhn, D.E. and Kolattukudy, P.E.
Biochem. J., ***333****, 633-640 (1996)*

Monocyte chemoattractant protein-1 (MCP-1) mediates monocyte migration into tissues in inflammatory diseases and atherosclerosis. We have investigated structure-activity relationships for human MCP-1. Mutations were introduced based upon differences between MCP-1 and the structurally related but functionally distinct molecule interleukin-8 (IL-8). Mutant proteins produced using the baculovirus/insect cell expression system were purified and their ability to stimulate monocyte chemotaxis and elevation of intracellular calcium in THP-1 monocytic leukaemia cells was measured. Two regions in MCP-1 were identified as important for its biological activity. One region consists of the sequence Thr-Cys-Cys-Tyr (amino acids 10-13). Point mutations of Thr-10 to Arg and Tyr-13 to Ile greatly lowered MCP-1 activity. The second functionally important region is formed by Ser-34 and Lys-35. Insertion of a Pro between these two residues, or their substitution by the sequence Gly-Pro-His, caused nearly complete loss of MCP-1 activity. Competition binding experiments showed that the mutations that affected activity also lowered the ability to compete with wild-type MCP-1 for receptors on THP-1 cells. Point mutations at positions 8, 15, 30, 37, 38 and 68 had little effect on MCP-1 activity. The important regions that we have identified in MCP-1 correspond with previously identified functionally important regions of IL-8, suggesting that the two molecules bind to their respective receptors by similar contacts.

85. Release of thrombomodulin from endothelial cells by concerted action of TNF-[alpha] and neutrophils: in vivo and in vitro studies
Boehne, M.W.J. et al
Immunology, ***87(1)****, 134-140 (1996)*

Inflammatory cytokines decrease the expression of thrombomodulin (TM) on the endothelial cell surface by suppression of TM transcription and translation or internalization with subsequent degradation. Nevertheless, elevated serum TM levels are found in diseases associated with systemical or locally increased levels of inflammatory cytokines. To study directly the in vivo effects of tumour necrosis factor-[alpha] (TNF-[alpha]) we determined the course of serum TM after systemic recombinant human (rh)TNF-[alpha] therapy. The TM levels were determined by enzyme-linked immunosorbent assay (ELISA). Systemic rhTNF-[alpha] therapy resulted in a marked and significant increase of serum TM. Using a mouse model we studied whether increased serum TM is associated with a decreased expression of TM on the endothelial surface in vivo. The immunohistochemical staining of the vasculature of meth-A sarcoma transplanted in mice showed a loss of TM immunoreactivity 4 hr after intravenous TNF-[alpha] application. To study the mechanism of TNF-[alpha] mediated release of TM, cultured endothelial cells were incubated with neutrophils and TNF-[alpha]. Incubation with TNF-[alpha] alone did not lead to an increase of TM in vitro. However TM was released into the culture supernatant when endothelial cells pretreated with TNF-[alpha] were exposed to neutrophils. This was associated with morphological evidence of endothelial cell damage. Therefore, the concerted action of cytokine-stimulated endothelial cells and neutrophils results in release of TM from cultured endothelial cells after rhTNF-[alpha] therapy. This might explain the increased serum TM levels observed in diseases associated with increased systemic or local levels of inflammatory cytokines despite the induced internalization and the direct inhibitory effects of TNF-[alpha] on TM transcription and translation.

86. Association of cytotoxin production and neutrophil activation by strains of Helicobacter pylori isolated from patients with peptic ulceration and chronic gastritis
Zhang, Q.B. et al
*Gut, **38(6)**, 841-845 (1996)*

Background: -Helicobacter pylori is associated with neutrophil infiltration within the gastroduodenal mucosa. Neutrophil activation provides a major source of oxygen free radicals, which have been implicated in the pathogenesis of peptic ulceration.
Aim: -To investigate if cytotoxin producing strains of H pylori are associated with the generation of oxidative burst in polymorphonuclear neutrophils (PMNs).
Patients: -76 patients undergoing endoscopy of whom 45 had peptic ulcer and 31 chronic gastritis only were studied.
Methods: -Strains of H pylori were cultured in Brucella broth. After 48 hours, bacteria were harvested by centrifugation and a bacterial suspension prepared as a stimulus for PMN oxidative burst using chemiluminescence. PMNs were prepared from healthy blood donors. To test the ability of strains to produce cytotoxin, culture supernatants of each were concentrated by polyethylene glycol and tested on cultured Vero cells for intracellular vacuolation.
Results: -30 of 45 (66.7 percent) peptic ulcer patients induced cell vacuolation versus nine of 31 (29 percent) strains from patients with chronic gastritis only (p less than 0.01). Cytotoxin positive strains of H pylori regardless of the presence or absence of peptic ulcer displayed an increased induction of respiratory burst in PMNs compared with toxin negative strains from patients with chronic gastritis only (p less than 0.05). Among the toxin negative strains, those from patients with peptic ulcer did not show a significant increase of the oxidative burst than those from patients without peptic ulcer (NS).
Conclusion: -Toxinogenicity of strains of H pylori seems to be correlated with neutrophil respiratory burst and peptic ulceration. The ability of some strains of H pylori to produce cytotoxin and to induce the oxidative burst in neutrophils may be important in the pathogenesis of peptic ulcer disease.

87. Azotemia, TNF[alpha], and LPS prime the human neutrophil oxidative burst by distinct mechanisms
McLeish, K.R. et al
*Kidney Int., **50(2)**, 407-416 (1996)*

Azotemia, TNF[alpha], and LPS prime the human neutrophil oxidative burst by distinct mechanisms. The oxidative burst of neutrophils from azotemic patients (AzoPMNs) is primed for an enhanced response compared to neutrophils from normal subjects (NorPMNs). The mechanism for this priming is unknown, although TNF[alpha] does not further prime AzoPMNs. The present study examines the hypothesis that azotemia and TNF[alpha] prime neutrophils by the same mechanism. Formyl peptide receptor expression and degranulation were not primed in AzoPMNs, but were primed by both LPS and TNF[alpha]. LPS was also able to prime the AzoPMN oxidative burst. Guanine nucleotide exchange by multiple guanine nucleotide binding proteins, including heterotrimeric G-proteins and low molecular weight GTP-binding proteins(LMWGs), was increased in AzoPMNs, as demonstrated by GTP[gamma]S binding and azidoanilide GTP photoaffinity labeling. The plasma membrane density of G-protein [alpha]i2, [alpha]i3, and [alpha]s subunits and the density in the cytosol of the LMWG, Rac2, did not differ between AzoPMNs and NorPMNs. However, the LMWG, Rap1A, was present in significantly greater amounts on plasma membranes from AzoPMNs. FMet-Leu-Phe-stimulated phospholipase D activity, but not basal activity, was significantly greater in AzoPMNs. Finally, incubation of NorPMNs in plasma from azotemic patients resulted in a significant increase in basal GTP[gamma]S binding. These results demonstrate that priming of AzoPMNs is restricted to oxidative burst activity and that it occurs by a mechanism distinct from that utilized by TNF[alpha] and LPS. While the exact mechanism remains unknown, it appears to involve a plasma factor and changes in LMWG expression or activity.

88. The Human Mast Cell Line HMC-1 Expresses C5a Receptors and Responds to C5a but not to C5a(desArg)
Werfel, T. et al
*Scand. J. Immunol., **44(1)**, 30-36 (1996)*

The expression of the receptor for the anaphylatoxin C5a (C5aR, CD88) on the human mast cell line HMC-1 was studied with four anti-C5aR monoclonal antibodies directed to the N-terminal domain of the receptor. All antibodies bound to the human mast cell line HMC-1. The binding could be blocked by recombinant C5a and by peptide EX-1 representing amino residues 1–31 on the N-terminal domain of the C5aR. In addition, FITC-labelled C5a bound to HMC-1, and this binding could be blocked by unlabelled C5a or C5aR antibodies. C5aR-specific mRNA was detected in HMC-1 cells by RT-PCR which confirmed the expression of the C5aR gene made by these cells. Lymphocyte-conditioned medium, interferon-γ or phorbol esters which have been shown to induce a down-regulation of C5aR on myeloid cells did not influence the expression of C5aR on HMC-1. C5a let to a transient mobilization of intracellular calcium in HMC-1 which could be inhibited by pre incubation of C5a with a C5a-specific antibody. In contrast to findings with granulocytes, HMC-1 did not respond to C5a(desArg), confirming previous findings with human skin mast cells. The findings show that (i) although HMC-1 differ from granulocytes in their responsiveness to C5a(desArg), they express similar C5aR and (ii) HMC-1 resemble skin mast cells in the expression and function of C5aR and may therefore serve as a model in future studies addressing the biology of this anaphylatoxin receptor on skin mast cells.

89. Neutrophils accentuate renal cold ischemia-reperfusion injury-dose-dependent protective effect of a platelet-activating factor receptor antagonist
Riera, M et al
*J. Pharmacol. Exp. Ther.,**280**, 786 (1997)*

This study was undertaken to evaluate whether the renal damage induced by cold ischemia-reperfusion was worsened by neutrophils (PMN), and if blockade of platelet-activating factor (PAF) could effectively decrease this injury. After flushing with EuroCollins, 85 kidneys from Sprague-Dawley rats underwent either no cold ischemia or a 4-h cold ischemia, and then were reperfused for 75 min at 37°C and 100 mm Hg in an isolated perfusion circuit. Reperfusion was performed with a Krebs-Henseleit solution containing 4.5% albumin, with and without human PMN (7.5×10^5 cells/ml) and with and without addition of a PAF receptor antagonist (BN 52021). Hemodynamic and functional parameters were continuously assessed during reperfusion. At end of the study, PAF production was evaluated. Presence of PMN during reperfusion of nonischemic kidneys produced no alteration of functional parameters or PAF production. After 4-h cold ischemia, the presence of PMN during reperfusion produced a significant worsening of plasma flow rate, glomerular filtration rate and sodium reabsorption in comparison with kidneys reperfused without PMN. Also, higher production of PAF was observed in the kidneys reperfused with PMN than in the kidneys reperfused without PMN. After 4-h cold ischemia, addition of BN 52021 during reperfusion in the presence of PMN significantly increased the plasma flow rate, glomerular filtration rate and sodium reabsorption in comparison with kidneys reperfused without this PAF antagonist. This effect was dose dependent. After 4-h cold ischemia, addition of BN 52021 during reperfusion in the absence of PMN produced no significant effect on functional parameters in comparison with kidneys reperfused without this PAF antagonist. These results indicate that PMN contribute to renal cold ischemia-reperfusion injury evaluated in the isolated perfused kidney. Treatment with a PAF receptor antagonist attenuated this injury in a dose-dependent manner, which suggests that it is mediated by PAF.

90. The human C3a receptor is expressed on neutrophils and monocytes, but not on B or T lymphocytes
Martin, U. et al
*J. Exp. Med., **186**, 199 (1997)*

The pathophysiological relevance of the complement split product C3a as a proinflammatory mediator is still ill defined. The expression pattern of the human C3a receptor (C3aR) can provide important clues for the role of this anaphylatoxin in inflammation. There is strong evidence for C3aR expression on basophils, and eosinophils, but additionally, only on tumor cell lines of leukemic or hepatic origin. It is unclear whether neutrophils also express the C3aR, but need a costimulus provided by eosinophils for certain biological responses, or whether neutrophils lack the C3aR and respond to C3a via a secondary stimulus generated by eosinophils, i.e., by an indirect mode. In the present study, polyclonal antiserum raised against the second extracellular loop of the C3aR was used to characterize C3aR expression on peripheral blood leukocytes. For high degree purification of neutrophils, a negative selection method was established that decreased the contamination with $CD9^{bright+}$ eosinophils down to <0.2%. Flow cytometric analyses, functional assays, and binding assays on highly purified neutrophils confirmed C3aR expression and coupling. Monocytes were identified as an additional C3aR-positive cell population of the peripheral blood. The expression of the C3aR on eosinophils could be confirmed. In contrast, the receptor could not be detected on unchallenged B or T lymphocytes (or lymphocyte-derived Raji cells).

91. Peroxidation of LDL from combined hyperlipidemic male smokers supplied with ¯–3 fatty acids and antioxidants
Brude, I.R. et al
*Arterioscler. Thromb. Vasc. Biol., **17**, 2576 (1997)*

Abstract The effects of marine ¯-3 polyunsaturated fatty acids (FAs) and antioxidants on the oxidative modification of LDL were studied in a randomized, double-blind, placebo-controlled trial. Male smokers (n=41) with combined hyperlipidemia were allocated to one of four groups receiving supplementation with ¯-3 FAs (5 g eicosapentaenoic acid and docosahexaenoic acid per day), antioxidants (75 mg vitamin E, 150 mg vitamin C, 15 mg ß-carotene, and 30 mg coenzyme Q_{10} per day), both ¯-3 FAs and antioxidants, or control oils. LDL and human mononuclear cells were isolated from the patients at baseline and after 6 weeks of supplementation. LDL was subjected to cell-mediated oxidation by the patients' own mononuclear cells, as well as to Cu^{2+}-catalyzed and 2,2'-azobis-(2-amidinopropane hydrochloride) (AAPH)–initiated oxidation. Extent of LDL modification was measured as lag time, the formation rate of conjugated dienes (CDs), the maximum amount of CDs formed, formation of lipid peroxides, and the relative electrophoretic mobility of LDL on agarose gels. Dietary supplementation with ¯-3 FAs increased the concentration of total ¯-3 FAs in LDL and reduced the concentration of vitamin E in serum. The ¯-3 FA–enriched LDL particles were not more susceptible to Cu^{2+}-catalyzed, AAPH-initiated, or autologous cell–mediated oxidation than control LDL. In fact, enrichment with ¯-3 FAs significantly reduced the formation rate of CDs when LDL was subjected to AAPH-induced oxidation. Supplementation with moderate amounts of antioxidants significantly increased the concentration of vitamin E in serum and increased the resistance of LDL to undergo Cu^{2+}-catalyzed oxidation, measured as

increased lag time, reduced formation of lipid peroxides, and reduced relative electrophoretic mobility compared with control LDL. Supplementation with ¯-3 FAs/antioxidants showed oxidizability of LDL similar to that of control LDL and ¯-3 FA–enriched LDL. In conclusion, ¯-3 FAs neither rendered the LDL particles more susceptible to undergo in vitro oxidation nor influenced mononuclear cells' ability to oxidize autologous LDL, whereas moderate amounts of antioxidants protected LDL against oxidative modification.

92. Interleukin 8 released after acute myocardial infarction is mainly bound to erythrocytes
De Winter, R. et al
Heart, 78, 598 (1997)

Objective—To determine whether rapid clearance of interleukin 8 (IL-8) from plasma through binding to the erythrocyte chemokine receptor may be responsible for failure to detect IL-8 consistently after acute myocardial infarction.
Design—Plasma concentrations of IL-8 were measured at frequent intervals in 43 consecutive patients. In 21 of these, erythrocyte bound IL-8 concentrations were also measured. The influence of infarct size, type of treatment, and the presence of early successful reperfusion on IL-8 release was assessed.
Results—Peak IL-8 concentrations in plasma were raised in 31 of the 43 patients (68%). Median plasma IL-8 concentrations were 16.0 pg/ml (range 2.4 to 225.0 pg/ml) six hours after the onset of chest pain. Twelve hours after the onset of symptoms, plasma IL-8 concentrations had already returned to normal in 27 patients. In contrast, in 18 of 21 patients (86%), erythrocyte bound IL-8 concentrations were raised at between 6 and 30 hours, with a median peak value of 59.8 pg/ml (range 19 to 148 pg/ml). No correlation between peak creatine kinase MB and peak IL-8 (plasma or erythrocyte bound) was observed. There was a significant difference in peak plasma IL-8 concentrations between patients who underwent direct PTCA (19.4 pg/ml) and those who received conservative treatment (9.9 pg/ml; p = 0.0206), but no correlation with the presence of early successful reperfusion.
Conclusions—IL-8 is released in plasma after acute myocardial infarction and subsequently binds to red blood cells, resulting in only a transient rise of plasma IL-8 and a more prolonged increase of erythrocyte bound IL-8.

93. Interaction of Ethanol with Inducible Nitric Oxide Synthase Messenger RNA and Protein: Direct Effects on Autacoids and Endotoxin In Vivo,
Greenberg, S.S. et al
J. Pharmacol. Exp. Ther., 282, 1044 (1997)

Inducible nitric oxide synthase (iNOS) mRNA is up-regulated *in vivo* by dibutyryl-cAMP (db-cAMP), the purine-2y receptor agonist 2-methylthio-ATP and *Escherichia coli* endotoxin lipopolysaccharide (LPS). Ethanol and diethyldithiocarbamate inhibit LPS-stimulated iNOS mRNA. Their effects on db-cAMP- and 2-methylthio-ATP-stimulated iNOS mRNA remain undefined. We examined the effect of ethanol (4.5 g/kg intraperitoneal) and intratracheal diethyldithiocarbamate (5 mg/kg) on intratracheal LPS (0.6 mg/kg), db-cAMP (0.1 and 1 mg/kg) or 2-methylthio-ATP (5 mg/kg)-stimulated rat alveolar macrophage (AM) iNOS mRNA and protein, reactive nitrogen intermediates nitrite and nitrate anion (RNI) and nuclear transcription factor-κB (NF-κB) *in vivo*. LPS and the autacoids increased iNOS mRNA and protein in rat AM and RNI in bronchoalveolar lavage fluid and in *ex vivo* incubates of AM compared with these parameters in control rats (n = 6-21/group). Only LPS up-regulated TNF-α mRNA and release of TNF-α in bronchoalveolar lavage fluid and AM. Ethanol inhibited LPS stimulation of the iNOS cascade at the level of transcription but inhibited only autacoid-stimulated iNOS protein and RNI. Diethyldithiocarbamate selectively inhibited the LPS-stimulated iNOS cascade at the level of transcription. Coadministration of ethanol and diethyldithiocarbamate inhibited LPS-stimulated iNOS mRNA, protein and RNI more than either inhibitor alone but did not differ from ethanol alone on autacoid-stimulated iNOS protein or RNI. LPS increased and db-cAMP did not affect NF-κB in AM. Ethanol inhibited LPS-stimulated NF-κB. Thus, two distinct pathways exist for induction of iNOS mRNA in rat AM *in vivo*: an NF-κB pathway for LPS and cytokines inhibitable by ethanol and diethyldithiocarbamate and an NF-kB-independent pathway, refractory to inhibition by ethanol and diethyldithiocarbamate for db-cAMP and 2-mes-ATP. Finally, ethanol inhibits iNOS at the level of transcription and at the level of the enzyme.

94. cAMP and purinergic P2y receptors upregulate and enhance inducible NO synthase mRNA and protein in vivo
Greenberg, S.S., Zhao, X., Wang, J-F., Hua, L. and Quyang, J.
Am. J. Lung Cell. Mol. Physiol., 273, 967 (1997)

Adenosine 3',5'-cyclic monophosphate (cAMP) and purinergic P2y receptor agonists upregulate inducible nitric oxide (NO) synthase (iNOS) but inhibit Escherichia coli endotoxin lipopolysaccharide (LPS)- and cytokine-mediated upregulation of iNOS in cultured cells. We examined the effects of cAMP and P2y receptor agonists on the iNOS system in vivo. Intratracheal administration of dibutyryl-cAMP (DBcAMP, 0.1 and 1 mg/kg), a P2y receptor agonist [2-methylthioadenosine 5'-triphosphate (MeS-ATP), 5 mg/kg], or LPS (0.6 mg/kg) to rats 2 h before bronchoalveolar lavage (BAL) increased iNOS mRNA (competitor-equalized reverse transcription-polymerase chain reaction) and iNOS protein (Western blot) in rat alveolar macrophages compared with the effects of sterile phosphate-buffered saline (0.5 ml it). At equal levels of upregulation of iNOS mRNA, 1) LPS, but not DBcAMP or MeS-ATP, upregulated nuclear transcription factor-kappa B (NF-kappa B) and 2) iNOS protein and formation of NO were greater in alveolar macrophages from LPS- and MeS-ATP-treated rats than from DBcAMP-treated rats. Administration of DBcAMP or MeS-AMP 15 min before LPS did not inhibit LPS-induced alveolar macrophage-derived iNOS mRNA, iNOS protein, and NO. Diethyldithiocarbamate (DETC, 5 mg/kg it)

inhibited LPS-induced iNOS mRNA but did not affect upregulation of iNOS mRNA produced by the other agonists. We conclude that an LPS-dependent and -independent pathway of iNOS mRNA induction exists in vivo. The former is activated by IPS and most cytokines, is associated with upregulation of NF-kappa B and inhibited by DETC, and elicits an inflammatory response. The latter, activated by DBcAMP and MeS-ATP, is not associated with upregulation of NF-kappa B, inhibition by DETC, or activation of inflammation. The two systems are additive in vivo rather than antagonistic. Speculatively, if the LPS-independent iNOS pathway exists in humans, the iNOS in tissues from patients taking drugs affecting cAMP or P2y receptors may be iatrogenic rather than pathogenetic in origin.

95. No deficiency in immunology tools
Horton, B.
Nature, 390, 316-318 (1997)

This week's new products selection describes systems for use in the study of immunity and systems that use immunological molecules to study other important areas of the life sciences.

96. Late and persistent up-regulation of intercellular adhesion molecule-1 (ICAM-1) expression by ionizing radiation in human endothelial cells in vitro
Gaugler, M.H. et al
Int. J. Rad. Biol., 72(2), 201-209 (1997)

Adhesion molecules play a key role in cellular traffic through vascular endothelium, in particular during the inflammatory response when leukocytes migrate from blood into tissues. Since inflammation is one of the major consequences of radiation injury, we investigated the effect of ionizing radiation on cellsurface expression of the intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in cultured human umbilical vein endothelial cells (HUVEC). Flow cytometry performed on irradiated HUVEC revealed both a time- (from 2 to 10 days) and dose- (from 2 to 10 Gy) dependent up-regulation of basal expression of ICAM-1, and no induction of VCAM-1 or E-selectin. The radiationinduced increase in ICAM-1 expression on HUVEC was correlated with augmented adhesion of neutrophils on irradiated endothelial cells. Interleukin-6 (Il-6) or other soluble factors released by irradiation were not involved in the enhanced ICAM-1 expression by irradiation. Northern blot analysis showed an overexpression of ICAM-1 mRNA from 1 to 6 days after a 10 Gy exposure. Our data suggest that ICAM-1 participates in the radiation-induced inflammatory reaction of the endothelium.

97. The systemic lupus erythematosus (SLE) disease autoantigen—calreticulin can inhibit C1q association with immune complexes
Kishore, U. et al
Clin. Exp. Immunol., 108(2), 181-190 (1997)

Following its release from cells during infection and inflammation, calreticulin (CRT) can act as an autoantigen in diseases such as SLE. Why CRT is a target of protective immunity and whether it may interfere with innate immunity once released from cells during inflammation is unclear. In the present study, we found that CRT was detected more frequently in SLE sera and in higher amounts than found in control sera. Approximately 40% of SLE sera tested contained autoantibodies against CRT as detected by ELISA and immunoblotting. CRT was found to be predominantly in the sera of SLE patients associated with immune complexes and C1q, and only bound to the surfaces of neutrophils in the presence of low levels of calcium and magnesium. In order to further investigate the C1q–CRT interaction, recombinant CRT and its discrete domains (N-, P-, and C-domains) were produced in *Escherichia coli*. CRT binds to globular head region of C1q primarily via its N- and P-domains. The N-domain was shown to be the most autoantigenic region of CRT, as the anti-CRT autoantibodies from most patients reacted against this region. CRT also altered C1q-mediated immune functions. The P-domain of CRT bound to C1q and reduced the binding of immune complexes in SLE sera to immobilized C1q. Full length CRT and its N- and P-domains were able to reduce the C1q-dependent binding of immune complexes to neutrophils and solid-phase bound C1q. We conclude that CRT, once released from leucocytes during inflammation, may not only induce an antigenic reaction, but also interfere with C1q-mediated inflammatory processes.

98. Prostate specific antigen reverse transcriptase-polymerase chain reaction assay in preoperative staging of prostate cancer
Ignatoff, J.M., Oefelein, M.G., Watkin, W., Chmiel, J.S. and Kaul, K.L.
J. Urol., 158(5), 1870-1874 (1997),

Purpose
Extracapsular extension of prostate cancer occurs in a significant number of men believed to have clinically localized disease. We report the ability of the reverse transcriptase-polymerase chain reaction (RT-PCR) assay to predict preoperatively the pathological stage of cases of clinically localized prostate cancer.
Materials and Methods
Since October 1994, 82 consecutive men with clinically localized prostate cancer had a venous blood RT-PCR assessment before radical retropubic prostatectomy. The extracted ribonucleic acid was reverse transcribed, amplified and the amplicon identity confirmed

by prostate specific antigen (PSA) directed probe hybridization. An additional 31 patients were enrolled to provide appropriate positive (T+Nx/1M2) and negative (human female and benign prostatic hyperplasia) controls. Histological examination of the entire prostatectomy specimen was performed.
Results
Positive RT-PCR assay results correlated significantly with skeletal metastases and elevated levels of serum PSA but they did not significantly improve our ability to identify prospectively patients with extracapsular extension over traditional predictors (serum PSA, Gleason score).
Conclusions
The role of molecular techniques in prostate cancer evaluation and prognosis continues to emerge. However, in our study we demonstrate no significant advantage in preoperative staging of prostate cancer using RT-PCR assay with PSA primers.

99. Rhenium-188 and technetium-99m nitridobis(N-ethoxy-N-ethyldithiocarbamate) leucocyte labelling radiopharmaceuticals: [188ReN(NOET)2] and [99mTcN(NOET)2], NOET = Et(EtO)NCS2: Their in vitro localization and chemical behaviour
Demaimay, F. et al
Nucl. Med. Biol., 24(8), 701-705 (1997)

In this study, we have investigated the preparation of rhenium-188 nitridobis(N-ethoxy-N-ethyldithiocarbamate) [$^{188}ReN(NOET)_2$] (NOET = $Et(EtO)NCS_2$), analogous to the known technetium-99m radiopharmaceutical. The new ^{188}Re complex was synthesized in good yield with a satisfactory radiochemical purity, using a kit method. The subcellular localization of both radiopharmaceuticals in granulocytes was observed by microautoradiography. The uptake was independent of the radionuclide and predominantly nuclear. Furthermore, HPLC was used to characterize the ^{99m}Tc complex before and after blood cell labelling and revealed that the intact radiopharmaceutical was involved.

100. Airborne particulate matter modulates the production of reactive oxygen species in human polymorphonuclear granulocytes
Hitzfeld, B., Friedrichs, K.H., Ring, J. and Behrendt, H.
Toxicology, 120(3), 185-195 (1997)

Causal relationships between airborne particles (especially particulate matter < 10 μm in diameter) and increases in prevalences and symptoms of respiratory diseases have been postulated in many epidemiologic studies. Polymorphonuclear leukocytes (PMN) in the nasal or bronchial epithelium can be exposed to particulate matter (PM) and may upon exposure produce reactive oxygen species (ROS). Release of ROS can result in cellular and tissue damage and initiate or exacerbate inflammation. To elucidate the effect of PM on inflammatory reactions, we exposed human PMN to PM extracts. PM were collected with high volume samplers in two cities, Düsseldorf and Duisburg, in Germany and reflect sites with high traffic and industrial emissions respectively. The collected particles were extracted using water and then dichloromethane, resulting in an aqueous and an organic extract. The production of ROS was determined using luminol-enhanced chemiluminescence (LCL) of resting and zymosan-stimulated PMN. The present study shows that extracts of PM alone significantly stimulated the production and release of ROS in resting PMN. The effects of the PM extracts were inhibited by superoxide dismutase (SOD), catalase and sodium azide (NaN_3). Zymosan-induced LCL was, however, diminished by coincubation with PM extracts. The chemical composition is important when considering the effects of particles. Our study shows that only organic substances adsorbed to particles stimulate LCL. SOZ-induced LCL is inhibited by both types of extracts, but aqueous extracts have a stronger inhibitory effect. It is at present unclear which substances are responsible for these effects.

101. New bis(dithiocarboxylato)nitridotechnetium-99m radiopharmaceuticals for leucocyte labelling: In vitro and In vivo studies
Demaimay, F. et al
Nucl. Med. Biol., 24(5), 439-445 (1997)

Dithiocarboxylate ligands were synthesized and characterized. New nitrido 99m-technetium complexes were obtained with these ligands and identified by thin layer chromatography. The nitrido complexes were tested *in vitro* in whole blood for leucocyte labelling and the design of the ligand was optimized. Best results were obtained with aliphatic linear ligands, containing 9 to 11 atoms of carbon. The *in vivo* experiment failed because an inflammated area could not be visualized by γ imaging, the cell labelling mechanism being probably different.

102. Reactive oxygen species generation by seminal cells during cryopreservation
Wang, A.W., Zhang, H., Ikemoto, I., Anderson, D.J. and Loughlin, K.R.
Urology, 49(6), 921-925 (1997)

Objectives. To test the hypothesis that conventionally used procedures for semen cryopreservation may cause an increase in the production of reactive oxygen species (ROS) by sperm or by seminal leukocytes, which may contribute to poor sperm function following cryopreservation.
Methods. Eighteen semen specimens with normal parameters from healthy male donors 22 to 40 years of age were each divided into two portions. The first portion was combined 1:1 with Test Yolk Buffer-Glycerol Freezing Medium and was frozen by gradual cooling

into liquid nitrogen (−196°C). The second portion was washed and the cells were resuspended in Sperm Washing Medium (SWM) and incubated at room temperature to serve as controls. After a period of treatment, frozen samples were thawed and semen cells were washed and resuspended in SWM. ROS generation by semen cells from each treatment group was measured on a luminometer. Sperm motility, sperm viability, and sperm membrane integrity were also measured in both control and freeze-thaw samples. To further assess ROS generation by semen cells during the cooling process, aliquots of washed semen cells and purified polymorphonuclear leukocytes (PMNs) were incubated separately at different temperature conditions (37°C, 22°C, 4°C, and −20°C). ROS activity in each treatment group was measured and compared with each other.
Results. In both semen cells and PMNs, ROS activity increased significantly during the cooling process. The highest ROS levels were recorded in both groups when cooled to 4°C. The ROS levels were extremely low in samples cooled to −20°C and in freeze-thaw samples, probably due to marked loss of cell viability.
Conclusions. Gradual reduction of temperature during the process of semen cryopreservation can cause a significant ROS generation by semen cells. ROS is particularly elevated during cooling if the semen sample is contaminated by more than 0.5×10^6 leukocytes. Removal of leukocytes from semen samples or treatment with antioxidants prior to cryopreservation may improve sperm viability and function.

103. Suppression of cytokine synthesis, integrin expression and chronic inflammation by inhibitors of cytosolic phospholipase A2
Amandi-Burgermeister, E., Tibes, U., Kaiser, B.M., Friebe, W.G. and Scheuer, W.V.
Eur. J. Pharmacol., 326(2-3), 237-250 (1997)

To define the isoform of phospholipases A_2 active in inflammation we evaluated the effects of low-molecular-weight inhibitors of secretory and cytosolic phospholipases A_2. We found that inhibitors of cytosolic phospholipase A_2 had therapeutic efficacy in an in vivo model of chronic inflammation (rat adjuvant arthritis), whereas inhibitors of secretory phospholipase A_2 had no beneficial effect. In vitro, inhibitors of cytosolic phospholipase A_2 diminished surface expression of Mac-1 (CD11b/CD18) β_2-integrin on calcium ionophore-stimulated human blood granulocytes and suppressed synthesis of interleukin-1β in lipopolysaccharide-stimulated human blood monocytes and U937 cells by reducing mRNA levels. Lipid mediators promote Mac-1 exocytosis and transcription of interleukin-1β, which further enhances cytosolic phospholipase A_2 activity and expression. Thus, superinduction of cytosolic phospholipase A_2 may establish a positive feedback loop, converting acute inflammation into chronic inflammation. Consequently, inhibitors of cytosolic phospholipase A_2 may prevent inflammation in vivo by interfering with cellular activation and infiltration. We conclude that cytosolic phospholipase A_2 but not secretory phospholipase A_2 is the predominant enzyme in inflammatory signalling.

104. Effect of protein kinase inhibitors on the superoxide generation and tyrosyl phosphorylation of prolylproline-primed human neutrophils
Sugahara, K., Zhang, J., Watanabe, Y., Sagara, Y. and Kodama, H.
Clin. Biochem., 30(1), 75-78 (1997)

No abstract available

105. Regulation of neutrophils in ulcerative colitis by colonic factors: A possible mechanism of neutrophil activation and tissue damage
Robinson, C.E. et al
J. Lab. Clin. Med., 130(6), 590-602 (1997)

The mucosal injury of active ulcerative colitis (UC) could involve enhanced migration and activation of neutrophils (PMNs). Because, in vitro, PMNs from patients with UC appear normal and are not therefore a likely cause for the enhancements, we hypothesized an abnormal colonic milieu. We previously found that factors in the UC colonic milieu markedly increase production of reactive oxygen species (ROS) by control PMNs. We now hypothesize that these factors also regulate PMN surface integrins, that regulation of UC PMNs is different than that of control PMNs, and that the integrin regulation is consistent with the ROS regulation. Using rectal dialysis, we sampled the colonic milieu in patients with active UC, in patients with inactive UC, and in control subjects. We monitored a key PMN adhesion molecule, CD1lb. When control PMNs were tested, active UC rectal dialysate was almost as effective (+115%) as N-formyl-methionyl-leucyl-phenylalanine (+132%) in up-regulating CD 11b. When inactive UC PMNs were tested, baseline CD 11b was 50% higher than that for control PMNs. In contrast, rectal dialysates failed to up-regulate CD11b of inactive UC PMNs and in fact down-regulated CD11b. Preincubating control PMNs with UC rectal dialysates converted their CD11b response to PMN activators from up-regulation to down-regulation, mimicking inactive UC PMNs. Changes in intracellular calcium levels paralleled these changes in CD11b. Rectal dialysate-induced changes in both CD11b and calcium paralleled our previous findings on rectal dialysate-induced changes in ROS production. Thus the net overall effect of factors in the colonic milieu is a consistent and predictable regulation of PMN function—proinflammatory in UC, anti-inflammatory in control subjects. These factors may be a critical part of the pathophysiology of UC.

106. Activation of the neutrophil and loss of plasma glutathione during Mg-deficiency – modulation by nitric oxide synthase inhibition
Mak, I.T., et al
Mol. Cell. Biochem., 176(1-2), 35-39 (1997)

Sprague-Dawley rats (200 g) were fed either a Mg-deficient or Mg-sufficient diet for 3 weeks. An enriched neutrophil fraction (>85%) was isolated from the blood by sodium metrizoate/dextran gradient centrifugation. Using the superoxide dismutase (SOD)-inhibitable cytochrome c reduction assay, the basal activity of neutrophils isolated from the Mg-deficient rats were found elevated 5 fold after two weeks, and up to ~7 fold after three weeks on the diet. Upon challenge by phorbol myristate acetate (PMA), unlike the Mg-sufficient cells, the Mg-deficient cells exhibited no significant activation. Treatment of the Mg-deficient rats with the nitric oxide (NO)-synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) in the drinking water, significantly attenuated the basal superoxide producing activity of the neutrophils and partially restored its response to PMA challenge. In association with the neutrophil activation. Mg-deficiency resulted in 70% decrease in plasma glutathione and 220% increase in Fe-promoted, thiobarbituric acid reactive substance (TBARS) levels; both changes were significantly attenuated by L-NAME treatment. The results suggest that neutrophils from Mg-deficient rats are activated endogenously to generate oxy-radicals which might directly mediate the in vivo peroxidative indices during Mg-deficiency. Furthermore, the neutrophil activity was lowered by NO-synthase inhibition suggesting that NO overproduction during Mg-deficiency participates in the neutrophil activation process.

107. Tissue Plasminogen Activator (tPA) Inhibits Human Neutrophil Superoxide Anion Production in Vitro
Stringer, K.A., Lindenfeld, J., Repine, A.J., Cohen, Z. And Repine, J.E.

Because neutrophils contribute to reperfusion injury associated with acute myocardial infarction (MI), and because tissue plasminogen activator (tPA) is often used in the management of MI, we evaluated the effect of tPA on superoxide ($O_2^{\cdot-}$) production by human neutrophils in vitro. We found that adding increasing amounts of tPA significantly ($r = 0.89$, $P < 0.025$) and progressively reduced $O_2^{\cdot-}$ generation by neutrophils treated with phorbol myristate acetate (PMA) in vitro. Furthermore, adding tPA that had been previously treated with the protease inhibitor, D-Phe-Pro-Arg-chloromethyl ketone HCl (PPACK), also decreased neutrophil $O_2^{\cdot-}$ generation in vitro ($P < 0.05$). In contrast, adding L-arginine, a component of the tPA preparation and a precursor of nitric oxide (NO), did not inhibit PMA-induced neutrophil $O_2^{\cdot-}$ production. Also, adding increasing concentrations of tPA did not reduce ($P > 0.05$) the concentrations of $O_2^{\cdot-}$ produced by xanthine oxidase (XO) in vitro. Our findings suggest that tPA reduces neutrophil $O_2^{\cdot-}$ generation by a mechanism that is not related to L-arginine, is not dependent on tPA proteolytic activity, and is not a function of direct scavenging. This property may account for some of the effectiveness of tPA in the treatment of MI and/or make tPA valuable for treating acute respiratory distress syndrome (ARDS) or other inflammatory disorders involving neutrophil $O_2^{\cdot-}$ production.

108. Effects of ropivacaine on eicosanoid release from human granulocytes and endothelial cells in vitro
Martinsson, T., Haegerstrand, A. and Dalsgaard, C.J.
Inflammation Res., 46(10), 398-403 (1997)

To examine the effects of ropivacaine, currently being investigated for treatment of ulcerative colitis, on the release of arachidonic acid metabolites.¶Material: Human granulocytes and endothelial cells.¶Treatment: Ropivacaine, lidocaine, hydrocortisone, 5-aminosalicylic acid or acetylsalicylic acid (10-1000 7M).¶Methods: Leukotriene B_4, 5-hydroxyeicosatetraenoic acid, 6-keto $PGF_{1!}$ and 15-hydroxyeicosatetraenoic acid were measured using immuno assays. Wilcoxon signed rank test was used for statistical calculations.¶Results: Ropivacaine dose-dependently inhibited zymosan-induced release of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid whereas the release after ionophore stimulation was not affected. Ropivacaine was more potent than 5-aminosalicylic acid but less potent compared to hydrocortisone. Ropivacaine had only a weak inhibitory effect on the release of 15-hydroxyeicosatetraenoic acid from zymosan- or ionophore-stimulated cells. In contrast to hydrocortisone and 5-aminosalicylic acid, ropivacaine only weakly affected the release of 6-keto $PGF_{1!}$ after stimulation with thrombin.¶Conclusions: The inhibited release of 5-lipoxygenase products may account for some of the anti-inflammatory effects of ropivacaine seen in the treatment of ulcerative colitis.

109. Ethanol Inhibits Inducible Nitric Oxide Synthese Transcription and Post-Transcriptional Processes in Vivo
Zhao, X. et al

Alcoholism Clin. Exp. Res., 21(7), 1246-1256 (1997)

The effects of ethanol (ETOH) on post-transcriptional regulation of inducible nitric oxide synthase (iNOS) in vivo has not been demonstrated. We examined the effect of ETOH on iNOS mRNA, protein, and the production of the nitrate and nitrite anion (RNI) in rat lung alveolar macrophages (AM) in vivo when stimulated by lipopolysaccharide (LPS) and dibutyryl cyclic AMP (DB-cAMP). Sprague-Dawley rats (225-250 g) (n = 5-7/gp) were given intratracheal LPS (0.6 mg/kg) or DB-cAMP (0.1 and 1 mg/kg) 30 min after ETOH (4 mg/kg, intraperitoneally (ip)) or phosphate-buffered sterile saline (PBS) (5 ml/kg, ip) or pyrrolidine dithiocarbamate (PDTC 10 mg/kg, intratracheally) or 15 min after diethyl dithiocarbamate (DETC) (5 mg/kg, intratracheally). At selected times after administration of LPS or DB-cAMP, the animals were anesthetized, the lungs with the heart attached were removed and the lungs subjected to bronchoalveolar lavage (BAL). The BAL fluid was assayed for tumor necrosis factor alpha (TNFα) and RNI. The BAL fluid AM were isolated and analyzed for iNOS mRNA and protein with competitor-equalized polymerase chain reaction (PCR) and Western blots, respectively. The ex vivo incubates of AM were assayed for RNI and TNFα. LPS and DB-cAMP each increased iNOS mRNA and protein in AM and RNI in the BAL fluid and *ex vivo* incubates of AM. However, the peak of the increase of iNOS mRNA occurred at 2 hr for DB-cAMP and 4 to 6 hr for LPS. Only LPS increased the concentrations of TNFα in BAL fluid and ex vivo incubates of AM. ETOH attenuated LPS-mediated up-regulation of iNOS mRNA and TNFα and iNOS protein and RNI produced by the AM. In contrast, pretreatment of rats with ETOH did not affect DB-cAMP-mediated increases of iNOS mRNA in AM at any time but suppressed the amount of iNOS protein and RNI produced in DB-cAMP-stimulated AM. DETC, but not PDTC, attenuated LPS-mediated up-regulation of iNOS mRNA without effects on that produced by DB-cAMP. Since ETOH and DETC, but not PDTC, suppressed LPS-mediated but not DB-cAMP-mediated transcription of iNOS, we conclude that two distinct pathways exist for induction of iNOS mRNA by these agonists. ETOH and DETC may inhibit the LPS-mediated activation pathway by acting as antioxidants. Also, since ETOH inhibited DB-cAMP-mediated increases in iNOS protein without affecting iNOS mRNA ETOH also acts at a post-transcriptional or translational site to inhibit iNOS protein in rat AM in vivo.

110. Release of calreticulin from neutrophils may alter C1q-mediated immune functions
Kishore, U. et al
*Biochem. J., **322**, 543-550 (1997)*

Calreticulin is an abundant intracellular protein which is involved in a number of cellular functions. During cytomegalovirus infection, as well as inflammatory episodes in autoimmune disease, calreticulin can be released from cells and detected in the circulation, where it may act as an immunodominant autoantigen in diseases such as systemic lupus erythematosus. Calreticulin is known to bind to the molecules of innate immunity, such as C1q, the first subcomponent of complement. However, the functional implications of C1q–calreticulin interactions are unknown. In the present study we sought to investigate, in greater detail, the interaction between these two proteins following the release of calreticulin from neutrophils upon stimulation. In order to pinpoint the regions of interaction, recombinant calreticulin and its discrete domains (N-, P- and C-domains) were produced in *Escherichia coli*. Both the N- and P-domains of calreticulin were shown to bind to the globular head regions of C1q. Calreticulin also appeared to alter C1q-mediated immune functions. Binding of calreticulin to C1q inhibited haemolysis of IgM-sensitized erythrocytes. Both the N- and P-domains of calreticulin were found to contain sites involved in the inhibition of C1q-induced haemolysis. Full-length calreticulin, and its N- and P-domains, were also able to reduce the C1q-dependent binding of immune complexes to neutrophils. We conclude that calreticulin, once released from neutrophils during inflammation, may not only induce an antigenic reaction, but, under defined conditions, may also interfere with C1q-mediated inflammatory processes.

111. Differential modulation of IL-1-induced endothelial adhesion molecules and transendothelial migration of granulocytes by G-CSF
Eissner, G., Lindner, H., Reisbach, G., Klauke, I. and Holler, E.
*Br. J. Hematol., **97(4-II)**, 726-733 (1997)*

Granulocyte colony stimulating factor (G-CSF) is widely used for mobilization of haemopoietic stem cells into the peripheral blood. However, little is known about the mechanisms involved in mobilization and the immune modulatory effects of this growth factor. In this report we show that G-CSF down-regulated intercellular adhesion molecule 1 (ICAM-1) induced by Interleukin-1 (IL-1) on human endothelial cells. Interestingly, the G-CSF-mediated down-modulation of IL-1-induced ICAM-1 appeared to be biphasic. In pharmacological concentrations (>300 ng/ml), and in dose ranges of plasma G-CSF levels above that of non-febrile healthy individuals (30 pg/ml), a significant decrease in surface ICAM-1 could be observed. This could be explained, at least in part, by an increased autocrine G-CSF production by endothelial cells in response to IL-1 and exogenous G-CSF. In contrast to ICAM-1, IL-1-triggered VCAM-1 expression was superinduced by G-CSF with the optimal concentration of 30 pg/ml. To evaluate the functional significance of these findings.51 Cr adhesion assays with peripheral blood mononuclear cells (PBMC) or granulocytes known to lack the VCAM-1 counter-receptor very late antigen 4 (VLA-4) and IL-1-stimulated endothelial cells, in the presence or absence of G-CSF, were performed. G-CSF could not inhibit the IL-1-induced adhesion of PBMC to endothelial cells, which may be due to the differential adhesion molecule modulation. In contrast, granulocyte adhesion induced by IL-1 could effectively be blocked by co-incubation with G-CSF. Finally, G-CSF also inhibited transendothelial migration of granulocytes through IL-1-activated endothelial cells in a concentration-dependent manner.

112. Modulatory effects of the colonic milieu on neutrophil oxidative burst: A possible pathogenic mechanism of ulcerative colitis
Keshavarzian, A., Haydek, J.M., Jacyno, M., Holmes, E.W. and harford, F.
*J. Lab. Clin. Med., **130(2)**, 216-225 (1997)*

An important hallmark of ulcerative colitis (UC) is mucosal neutrophil (PMN) infiltration associated with mucosal damage. This suggests that colonic chemoattractants such as bacterial products (e.g., N-formyl-methionyl-leucyl-phenylalanine (fMLP), lipopolysaccharide (LPS)) reach systemic circulation and attract PMNs to the colon. PMNs are then activated in the colonic mucosa and release their toxic oxidative metabolites. However, bacterial products are also present in the systemic circulation of healthy subjects. Thus we hypothesized that PMNs develop tolerance to colonic factors in the normal state and that this tolerance is absent in UC. We evaluated the PMN respiratory burst in response to stimulation with fMLP, LPS, or phorbol 12-myristate 13-acetate (PMA) by measuring the production of reactive oxygen species (ROS) with both luminol-enhanced chemiluminescence and a cytochrome C reduction assay. PMNs were obtained from control subjects, inactive UC patients, patients with UC who had undergone colectomies, and non-UC patients with colectomies. All three stimuli induced a significant rise in ROS. PMNs from non-UC colectomy subjects produced significantly higher ROS than PMNs from control subjects with intact colons in response to both fMLP and LPS. In contrast, PMNs from UC colectomy patients produced levels of ROS similar to those produced by PMNs from UC patients with intact colons in response to fMLP and LPS. Colectomy had no effect on PMA-induced ROS production in controls. The observed difference in fMLP-induced ROS production in control subjects with intact colons was not due to fMLP receptor down-regulation because a competition assay performed with the fMLP blocker BMLP showed a similar receptor apparent affinity in all four groups. We conclude the following: (1) the normal colonic milieu modulates the PMN respiratory burst, resulting in hyporesponsiveness of PMNs to "physiologic" but not "pharmacologic" stimulation. This effect is not due to receptor down-regulation. (2) UC colonic milieu does not appear to modulate PMN respiratory burst. This loss of PMN "tolerance" to colonic factors may have a pathogenic role in the sustained inflammation and tissue damage in UC.

113. Patterns of Azathioprine Metabolites in Neutrophils, Lymphocytes, Reticulocytes, and Erythrocytes: Relevance to Toxicity and Monitoring in Recipients of Renal Allografts
Bergan, S. et al
*Therapeutic Drug Monitoring, **19(5)**, 502-509 (1997)*

Monitoring of azathioprine (AZA) therapy by the measurement of 6-thioguanine nucleotides (6-TGN) concentrations in red blood cells (RBC) may improve safety and ensure optimal immunosuppressive effects of AZA in organ transplantation. The authors explored the rationale for such monitoring by measuring thiopurine metabolites in peripheral blood cell types that are more relevant to the effects and kinetics of AZA and its active metabolites. Neutrophil granulocytes were isolated by density gradient centrifugation, and CD4+ lymphocytes and reticulocytes by using specific immunomagnetic beads. In neutrophils, 6-TGN concentrations had median measurements 31 times higher than in RBCs. In contrast to the high methylated mercaptopurine (me-MP) concentrations in RBCs, these metabolites were not detected in the neutrophils. Thiopurine metabolite levels were lower than the analytic limit of detection in all the CD4+ samples. The concentrations of 6-TGN and me-MPs were lower in reticulocytes than in RBCs in general, indicating that thiopurine metabolites are taken up by RBCs in the circulation. This study's findings, that 6-TGN concentrations are very high in neutrophils, whereas me-MPs are undetectable, may explain the specific neutropenic adverse effect of AZA. The results also add support to monitoring AZA through measurements of 6-TGN and me-MPs in RBCs

114. Differential modulation of IL-1-induced endothelial adhesion molecules and transendothelial migration of granulocytes by G-CSF
Eissner, G., Lindner, H., Reisbach, G., Klauke, I. and Holler, E.
*Br. J. Hematol., **97(4)**, 726-733 (1997)*

Granulocyte colony stimulating factor (G-CSF) is widely used for mobilization of haemopoietic stem cells into the peripheral blood. However, little is known about the mechanisms involved in mobilization and the immune modulatory effects of this growth factor. In this report we show that G-CSF down-regulated intercellular adhesion molecule 1 (ICAM-1) induced by Interleukin-1 (IL-1) on human endothelial cells. Interestingly, the G-CSF-mediated down-modulation of IL-1-induced ICAM-1 appeared to be biphasic. In pharmacological concentrations (>300 ng/ml), and in dose ranges of plasma G-CSF levels above that of non-febrile healthy individuals (30 pg/ml), a significant decrease in surface ICAM-1 could be observed. This could be explained, at least in part, by an increased autocrine G-CSF production by endothelial cells in response to IL-1 and exogenous G-CSF. In contrast to ICAM-1, IL-1-triggered VCAM-1 expression was superinduced by G-CSF with the optimal concentration of 30 pg/ml. To evaluate the functional significance of these findings, ^{51}Cr adhesion assays with peripheral blood mononuclear cells (PBMC) or granulocytes known to lack the VCAM-1 counter-receptor very late antigen 4 (VLA-4) and IL-1-stimulated endothelial cells, in the presence or absence of G-CSF, were performed. G-CSF could not inhibit the IL-1-induced adhesion of PBMC to endothelial cells, which may be due to the differential adhesion molecule modulation. In contrast, granulocyte adhesion induced by IL-1 could effectively be blocked by co-incubation with G-CSF. Finally, G-CSF also inhibited transendothelial migration of granulocytes through IL-1-activated endothelial cells in a concentration-dependent manner.

115. Activation of tumor cell matrix metalloproteinase-2 by neutrophil proteinases requires expression of membrane-type 1 matrix metalloproteinase
Schwartz, J.D. et al
Surgery, ***124(2),*** *232-238 (1998)*

Background: Matrix metalloproteinase-2 (MMP-2), an enzyme involved in tumor invasion, is secreted as an inactive proenzyme and requires interaction with membrane-type 1 MMP (MT1-MMP) for activation. We have previously demonstrated that polymorphonuclear neutrophils (PMNs) release a soluble factor(s) that activates pro-MMP-2. Therefore, we tested the hypothesis that PMN-derived proteinases act in concert with MT1-MMP to activate pro-MMP-2. **Methods:** Human HT-1080 cells transfected with MT1-MMP cDNA (HT-SE) or the corresponding antisense cDNA (HT-AS) or an empty vector (HT-V), which expressed differing levels of MT1-MMP, were incubated with serum-free, human PMN-conditioned medium with or without proteinase inhibitors. The culture supernatants were analyzed by gelatin zymography. **Results:** HT-1080 cells expressing basal (HT-V) or low levels (HT-AS) of MT1-MMP secreted MMP-2 in proenzyme form (72 kd). HT-1080 cells with high levels of MT1-MMP (HT-SE) secreted proMMP-2 and a 68 kd intermediate activation product. Addition of PMN-conditioned medium to either HT-SE or HT-V clones resulted in dose-dependent generation of active, 62 kd MMP-2. In contrast, when PMN-conditioned medium was added to HT-AS clones, no MMP-2 activation occurred. **Conclusions:** PMN-derived serine proteinases act in concert with MT1-MMP to activate proMMP-2. This finding indicates a potential role for inflammatory cells in promoting extracellular matrix breakdown during tumor invasion.

116. Acute regulation of glucose transport after activation of human peripheral blood neutrophils by phorbol myristate acetate, fMLP, and granulocyte-macrophage colony-stimulating factor
Tan, A.S., Ahmed, N. and Berridge, M.V.
Blood, 91, 655 (1998)

Activation of human peripheral blood neutrophils by pathogens or by phorbol myristate acetate (PMA), fMLP, or myeloid growth factors generates a respiratory burst in which superoxide production plays an important role in killing invading microorganisms. Although the increased energy demands of activated neutrophils would be expected to be associated with increased glucose uptake and utilization, previous studies have shown that PMA inhibits 2-deoxyglucose (2-DOG) uptake. In this study, we show that PMA activation of neutrophils, isolated by methods not involving hypotonic lysis, increases the rate of 2-DOG uptake and results in a 1.6-fold to 2.1-fold increase in transporter affinity for glucose without changing V_{max}. Increased transporter affinity in response to PMA was also observed with 3-O-methyglucose, which is not phosphorylated, and inclusion of glucose in the activation medium further increased respiratory burst activity. Increased 2-DOG uptake and increased transporter affinity for glucose were also observed with the peptide activator, fMLP, and with granulocyte-macrophage colony-stimulating factor (GM-CSF). The protein kinase C (PKC) inhibitor, calphostin C, and the tyrosine kinase inhibitor, genistein, inhibited both PMA- and fMLP-stimulated 2-DOG uptake. In contrast, genistein inhibited fMLP-induced superoxide production, but had little effect on the PMA-induced response, while staurosporine differentially inhibited PMA-induced superoxide production. These results show that neutrophil activation involves increased glucose transport and intrinsic activation of glucose transporter molecules. Both tyrosine kinases and PKC are implicated in the activation process.

117. Assay of β-*N*-acetylhexosaminidase isoenzymes in different biological specimens by means of determination of their activation energies
Perez, L.F. and Tutor, J.C.
Clin. Chem., 44, 226-231 (1998)

The activation energy (E_a) of ß-***N***-acetylhexosaminidase (Hex, EC 3.2.1.52) was determined with 3,3'-dichlorophenylsulfonphthaleinyl-***N***-acetyl-ß-D-glucosaminide as substrate, with a much higher value being found for the Hex B isoenzyme (E_a = 75.1 kJ/mol) than for the Hex A isoenzyme (E_a = 41.8 kJ/mol). This fact allowed for the development of a fast and reliable thermodynamic method to determine the isoenzyme composition of Hex in different biological specimens (serum/plasma, saliva, cerebrospinal fluid, seminal plasma, urine, and leukocyte lysates). The results in serum given by the proposed method may be superimposed upon those obtained by the heat inactivation assay of O'Brien et al. (***N Engl J Med*** 1970;273:15–20), and the catalytic activity calculated for Hex A offers a good correlation with that obtained by using the specific substrate 4-methylumbelliferyl-***N***-acetyl-ß-D-glucosaminide-6 sulfate (n = 25, ***r*** = 0.953).

118. Coiling phagocytosis discriminates between different spirochetes and is enhanced by phorbol myristate acetate and granulocyte-macrophage colony-stimulating factor
Rittig, M.G. et al
Infect. Immun., 66, 627-635 (1998)

The mechanisms involved in coiling phagocytosis are not yet known, and it is not even clear whether this phenomenon is either an incidental event or a specific response. Therefore, the phagocytic uptake of *Borrelia burgdorferi* and other spirochetes by human monocytes in vitro was used to investigate the involvement of both sides—microbes and phagocytes—in coiling phagocytosis. As seen with electron microscopy, morphologically similar *Borrelia*, *Leptospira* and *Treponema* strains induced markedly different frequencies of coiling phagocytosis. The monocytes used coiling phagocytosis for both live (motile) and killed (nonmotile) *B. burgdorferi*, but pseudopod coils were observed neither with fragmented *B. burgdorferi* nor with cell-free supernatant from *B. burgdorferi* cultures. Investigation of the relationship of coiling phagocytosis with other pseudopod-based cellular mechanisms revealed that the use of bioreagents that inhibit conventional phagocytosis also inhibited coiling phagocytis but did not affect membrane ruffling. Bioreagents that increase membrane ruffling did not affect phagocytosis of *B. burgdorferi*, except for granulocyte-macrophage colony-stimulating factor and phorbol myristate acetate, which increased coiling phagocytosis selectively. These results demonstrate that coiling phagocytosis is not induced by microbial motility, viability, or a certain morphology and that it is not a random event. Rather, it is a selective uptake mechanism actively driven by the phagocytes. However, whether coiling phagocytosis represents an independent alternative to conventional phagocytosis or, alternatively, a fault in conventional phagocytosis remains to be determined.

119. Rapid cytomegalovirus pp65 antigenemia assay by direct erythrocyte lysis and immunofluorescence staining
Ho, S.K.N., Lo, C-Y., Cheng, I.K.P. and Chan, T-M.
*J. Clin. Microbiol., **36**, 638-640 (1998)*

A rapid cytomegalovirus (CMV) pp65 antigenemia assay with direct erythrocyte lysis (DL) with 0.8% NH_4Cl, followed by indirect immunofluorescence staining (IF), was evaluated with 82 blood samples from renal transplant recipients, and the results were compared to those of the conventional antigenemia assay with dextran sedimentation and two-cycle alkaline phosphatase, anti-alkaline phosphatase staining (DS-APAAP). The DL-IF modification gave a higher leukocyte yield compared to DS-APAAP (75.4 versus 54.9%; $P < 0.05$), with similar leukocyte viability rates of >95%. The DL-IF methodology involved fewer technical steps, and the assay time was shortened from 5 h to less than 3 h. Nineteen of the 82 samples concordantly tested positive for pp65 antigenemia by both assays, and the readings showed a good correlation ($r = 0.996$; $P < 0.01$). No discordant results were observed. We conclude that the CMV pp65 antigenemia assay by this novel DL-IF modification is technically simpler, cheaper, and less time-consuming but yields results comparable to those of the conventional DS-APAAP assay. The shortened assay time and increased capacity to handle more samples confer distinct advantages in the rapid diagnosis and prompt treatment of CMV disease in immunosuppressed patients.

120. Site-directed C3a receptor antibodies from phage display libraries
Hawlisch, H. et al
*J. Immunol., **160,** 2947 (1998)*

Recent cloning of the human C3a receptor (C3aR) revealed that this receptor belongs to the large family of rhodopsin-type receptors. A unique feature of the C3aR is the large second extracellular loop comprising about 175 amino acid residues. We constructed combinatorial phage Ab libraries expressing single chain Fv Abs from BALB/c mice immunized with the affinity-purified second extracellular loop of the C3aR, fused to glutathione-*S*-transferase. A panel of anti-C3aR single chain Fv fragments (scFvs) was selected after four rounds of panning using the second extracellular loop of the C3aR, fused to the maltose binding protein as Ag. Sequencing of the clones obtained revealed three different groups of scFvs, the epitopes of which were mapped to two distinct regions within the loop, i.e., positions 185 to 193 and 218 to 226, representing the immunodominant domains of the loop. By flow cyotmetric analyses, the scFvs bound to RBL-2H3 cells transfected with the C3aR, but not to cells transfected with the C5aR or to nontransfected RBL-2H3 cells. In addition, the scFvs bound to the human mast cell line HMC-1. Immunofluorescence studies showed C3aR expression on polymorphonuclear granulocytes and monocytes, but not on lymphocytes. In addition, no C3aR expression was observed on human erythrocytes or platelets. Surprisingly, none of the scFvs alone or in combination inhibited C3a-induced Ca^{2+} mobilization from RBL-2H3 cells transfected with the C3aR. In addition, C3a did not displace binding of the scFvs to the receptor, strongly suggesting that the N-terminal part of the second extracellular loop is not involved in ligand binding.

121. Utility of major leukocyte subpopulations for monitoring secondary cytomegalovirus infections in renal.allograft recipients by PCR
Schäfer, P., Tenschert, W., Cremaschi, L., Gutensohn, K. and Laufs, R.
J. Clin. Microbiol., 36, 1008-1014 (1998)

The feasibility of the major peripheral blood leukocyte (PBL) subsets for use in qualitative and quantitative PCR to monitor secondary cytomegalovirus (CMV) infection and ganciclovir therapy was assessed with 188 blood samples derived from 40 CMV immunoglobulin G-positive renal-allograft recipients. In pp65 antigen-positive patients all leukocyte fractions, but only 79.5% of plasma preparations, were PCR positive. In pp65 antigen-negative samples from patients after antiviral treatment only 7.3% of polymorphonuclear cell (PMNL) samples, but 81.8% of peripheral blood mononuclear cells (PBMC), and 10.9% of plasma samples remained PCR positive. Similarly, in patients with latent infections only 5.0% of PMNL, but 51.7% of PBMC preparations, and 8.0% of plasma samples were PCR positive. Regarding patients with active CMV infection, CMV DNA copy numbers in PMNL correlated significantly with pp65 antigen-positive cell counts before and after onset of ganciclovir therapy. Significant differences in CMV DNA copy numbers in PMNL

and plasma were observed (i) between patients with symptomatic infection and those with asymptomatic infection and (ii) between patients with active infection and those with latent infection. In contrast, PBMC harbored equally low CMV DNA levels both in patients with active infection and those with latent infections, and no decline of CMV DNA load in PBMC was observed during antiviral treatment. We conclude that detection of CMV DNA in PMNL, not in PBMC, is associated with active infections and is more sensitive than detection of CMV DNA in plasma. Negative PCR results for PMNL after antiviral therapy indicate recovery, and fewer unwanted positive results occur compared to PBMC and plasma. Therefore, purified PMNL should be preferred for analysis by qualitative CMV PCR to avoid unwanted positive results. The CMV DNA load in PBMC compared with that in PMNL is negligible during active infection, so mixed PBL are sufficient for use in quantitative PCR.

122. Progressive telomere shortening in aplastic anemia
Ball, S.E.
Blood, 91, 3582-3592 (1998)

Improved survival in aplastic anemia (AA) has shown a high incidence of late clonal marrow disorders. To investigate whether accelerated senescence of hematopoietic stem cells might underlie the pathophysiology of myelodysplasia (MDS) or paroxysmal nocturnal hemoglobinuria (PNH) occurring as a late complication of AA, we studied mean telomere length (TRF) in peripheral blood leukocytes from 79 patients with AA, Fanconi anemia, or PNH in comparison with normal controls. TRF lengths in the patient group were significantly shorter for age than normals ($P < .0001$). Telomere shortening was apparent in both granulocyte and mononuclear cell fractions, suggesting loss at the level of the hematopoietic stem cell. In patients with acquired AA with persistent cytopenias (n = 40), there was significant correlation between telomere loss and disease duration ($r = -.685$; $P < .0001$), equivalent to progressive telomere erosion at 216 bp/yr, in addition to the normal age-related loss. In patients who had achieved normal full blood counts (n = 20), the rate of telomere loss had apparently stabilised. There was no apparent association between telomere loss and secondary PNH (n = 13). However, of the 5 patients in the study with TRF less than 5.0 kb, 3 had acquired cytogenetic abnormalities, suggesting that telomere erosion may be relevant to the pathogenesis of MDS in aplastic anemia.

123. A low concentration of ethanol reduces the chemiluminiscence of human granulocytes and monocytes but not the tumor necrosis factor alpha production by monocytes after endotoxin stimulation
Parlesak, A., Diedrich, J.P., Schåfer, C. and Bode, C.
Infect. Immun.,66, 2809-2813 (1998)

The ability of polymorphonuclear neutrophils (PMNs) and monocytes (MΦ) to produce reactive oxygen species (ROS) has been related closely to their potential in the killing of microorganisms. Ethanol has been shown to impair the generation of ROS in these phagocytes after stimulation with some immunogens and to increase the susceptibility of alcohol abusers to infectious diseases. As endotoxemia is common in alcohol abusers, we investigated the effect of ethanol (21.7 mmol/liter) on the luminol-amplified chemiluminescence of PMNs and MΦ after endotoxin stimulation and the release of tumor necrosis factor alpha (TNF-α) from MΦ. Further, the efficiency of ethanol to inactivate chemically generated ROS was tested. Significant stimulation of ROS release occurred at endotoxin concentrations of 1 ng/ml or higher in both PMNs and MΦ. Ethanol significantly suppressed the formation of ROS in both cell types, the decrease being more pronounced in MΦ (−73.8%) than in PMNs (−45.7%). The correlations between endotoxin concentration and the amount of released ROS showed a dose-dependent, sigmoidal course. Concentrations of endotoxin necessary for half-maximum stimulation were nearly identical (6 to 8 ng/ml) in both PMNs and MΦ, independent of the presence of ethanol. In contrast to ROS formation, ethanol had no effect on the amount of TNF-α produced by endotoxin-stimulated MΦ. Ethanol was shown to be unable to decrease the levels of chemically generated ROS under physiological conditions. Therefore, ethanol cannot be assumed to be an "antioxidative" compound but rather seems to modify processes of endotoxin recognition, intracellular signal transduction, or metabolism.

124. Quantification of prostate-specific antigen mRNA by coamplification with a recombinant RNA internal standard and microtiterwell-based hybridisation
Verhaegen, M., Joannou, P.C. and Christopoulos, T.K.
Clin. Chem., 44, 1170-1176 (1998)

We report a quantitative analytical methodology for prostate-specific antigen (PSA) mRNA, which is based on the coamplification of the target with a recombinant RNA internal standard (IS) using reverse transcriptase-polymerase chain reaction. PSA mRNA and the RNA IS contain the same primer recognition sites and generate amplification products that have identical sizes but differ in a 24-bp sequence located in the center of the molecule. Amplified sequences are labeled with biotin using a biotinylated upstream primer. The products are captured on streptavidin-coated microtiter wells and hybridized to specific probes labeled with the hapten digoxigenin. The hybrids are determined using alkaline phosphatase-labeled anti-digoxigenin antibody and time-resolved fluorometry. The ratio of the fluorescence values obtained for the PSA mRNA and the RNA IS is a linear function of the amount of PSA mRNA present in the sample. Samples containing total RNA from PSA-expressing cells (LNCaP cells) in addition to 1 μg of RNA from healthy cells give fluorescence ratios related linearly to the number of cells in the range of 4 to 3000 cells.

125. Molecular defects in ferrochelatase in patients with protoporhyria requiring liver transplantation
Blomer, J. et al
J. Clin. Inverst., 102, 107 (1998)

Protoporphyria is a genetic disorder in which a deficiency of mitochondrial ferrochelatase activity causes accumulation of protoporphyrin that produces severe liver damage in some patients. In this study, mutations of the ferrochelatase gene were examined in eight unrelated patients who had liver transplantation. RNA was prepared from liver and/ or lymphoblasts, and specific reverse transcriptase-nested polymerase chain reactions amplified and sequenced ferrochelatase cDNAs. Products shorter than normal resulted from an exon 3 deletion in three patients, exon 10 deletion in two, exon 2 deletion in one, and deletion of five nucleotides in exon 5 in one. Sequence of normal-size products revealed no other mutations. Western blot showed a reduced quantity of normal-size ferrochelatase protein in protoporphyria liver compared with normal liver (19-51%, mean 32% of normal). Levels of the mitochondrial protein F_1-ATPase β-subunit were not decreased to a similar degree. Liver ferrochelatase activity was reduced more than could be explained by the decrease in ferrochelatase protein (4-20%, mean 9% of normal). These results establish genetic heterogeneity in the most severe phenotype of protoporphyria. However, the gene mutations found share the property of causing a major structural alteration in the ferrochelatase protein.

126. Molecular detection of circulating prostate cells in cancer II: comparison of prostate epithelial cells isolation procedures
Berteau, P. et al
Clin. Chem., 44, 1750-1753 (1998)

The sensitive and specific detection of circulating tumor cells holds great promise for more accurate staging of cancer patients. Several reverse transcription-PCR (RT-PCR) procedures based on tissue-specific mRNA expression are now able to detect one cell derived from a given tissue among >10 peripheral nucleated blood cells [PNBCs; for a review, see (1)]. However, even for a single marker, highly discrepant results have been observed among the available clinical studies; e.g., the frequencies of positive prostate-specific antigen (PSA) RT-PCR results range from 25% (2) to 80% (3) in patients with metastatic prostate cancer (CaP), blurring the clinical relevance of these assays. Standardization and quality control in molecular diagnosis are crucial to the solution of this issue. We have previously studied factors potentially affecting RT-PCR results (4), and this current work focuses on the approaches for harvesting prostate cells among nucleated blood cells.
Since the first 1992 clinical report describing the RT-PCR detection of circulating prostatic cells in CaP, the majority of reported assays have used gradient separations to recover nucleated cells from the peripheral blood (1). We (5) and others (6) rather choose overnight hypo-osmotic red blood cell lysis as an easier and more cost-effective protocol. Because the approach used to harvest PNBCs may account for discrepancies in clinical results, we have compared a panel of nucleated blood cell separation methods.

127. Chemotactic peptide-induced changes of intermediate filament organization in neutrophils during granule secretion: role of cyclic guanosine monophosphate
Pryzwansky, K.B. and Merricks, E.P.
Mol. Biol. Cell, 9, 2933 (1998)

In neutrophils activated to secrete with formyl-methionyl-leucyl-phenylalanine, intermediate filaments are phosphorylated transiently by cyclic guanosine monophosphate (cGMP)-dependent protein kinase (G-kinase). cGMP regulation of vimentin organization was investigated. During granule secretion, cGMP levels were elevated and intermediate filaments were transiently assembled at the pericortex to areas devoid of granules and microfilaments. Microtubule and microfilament inhibitors affected intermediate filament organization, granule secretion, and cGMP levels. Cytochalasin D and nocodazole caused intermediate filaments to assemble at the nucleus, rather than at the pericortex. cGMP levels were elevated in neutrophils by both inhibitors; however, with cytochalasin D, cGMP was elevated earlier and granule secretion was excessive. Nocodazole did not affect normal cGMP elevations, but specific granule secretion was delayed. LY83583, a guanylyl cyclase antagonist, inhibited granule secretion and intermediate filament organization, but not microtubule or microfilament organization. Intermediate filament assembly at the pericortex and secretion were partially restored by 8-bromo-cGMP in LY83583-treated neutrophils, suggesting that cGMP regulates these functions. G-kinase directly induced intermediate filament assembly in situ, and protein phosphatase 1 disassembled filaments. However, in intact cells stimulated with formyl-methionyl-leucyl-phenylalanine, intermediate filament assembly is focal and transient, suggesting that vimentin phosphorylation is compartmentalized. We propose that, in addition to changes in microfilament and microtubule organization, granule secretion is also accompanied by changes in intermediate filament organization, and that cGMP regulates vimentin filament organization via activation of G-kinase.

128. Endothelin-1 induces production of the neutrophil chemotactic factor interleukin-8 by human brain-derived endothelial cells
Hoffman, F.M. et al
Blood, 92, 3064-3072 (1998)

Increased levels of endothelin-1 (Et-1), a potent vasoconstrictor, have been correlated with hypertension and neuronal damage in ischemic/reperfusion injury. The presence of polymorphonuclear cells (PMNs) in the brain has been shown to be directly responsible for

this observed pathology. To address the question of whether Et-1 plays a role in this process, human brain-derived endothelial cells (CNS-ECs) were cultured with Et-1. The results demonstrate that Et-1 induces production of the neutrophil chemoattractant interleukin-8 (IL-8) twofold to threefold after 72 hours; mRNA was maximal after 1 hour of stimulation. Conditioned culture medium derived from Et-1-stimulated CNS-ECs induced a chemotactic response in the PMN migration assay. The inflammatory cytokines tumor necrosis factor-α (TNF) and IL-1β functioned additively with Et-1 in increasing IL-8 production. In contrast, transforming growth factor-β (TGF-β), but not IL-10, completely abolished the effect of Et-1 on IL-8 production. However, Et-1 did not modulate intercellular adhesion molecule-1 (ICAM-1) expression. These data demonstrate that Et-1 may be a risk factor in ischemic/reperfusion injury by inducing increased levels of the neutrophil chemoattractant IL-8.

129. In vivo treatment with granulocyte colony-stimulating factor results in divergent effects on neutrophil functions measured in vitro

Leavey, P.J. et al

Blood, 92, 4366-4374 (1998)

We have studied the effects of granulocyte colony-stimulating factor (G-CSF) administration to normal individuals on a variety of functional and biochemical neutrophil characteristics that relate to host defense. G-CSF adversely affected neutrophil (polymorphonuclear leukocyte [PMN]) chemotaxis. While this could be partially explained by reduced assembly of neutrophil F-actin, we also recognized an elevated cytosolic calcium mobilization and a normal upregulation of neutrophil CD11b. G-CSF resulted in reduced PMN killing of Staphylococcus aureus with a 10:1 (bacteria:neutrophil) ratio and normal killing with a 1:1 ratio. In association with this, we demonstrated divergent effects on the respiratory burst of intact cells and divergent effects on the content of marker proteins for neutrophil granules. While G-CSF may have resulted in increased content of cytochrome b_{558} in the cell membrane, it did not alter the amounts of cytosolic oxidase components. After therapy, there was normal content of the azurophilic granule marker, myeloperoxidase, decreased content of the specific granule marker, lactoferrin, and normal content of lysozyme (found in both granules classes). Finally, G-CSF therapy markedly reduced the apoptotic rate of the isolated neutrophil. Therefore, considering disparate functional and biochemical activities, the real benefit of G-CSF therapy may lie in enhanced number and survival of neutrophils.

130. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic soerm injection

Twigg, J.P., Irvine, D.S. and Aitken, R.J.

Human. Reprod., 13, 1864-1871 (1998)

We present the first evidence that genetically damaged human spermatozoa are able to form normal pronuclei in oocytes after intracytoplasmic sperm injection (ICSI). The role of reactive oxygen species (ROS) as a cause of chromatin and DNA damage is well recognized. The same class of molecule can be found in the semen of males with severe infertility, who remained infertile until the advent of ICSI. In this study we have investigated the role of ROS in the induction of chromatin damage, DNA strand breakage and the subsequent ability of spermatozoa to decondense and form pronuclei after ICSI. Spermatozoa from normozoospermic men participating in our research programme were exposed to oxidizing environments created by co- incubation with hydrogen peroxide, reduced nicotinamide adenine dinucleotide phosphate (NADPH) or activated white cells. The subsequent ability of the spermatozoa to decondense in vitro was examined using sequential incubations in EDTA, dithiothreitol and sodium dodecyl sulphate, and the amounts of DNA strand breakage were assessed using an in-situ nick translation protocol. Finally, cells exposed to hydrogen peroxide, NADPH and activated leukocytes were microinjected into hamster oocytes, and their ability to decondense and form normal pronuclei was determined. The results indicate that human sperm chromatin becomes cross-linked under conditions of oxidative stress and exhibits increased DNA strand breakage, yet the rate of pronucleus formation is no different from that of untreated control cells. The ability of genetically damaged spermatozoa to achieve normal fertilization following ICSI has implications for the practice of this form of assisted conception therapy.

131. Defining an antigenic epitope on platelet factor 4 associated with heparin-induced thrombocytopenia

Ziporen, Z.Q. et al

Blood, 92, 3250-3259 (1998)

Heparin-induced thrombocytopenia (HIT) is a potentially serious complication of heparin therapy. Antibodies to platelet factor 4 (PF4)/heparin complexes have been implicated in the pathogenesis of this disorder, but the antigenic epitope(s) on the protein have not been defined. To address this issue, we studied the binding of HIT antibodies to a series of recombinant proteins containing either point mutations in PF4 or chimeras containing various domains of PF4 and the related protein, neutrophil activating peptide-2 (NAP-2). Serum samples from 50 patients with a positive ^{14}C-serotonin release assay (^{14}C-SRA) and a clinical diagnosis of HIT and 20 normal controls were studied. HIT antibodies reacted strongly with wild-type (WT) PF4/heparin complexes, but reacted little, if at all, with NAP-2/heparin complexes (optical density $[OD]_{405}$ = 2.5 and 0.2, respectively). Alanine substitutions at three of the four lysine residues implicated in heparin binding, K62, K65, and K66, had little effect on recognition by HIT antibodies (OD_{405} = 2.2, 2.8, and 2.0, respectively), whereas an alanine substitution at position K61 led to reduced, but still significant binding (OD_{405} = 1.0). Similar studies involving chimeras between PF4 and NAP-2 localized a major antigenic site to the region between the third and fourth cysteine residues for more than half of the sera tested. This site appears to involve a series of amino acids immediately after the third cysteine

residue beginning with P37. Thus our studies suggest that whereas the C-terminal lysine residues of PF4 are important for heparin binding, they do not comprise a critical antigenic site for most HIT antibodies. Rather, we propose that maintaining a region near the third cysteine residue of PF4, distal from the proposed heparin-binding domain, is required to form the epitope recognized by many HIT antibodies.

132. *In vivo* induction of functional FcγRI (CD64) on neutrophils and modulation of blood cytokine mRNA levels in cancer patients treated with G-CSF (rMetHuG-CSF)
Michon, J.M. et al
Br. J. Hematol., 100(3), 550-556 (1998)

Neutrophils from 13 children who received G-CSF for the collection of peripheral blood progenitors while they were in haematological steady state were studied at various times after G-CSF injection for FcγR expression (FcγRI or CD 64, FcγRII or CD32, and FcγRIII or CD16) and for their ability to exert antibody-dependent cell cytotoxicity (ADCC) through FcγRI. Changes in IFNγ, IL8, IL10, MCP1 and TNFα mRNA levels in peripheral blood cells were also studied 4 h and 24 h after the first G-CSF injection. FcγRI expression increased strongly after 24 h and then remained at the same level throughout treatment. In contrast, FcγRIII expression sharply decreased at day 1 and diminished even further thereafter. No change in FcγRII was observed. ADCC exerted by neutrophils through FcγRI started to increase after 24 h with the peak level at day 5. Cytokine mRNA analyses indicated a reproducible and strong increase of IL8 mRNA (11/13 children) after 24 h, whereas the changes in the mRNA levels of the other cytokines tested were more heterogenous (IFNγ: three; IL10: six; MCP1: five; TNFα: four, of the 13 children). Therefore this study opens the way to an optimized therapeutic schedule for the combined use of G-CSF and monoclonal antibodies in adjuvant immuno-intervention.

133. Patients with high-risk myelodysplastic syndrome can have polyclonal or clonal haemopoiesis in complete haematological remission
Delforge, M. et al
Br. J. Hematol., 102(2), 486-494 (1998)

The clonality of mature peripheral blood-derived myeloid and lymphoid cells and bone marrow haemopoietic progenitors from 18 females with myelodysplasia (MDS) (five refractory anaemia, RA; one RA with ringed sideroblasts, RARS; three chronic myelomonocytic leukaemia, CMML; four RA with excess of blasts, RAEB; five RAEB in transformation, RAEB-t) was studied by X-chromosome inactivation analysis. Using the human androgen-receptor (HUMARA) assay, we analysed the clonal patterns of highly purified immature $CD34^+38$ and committed $CD34^+38^+$ marrow-derived progenitors, and $CD16^+14$ granulocytes, $CD14^+$ monocytes, $CD3^+$ T and $CD19^+$ B lymphocytes from peripheral blood. In high-risk patients (RAEB, RAEB-t), clonality analysis was performed before and after intensive remission-induction treatment. All patients, except one with RA, had predominance of a single clone in their granulocytes and monocytes. The same clonal pattern was found in $CD34^+$ progenitor cells. In contrast, $CD3^+$ T lymphocytes were polyclonal or oligoclonal in 14/18 patients. X-chromosome inactivation patterns of $CD19^+$B cells were highly concordant with $CD3^+$ T cells except for two patients (one RA, one CMML) with monoclonal B and polyclonal T lymphocytes, therefore suggesting a clonal mutation in a progenitor common to the myeloid and B-lymphoid lineages or the coexistence of MDS and a B-cell disorder in these particular patients. After high-dose non-myeloablative chemotherapy, polyclonal haemopoiesis was reinstalled in the mature myeloid cells and immature and committed marrow progenitors in three of four patients achieving complete haematological remission. Therefore we conclude that most haematological remissions in MDS are associated with restoration of polyclonal haemopoiesis.

134. Role of fimbriae-mediated adherence for neutrophil migration across *Escherichia coli*-infected epithelial cell layers
Godaly, G. et al
Mol. Microbiol., 30(4), 725-735 (1998)

This study examined the role of P and type 1 fimbriae for neutrophil migration across Escherichia coli-infected uroepithelial cell layers in vitro and for neutrophil recruitment to the urinary tract in vivo. Recombinant E. coli K-12 strains differing in P or type 1 fimbrial expression were used to infect confluent epithelial layers on the underside of transwell inserts. Neutrophils were added to the upper well, and their passage across the epithelial cell layers was quantified. Infection with the P- and type 1-fimbriated recombinant E. coli strains stimulated neutrophil migration to the same extent as a fully virulent clinical E. coli isolate, but the isogenic non-fimbriated vector control strains had no stimulatory effect. The enhancement of neutrophil migration was adhesion dependent; it was inhibited by soluble receptor analogues blocking the binding of P fimbriae to the globoseries of glycosphingolipids or of type 1 fimbriae to mannosylated glycoprotein receptors. P- and type 1-fimbriated E. coli triggered higher interleukin (IL) 8 secretion and expression of functional IL-8 receptors than non-fimbriated controls, and the increase in neutrophil migration across infected cell layers was inhibited by anti-IL-8 antibodies. In a mouse infection model, P- or type 1-fimbriated E. coli stimulated higher chemokine (MIP-2) and neutrophil responses than the non-fimbriated vector controls. The results demonstrated that transformation with the pap or fim DNA sequences is sufficient to convert an E. coli K-12 strain to a host response inducer, and that fimbriation enhances neutrophil recruitment in vitro and in vivo. Epithelial chemokine production provides a molecular link between the fimbriated bacteria that adhere to epithelial cells and tissue inflammation.

135. The Effect of Midazolam and Propofol on Interleukin-8 from Human Polymorphonuclear Leukocytes
Galley.H., Dubbels, A.M. and Webster N.R.
Anesthesia & Analgesia, 86(6), 1289-1293 (1998)

Anesthetics and sedatives contribute to postoperative immunosuppression.Interleukin-8 (IL-8) is a chemotactic and activating factor that mediates neutrophil adhesion and margination and is essential for host defense. We investigated the effect of anesthetics on isolated human polymorphonuclear leukocyte production of IL-8. Healthy human polymorphonuclear leukocytes were isolated using a single-step density gradient and stimulated with lipopolysaccharide in the presence of varying concentrations of propofol or midazolam for up to 20 h. IL-8 was measured in both culture supernatants and cell lysates using enzyme immunoassay, and IL-8 mRNA in cells was measured using Northern blotting and phosphorimaging. Data were analyzed using Kruskal-Wallis analysis of variance or the Mann-Whitney U-test as appropriate. Lipopolysaccharide increased extracellular accumulation of interleukin-8, which was suppressed by both propofol (P = 0.025) and midazolam (P = 0.028). However, intracellular IL-8 increased with exposure to lipopolysaccharide (P = 0.028) and remained increased with both anesthetics. Northern blot analysis also revealed increased IL-8 mRNA levels in the presence of both midazolam and propofol, which was confirmed by molecular imaging. These data strongly suggest that the anesthetics modulate transport or secretion of IL-8 protein from the cell. Suppression of IL-8 by anesthetics and sedatives may predispose postoperative and intensive care patients to infection. Implications: Anesthesia causes immune suppression and alters neutrophil function. We investigated the effect of propofol and midazolam on interleukin-8, a neutrophil chemotactic agent in human neutrophils. Both anesthetics decreased extracellular interleukin-8 accumulation, but intracellular levels and mRNA remained high. This suggests that propofol and midazolam alter interleukin-8 secretion from cells.

136. Lysophosphatidylcholine generates superoxide anions through activation of phosphatidylinositol 3-kinase in human neutrophils
Hishioka, H., Horiuchi, H., Arai, H. and Kita, T.
FEBS Lett., 441(1), 63-66 (1998)

Lysophosphatidylcholine (LPC) accumulates in inflammatory tissues, where neutrophils are recruited to generate superoxide anions ($O^{\bullet}_{2}$). Here, we show that LPC stimulates $O^{\bullet}_{2}$ generation in human neutrophils and that the activity is inhibited with phosphatidylinositol 3-kinase (PI3 kinase) inhibitors, but not with protein kinase C (PKC) inhibitors. Furthermore, we demonstrate that LPC activates PI3 kinase in neutrophils. Thus, LPC might contribute to host defense by generating $O^{\bullet}_{2}$ in neutrophils through PI3 kinase activation, but not through PKC activation.

137. Study of Citrus taitensis and radical scavenger activity of the flavonoids isolated
Saleh, M.M., El-Megeed, F.A. and Glombitza, K.W.
Food Chem., 63(3), 397-400 (1998)

The GC-MS study of the head space volatiles of *Citrus taitensis* Risso revealed that the main component, constituting 60% of the oil, is linalool. Three N-containing compounds, benzyl cyanide, indole and methyl anthranilate, constituted a considerable additional percentage of the volatiles (16.9%). Investigation of the flavonoids indicated that the two major components were dihydrorobinetin and genistein. Both compounds give a high percentage inhibition of the chemiluminescence in polymorphonuclear cells stimulated by *N*-formylmethionyl-leucyl-phenylalanine, but a much lower inhibition with stimulation by opsonized zymosan.

138. The effect of nitric oxide and peroxynitrite on apoptosis in human polymorphonuclear leukocytes
Blaylock, M.G., Cuthbertson, B.H., Galley, H.F., Ferguson, N.R. and Webster, N.R.
Free Rad. Biol. Med., 25(6), 748-752 (1998)

In acute lung injury, neutrophil apoptosis may be important in regulating the inflammatory process by controlling neutrophil numbers and thus activity. Exogenous inhaled nitric oxide is now a widely used therapy in patients with acute lung injury, and its effects on apoptosis may be important. We investigated the effect of nitric oxide and peroxynitrite on apoptosis in lipopolysaccharide stimulated polymorphonuclear leukocytes as a model of nitric oxide-treated lung injury. Cells were incubated for up to 16 h with and without 1.7 μg/ml lipopolysaccharide and the nitric oxide donor GEA-3162 or the peroxynitrite donor SIN-1. Apoptosis was assessed using flow cytometry following annexin-V staining, after 4, 6, 8, and 16 h. Data were assessed using Kruskal–Wallis analysis of variance or Mann–Whitney *U*-test as appropriate. Annexin-V staining increased spontaneously over 16 h in untreated cells ($p = .0002$) and incubation with either 1000 μM SIN-1 or 10 μM GEA-3162 increased annexin staining at early time points in nonactivated cells. Apoptosis was attenuated when cells were exposed to lipopolysaccharide and both nitric oxide and peroxynitrite dose dependently inhibited this suppression at all time points and was most apparent at 16 h ($p = .004$ and .001, respectively). Exposure of activated neutrophils to exogenous nitric oxide or peroxynitrite has marked influences on apoptosis. This work has implications for the modulation of neutrophil function within the lung in patients with lung injury who receive inhaled nitric oxide therapy.

139. Neutrophil Adhesion and Activation during Systemic Thrombolysis in Acute Myocardial Infarction
Link, B. et al

Thromb. Res., 91/4), 183-190 (1998)

In a pilot study, alterations of polymorphonuclear neutrophil function during systemic thrombolysis in acute myocardial infarction have been investigated in humans. The following parameters of neutrophil function were measured before and at 15 and 45 minutes after initiation of systemic thrombolysis with a recombinant tissue-type plasminogen activator in 20 patients with acute myocardial infarction: (1) neutrophil adhesion and (2) neutrophil activation. During systemic thrombolysis a significant decrease was observed in neutrophil adhesion (5.5±6.4 to 3.2±3.3; $p<0.05$), in phagocyting neutrophil activation (39±18 to 25±14%; $p<0.05$), and in resting neutrophil activation (9±7 to 3±4%; $p<0.05$). Successful reperfusion coincided with a significantly higher reduction of phagocyting neutrophil activation (40±14 to 20±12% vs. 39±24 to 26±19% in unsuccessful reperfusion; $p<0.05$), and of neutrophil adhesion (6.2±5.7 to 2.7±3.0 vs. 4.1±3.8 to 3.5±4.0 in unsuccessful reperfusion; $p<0.05$) during thrombolysis. Systemic thrombolysis in acute myocardial infarction is accompanied by a reduction in neutrophil adhesion and activation dependent on thrombolytic success.

140. Fluorescein isothiocyanate staining and characterization of avian heterophils
Rath, N.C., Huff, G.R., Balog, J.M. and Huff, W.E.
Vet. Immunol. Immunopathol., 64(1), 83-95 (1998)

Fluorescein isothiocyanate (FITC) was found to stain cytoplasmic granules of avian heterophil-granulocytes. In tissue sections, the fluorescent granulocytes were predominantly distributed adjacent to trabecular bones. The fluorescein stained granulocytes were abundant in synovial fluids of chickens with synovitis. A significant correlation was observed in the percent of fluorescein labeled granulocytes in blood smears and the percent of heterophils determined using an automated counting method, in unstained blood from normal and *Escherichia coli*-infected turkeys. The fluorescein-binding heterophils purified from chickens showed a time dependent increases in the oxidation of 2′,7′-dichlorofluorescin diacetate (DCF-DA) and the reduction of nitroblue tetrazolium (NBT) which were indicative of changes in oxidative burst in response to phorbol 12-myristate 13-acetate (PMA), *Salmonella typhimurium* lipopolysaccharide (LPS), and zymosan A (ZA). These heterophil-activating agents, also, caused significant degranulation at 16 h post-treatment, as indicated by the loss fluorescence. There were microscopically visible alterations in the cell shapes and a decrease in the density of granules due to treatment with LPS, PMA or ZA. In addition, these cells also showed phagocytic response which was evident at 30 min of incubation with fluorescent latex particles. Both chicken and turkey heterophils produced interleukin-6 in vitro at 24 h in response to LPS but not to PMA, FMLP or ZA. The chicken heterophils showed spontaneous production of matrix metalloproteinases (MMP) which was significantly enhanced by treatment with LPS, PMA, and ZA; however, LPS appeared to be most effective in inducing MMP production. These results demonstrate that the functions of heterophils can be differentially regulated by different activating agents and the fluorescein binding property of these cells may be useful for their histochemical identification.

141. CD40L activation of dendritic cells down-regulates DORA, a novel member of the immunoglobulin superfamily
Bates, E.E.M. et al
Mol. Immunol., 35(9), 513-524 (1998)

Using a cDNA subtraction technique, a novel member of the immunoglobulin superfamily was isolated from human Dendritic cells (DC). This cDNA which we named DORA, for *DO*wn-*R*egulated by *A*ctivation encodes a protein belonging to the CD8 family of receptors containing a single V type loop domain with an associated J chain region, a transmembrane region containing an atypical tyrosine residue and a cytoplasmic domain containing three putative tyrosine phosphorylation sites. The hDORA gene has been localised to chromosome 16. From database searches a rat cDNA was identified that encoded a polypeptide with 63% identity to hDORA. The expression of the human cDNA was studied in detail. Northern blot analysis revealed 1.0 kb and 2.5 kb mRNAs in peripheral blood lymphocytes, spleen and lymph node, while low levels were observed in thymus, appendix, bone marrow and fetal liver. No signal was noted in non-immune system tissues. By RT-PCR analysis of hDORA revealed expression in cells committed to the myeloid lineage but not in $CD34^+$ precursors or B cells and low expression in T cells. Expression was also observed in DC, purified ex vivo or generated in vitro from either monocytes or $CD34^+$ progenitors. This was down-regulated following activation both by PMA and Ionomycin treatment and also by CD40L engagement. In situ hybridisation performed on tonsil sections showed the presence of hDORA in cells within Germinal Centers. This structure and expression suggests a function as a co-receptor, perhaps in an antigen uptake complex, or in homing or recirculation of DC.

142. Differential gene expression of a human α2,3-sialyltransferase in leukaemic cell lines and leucocytes
Gassen, U., Kelm, S. and Schauer, R.
FEBS Lett., 427(1), 91-95 (1998)

The gene expression of the human Galβ1,4(3)GlcNAc/Galβ1,3GalNAcα-2,3-sialyltransferase was investigated in the leukaemic cell lines HL60, K-562, MOLT-4, THP-1 and in blood leucocytes. Five different transcripts were identified. In HL60 and THP-1 cells the expression levels of two of these changed during differentiation. Two potential AP1 binding sites were detected in the promoter regions of the gene. THP-1 cells contain proteins binding with higher affinities to these sequences in the sialyltransferase gene than to the AP1 consensus sequence, whereas nuclear extracts from HL60 cells have the opposite affinity.

143. Effect of plasma and LPS on respiratory burst of neutrophils in septic patients
Pascual, C. et al
Intensive Care Med., 24(11), 1181-1186 (1998)

To compare the respiratory burst of neutrophils in sepsis and control patients using lipopolysaccharide (LPS), autologous plasma, and a combination of the two. Design: Prospective, consecutive case study. Setting: A 16-bed intensive care unit (ICU) in a university teaching hospital. Interventions: None. Patients: Plasma was obtained from 23 healthy patients scheduled for minor surgery immediately prior to induction of anesthesia (controls) and from 23 ICU patients within 24 h of diagnosis of sepsis or septic shock. Measurements and main results: Respiratory burst was determined by lucigenin chemiluminescence expressed as mean - SEM of peak values of relative light units per neutrophil. There were no significant differences between neutrophils of septic patients and controls for the stimuli saline, phorbol myristate acetate, formyl-methionyl-leucyl-phenylalenine, and LPS alone. Septic patients showed a lower respiratory burst than controls ($p < 0.05$) under the following stimuli: plasma alone (5911 - 803 vs 15 397 - 3038) and LPS and plasma combined (13 857 - 1537 vs 23 026 - 2640). However, when stimulated with plasma after priming with LPS, septic patients elicited a higher value than control subjects (11 373 - 1758 vs 5987 - 1234, $p < 0.05$). Conclusions: (1) Some components of the plasma of septic patients may have a profound effect on neutrophil response; (2) plasma as a respiratory burst stimulus differentiates between sepsis and non-sepsis samples better than other common stimuli; (3) precautions must be taken when using plasma together with LPS because of the different response depending on whether LPS-priming precedes the plasma stimulus or both are introduced simultaneously and whether septic or nonseptic plasma

144. Leukocyte Transfusion-Associated Granulocyte Responses in a Patient with X-Linked Hyper-IgM Syndrome
Prescott Atkinson, T. et al
J. Clin. Immunol., 18(6), 430-439 (1998)

X-linked hyper-IgM syndrome (XHIM) is a severe congenital immunodeficiency caused by mutations in CD154 (CD40 ligand, gp39), the T cell ligand for CD40 on B cells. Chronic or cyclic neutropenia is a frequent complicating feature that heightens susceptibility to severe infections. We describe a patient with a variant of XHIM who produced elevated levels of serum IgA as well as IgM and suffered from chronic severe neutropenia. Eight of ten leukocyte transfusions with cells from a maternal aunt, performed because of mucosal infections, resulted in similar episodes of endogenous granulocyte production. Transfection studies with the mutant CD154 protein indicate that the protein is expressed at the cell surface and forms an aberrant trimer that does not interact with CD40. The data suggest that allogeneic cells from the patient's aunt, probably activated T cells bearing functional CD154, may interact with $CD40^+$ recipient cells to produce maturation of myeloid precursors in the bone marrow.

145. Serum proteins facilitate neutrophil induction of endothelial leukocyte adhesion molecule 1
Van den Bogaerde, J.M., Hynes, K.L., Clark, E.T. and Gewertz, B.L.
Surgery, 123, 199-204 (1998)

Although the individual actions of neutrophils and serum proteins such as complement in acute inflammation are well characterized, less is known about their effects in combination. We investigated the combined effects of neutrophil contact and active serum proteins on the expression of endothelial leukocyte adhesion molecule 1 (ELAM-1). **Methods:** Confluent monolayers of human umbilical vein endothelial cells were incubated with neutrophils in the presence and absence of fresh human serum. Flow cytometry was used to assess expression of endothelial intercellular adhesion molecule 1 (ICAM-1) and ELAM-1. In addition, neutrophils were retained in a semipermeable insert, which allowed their secretions to contact the endothelium but restricted neutrophil-endothelial contact. **Results:** ELAM-1 expression was significantly increased on the cells coincubated with neutrophils and fresh human serum (25.8%; $p < 0.01$). There was no significant change in ELAM-1 expression on endothelial cells incubated with fresh human serum alone (3.9%; 0.01) or in those incubated with neutrophils and heat-inactivated serum (9.3%; $p > 0.01$). In the absence of neutrophil contact, ELAM-1 expression was increased only in the presence of fresh human serum (9.6%; $p < 0.05$). **Conclusions:** These findings suggest that serum proteins may potentiate the volume or potency of neutrophil-derived diffusable mediators of ELAM-1 expression. These effects are eliminated with the heat inactivation of serum proteins, implicating a heat sensitive mediator such as the complement cascade.

146. C1q-mediated chemotaxis by human neutrophils: involvement of gClqR and G-protein signalling mechanisms
Leigh, L.E.A. et al
*Biochem. J., **330**, 247-254 (1998)*

C1q, the first component of the classical pathway of the complement system, interacts with various cell types and triggers a variety of cell-specific cellular responses, such as oxidative burst, chemotaxis, phagocytosis, etc. Different biological responses are attributed to the interaction of C1q with more than one putative cell-surface C1q receptor/C1q-binding protein. Previously, it has been shown that C1q-mediated oxidative burst by neutrophils is not linked to G-protein-coupled fMet-Leu-Phe-mediated response. In the present study, we have investigated neutrophil migration brought about by C1q and tried to identify the signal-transduction pathways involved in the chemotactic response. We found that C1q stimulated neutrophil migration in a dose-dependent manner, primarily by enhancing chemotaxis (directed movement) rather than chemokinesis (random movement). This C1q-induced chemotaxis could be abolished by an inhibitor of G-proteins (pertussis toxin) and PtdIns(3,4,5)P3 kinase (wortmannin and LY294002). The collagen tail of C1q appeared to mediate chemotaxis. gC1qR, a C1q-binding protein, has recently been reported to participate in C1q-mediated chemotaxis of murine mast cells and human eosinophils. We observed that gC1qR enhanced binding of free C1q to adherent neutrophils and promoted C1q-mediated

chemotaxis of neutrophils by nearly seven-fold. Our results suggests C1q-mediated chemotaxis involves gC1qR as well as G-protein-coupled signal-transduction mechanisms operating downstream to neutrophil chemotaxis.

147. Carnosine and anserine as modulators of neutrophil function
Tan, K.M.L. and Candlish, J.K.
*Clin. Lab. Hematol., **20(4)**, 239-244 (1998)*

Carnosine and anserine, the bioactive peptides found in most meats and fish, were tested for their ability to modulate neutrophil and U937 cell function, specifically with respect to respiratory burst, interleukin-1β production and apoptosis. Both peptides increased the respiratory burst and interleukin-1β production of human neutrophils but not of U937 cells. They suppressed apoptosis of human neutrophils but enhanced apoptosis of U937 cells as assessed by DNA strand breaks. These results suggest that carnosine and anserine have the capacity to modulate the immune response at least in human neutrophils.

148. Compartmentalization of PDE-4 and cAMP-dependent protein kinase in neutrophils and macrophages during phagocytosis
Pryzwansky, K.B., Kidao, S. and Merricks, E.P.
*Cell Biochem. Biophys., **28(2-3)**, 251-175 (1998)*

The compartmentalization of cAMP in human neutrophils during phagocytosis of serum-opsonized zymosan suggests that cAMP is an important second messenger for regulating phagocytosis. Type 4 cAMP-specific phosphodiesterase (PDE-4), cAMP-dependent protein kinase (PKA), and adenylate cyclase are the principal effector molecules for cAMP regulation in phagocytes. Immunofluorescence microscopy demonstrated that PDE-4 isoforms (HSPDE-4A, HSPDE-4B, HSPDE-4D) were targeted to the forming phagosome in neutrophils, and were colocalized with the catalytic subunit of PKA and degranulated myeloperoxidase. Phagocytosis and accumulation of PDE-4 and PKA near adherent zymosan were inhibited by elevating cAMP levels with forskolin or rolipram. cAMP, PDE-4, and PKA were localized at sites of zymosan adherence in cells treated with cytochalasin D to inhibit phagosome formation, suggesting that zymosan engagement to Fc/CR3 receptors triggers cAMP elevations at sites of phagocytosis. HSPDE-4A, HSPDE-4B, HSPDE-4D, and PKA also were localized at the forming phagosome in monocyte-derived macrophages, and the lysosomal marker CD63 demonstrated the absence of PDE-4 around internalized phagolysosomes. These results suggest that cAMP levels are focally regulated by PDE-4 at the nascent phagosome, and that PKA may phosphorylate proteins associated with pseudopodia formation and phagosome internalization.

149. Hprt mutant frequency and aromatic DNA adduct level in non-smoking and smoking lung cancer patients and population controls
Hou, S-M. et al
Carcinogenesis, 20, 437-444 (1999)

T cell cloning and ^{32}P-post-labelling methods were used to study the mutant frequency (MF) at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus and the aromatic DNA adduct level (AL) in peripheral lymphocytes of newly diagnosed lung cancer patients (92 ever-smokers and 87 never-smokers) and matched population controls (82 ever-smokers and 79 never-smokers). Overall, the MF (total mean 20.6×10^{-6}) and AL (4.1×10^{-8}) were similar in cases and controls with the same smoking status, indicating that the disease has limited effect on the two endpoints. When cases and controls were combined, the AL was significantly higher in current smokers than in former or never-smokers ($P = 0.0003$) and the MF was significantly higher in ever-smokers than in never-smokers ($P = 0.004$). Age affected the MF significantly in ever-smokers (1.6%/year, 95% CI 0.6–2.5, adjusted for packyears and years since last smoking), especially among cases (2.1%/year, 95% CI 0.5–3.7). An increase of AL with age was observed in currently smoking cases only (2.3%/year, 95% CI 0.3–4.2, adjusted for smoking dose). For currently smoking cases, there was also a more pronounced effect of smoking dose on both endpoints and a significant correlation between AL and MF ($r = 0.52$, $P = 0.04$) was observed among those with the highest dose. Our data also provide additional evidence for the different turnover times of smoking-induced DNA adducts and *hprt* mutations. The stronger increase of MF and AL with age and dose in currently smoking patients compared with controls is consistent with an interaction between smoking and genetic host factors.

150. Capsular sialic acid limits C5a production on type III group B streptococci
Takahashi, S., Aoyagi, Y., Adderson, E.E., Okuwaki, Y. and Bohnsack, J.F.
Infect. Immun., 67, 1866-1870 (1999)

The majority of type III group B streptococcus (GBS) human neonatal infections are caused by a genetically related subgroup called III-3. We have proposed that a bacterial enzyme, C5a-ase, contributes to the pathogenesis of neonatal infections with GBS by rapidly inactivating C5a, a potent pro-inflammatory molecule, but many III-3 strains do not express C5a-ase. The amount of C5a produced in serum following incubation with representative type III strains was quantitated in order to better understand the relationship between C5a production and C5a-ase expression. C5a production following incubation of bacteria with serum depleted of antibody to the

bacterial surface was inversely proportional to the sialic acid content of the bacterial capsule, with the more heavily sialylated III-3 strains generating less C5a than the less-virulent, less-sialylated III-2 strains. The amount of C5a produced correlated significantly with C3 deposition on each bacterial strain. Repletion with type-specific antibody caused increased C3b deposition and C5a production through alternative pathway activation, but C5a was functionally inactivated by strains that expressed C5a-ase. The increased virulence of III-3 strains compared to that of III-2 strains results at least partially from the higher sialic acid content of III-3 strains, which inhibits both opsonophagocytic killing and C5a production in the absence of type-specific antibody. We propose that C5a-ase is not necessary for III-3 strains to cause invasive disease because the high sialic acid content of III-3 strains inhibits C5a production.

151. Functional analysis of IgA antibodies specific for a conserved epitope within the M protein of group A streptococci from Australian aboriginal endemic communities
Brandt, E.R. et al
Int. Immunol., 11, 569-576 (1999)

The mucosa is one of the initial sites of group A streptococcal (GAS) infection and salivary IgA (sIgA) is thought to be critical to immunity. However, the target epitopes of sIgA and the function of sIgA in GAS immunity, in particular the role of accessory cells and complement, is largely unknown. We studied the aquisition and the function of sIgA specific for a conserved region epitope, p145 (sequence: LRRDLDASREAKKQVEKALE) of the M protein. Peptide 145-specific sIgA is highly prevalent within an Aboriginal population living in an area endemic for GAS and acquisition of p145-specific sIgA increases with age, consistent with a role for such antibodies in immunity to GAS. Human sIgA and IgG specific for p145 were affinity purified and shown to opsonize M5 GAS *in vitro.* Opsonization could be specifically inhibited by the addition of free p145 to the antibodies during assay. Opsonization of GAS was totally dependent on the presence of both complement and polymorphonuclear leukocytes, and, moreover, affinity-purified p145-specific sIgA was shown to fix complement in the presence of M5 GAS. These data show that mucosal IgA to this conserved region peptide within the M protein has an important role in human immunity against GAS and may be useful in a broad-based cross-protective anti-streptococcal vaccine.

152. Insulin infusion improves neutrophil function in diabetic cardiac surgery patients
Rassias, A.J. et al
Anesth. Analg., 88, 1011 (1999)

Diabetic patients are at increased risk of wound infection after major surgery, but the effect of perioperative glucose control on postoperative wound infection rates after surgery is uncertain. We tested the effect of an insulin infusion on perioperative neutrophil function in diabetic patients scheduled for coronary artery bypass surgery. Participants (n = 26) were randomly allocated to receive either aggressive insulin therapy (AIT) or standard insulin therapy (SIT) during surgery. Blood was drawn for neutrophil testing before surgery, 1 h after the completion of cardiopulmonary bypass, and on the first postoperative day. Neutrophil phagocytic activity decreased to 75% of baseline activity in the AIT group and to 47% of baseline activity in the SIT group ($P < 0.05$ between groups). No important differences in neutrophil antibody-dependent cell cytotoxicity were found. This study documents a potentially beneficial effect of continuous insulin therapy in diabetic patients who require major surgery.
Implications: A continuous insulin infusion and glucose control during surgery improves white cell function in diabetic patients and may increase resistance to infection after surgery.

153. Inhibition of a membrane complement regulatory protein by a monoclonal antibody induces acute lethal shock in rats primed with lipopolysaccharide
Mizuno, M. et al
*J. Immunol., **162**, 5477 (1999)*

Rats pretreated with traces of LPS developed acute fatal shock syndrome after i.v. administration of a mAb that inhibits the function of a membrane complement regulatory molecule. Such a shock was not observed after the administration of large amounts of LPS instead of the mAb following LPS pretreatment. The lethal response did not occur in rats depleted of either leukocytes or complement, and a C5a receptor antagonist was found to inhibit the reaction. Furthermore, LPS-treated rats did not suffer fatal shock following the injection of cobra venom factor, which activates complement in the fluid phase so extensively as to exhaust complement capacity. Therefore, complement activation on cell membranes is a requirement for this type of acute reaction.

154. Antimicrobial defensin peptides of the human ocular surface
Haynes, R.J., Tighe, P.J. and Dua, H.S.
Br. J. Ophthalmol., 83, 737 (1999)

BACKGROUND/AIMS—The antimicrobial activity of the tear film exceeds the activity of its known constituents. The authors postulate that this excess activity is the result of antimicrobial peptides called defensins, and they aimed to look for defensins in the human eye. *METHODS*—Evidence of defensin production was sought by reverse transcriptase polymerase chain reaction (RT-PCR). Intron spanning primers were designed for β defensins 1 and 2, and α defensins 5 and 6. RT-PCR was performed on cornea, conjunctiva, and

lacrimal gland samples, and reaction products were size fractionated and sequenced to confirm their identity. A monoclonal antibody was utilised for the detection of α defensins 1, 2, and 3 in tissue sections and in immunoblots of tears.
RESULTS—RT-PCR revealed β defensin 1 message in samples of conjunctiva, cornea, and lacrimal gland. β Defensin 2 message was detected in the conjunctiva and cornea but was absent from the lacrimal gland. α Defensin 5 and 6 message was absent in these tissues but α defensins 1, 2, and 3 were detected in normal tears, lacrimal gland, and inflamed conjunctiva by immunochemistry.
CONCLUSION—The data suggest the human eye innately produces a spectrum of antimicrobial defensin peptides. Defensins hold therapeutic potential in ocular infections as they have a broad spectrum of antimicrobial activity (bacteria fungi and viruses) and accelerate epithelial healing.

155. A large subset of neutrophils expressing membrane proteinase 3 is a risk factor for vasculitis and rheumatoid arthritis
Witko-Sarsat, V. et al
J. Am. Soc. Nephrol., 10, 1224 (1999)

Abstract. It has been shown previously that proteinase 3 (PR3), a neutrophil intracellular protease that is the main antigen of antineutrophil cytoplasm (ANCA) autoantibodies, is present on the plasma membrane of a subset of freshly isolated neutrophils. This study shows that the size of this subset of membrane PR3-positive ($mPR3^+$) neutrophils is a stable feature of a given individual, most likely genetically controlled. It ranges from 0 to 100% of neutrophils and allows us to define a new polymorphism in the healthy population, with three discrete phenotypes corresponding respectively to less than 20% $mPR3^+$ neutrophils ($mPR3^{low}$) or to a mean percentage of 47% ($mPR3^{intermediate}$) and 71.5% ($mPR3^{high}$) $mPR3^+$ neutrophils. The frequency of the $mPR3^{high}$ phenotype was significantly increased in patients with ANCA-associated vasculitis (85% *versus* 55% in healthy subjects). The percentage of $mPR3^+$ neutrophils was not affected by disease activity, relapses, or therapy, and did not reflect *in vivo* cell activation. In addition, $mPR3^+$ phenotypes were normally distributed in cystic fibrosis patients, indicating that infection and/or inflammation *per se* do not lead to a high percentage of $mPR3^+$ neutrophils. The frequency of the $mPR3^{high}$ phenotype was not related to anti-PR3 autoimmunization, since it was increased in vasculitic patients regardless of the ANCA specificity (anti-PR3, anti-myeloperoxidase, or unknown). Interestingly, the frequency of the $mPR3^{high}$ phenotype was also increased in patients with rheumatoid arthritis. It was normal in type I-diabetes, a T cell-dependent autoimmune disease. It is proposed here that a high proportion of membrane PR3-positive neutrophils could favor the occurrence or the progression of chronic inflammatory diseases.

156. Migration of neutrophils across human pulmonary endothelial cells is not blocked by matrix metalloproteinase or serine protease inhibitors
Mackarel, A.J., Cottell, D.C., Russell, K.J., FitzGerald, M.X. and O'Connor, C.M.
Am. J. Respir. Cell Mol. Biol., 20, 1209 (1999)

It has long been speculated that neutrophils deploy proteases to digest subendothelial matrix as they migrate from the bloodstream. Direct evidence for the involvement of proteases in neutrophil transendothelial migration is, however, lacking. To address this issue we used transmission electron microscopy to verify the presence of continuous basal lamina beneath pulmonary endothelial cells grown on microporous filters, and then examined the effects of protease inhibitors on neutrophil migration through the endothelial cells and their associated subcellular matrix. Inhibitors of the two major matrix-degrading protease groups present in neutrophils, the matrix metalloproteinases (MMPs) and serine proteases, were assessed for their ability to modulate neutrophil transendothelial migration in response to the chemoattractant *n*-formylmethionyl leucylphenylalanine (FMLP). Neither the naturally occurring MMP inhibitor, tissue inhibitor of metalloproteinase-1, nor the hydroxamic acid-based inhibitors GM-6001, BB-3103, or Ro 31-9790 had any significant effect on FMLP-stimulated neutrophil migration across endothelial cells and associated basal lamina, with ≥ 80% of neutrophils migrating through the system, even in the presence of inhibitors, at concentrations that totally inhibited all the gelatinase B (MMP-9) released upon stimulation with FMLP. Similarly, with serine protease inhibitors no significant inhibition of neutrophil migration was observed with a naturally occurring inhibitor, secretory leukocyte protease inhibitor, or a low molecular-weight synthetic inhibitor, Pefabloc SC. These results indicate that neither MMP nor serine protease digestion of sub-endothelial matrix is required for successful neutrophil transendothelial migration.

157. Humanized anti-HM1.24 antibody mediates myeloma cell cytotoxicity that is enhanced by cytokine stimulation of effector cells
Ozaki, S. et al
Blood, 93, 3922-3930 (1999)

To develop a new immunotherapy for multiple myeloma, we have generated a monoclonal antibody (MoAb) that detects a human plasma cell-specific antigen, HM1.24. Our previous study has shown that mouse anti-HM1.24 MoAb inhibits the proliferation of human myeloma cells implanted into severe combined immunodeficiency mice. In this report, we evaluated the antitumor activity of the humanized anti-HM1.24 MoAb (IgG1κ), which was constructed by grafting the complementarity-determining regions. In contrast to the parent mouse MoAb, humanized anti-HM1.24 MoAb mediated antibody-dependent cellular cytotoxicity (ADCC) against both myeloma cell lines and myeloma cells from patients in the presence of human peripheral blood mononuclear cells (PBMCs). The PBMCs from untreated myeloma patients exhibited ADCC activity as efficiently as those of healthy donors. Although decreased ADCC activity of PBMCs was observed in patients who responded poorly to conventional chemotherapy, it could be significantly augmented by the

stimulation with interleukin-2 (IL-2), IL-12, or IL-15. There was a strong correlation between the percentage of $CD16^+$ cells and ADCC activity in the PBMCs of myeloma patients. Moreover, peripheral blood stem cell collections from myeloma patients contained higher numbers of $CD16^+$ cells than PBMCs and exhibited ADCC activity that was enhanced by IL-2. These results indicate that humanized anti-HM1.24 MoAb has potential as a new therapeutic strategy in multiple myeloma and that treatment of effector cells with immunomodulating cytokines can restore the effect of humanized anti-HM1.24 MoAb in patients with diminished ADCC activity.

158. Variation of bronchoalveolar lymphocyte phenotypes with age in the physiologically normal human lung
Meyer, K.C. and Soergel, P.
Thorax, 54, 697 (1999)

BACKGROUND—Changes in T lymphocyte subsets have been observed in various forms of pulmonary disease. However, bronchoalveolar lymphocyte subsets have not been well characterised for healthy individuals differing in age. A study was undertaken to investigate the bronchoalveolar lavage (BAL) and peripheral blood lymphocyte subsets in clinically normal volunteers of two different age groups (19-36 and 64-83 years).
METHODS—Bronchoalveolar lavage was performed on all individuals in both age groups and peripheral venous blood was drawn just prior to BAL. Bronchoalveolar cell profiles were characterised by morphological criteria, and cell surface antigen expression of lymphocytes was determined by flow cytometry.
RESULTS—A significant increase in total BAL lymphocytes was observed for the oldest group compared with the youngest age group. Mean lymphocyte subset (CD4+/CD8+) ratios were significantly increased in BAL fluid from the older group compared with the younger group (mean (SE) 7.6 (1.5) vs 1.9 (0.2); $p<0.0001$). The increase in the BAL CD4+/CD8+ T cell ratio was mostly due to an increase in relative numbers of CD4+ lymphocytes, and the BAL CD4/CD8 ratio was disproportionately increased compared with peripheral blood in the older group. Increased expression of HLA-DR and CD69 on CD4+ T lymphocytes was observed in the oldest age group. Relative numbers of natural killer (NK) cells did not vary with age, and γδT cells and CD5+ B cells were present in very low numbers in both age groups.
CONCLUSIONS—CD4+ T cells accumulate in air spaces of the lower respiratory tract with age in healthy adults and express increased amounts of HLA-DR and CD69 on their surfaces, suggesting a relative degree of CD4+ T lymphocyte activation for healthy older individuals who have normal lung function.

159. Pathogenesis of Borna disease virus: Granulocyte fractions of phsychiatric patients harbor infectious virus in the absence of antiviral antibodies
Planz, O. et al
J. Virol., 73, 6251-6256 (1999)

Borna disease virus (BDV) causes acute and persistent infections in various vertebrates. During recent years, BDV-specific serum antibodies, BDV antigen, and BDV-specific nucleic acid were found in humans suffering from psychiatric disorders. Furthermore, viral antigen was detected in human autopsy brain tissue by immunohistochemical staining. Whether BDV infection can be associated with psychiatric disorders is still a matter of debate; no direct evidence has ever been presented. In the present study we report on (i) the detection of BDV-specific nucleic acid in human granulocyte cell fraction from three different psychiatric patients and (ii) the isolation of infectious BDV from these cells obtained from a patient with multiple psychiatric disorders. In leukocyte preparations other than granulocytes, either no BDV RNA was detected or positive PCR results were obtained only if there was at least 20% contamination with granulocytes. Parts of the antigenome of the isolated virus were sequenced, demonstrating the close relationship to the prototype BDV strains (He/80 and strain V) as well as to other human virus sequences. Our data provide strong evidence that cells in the granulocyte fraction represent the major if not the sole cell type harboring BDV-specific nucleic acid in human blood and contain infectious virus. In contrast to most other reports of putative human isolates, where sequences are virtually identical to those of the established laboratory strains, this isolate shows divergence in the region previously defined as variable in BDV from naturally infected animals.

160. APCs express DCIR, a novel C-type lectin receptor containing an immunoreceptor tyrosine-based inhibitory motif
Bates, E.E. et al
J. Immunol., 163, 1973 (1999)

We have identified a novel member of the calcium-dependent (C-type) lectin family. This molecule, designated DCIR (for dendritic cell (DC) immunoreceptor), is a type II membrane glycoprotein of 237 aa with a single carbohydrate recognition domain (CRD), closest in homology to those of the macrophage lectin and hepatic asialoglycoprotein receptors. The intracellular domain of DCIR contains a consensus immunoreceptor tyrosine-based inhibitory motif. A mouse cDNA, encoding a homologous protein has been identified. Northern blot analysis showed DCIR mRNA to be predominantly transcribed in hematopoietic tissues. The gene encoding human DCIR was localized to chromosome 12p13, in a region close to the NK gene complex. Unlike members of this complex, DCIR displays a typical lectin CRD rather than an NK cell type extracellular domain, and was expressed on DC, monocytes, macrophages, B lymphocytes, and granulocytes, but not detected on NK and T cells. DCIR was strongly expressed by DC derived from blood monocytes cultured with GM-CSF and IL-4. DCIR was mostly expressed by monocyte-related rather than Langerhans cell related DC obtained from $CD34^+$ progenitor cells. Finally, DCIR expression was down-regulated by signals inducing DC maturation such as CD40 ligand,

LPS, or TNF-α. Thus, DCIR is differentially expressed on DC depending on their origin and stage of maturation/activation. DCIR represents a novel surface molecule expressed by Ag presenting cells, and of potential importance in regulation of DC function.

161. Tropical estrogen accelerate cutaneous wound healing in aged humans associated with an altered inflammatory response
Ashcroft, G.S., Greenwell-Wild, T., Horan, M.A., Wahl, S.M. and Ferguson, M.W.J.
Am. J. Pathol., 155, 1137 (1999)

The effects of intrinsic aging on the cutaneous wound healing process are profound, and the resulting acute and chronic wound morbidity imposes a substantial burden on health services. We have investigated the effects of topical estrogen on cutaneous wound healing in healthy elderly men and women, and related these effects to the inflammatory response and local elastase levels, an enzyme known to be up-regulated in impaired wound healing states. Eighteen health status-defined females (mean age, 74.4 years) and eighteen males (mean age, 70.7 years) were randomized in a double-blind study to either active estrogen patch or identical placebo patch attached for 24 hours to the upper inner arm, through which two 4-mm punch biopsies were made. The wounds were excised at either day 7 or day 80 post-wounding. Compared to placebo, estrogen treatment increased the extent of wound healing in both males and females with a decrease in wound size at day 7, increased collagen levels at both days 7 and 80, and increased day 7 fibronectin levels. In addition, estrogen enhanced the strength of day 80 wounds. Estrogen treatment was associated with a decrease in wound elastase levels secondary to reduced neutrophil numbers, and decreased fibronectin degradation. *In vitro* studies using isolated human neutrophils indicate that one mechanism underlying the altered inflammatory response involves both a direct inhibition of neutrophil chemotaxis by estrogen and an altered expression of neutrophil adhesion molecules. These data demonstrate that delays in wound healing in the elderly can be significantly diminished by topical estrogen in both male and female subjects.

162. Apoptosis induction of human lung cancer cell line in multicellular heterospheroids with humanized antiganglioside GM2 monoclonal antibody
Nakamura, K. et al
Cancer Res., 59, 5323 (1999)

The chimeric antiganglioside GM2 monoclonal antibody (MAb) KM966, which showed high effector functions such as complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC), potently suppressed growth and metastases of GM2-positive human cancer cells inoculated into mice. To further improve the therapeutic efficacy of the anti-GM2 MAb in humans, we constructed a humanized anti-GM2 MAb, KM8969. The humanized KM8969 was more efficient in supporting ADCC against GM2-positive human cancer cell lines than the chimeric KM966, whereas complement-dependent cytotoxicity was slightly reduced in the humanized KM8969. In addition, the humanized KM8969 was shown to exert potent ADCC mediated by both lymphocytes and monocytes. To investigate the effect of the humanized KM8969 on the biological function of GM2 in the condition physiologically mimicking formation and growth of cancer masses, the heterospheroids composed of normal human dermal fibroblasts and GM2-positive human lung cancer cells were developed. Interestingly, the humanized KM8969 gave rise to growth inhibition of heterospheroids without dependence of the effector functions. Morphological and immunocytochemical analysis suggested that the inhibitory effect was due to the apoptosis of GM2-positive cancer cells in the heterospheroids. The result indicates that GM2 captured by the antibody on the cell surface loses its physiological function that plays a critical role in maintaining the three-dimensional growth of cancer cells in contact with its own cells or other type of cells in a microenvironment. The humanized KM8969, which can destroy the cancer cells via blocking functional GM2 on the cell surface as well as the effector functions, would have extraordinary potential in human cancer therapy.

163. Presence of proteinase 3 in secretory vesicles: evidence of a novel, highly mobilizable intracellular pool distinct from azurophil granules
Witko-Sarsat, V. et al
Blood, 94, 2487-2496 (1999)

Proteinase 3 (PR3), which is also called myeloblastin, the target autoantigen for antineutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis, is a serine proteinase stored in azurophil granules of human neutrophils. We have previously shown that, in contrast to elastase or myeloperoxidase, PR3 is also expressed at the plasma membrane of a subset of unactivated neutrophils and that a high proportion of neutrophils expressing membrane PR3 is a risk factor for vasculitis. The present study demonstrates that the association of PR3 with the plasma membrane is not an ionic interaction and seems to be covalent. Fractionation of neutrophils shows that, besides the azurophil granules, PR3 could be detected both in specific granules and in the plasma membrane-enriched fraction containing secretory vesicles, whereas elastase and myeloperoxidase were exclusively located in azurophil granules. Electron microscopy confirms that PR3 is present along with CR1 in secretory vesicles as well as in some specific granules. In neutrophils stimulated with an increasing dose of FMLP, membrane PR3 expression increased with the degranulation of secretory vesicles, followed by specific granules, and culminated after azurophil granules mobilization. The presence of a readily plasma membrane-mobilizable pool of PR3 contained in the secretory vesicles might play a relevant role in the pathophysiological mechanisms of ANCA-associated vasculitis.

164. Neutrophil inhibitory factor abrogates neutrophil adhesion by blockade of CD11a and CD11bβ_2 integrins
Lo, S.K. et al
Mol. Pharmacol., 56, 926 (1999)

We studied the basis of inhibition of polymorphonuclear leukocyte (PMN) adhesion induced by neutrophil inhibitory factor (NIF), a 41-kDa CD11/CD18 β_2 integrin-binding protein isolated from the canine hookworm (*Ancylostoma caninum*). NIF blocked PMN adhesion in a concentration-dependent manner with complete blockade occurring at ~10 nM NIF. Because CD11a and CD11b β_2 integrins are functionally active on stimulated PMNs, and yet NIF is postulated to inhibit only CD11b integrin by binding to its I domain, we evaluated the contributions of CD11a and CD11b β_2 integrins in the mechanism of inhibition of PMN adhesion to endothelial cells. We observed an additive inhibitory effect (>90% inhibition) of PMN adhesion to endothelial cells when NIF was used in combination with anti-CD11b monoclonal antibodies, which alone at saturating concentrations reduced PMN adhesion by only 50%. NIF also prevented aggregation of phorbol ester-stimulated JY lymphoblastoid cells that expressed only the functionally active CD11a, suggesting that NIF also can inhibit CD11a-dependent response. We transduced the NIF cDNA into human dermal microvessel endothelial cells in which NIF synthesis and release prevented PMN adhesion to the transduced human dermal microvessel endothelial cells. These data indicated that the potent antiadhesive effect of NIF may be the result of inhibition of CD11a and CD11b β_2 integrins on PMNs. Moreover, the strategy of NIF release from transduced endothelial cells suggests the feasibility of blocking the CD11a- and CD11b β_2 integrin-dependent PMN adhesion and PMN migration responses specifically at sites of endothelial cell activation.

165. Pharmacokinetics in Serum and Leukocyte Exposures of Oral Azithromycin, 1,500 Milligrams, Given over a 3- or 5-Day Period in Healthy Subjects
Amsden, G.W., Nafziger, A.N. and Foulds, G.
Antimicrob. Agents Chemother., 43, 163-165 (1999)

The pharmacokinetics in serum and leukocyte (WBC) exposures of 1,500 mg of oral azithromycin administered as 3-day (500 mg/day, days 1 to 3) and 5-day (500 mg on day 1 and 250 mg/day on days 2 to 5) regimens were compared in 12 healthy volunteers. Serum, polymorphonuclear leukocytes, and mononuclear leukocytes were collected over a 12-day period from the start of each regimen. Results of the study indicate that the exposures of serum and both types of WBCs were similar with both regimens. Drug concentrations in day 12 WBCs were well above the MICs for all relevant community-acquired respiratory tract pathogens. Terminal half-lives in serum obtained by both regimens were essentially equal at 66 h and consistent with past reports. These results indicate that the standard 1,500-mg dose of oral azithromycin can be administered over either 5 or 3 days.

166. *Eesherichia coli msbB* gene as a virulence factor and a therapeutic target
Somerville, J.E., Cassiano, L. and Darveau, R.P.
Infect. Immun., 67, 6583-6590 (1999)

A mutation in the *msbB* gene of *Escherichia coli* results in the synthesis of *E. coli* lipopolysaccharide (LPS) that lacks the myristic acid moiety of lipid A. Although such mutant *E. coli* cells and their purified LPS have a greatly reduced ability to stimulate human immune cells, a minor reduction in the mouse inflammatory response is observed. When the *msbB* mutation is transferred into a clinical isolate of *E. coli*, there is a significant loss in virulence, as assessed by lethality in BALB/c mice. When a cloned *msbB* gene is provided to functionally complement the *msbB* mutant, virulence returns, providing direct evidence that the *msbB* gene product is an important virulence factor in a murine model of *E. coli* pathogenicity. In the genetic background of the clinical *E. coli* isolate, the *msbB* mutation also results in filamentation of the cells at 37°C but not at 30°C, a reduction in the level of the K1 capsule, an increase in the level of complement C3 deposition, and an increase in both opsonic and nonopsonic phagocytosis of the *msbB* mutant, phenotypes that can help to explain the loss in virulence. The demonstration that the inhibition of *msbB* gene function reduces the virulence of *E. coli* in a mouse infection model warrants further investigation of the *msbB* gene product as a novel target for antibiotic therapy.

167. Multicenter Comparison of the Digene Hybrid Capture CMV DNA Assay (Version 2.0), the pp65 Antigenemia Assay, and Cell Culture for Detection of Cytomegalovirus Viremia
Mazulli, T. et al
J. Clin. Microbiol., 37, 958-963 (1999)

We compared the Digene Hybrid Capture CMV DNA Assay version 2.0, the pp65 antigenemia assay, traditional tube culture, and shell vial culture for the detection of cytomegalovirus (CMV) viremia in several patient populations at three centers. Of 561 blood specimens collected from 402 patients, complete clinical and laboratory data were available for 489. Using consensus definitions for true positives and true negatives, the sensitivities of the Hybrid Capture assay, antigenemia, shell vial, and tube culture were 95, 94, 43, and 46%, respectively. The specificities of the Hybrid Capture assay and antigenemia were 95 and 94%, respectively. At all three study sites, the detected level of CMV viremia was significantly higher with the Hybrid Capture assay or antigenemia than with shell vial and tube culture. In a group of 131 healthy nonimmunosuppressed volunteers, the Hybrid Capture assay demonstrated a specificity of over 99%. The Hybrid Capture assay is a standardized assay that is simple to perform and can utilize whole blood specimens that have been stored for up to 48 h. The high sensitivity and specificity of the Hybrid Capture assay along with its simplicity and flexibility make it a

clinically useful assay for the detection of CMV viremia in immunocompromised or immunosuppressed patients. Further evaluation to determine its role in predicting CMV disease and for monitoring the therapeutic response to anti-CMV therapy is needed.

168. Studies of technetium-99m nitridobisdithiocarboxylate leucocyte specific radiopharmaceutical: [99mTcN(DTCX)2], DTCX = CH3(CH2)8CS2. The cellular and subcellular distribution in human blood cells, and chemical behaviour. Synthesis of the analogous rhenium-188 radiopharmaceutical.
Demaimay, F. et al
Nucl. Med. Biol., 26(2), 225-231 (1999)

The distribution of the radiopharmaceutical ([99mTcN(DTCX)2], DTCX = CH3(CH2)8CS2) in the leucocyte population determined by a density separation with double gradient Polymorphprep was studied. Microautoradiographic analysis showed a subcellular distribution of the radiomarker in human blood cells. This technique confirmed the observed lymphocyte selectivity (69%) and revealed that the uptake was predominantly cytoplasmic around the nucleus. A labeling mechanism by passive endocytosis could be proposed involving a required lipophilicity of the radiopharmaceutical for lymphocyte targeting. Finally, we describe the new synthesis with an efficient yield and radiochemical purity of the analogous radiopharmaceutical

169. Endothelial degradation of extracellular lyso-phosphatidylcholine
Tøsti, E., Dahl, L., Endresen, M.J: and Henriksen, T.
Scand. J. Clin. Lab. Invest., 59(4), 249-257 (1999)

Formation of lysophospholipids, including lyso-phosphatidylcholine (lysoPC), is enhanced during oxidation of low-density lipoprotein, in ischaemic tissue and under inflammatory conditions. Besides being potentially cytotoxic, extracellular lysoPC induces changes in several properties of vascular endothelial cells. These include expression of endothelial adhesion molecules and interference with the endothelial production of nitrogen monoxide, prostacyclin and growth factors. One way of controlling the concentration of extracellular lysoPC is by the action of lysophospholipases, which degrade lysoPC into a free fatty acid and glycerophosphocholine. We therefore tested whether vascular endothelial cells have the ability to degrade extracellular lysoPC. Monolayers of primary cultures of human umbilical vein endothelial cells degraded an average of 84±24 nmol lysoPC/106 cells/2 h. By comparison, monocytes degraded 9.7±3.7 nmol lysoPC/106 cells/2 h, and erythrocytes and platelets <1 nmol lysoPC/106 cells/2 h. The ability of endothelial cells to degrade extracellular phospholipids (diacylphosphatidyl choline) was found to be relatively low (9.5±6.4 nmol/106 cells/2 h). Triacylglycerol hydrolase activity was just above detection level. In conclusion, endothelial cells seem to degrade extracellular lysoPC effectively. This endothelial property may be important in controlling plasma and tissue levels of extracellular lysoPC as well as in the interaction between lysoPC and the vascular endothelium.

170. Plasma homocysteine concentration related to diet, endothelial function and mononuclear cell gene expression among male hyperlipidaemic smokers
Brude, I.R. et al
Eur. J. Clin. Invest., 29(2), 100-108 (1999)

Background
Elevated plasma concentration of homocysteine is an independent risk factor for development of cardiovascular diseases.
Materials and methods
We evaluated potential links between homocysteine and atherothrombogenesis by relating the plasma concentration of homocysteine to (i) dietary antioxidants and omega-3 fatty acids (and determined influence of intervention with antioxidants or omega-3 fatty acids); (ii) markers of endothelial cell function; and (iii) peripheral blood mononuclear cell mRNA levels.
Results
We observed an inverse relationship between the plasma homocysteine concentration and dietary intake of vegetables, vitamin C and β-carotene and between homocysteine and the serum concentration of folate, vitamin B_{12} and omega-3 fatty acids. Intervention with antioxidants or omega-3 fatty acids did not affect plasma homocysteine concentration. The plasma levels of cysteinylglycine and vitamin B_{12} correlated positively with circulating E-selectin and VCAM-1, respectively, whereas folate in serum and blood correlated negatively with P-selectin. A negative correlation was found between the concentrations of homocysteine and von Willebrand factor. Negative and positive correlations were found between plasma homocysteine and the mononuclear cell mRNA levels of peroxisome proliferator activated receptor δ (PPARδ) and c-*myc* respectively. A negative correlation was also found between plasma homocysteine and mononuclear cell mRNA levels of the proteoglycan serglycin. Homocysteine was not correlated with serum activity of glutathione peroxidase or with the mRNA level of glutathione peroxidase in mononuclear cells.
Conclusion
The plasma homocysteine level was negatively correlated with dietary intake of vegetables, including vitamins C and E, and serum omega-3 fatty acids, whereas supplementation with antioxidants or omega-3 fatty acids did not affect plasma homocysteine concentration. Homocysteine was not associated with circulating adhesion molecules or increased procoagulant activity, but homocysteine may alter mononuclear cell gene expression. Cysteine showed no significant correlation with these parameters.

171. Increased interleukin-8 (IL-8) in rectal dialysate from patients with ulcerative colitis: evidence for a biological role for IL-8 in inflammation of the colon
Keshavarzian, A. et al
Am. J. Gastroenterol., 94(3), 704-712 (1999)

OBJECTIVE: Infiltration of neutrophils and their release of toxic reactive oxygen species (ROS) in the colonic mucosa are associated with tissue damage in ulcerative colitis (UC). This neutrophil migration may be induced by chemoattractants, such as cytokines, in the colonic milieu. One such chemoattractant is interleukin-8 (IL-8), a neutrophil chemokine that is present at high concentrations in inflamed mucosa. However, the functional significance of IL-8 in neutrophil attraction and activation in UC has not been established. We hypothesized that IL-8 in the colonic lumen of patients with UC primes neutrophils, leading to their attraction and activation.
METHODS: The colonic milieu was sampled by rectal dialysis. Using a semi-permeable membrane with a molecular weight cut-off of 12 kDa, dialysis solution was placed in the rectum and allowed to equilibrate over a 4-h period with the colonic milieu of controls or of patients with UC. IL-8 concentrations were measured by ELISA. Two functions of healthy neutrophils (PMN) were measured: expression of CD11-b surface adhesion molecules (by flow cytometry), and production of ROS (by both chemiluminescence and cytochrome C reduction assays). Neutrophil functions after exposure to rectal dialysates or n-formyl-methionyl-leucyl-phenylalanine (fMLP) were assessed before and after adding anti–IL-8 antibody or the fMLP blocker BMLP.
RESULTS: IL-8 concentrations in dialysates from patients with active UC were significantly higher than in controls and correlated with disease activity. UC dialysates significantly increased ROS production and CD11-b expression by neutrophils and anti–IL-8 antibody partially (50%) inhibited these stimulatory effects of UC dialysates. Preincubation of neutrophils with UC dialysates significantly potentiated the fMLP-induced rise in ROS and anti–IL-8 antibody completely abolished this priming effect.
CONCLUSIONS: The colonic milieu, sampled by rectal dialysis, from patients with active UC can both activate and prime neutrophils *in vitro*. High concentrations of IL-8 in the colonic lumen of UC patients are partially responsible for the activating effects of rectal dialysates, and account for all of its priming effects. These findings provide direct evidence for a role for IL-8 in inflammatory bowel disease.

172. P fimbriae-dependent, lipopolysaccharide-independent activation of epithelial cytokine responses
Hedlund, M. et al
Mol. Microbiol., 33(4), 693-703 (1999)

Cells in the mucosal barrier are equipped to sense and respond to microbes in the lumen and translate this molecular information into signals that can reach local or distant sites. The interaction of P-fimbriated *Escherichia coli* with human uroepithelial cells is a model to study the molecular mechanism of epithelial cell activation by mucosal pathogens. Here, we examine the role of lipopolysaccharide (LPS) as a co-stimulatory molecule in epithelial cell activation by P-fimbriated *E. coli*. P-fimbriated clinical isolates or recombinant strains were shown to trigger a fimbriae-dependent epithelial cell cytokine response. Mutational inactivation of the *msbB* sequences that control lipid A myristoylation drastically impaired monocyte stimulation but not epithelial responses to P-fimbriated bacteria. Polymyxin B or bactericidal/permeability increasing factor (BPI) neutralized the effects of lipid A in the monocyte assay, but did not reduce epithelial responses. Finally, isolated LPS of the smooth, rough and deep rough chemotypes were poor epithelial cell activators. The cells were shown to lack surface CD14 or CD14 mRNA as well as the CD14 co-receptor function and were also very poor LPS responders in the presence of human serum. These results demonstrate that epithelial cell responses to P-fimbriated *E. coli* are CD14 and LPS independent, and suggest that attaching pathogens can overcome the LPS unresponsiveness of epithelial cells by fimbriae-dependent activation mechanisms.

173. Biased T cell receptor Vβ gene expression in bronchoalveolar lavage fluid from Japanese patients with sarcoidosis
Yoshitomi, A. et al
Respirology, 4(4), 339-347 (1999)

Objective:
Sarcoidosis is believed to be one of the T cell-mediated granulomatous diseases with unknown aetiology. We attempt to search for the causative T cell clones of sarcoidosis.
Methods: We study T cell receptor β-chain variable region (Vβ) repertoire in peripheral blood (PB) and bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis, using semi-quantitative reverse transcriptase–polymerase chain reaction method. The expression of 22 kinds of Vβ genes is examined in 17 patients with sarcoidosis and nine normal subjects.
Results: Compared with control subjects, the group with sarcoidosis exhibits significantly high expressions of the Vβ2 ($P< 0.005$, Wilcoxon's test) and Vβ6 ($P = 0.005$) genes in BALF. In each BALF sample, the Vβ2 ($P< 0.01$, χ^2 test) and Vβ6 ($P< 0.01$) genes were overexpressed (> 2 SD above the mean value for each Vβ observed in control subjects) in 11 and 10 of 17 patients with sarcoidosis, respectively. Furthermore, the amino acid sequences of $V\beta6^+$ complementarity determining region 3 were conserved in one of three patients. There is, however, no disposition of Vβ gene usage in PB from patients with sarcoidosis compared with control subjects.
Conclusions: The T lymphocytes with Vβ2 and/or Vβ6 are associated with the pathogenesis of sarcoidosis. The possibility exists that these T lymphocytes might be capable of recognizing the restricted antigens, thereby inducing oligoclonal expansion.

174. Insulin Infusion Improves Neutrophil Function in Diabetic Cardiac Surgery Patients
Rassias, A.J. et al
Anesthesia & Anagesia, 88(5), 1011-1016 (1999)

Diabetic patients are at increased risk of wound infection after major surgery, but the effect of perioperative glucose control on postoperative wound infection rates after surgery is uncertain.We tested the effect of an insulin infusion on perioperative neutrophil function in diabetic patients scheduled for coronary artery bypass surgery. Participants (n = 26) were randomly allocated to receive either aggressive insulin therapy (AIT) or standard insulin therapy (SIT) during surgery. Blood was drawn for neutrophil testing before surgery, 1 h after the completion of cardiopulmonary bypass, and on the first postoperative day. Neutrophil phagocytic activity decreased to 75% of baseline activity in the AIT group and to 47% of baseline activity in the SIT group ($P < 0.05$ between groups). No important differences in neutrophil antibody-dependent cell cytotoxicity were found. This study documents a potentially beneficial effect of continuous insulin therapy in diabetic patients who require major surgery. Implications: A continuous insulin infusion and glucose control during surgery improves white cell function in diabetic patients and may increase resistance to infection after surgery.

175. **CR3, FcγRIIA and FcγRIIIB induce activation of the respiratory burst in human neutrophils: the role of intracellular Ca2+, phospholipase D and tyrosine phosphorylation**
Löfgren, R., Serrander, L., Forsberg, M., Wilsson, Å. and Stendahl, O.
Biochim. Biophys. Acta, 1452(1), 46-59 (1999)

Human neutrophils express two different types of phagocytic receptors, complement receptors (CR) and Fc receptors. In order to characterize the different signaling properties of each receptor we have used non-adherent human neutrophils and investigated CR3, FcγRIIA and FcγRIIIB for their signaling capacity. Selective activation of each receptor was achieved by coupling specific antibodies to heat-killed *Staphylococcus aureus* particles, Pansorbins, through their Fc moiety. Despite the fact that these particles are not phagocytosed, we show that addition of Pansorbins with anti-CD18 antibodies recognizing CR3 induced prominent signals leading to a respiratory burst. Stimulation with anti-FcγRIIIB Pansorbins induced about half of the response induced by anti-CR3 Pansorbins, whereas anti-FcγRIIA Pansorbins induced an even weaker signal. However, FcγRIIA induced strong phosphorylation of $p72^{syk}$ whereas FcγRIIIB induced only a very weak $p72^{syk}$ phosphorylation. During CR3 stimulation no tyrosine phosphorylation of $p72^{syk}$ was seen. Both phospholipase D and NADPH oxidase activities were dependent on intracellular calcium. This is in contrast to tyrosine phosphorylation of $p72^{syk}$ that occurred even in calcium-depleted cells, indicating that oxygen metabolism does not affect $p72^{syk}$ phosphorylation. Inhibitors of tyrosine phosphorylation blocked the respiratory burst induced by both FcγRIIA and FcγRIIIB as well as CR3. This shows that tyrosine phosphorylation of $p72^{syk}$ is an early signal in the cascade induced by FcγRIIA but not by CR3.

176. Doxazosin treatment and peroxidation of low-density lipoprotein among male hypertensive subjects: in vitro and ex vivo studies
Brude, I.R. et al
Biochem. Pharmacol., 58(1), 183-191 (1999)

Doxazosin is an antihypertensive drug that gives rise to 6- and 7-hydroxydoxazosin during hepatic metabolism. The structures of the hydroxymetabolites suggest that they may possess antioxidative properties. The aim of the present study was to examine whether doxazosin and 6- and 7-hydroxydoxazosin were able to scavenge free radicals and whether these compounds might protect low-density lipoprotein (LDL) against *in vitro* and *ex vivo* oxidation. Both 6- and 7-hydroxydoxazosin showed radical scavenging capacity as assessed by measuring scavenging of 1,1-diphenyl-2-picrylhydrazyl radicals. *In vitro* incubation with 10 μM 6- and 7-hydroxydoxazosin significantly reduced human mononuclear cell-mediated oxidation of LDL, measured as the formation of lipid peroxides and the relative electrophoretic mobility of LDL (to 10 and 6% of the control, respectively). Furthermore, formation of conjugated dienes in LDL during Cu^{2+}-induced oxidation was significantly reduced in the presence of 5 μM 6- and 7-hydroxydoxazosin (to 28% of t_{max} [time to maximum] of control). However, treatment of hypertensive patients with increasing doses of doxazosin (from 1 to 8 mg/day) for 8 weeks altered neither Cu^{2+}-catalyzed, 2,2′azobis-(2-amidinopropane hydrochloride)-initiated, nor cell-mediated oxidation of patient LDL *ex vivo*. Furthermore, the total antioxidative capacity of plasma was unaffected by treatment. In conclusion, the present study shows that 6- and 7-hydroxydoxazosin have radical scavenging properties and protect LDL against *in vitro* oxidation. However, treatment of hypertensive male subjects with increasing doses of doxazosin for 8 weeks did not affect *ex vivo* oxidation of LDL.

177. Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis
McCall, A.M. et al
Mol. Immunol., 36(7), 433-446 (1999)

Bispecific antibody (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal antibody (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of antibody Fc domains with cellular Fc receptors has limited the doses of antibody (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the

HER2/*neu* protooncogene product and the human FcγRIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from single-chain Fv (scFv) molecules specific for HER2/*neu* and CD16. The human anti-CD16 scFv component, NM3E2, was isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fc binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/*neu* scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as fusion partner for the creation of a bispecific scFv (bs-scFv). In the presence of the C6.5×NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian cancer cells overexpressing HER2/*neu*. Biodistribution studies performed in SK-OV-3 tumor-bearing *scid* mice revealed that 1% ID/g of ^{125}I-labeled C6.5×NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the fusion protein. This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5×NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins with potential clinical applications as a result of its human origin, lack of an Fc domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting.

178. Matrix metalloproteinases in liver and serum in chronic active hepatitis C and HCV-induced cirrhosis
Lichtinghagen, R. et al
Hepatol. Res., 14(2), 119-134 (1999)

To study the value of matrix metalloproteinases as indicators of hepatic fibroproliferation, expression of MMP-1, MMP-2, MMP-7 and MMP-9 was studied in healthy controls, patients with chronic hepatitis C (CAH) and in HCV-induced cirrhosis. Western blot revealed an increase for MMP-1 in CAH with histologically detectable fibrosis and diminished amounts in cirrhosis. MMP-2 was unchanged in CAH and cirrhosis, while there was an increase of MMP-7 in 8 of 18 cirrhotic liver samples. Northern analysis demonstrated in cirrhosis an increase of MMP-7 mRNA more strongly than MMP-2 mRNA. ELISA studies demonstrated a trend towards an increase in circulating MMP-2 levels and a reduction in circulating MMP-9 levels with advancing fibrosis. RT-PCR demonstrated transcripts for MMP-1, MMP-2 and MMP-7 in hepatic tissue and isolated stellate cells. MMP-9 transcripts were only found in white blood cells. Hepatic expression of MMP-1, MMP-2 and MMP-7 is increased in chronic hepatitis C, but the resulting proenzyme levels are not sufficiently different to allow discrimination between patients with and without fibroproliferation. MMP-9 shows significant changes in serum, with a progressive reduction from controls to cirrhosis, but is most likely derived from extrahepatic sources.

179. Detection of cytomegalovirus proteins by flow cytometry in the blood of patients undergoing hematopoietic stem cell transplantation
Detrick, B., Hooks, J.J., Keiser, J. and Tabbara, I.
Exp. Hematol., 2783), 569-575 (1999)

Cytomegalovirus (CMV) infection and associated diseases continue to be a major complication encountered by patients undergoing high-dose chemoradiotherapy and hematopoietic stem cell transplantation (HSCT). A number of studies revealed that identification of CMV in the blood of HSCT patients was a predictor of future CMV disease. The purpose of this study was to determine if CMV proteins detected by flow cytometry could be a rapid and more quantitative way to monitor CMV infections and CMV antigenemia in HSCT patients. Preliminary studies showed that CMV immediate early (IE), early (E), and late (L) tegument proteins were specifically identified in CMV-infected cell lines and not in uninfected cells. We evaluated CMV antigen detection by flow cytometry in blood samples collected before and after transplantation in 56 serially collected blood samples from 17 HSCT patients and CMV protein expression was compared to CMV isolation. CMV IE and E proteins were not detected in any of the samples analyzed. However, CMV L protein detection by flow cytometry correlated with virus isolation in serially collected blood samples. Samples from 14 patients were evaluated by both techniques, at the same time intervals. There was a 100% correlation (8/8) between the lack of CMV antigen detection by flow cytometry and the failure to isolate infectious virus. Moreover, 5 of 6 patients who were positive for CMV L antigen by flow cytometry also were positive by virus isolation techniques. When flow cytometry and virus isolation did not detect CMV antigen on the same day, CMV positivity was first detected by flow cytometry. Then, 1–2 weeks later, positive virus isolation was documented. This study indicates that flow cytometric identification of CMV antigenemia correlates with isolation of CMV in HSCT patients and may be a predictive test for the rapid detection of CMV in the blood.

180. Increased interleukin-8 (IL-8) in rectal dialysate from patients with ulcerative colitis: evidence for a biological role for IL-8 in inflammation of the colon
Keshavarzian, A. et al
Am. J. Gastrolenterol., 94(3), 704-712 (1999)

OBJECTIVE:
Infiltration of neutrophils and their release of toxic reactive oxygen species (ROS) in the colonic mucosa are associated with tissue damage in ulcerative colitis (UC). This neutrophil migration may be induced by chemoattractants, such as cytokines, in the colonic milieu. One such chemoattractant is interleukin-8 (IL-8), a neutrophil chemokine that is present at high concentrations in inflamed

mucosa. However, the functional significance of IL-8 in neutrophil attraction and activation in UC has not been established. We hypothesized that IL-8 in the colonic lumen of patients with UC primes neutrophils, leading to their attraction and activation.
METHODS:
The colonic milieu was sampled by rectal dialysis. Using a semi-permeable membrane with a molecular weight cut-off of 12 kDa, dialysis solution was placed in the rectum and allowed to equilibrate over a 4-h period with the colonic milieu of controls or of patients with UC. IL-8 concentrations were measured by ELISA. Two functions of healthy neutrophils (PMN) were measured: expression of CD11-b surface adhesion molecules (by flow cytometry), and production of ROS (by both chemiluminescence and cytochrome C reduction assays). Neutrophil functions after exposure to rectal dialysates or n-formyl-methionyl-leucyl-phenylalanine (fMLP) were assessed before and after adding anti–IL-8 antibody or the fMLP blocker BMLP.
RESULTS:
IL-8 concentrations in dialysates from patients with active UC were significantly higher than in controls and correlated with disease activity. UC dialysates significantly increased ROS production and CD11-b expression by neutrophils and anti–IL-8 antibody partially (50%) inhibited these stimulatory effects of UC dialysates. Preincubation of neutrophils with UC dialysates significantly potentiated the fMLP-induced rise in ROS and anti–IL-8 antibody completely abolished this priming effect.
CONCLUSIONS:
The colonic milieu, sampled by rectal dialysis, from patients with active UC can both activate and prime neutrophils *in vitro*. High concentrations of IL-8 in the colonic lumen of UC patients are partially responsible for the activating effects of rectal dialysates, and account for all of its priming effects. These findings provide direct evidence for a role for IL-8 in inflammatory bowel disease.

181. HIV-1 tat protein induces the production of interleukin-8 by human brain-derived endothelial cells
Hofman, F.M., Chen, P., Incardona, F., Zidovetski, R. and Hinton, D.R.
J. Neuroimmunol., 94(1-2), 28-39 (1999)

This study focused on the role of the HIV-derived viral protein, tat, in activating central nervous system (CNS)-derived endothelial cells (EC) to produce interleukin-8 (IL-8), a stimulator and chemoattractant for neutrophils and lymphocytes. Human CNS-EC treated with tat (100 ng/ml) demonstrated a 2 to 3 fold upregulation in IL-8 mRNA and protein. Tumor necrosis factor-α (TNF) and tat were found to act additively in upregulating IL-8 production. In contrast, transforming growth factor β (TGF β), appeared to down modulate tat-induced IL-8 production. These data suggest that extracellular tat, especially in the presence of TNF, may be responsible for the local production of IL-8.

182. The Glucose Concentration Modulates *N*-Formyl-Methionyl-Leucyl-Phenylalanine (fMet-Leu-Phe)-Stimulated Chemokinesis in Normal Human Neutrophils
Oldenborg, P-A. and Sehlin, J.
Bioscience Reports, 19(6), 511-523 (1999)

The effects of glucose concentration on the chemokinetic effects of the chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe) was evaluated for normal human neutrophils using a direct microscopic assay. fMet-Leu-Phe increased the rate of locomotion in the absence of glucose, but the chemokinetic effect of fMet-Leu-Phe was most potent at 5mM glucose and not further changed at 15 mM glucose. The chemokinetic effects of fMet-Leu-Phe and glucose were essentially the same in blood clot-isolated and gradient-isolated neutrophils. However, in gradient-isolated neutrophils, the rate of locomotion under different experimental conditions was strictly negatively correlated to the fraction of non-locomoting cells and the degree of adhesion to the substratum. These results indicate that the chemokinetic effects of fMet-Leu-Phe are regulated by the glucose concentration by inducing locomotor activity in otherwise non-locomoting cells and by improving adhesion to the substratum.

183. Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-kB
Hofman, M.A. et al
Diabetologia, 42(2), 222-232 (1999)

Summary Increased oxidative stress and subsequent activation of the transcription factor NF-kB has been linked to the development of late diabetic complications. To determine whether oxidative stress dependent NF-kB activation is evident in patients with diabetic nephropathy we used an Electrophoretic Mobility Shift Assay based semiquantitative detection system which enabled us to determine NF-kB activation in ex vivo isolated peripheral blood mononuclear cells. We examined 33 patients with diabetes mellitus (Type I and Type II). Patients with diabetic nephropathy showed higher NF-kB binding activity in Electrophoretic Mobility Shift Assays and stronger immunohistological staining for activated NF-kBp65 than patients without renal complications. NF-kB binding activity correlated with the degree of albuminuria (r = 0.316) and with thrombomodulin plasma concentrations (r = 0.33), indicative for albuminuria associated endothelial dysfunction. In a 3 day intervention study in which 600 mg of the antioxidant thioctic acid (f-lipoic acid) per day were given to nine patients with diabetic nephropathy oxidative stress in plasma samples was decreased by 48 % and NF-kB binding activity in ex vivo isolated peripheral blood mononuclear cells by 38 %.

In conclusion, activation of the transcription factor NF-kB in ex vivo isolated peripheral blood mononuclear cells of patients with diabetes mellitus correlates with the degree of diabetic nephropathy. NF-kB activation is at least in part dependent on oxidative stress since thioctic acid (f-lipoic acid) reduced NF-kB binding activity.

184. Superoxide Anion Mediates Pulmonary Vascular Permeability Caused by Neutrophils in Cardiopulmonary Bypass
Tanita, T. et al
Surgery Today, 29(8), 755-761 (1999)

During cardiopulmonary bypass (CPB), neutrophils (PMNs) may be stimulated by shear stress which could contribute to the pulmonary injury that occurs after CPB. To elucidate whether mechanically stimulated PMNs increase pulmonary vascular permeability, measured as the pulmonary filtration coefficient (K) and pulmonary vascular resistance, and to elucidate whether superoxide anion mediates this increase, we assessed the effects of stimulated and unstimulated PMNs, and of superoxide dismutase (SOD) on K and resistance in isolated perfused lungs from Sprague-Dawley rats. PMNs were stimulated by gentle agitation in a glass vial for 10 s. Lungs perfused with the stimulated PMNs, being the stimulated group ($n = 6$), elicited a 5-fold increase in the filtration coefficient compared with lungs perfused with unstimulated cells, being the unstimulated group ($n = 6$). This increase in filtration was completely blocked by the pre-incubation of stimulated PMNs with CD18 monoclonal antibody, being the Ab group ($n = 6$), and also by superoxide dismutase, being the SOD group ($n = 6$). Pulmonary vascular resistance was not increased by stimulated PMNs, and the accumulation of stimulated PMNs was not blocked by SOD. These findings suggest that stimulated PMNs increase K and that superoxide anion may injure the pulmonary vascular endothelial cells.

185. Expression of heat shock protein 70 mRNA in polymorphonuclear cells responding to surgical stress
Ohse, K., Yashiki, S., Fujiyoshi, T., Sonoda, S. and Yoshimura, N.
J. Anesthesia, 13(3), 144-149 (1999)

This study was performed to investigate the expression of heat shock protein (HSP) 70 mRNA in polymorphonuclear neustrophils (PMN) as a possible new biomarker for surgical stress. *Methods.* The HSP70 mRNA in PMN of 10 patients who underwent lobectomy was evaluated by Northern blot analysis. Their leukocyte counts, including white blood cells (WBC) and PMN, plasma cortisol levels, and plasma interleukin-6 (IL-6) levels, were obtained by cell counting, radioimmunoassay, and enzyme-linked immunosorbent assay, respectively. *Results.* The level of HSP70 mRNA in PMN slightly increased at the end of surgery and showed a significant increase 6 h after surgery. It promptly decreased at 24 h postoperatively and returned to the basal preanesthetic level 48 h after surgery. On the other hand, WBC/PMN counts, plasma cortisol, and IL-6 significantly increased at the end of surgery. WBC/PMN counts remained at increased levels until 48 h postoperatively. Cortisol peaked at 6 h postoperatively and gradually decreased. IL-6 reached a maximum at 1 h postoperatively, then tapered down to its basal level at 48 h postoperatively. *Conclusion.* Expression of HSP70 mRNA in PMN that is induced after thoracic surgery appears to be a promising candidate as a marker for evaluating surgical stress.

186. Pharmacological considerations in the emergence of resistance
Amsden, G.W.
Int. J. Antimicrob. Agents,11 Suppl. 1, S7-S14 (1999)

Resistance to macrolides in vitro is increasingly being reported. However, there has been no corresponding increase in clinical failures noted. Lack of clinical failures due to resistance is most likely the result of the high intracellular concentrations that these drugs achieve in phagocytes. In the case of clarithromycin, concentrations in both monocytes and granulocytes fluctuate between peaks of ≈22–25 mg/l and troughs of ≈5 mg/l during a standard dosing interval. In contrast, azithromycin attains concentrations of over 60 mg/l in granulocytes and at least 100 mg/l in monocytes. After 7 days, azithromycin concentrations of >32 mg/l are still observed. These data also imply that against pathogens with increasing minimum inhibitory concentrations (MICs), macrolides with relatively lower or less sustained intracellular concentrations will become ineffective clinically much sooner than compounds, such as azithromycin, that concentrate to a high degree and are retained in white blood cells for prolonged periods.

187. Heparin Inhibits Reactive Oxygen Species Generation by Polymorphonuclear and Mononuclear Leucocytes
Dandona, P. et al
Thromb. Res., 96(6), 437-443 (1999)

To examine the hypothesis that heparin may affect leukocyte function and that it may have anti-inflammatory properties, we investigated the effect of heparin on reactive oxygen species (ROS) generation by leucocytes. Heparin was injected intravenously at a dose of 10000 units into eight normal subjects. Blood samples were collected from the antecubital vein sequentially, prior to and following heparin at 0, 0.5, 1, 2, and 4 hours. ROS generation was inhibited significantly by polymorphonuclear cells (PMNL) at 0.5, 1, and 2 hours and returned to baseline level at 4 hours. Similarly, ROS generation was inhibited markedly by mononuclear cells (MNC) at 0.5 hours, with a peak inhibition at 1 hour; it returned to baseline level by 4 hours. The maximum inhibition of ROS generation by PMNL was 57.3±19% of the basal, while that by MNC was 56.4±11% of the basal. Since ROS are proinflammatory and cause tissue damage, it is possible that heparin may have an anti-inflammatory effect in vivo, apart from its antithrombotic effect. Since ROS also

bind to nitric oxide (NO) and reduce the bioavailability of NO, heparin may indirectly increase the bioavailability of NO and thus act as a vasodilator. This effect of heparin may be of particular relevance to its use in unstable angina and following thrombolysis in acute myocardial infarction in preventing reperfusion injury.

188. Mononuclear and Polymorphonuclear Leukocyte Dispositions of Clarithromycin and Azithromycin in AIDS Patients Requiring Mycobacterium avium Complex Prophylaxis
Bui, K.Q., McNabb, JC., Li, C., Nightingale, C.H. and Nicolau, D.P.
Antimicrob. Agents Chemother., 43(9), 2302-2304 (1999)

The intracellular dispositions of clarithromycin and azithromycin in AIDS patients requiring *Mycobacterium avium* complex (MAC) prophylaxis were studied. The dispositions of both drugs in mononuclear and polymorphonuclear leukocytes were markedly different. Our data support the proven efficacy of these agents for MAC prophylaxis since clarithromycin and azithromycin displayed sustained intracellular concentrations which exceeded their MICs for MAC throughout the dosing periods.

189. Identification of oligopeptide sequences which inhibit migration induced by a wide range of chemokines
Reckless, J. and Grainger, D.J.
*Biochem. J., **340**, 803-811 (1999)*

We have identified an amino acid sequence, termed peptide 3, corresponding to amino acids 51–62 of the mature human monocyte chemoattractant protein-1 (MCP-1), which inhibits human mononuclear-cell and THP-1-cell migration induced by a wide range of chemokines. For example, peptide 3 inhibited MCP-1-induced THP-1 migration in a transwell assay with an ED_{50} of approx. 8 μM. Peptide 3 binds directly to THP-1 cells with an association constant of approx. 10 μM, and is therefore likely to be a direct receptor antagonist for CC and CXC chemokine receptors. By performing a structure–function analysis of this peptide, we have identified a sequence variant that shows an approx. 3–4-fold greater potency as an inhibitor of chemokine-induced migration [Leu_4Ile_{11} peptide 3 (1–12)]. Furthermore, unlike peptide 3, which binds to the Duffy antigen receptor for chemokines on human erythrocytes with a similar affinity to the specific chemokine receptors on THP-1 cells, the Leu_4Ile_{11} peptide 3 (1–12) sequence variant shows at least 20-fold greater selectivity for the specific receptors. Derivatives of Leu_4Ile_{11} peptide 3 (1–12) are therefore the best candidates among the molecules we have investigated for use as a chemokine inhibitor *in vivo*.

190. Biased T cell receptor Vβ gene expression in bronchoalveolar lavage fluid from Japanese patients with sarcoidosis
Yoshitomi, A. et al
*Respirology, **4(4)**, 339-347 (1999)*

Objective:
Sarcoidosis is believed to be one of the T cell-mediated granulomatous diseases with unknown aetiology. We attempt to search for the causative T cell clones of sarcoidosis.
Methods: We study T cell receptor β-chain variable region (Vβ) repertoire in peripheral blood (PB) and bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis, using semi-quantitative reverse transcriptase–polymerase chain reaction method. The expression of 22 kinds of Vβ genes is examined in 17 patients with sarcoidosis and nine normal subjects.
Results: Compared with control subjects, the group with sarcoidosis exhibits significantly high expressions of the Vβ2 ($P< 0.005$, Wilcoxon's test) and Vβ6 ($P = 0.005$) genes in BALF. In each BALF sample, the Vβ2 ($P< 0.01$, χ^2 test) and Vβ6 ($P< 0.01$) genes were overexpressed (> 2 SD above the mean value for each Vβ observed in control subjects) in 11 and 10 of 17 patients with sarcoidosis, respectively. Furthermore, the amino acid sequences of $V\beta6^+$ complementarity determining region 3 were conserved in one of three patients. There is, however, no disposition of Vβ gene usage in PB from patients with sarcoidosis compared with control subjects.
Conclusions: The T lymphocytes with Vβ2 and/or Vβ6 are associated with the pathogenesis of sarcoidosis. The possibility exists that these T lymphocytes might be capable of recognizing the restricted antigens, thereby inducing oligoclonal expansion.

191. Ethanol Inhibits Lung Clearance of Pseudomonas aeruginosa by a Neutrophil and Nitric Oxide-Dependent Mechanism, In Vivo
Greenberg, S.S. et al
*Alcoholism: Clin. Exp. Res., **23(4)**, 735-744 (1999)*

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic pathogen that can be found in individuals in which the immune system has been suppressed by HIV/AIDS or chronic alcoholism. We evaluated the role of inducible nitric oxide synthase (NOSII) as a modulator of lung concentrations of P. aeruginosa in normal rats and rats given a single dose of ethanol (ETOH). Rats were pretreated with either sterile saline (PBS, 0.1 ml/kg, iv) or the NOS II inhibitor L-N^6-iminoethyl lysine (LNIL, 10 mg/kg, iv) 15 min before intraperitoneal administration of either PBS (4.5 ml/kg) or ETOH (4.5 g/kg). Thirty min after administration of PBS or ETOH the rats were placed in inhalation chambers and exposed to 45 min of an aerosol containing P. aeruginosa (5×10^4 colony forming units, CFU). A group of rats (n = 5-6/treatment/time period) were killed immediately (0 hr) or 4 hr after inhalation of P. aeruginosa. The lungs were homogenized and the *P. aeruginosa* were grown in nutrient broth to determine the number of viable CFU remaining in the lung. The NOS II and TNFα mRNA and protein content lung alveolar macrophages (AM) and neutrophils (PMN) were measured with RT-PCR and Western

blot. The concentration of nitrate and nitrite anion in the bronchoalveolar lavage fluid (BALf) and ex vivo incubates of PMN were also measured. The CFU of *P. aeruginosa* present in the lungs of the four groups of rats at 0 hr did not differ. The CFU of *P. aeruginosa* in the lung increased ($p < 0.05$) in rats pretreated with ETOH when compared with that obtained from rats pretreated with PBS. However, pretreatment of rats with LNIL decreased ($p<0.05$) the 4 hr lung content of *P. aeruginosa.* Coadministration of LNIL and ETOH to rats augmented the CFU of *P. aeruginosa* in lungs to amounts which did not differ from that of rats pretreated with ETOH. Inhalation of *P. aeruginosa* increased NOS II mRNA and protein in rat AM and PMN. Pretreatment of rats with ETOH alone, or in combination with LNIL, inhibited *P. aeruginosa*-induced NOS II transcription and translation and AM and PMN nitrate and nitrite generation whereas pretreatment with LNIL alone only inhibited nitrate and nitrite generation. Pretreatment of rats with ETOH suppressed *P. aeruginosa* stimulated PMN recruitment into the lung whereas LNIL enhanced ($p<0.05$) *P. aeruginosa*-stimulated PMN recruitment into the lung. ETOH-induced increases of the lung content of *P. aeruginosa* were associated with increased PKC delta isozyme in the membrane of the PMN but could not be explained by altered plasma concentrations of hydrocortisone or ETOH. The data demonstrate that selective inhibition of NOS II-derived NO by LNIL decreases the lung content of *P. aeruginosa* whereas ETOH inhibits the lung clearance of *P. aeruginosa.* Speculatively, the difference between these effects of LNIL and ETOH may result from differences in drug-induced changes in lung recruitment of PMN.

192. The effect of melatonin on cellular activation processes in human blood

Fjærli, O., Lund, T. And Østerud, B.

*J. Pineal Res., **26(1)**, 50-55 (1999)*

The pineal hormone melatonin, due to its lipophilic nature, has access to every cell and every part of a cell in the body, suggesting that it could exert effects on blood immune cells. The regulation of the activation of monocytes may be important in a number of diseases, especially pathophysiological conditions associated with inflammatory reactions. Considering this, a study on the effect of melatonin on monocytes in whole blood was carried out. Melatonin added at a final concentration of 5 ng/mL to whole blood in vitro reduced lipopolysaccharide (LPS)-induced tissue factor (TF) activity in monocytes by 55% in blood from a group of subjects with melatonin-sensitive cells. At even lower concentrations of melatonin (20-50 pg/mL) and in the physiological range, a trend of suppressed LPS-induced TF activity by ~20% was seen. A further indication of a downregulation of LPS-stimulated monocytes by melatonin was shown by its reduction of LPS-induced tumor necrosis factor (TNF). Twenty to one hundred pg/mL melatonin caused a significant reduction of LPS-induced TNF production by ~25–30%. In contrast, melatonin at a final concentration of 10 pg/mL, added to whole blood incubated with LPS and also the phorbol ester, PMA, caused a signficant rise of 25%; whereas 100 pg/mL enhanced LPS + PMA-induced TNF by ~80% as compared to LPS + PMA alone. These effects were not detectable during the winter darkness of Tromsø (70°N), probably due to the high content of melatonin in the blood even at daytime. These results show that melatonin may have a beneficial effect by suppressing the expression of TF activity in LPS-stimulated monocytes. Furthermore, the results indicate that LPS-induced TF in monocytes of whole blood is independent of protein kinase C (PKC) activation. Melatonin is probably amplifying cellular activation reactions that are PKC-dependent. This may be physiologically important in upregulation of the immune system.

193. Human endothelial cells cultured on microporous filters used for leukocyte transmigration studies form monolayers on both sides of the filter

Mackarel, A.J., Cottell, D.C., Fitzgerald, M.X. and O'Connor, C.M.

*In Vitro Cell. Development. Biol. – Animal, **35(6)**, 346-351 (1999)*

A growing number of studies on the mechanism of leukocyte transendothelial migration use endothelial cells grown on microporous filters as an in vitro model of endothelium. Ultrastructural examination of such a model system previously demonstrated that human pulmonary artery endothelial cells (HPAEC) formed confluent monolayers on both sides of the 3-µm-pore filter (Mackarel et al., 1999). To determine whether this was a characteristic specific to pulmonary artery endothelial cells, the growth characteristics of a human pulmonary microvascular endothelial cell type (HMVEC-L) and the widely used human umbilical vein endothelial cells (HUVEC) on 3-µm microporous filters were examined by transmission electron microscopy (TEM). Similar to HPAEC, HMVEC-L and HUVEC were also found to grow on both sides of the filter. All three endothelial cell types were capable of migrating through the 3 µm pores of the filter to form a monolayer on the filter underside. The endothelial cells on the underside were orientated in an inverted position with the luminal surface facing away from the filter. Such 'bilayer' formation was observed at a range of seeding densities and in different culture media. Despite the presence of a bilayer of endothelial cells, TEM demonstrated that neutrophils migrated successfully across the cell-filter-cell system. Previous transmigration reports in which an in vitro model similar to ours was used have often assumed only one layer of endothelial cells. The observations reported here indicate that while endothelial cells on microporous filters are useful models for examining leukocyte-endothelial interactions, they are not appropriate for studies examining endothelial cell 'sidedness.'

194. Convergence of Fcγ receptor IIA and Fcγ receptor IIIB signalling pathways in human neutrophils

Chuang, F.Y.S., Sassaroli, M. and Unkeless, J.C.

J. Immunol., 164, 350 (2000)

Human neutrophils (PMNs) express two receptors for the Fc domain of IgG: the transmembrane FcγRIIA, whose cytosolic sequence contains an immunoreceptor tyrosine-based activation motif, and the GPI-anchored FcγRIIIB. Cross-linking of FcγRIIIB induces cell activation, but the mechanism is still uncertain. We have used mAbs to cross-link selectively each of the two receptors and to assess their signaling phenotypes and functional relation. Cross-linking of FcγRIIIB induces intracellular Ca^{2+} release and receptor capping. The Ca^{2+} response is blocked by wortmannin and by *N*,*N*-dimethylsphingosine, inhibitors of phosphatidylinositol 3-kinase and sphingosine kinase, respectively. Identical dose-response curves are obtained for the Ca^{2+} release stimulated by cross-linking FcγRIIA, implicating these two enzymes in a common signaling pathway. Wortmannin also inhibits capping of both receptors, but not receptor endocytosis. Fluorescence microscopy in double-labeled PMNs demonstrates that FcγRIIA colocalizes with cross-linked FcγRIIIB. The signaling phenotypes of the two receptors diverge only under frustrated phagocytosis conditions, where FcγRIIIB bound to substrate-immobilized Ab does not elicit cell spreading. We propose that FcγRIIIB signaling is conducted by molecules of FcγRIIA that are recruited to protein/lipid domains induced by clustered FcγRIIIB and, thus, are brought into juxtaposition for immunoreceptor tyrosine-based activation motif phosphorylation and activation of PMNs.

195. Ketamine modulates the stimulated adhesion molecule expression on human neutrophils in vitro
Weigand, M.A. et al
Anesth. Analg., 90, 206 (2000)

Cytokine production, neutrophil adhesion to endothelial cells, and release of reactive oxygen species are thought to be critical events in sepsis or ischemia/reperfusion. Modulation of leukocyte responses by anesthetics may have an important role in limiting tissue injury under these conditions. Therefore, we investigated the effect of ketamine on the expression of CD18, CD62L, and oxygen radical production of human neutrophils *in vitro* and on interleukin-6 production in endotoxin-stimulated human whole blood. Ketamine inhibited both the *N*- formyl-methionyl-leucyl-phenylalanine- and phorbol 12-myristate 13-acetate-induced up-regulation of CD18 and shedding of CD62L, determined by flow cytometry, in a concentration-dependent manner. Ketamine also caused a significant suppression of oxygen radical generation of isolated human neutrophils. In addition, there was a significant decrease in endotoxin-stimulated interleukin-6 production in human whole blood. The inhibitory effects were similar for racemic ketamine and its isomers S(+)-ketamine and R(-)-ketamine, suggesting that the inhibition of stimulated neutrophil function is most likely not mediated through specific receptor interactions.
Implications: Modulation of leukocyte responses by anesthetics may have an important role in limiting tissue injury in sepsis or ischemia/reperfusion. Therefore, we examined the effect of ketamine on stimulated neutrophil functions *in vitro.* These neutrophil functions were significantly inhibited by ketamine, independent of whether the racemic mixture or isomers were tested.

196. Activation of human neutrophils by *Mycobacterium tuberculosis* H37Ra involves phospholipase Cγ2, Shc adapter protein, and p38 mitogen-activated protein kinase
Persqvist, N., Zheng, L. and Stendahl, O.
J. Immunol., 164, 959 (2000)

Recent studies have shown that human neutrophils play a significant protective role in mycobacteria infection. When encountered with mycobacteria, neutrophils exhibit the typical early bactericidal responses including phagocytosis and generation of reactive oxygen intermediates (ROI), but the underlying mechanisms are largely unknown. The present study shows that stimulation of neutrophils with an attenuated strain of *Mycobacterium tuberculosis* H37Ra (Mtb) led to a tyrosine kinase-dependent ROI production in these cells. Stimulation with Mtb induces a rapid and transient tyrosine phosphorylation of several proteins, one of which was identified as phospholipase Cγ2 (PLCγ2). Several tyrosine-phosphorylated proteins were associated with the PLCγ2 precipitates from Mtb-stimulated neutrophils, of which pp46 was characterized as the Shc adapter protein. A role for PLCγ2-Shc association in the generation of ROI is supported by the observations that stimulation with Mtb causes the activation of p38 mitogen-activated protein kinase (MAPK), a downstream target of the Shc/Ras signaling cascade, and that the effect of genistein on ROI production coincided with its ability to inhibit both PLCγ2-Shc association and p38 MAPK activation. Moreover, pretreatment of neutrophils with a PLC inhibitor markedly suppresses the Mtb-stimulated ROI production as well as p38 MAPK activation in these cells. Taken together, these results indicate that stimulation of neutrophils with Mtb triggers the tyrosine phosphorylation of PLCγ2 and its association with Shc, and that such association is critical for the Mtb-stimulated ROI production through activating p38 MAPK.

197. Expression of matrix metalloproteinase-2 and –9 and their inhibitors in peripheral blood cells of patients with chronic hepatitis C
Lichtinghagen, R. et al
Clin. Chem., 46, 183-192 (2000)

Background: To clarify whether circulating matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) can be used as serum markers of fibroproliferation in chronic liver diseases, we studied the expression of MMP-2 and MMP-9 in relation to TIMP-1 and TIMP-2 in peripheral blood mononuclear leukocytes (MNLs) and polymorphonuclear leukocytes (PMLs), and compared this expression to circulating concentrations and hepatic histology in patients with chronic active hepatitis C (CAH).

Methods: Quantitative reverse transcription-PCR/ELISA assays were performed for MMP and TIMP RNA, and corresponding circulating protein concentrations were studied by ELISA in 20 healthy controls, 40 patients with CAH, and 20 patients with hepatitis C-induced cirrhosis (Ci).
Results: MMP-2 mRNA was found almost exclusively in the liver, MMP-9 mRNA in leukocytes. TIMP RNA-equivalents were decreased in MNLs of CAH patients, but neither MMP-9 nor TIMP RNA expression showed any correlation to the extent of inflammation and fibrosis. MMP-2 and TIMP-1 protein concentrations were increased in Ci patients and showed a wide overlap in CAH patients and healthy controls. MMP-9 values were lower in CAH and Ci patients than in healthy controls. TIMP-2 values showed a wide overlap in all three groups. The MMP-2/TIMP-1 and MMP-9/TIMP-1 ratios were lower in Ci patients than in healthy controls; the MMP-2/TIMP-2 and MMP-9/TIMP-2 ratios were not different. Circulating TIMP-1 and the MMP-2/TIMP-1 ratio correlated to the inflammatory activity in liver biopsies, but only the circulating MMP-2/TIMP-1 ratio also correlated with the degree of fibrosis.
Conclusions: Peripheral blood cell expression of MMP-2, MMP-9, and TIMP revealed no correlation with the circulating concentrations of these proteins. Only the circulating MMP-2/TIMP-1 ratio correlated to the histological degree of fibrosis in hepatitis C and should be further evaluated as a progression marker in patients with chronic liver disease.

198. Superoxide possibly produced in endothelial cells mediates the neutrophil-induced lung injury
Tanita, T. et al
Ann. Thorac. Surg., 69, 402 (2000)

Background. The mechanism by which stimulated neutrophils (polymorphonuclear leukocytes [PMNs]) damage pulmonary vascular endothelium was investigated.
Methods. The ability of unstimulated and mechanically stimulated PMNs to adhere to pulmonary endothelial cells and, thereby, alter pulmonary vascular permeability was tested. Each series was conducted on 6 rats. To stimulate PMNs, they were agitated gently in a glass vial for 10 seconds.
Results. Perfusing lungs with the stimulated PMNs elicited a fivefold increase in permeability compared with lungs perfused with the unstimulated cells. This increase in permeability was blocked completely by preincubation of stimulated PMNs with CD18 monoclonal antibody. This increase in permeability was also blocked completely by superoxide dismutase (SOD) or the xanthine oxidase (XO) inhibitor allopurinol. Pulmonary vascular hemodynamics were unaffected by any treatment protocol. The accumulation of stimulated PMNs within the lungs was not inhibited by SOD but was partially blocked by allopurinol.
Conclusions. These findings suggest that stimulated PMN-induced increases in pulmonary vascular filtration resulted from endothelial cell injury caused by superoxide anion possibly generated by XO, exclusively present in the endothelial cells.

199. Comparison of polymorphonuclear cells from donors and differentiated HL-60 cells as phagocytes in an opsonophagocytic assay using antigen-coated fluorescent beads
Guy, B. et al
Clin. Diagn. Lab. Immunol., 7, 314-317 (2000)

Polymorphonuclear cells (PMNs) from healthy donors and differentiated HL-60 cells were compared in an opsonophagocytic assay using fluorescent latex beads coated with *Streptococcus pneumoniae* polysaccharide conjugates. Serum-specific phagocytosis was efficiently mediated by both sources of cells, as measured by flow cytometry, but the mean number of beads ingested per cell was three- to fivefold higher when PMNs were used than when HL-60 cells were used. Nevertheless, differentiated HL-60 cells could be a convenient and standardized source of cells to evaluate the functionality of specific antibodies to vaccine candidates as a coating on fluorescent beads.

200. Integrin $\alpha_2\beta_1$ (VLA-2) is a principal receptor used by neutrophils for locomotion in extravascular tissue
Werr, J. et al
Blood, 95, 1804-1809 (2000)

Cell adhesion molecules are critically involved in the multistep process of leukocyte recruitment in inflammation. The specific receptors used by polymorphonuclear leukocytes (PMN) for locomotion in extravascular tissue have as yet not been identified. By means of immunofluorescence flow cytometry and laser scanning confocal microscopy, this study demonstrated that surface expression of the $\alpha_2\beta_1$ (VLA-2) integrin, though absent on blood PMN, is induced in extravasated PMN collected from human skin blister chambers, and rat PMN accumulated in the peritoneal cavity after chemotactic stimulation. Intravital time-lapse videomicroscopy was used to investigate chemoattractant-induced PMN locomotion in the rat mesentery in vivo. Local administration of function-blocking monoclonal antibody or peptide recognizing the $\alpha_2\beta_1$ integrin reduced PMN migration velocity in the extravascular tissue by 73% ± 3% and 70% ± 10%, respectively (means ± SD). The distance f-met-leu-phe peptide (fMLP)-stimulated human PMN migrated in a collagen gel in vitro was markedly reduced by treatment with anti-α_2 mAbs or peptide, whereas no effect was observed with antibodies or peptides recognizing the $\alpha_4\beta_1$ or $\alpha_5\beta_1$ integrins. Further evidence for a critical role of expression of $\alpha_2\beta_1$ integrin in PMN locomotion in extravascular tissue was obtained in the mouse air pouch model of acute inflammation where chemoattractant-induced PMN recruitment was substantially inhibited by local anti-α_2 mAb treatment. Thus, expression of $\alpha_2\beta_1$ integrin on extravasated PMN has been identified and a novel role of this receptor in regulating the extravascular phase of leukocyte trafficking in inflammation has been formulated.

201. Neutrophil polarity and locomotion are associated with surface redistribution of leukosialin (CD43), an antiadhesive membrane molecule
Seveau, S. et al
Blood, 95, 2462-2470 (2000)

This study analyzed the behavior of an antiadhesive membrane molecule, CD43, in neutrophil polarization and locomotion. CD43 cross-linking by antibodies induced neutrophil locomotion, with CD43 molecules clustered at the uropod of polarized neutrophils. In contrast, CD11b/CD18 cross-linking by antibodies did not affect either cell polarization or locomotion. Stimulation of suspended or adherent neutrophils with chemotactic peptide results in cell polarization and locomotion and a concomitant redistribution of CD43 to the uropod. This process is entirely reversible. The study also investigated which actin-binding protein could be involved in CD43 lateral redistribution. α-Actinin and moesin are preferentially adsorbed on Sepharose beads bearing a recombinant CD43 intracellular domain. Analysis by immunofluorescence confocal microscopy shows a codistribution of moesin during CD43 lateral redistribution. By contrast, α-actinin is located at the leading edge, an area devoid of CD43. These results shed new light on the role of CD43 membrane redistribution, which appears to be directly related to neutrophil polarity and locomotion.

202. Perflubron attenuates neutrophil adhesion to activated endothelial cells in vitro
Woods, C.M., Neslund, G., Kornbrust, E. and Flaim, S.F.
*Am. J. Physiol. Lung Cell. Mol. Physiol., **278**, 1008 (2000)*

Infiltration of activated neutrophils into the lung appears to be a key element in the severe lung injury that develops in animal models of acute lung injury. Partial liquid ventilation with perflubron has been shown to ameliorate tissue damage compared with conventional mechanical ventilation in acute lung injury models. Pilot experiments indicated that indirect exposure to perflubron could modulate the degree to which subsequent neutrophil binding to endothelial cell monolayers was upregulated after lipopolysaccharide activation. Endothelial cell monolayers preexposed to perflubron showed >40% reductions in the surface steady-state levels of E-selectin and intercellular adhesion molecule-1 achieved after proinflammatory activation ($P < 0.05$), which correlated with a reduction in the real-time association constants measured by biosensor techniques. These results indicate that direct contact with the perflubron liquid phase is not necessary to attenuate inflammatory responses. Rather, diffusion of perflubron from the alveolar space into the adjacent pulmonary vascular endothelial layer may modulate neutrophil adhesion and thereby reduce the rate of infiltration of activated neutrophils into the injured lung.

203. Interleukin-8 and leukotriene-B_4, but not formylmethionyl leucylphenylalanine, stimulate CD18-independent migration of neutrophils across human pulmonary endothelial cells in vitro
Mackarel, A.J., Russell, K.J., Brady, C.S., FitzGerald, M.X. and O'Connor, C.M.
Am. J. Cell. Mol. Biol., 23, 154 (2000)

Although neutrophil migration from the systemic circulation involves the β2- (or CD18) integrin family, the existence of an alternative, CD18-independent route of neutrophil extravasation to tissues has been demonstrated in animal models. The molecular interactions involved in this alternative migratory route have not yet been characterized. The objective of this study was to assess the CD18-dependency of neutrophil migration across human endothelial cells from an organ known to support CD18-independent migration, the lung, with a view to establishing an *in vitro* model to facilitate study of CD18-independent migration. Neutrophil migration across human pulmonary artery endothelial cells (HPAECs) in response to three different chemoattractants, formylmethionyl leucylphenyl-alanine (FMLP), interleukin (IL)-8, and leukotriene (LT) B_4, was examined. Results demonstrated that a function-blocking antibody to CD18 decreased FMLP-stimulated migration by 71.7 ± 4.4% ($P < 0.001$). In contrast, migration in response to LTB_4 was decreased by only 20.5 ± 10.2% ($P < 0.01$), and no significant decrease was observed with migration to IL-8. Neutrophils that migrated to FMLP had 1.7-fold more surface CD11b/CD18 compared with nonmigrated neutrophils ($P < 0.01$), whereas this integrin complex was not significantly upregulated on neutrophils that had migrated to IL-8 or LTB_4. Further investigation of this migratory route indicated that it did not involve the β1 integrins (CD29) or the endothelial selectins, E- or P-selectin, nor did it require the activity of either metalloproteinases or neutrophil elastase. These results indicate that neutrophil migration across HPAECs *in vitro* to IL-8 and LTB_4 is predominantly CD18-independent and provides a much-needed *in vitro* system for examination of the neutrophil-endothelial interactions involved in this alternative migratory route.

204. Characterization of naturally occurring and recombinant human N-acyltransferase variants encoded by NAT1
De Leon, J.H., Vatsis, K.P. and Weber, W.K.
Mol. Pharmacol., 58, 288 (2000)

The genotype at the *NAT1** locus of an interethnic population of 38 unrelated subjects was determined by direct sequencing of 1.6-kb fragments amplified by PCR. The coding exon alone and together with the 3' noncoding exon of the wild-type (*NAT1*4*) and the three mutant alleles (*NAT1*10*, *11*, and *16*) detected was expressed in *Escherichia coli* and COS-1 cells, respectively, and the cytosolic fraction of mononuclear leukocytes from *NAT1*4/*4* and *NAT1*10/*10* homozygotes was also isolated. Recombinant and leukocyte

cytosolic preparations were thoroughly characterized by *N*-acetylation activity with several NAT1-specific and -selective substrates, as well as by steady-state kinetics with varying amounts of the substrate (fixed acetyl CoA) and acetyl CoA (fixed substrate), thermodynamics, stability, and protein immunoreactivity with a polyclonal human anti-NAT1. The polyadenylation signal mutation in the 3' noncoding sequence of *NAT1*10* affected none of the aforementioned parameters evaluated both with recombinant *NAT1*10* and with the naturally occurring allele. Function was also unaffected by the coding and 3' noncoding exon mutations in *NAT1*11*. In contrast, the three extra adenosines located immediately after the sixth position of the polyadenylation signal in the 3' untranslated region of *NAT1*16* ostensibly caused disruption of the predicted secondary structure of the pre-mRNA for NAT1 16, culminating in parallel 2-fold decreases in the amount and catalytic activity of NAT1 16 in COS-1 cell cytosol. This novel finding in *N*-acetylation pharmacogenetics clearly demonstrates a direct link between reduced catalytic activity and structural alteration in the 3' untranslated region of an *NAT* variant (*NAT1*16*) brought about by mutation.

205. Immunologic characterization of normal human pleural macrophages
Frankenberger, M. Et al
Am. J. Respir. Cell Mol. Biol., 23, 419 (2000)

Human pleural macrophages (PLM) have been studied in effusions, but little is known about normal human PLM. We therefore analyzed resting human PLM recovered by lavage before lobe resection from patients with a central bronchial tumor, not involving the pleura, and from patients with pulmonary chondroma, intrapulmonary hemorrhage, and pneumothorax. Analysis of surface antigens, phagocytosis capacity, and cytokine production was done in comparison to the regular $CD14^{++}$ blood monocytes and the recently described blood monocyte subset $CD14^{+}CD16^{+}$ monocytes. When defining fluorescence intensity for the various markers on $CD14^{++}$ monocytes as 100%, the PLM gave the following pattern: CD14, 45%; CD32, 200%; CD64, 72%; CD11b, 128%; CD33, 74%; CD54, 299%; and HLA-DR, 1,906%. When CD16 on the $CD14^{+}CD16^{+}$ monocytes was set as 100%, the level of CD16 expression on PLM was 7.7%. Taken together, when compared to blood monocytes, PLM appear to represent a cell-type intermediate of regular $CD14^{++}$ monocytes and the $CD14^{+}CD16^{+}$ subset. In functional studies, we demonstrate that PLM can perform efficient Fc-receptor-mediated phagocytosis of antibody-coated sheep red blood cells. Compared with blood monocytes, the capacity of PLM to produce tumor necrosis factor is similar, but a striking finding in PLM was the constitutive interleukin-10 messenger RNA expression that could not be substantially increased by lipopolysaccharide stimulation. This first characterization of normal, noneffusion human PLM can form the basis for a better interpretation of findings in malignant and inflammatory exsudates.

206. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart
Frendeus, B. et al
J. Exp. Med., 192, 881 (2000)

Neutrophils migrate to infected mucosal sites that they protect against invading pathogens. Their interaction with the epithelial barrier is controlled by CXC chemokines and by their receptors. This study examined the change in susceptibility to urinary tract infection (UTI) after deletion of the murine interleukin 8 receptor homologue (mIL-8Rh). Experimental UTIs in control mice stimulated an epithelial chemokine response and increased chemokine receptor expression. Neutrophils migrated through the tissues to the epithelial barrier that they crossed into the lumen, and the mice developed pyuria. In mIL-8Rh knockout (KO) mice, the chemokine response was intact, but the epithelial cells failed to express IL-8R, and neutrophils accumulated in the tissues. The KO mice were unable to clear bacteria from kidneys and bladders and developed bacteremia and symptoms of systemic disease, but control mice were fully resistant to infection. The experimental UTI model demonstrated that IL-8R–dependent mechanisms control the urinary tract defense, and that neutrophils are essential host effector cells. Patients prone to acute pyelonephritis also showed low CXC chemokine receptor 1 expression compared with age-matched controls, suggesting that chemokine receptor expression may also influence the susceptibility to UTIs in humans. The results provide a first molecular clue to disease susceptibility of patients prone to acute pyelonephritis.

207. Na^{+}/H^{+} exchange inhibition-induced cardioprotection in dogs: effects on neutrophils versus cardiomyocytes
Gumina, R.J. et al
Am. J. Physiol. Heart Circ. Physiol., 279, 1563 (2000)

Numerous studies have examined the effect of Na^{+}/H^{+} exchanger (NHE) inhibition on the myocardium; however, the effect of NHE-1 inhibition on neutrophil function has not been adequately examined. An in vivo canine model of myocardial ischemia-reperfusion injury in which 60 min of left anterior descending coronary artery occlusion followed by 3 h of reperfusion was used to examine the effect of NHE-1 inhibition on infarct size (IS) and neutrophil function. BIIB-513, a selective inhibitor of NHE-1, was infused before ischemia. IS was expressed as a percentage of area at risk (IS/AAR). NHE-1 inhibition significantly reduced IS/AAR and reduced neutrophil accumulation in the ischemic myocardium. NHE-1 inhibition attenuated both phorbol 12-myristate 13-acetate- and platelet-activating factor-induced neutrophil respiratory burst but not CD18 upregulation. Furthermore, NHE-1 inhibition directly protected cardiomyocytes against metabolic inhibition-induced lactate dehydrogenase release and hypercontracture. This study provides evidence that the cardioprotection induced by NHE-1 inhibition is likely due to specific protection of cardiomyocytes and attenuation of neutrophil activity.

208. Engagement of B_2 integrins induces surface expression of B_1 integrin receptors in human neutrophils
Werr, J., Eriksson, E.E., Hedqvist, P. and Lindbom, L.
J. Leukoc. Biol., 68, 553 (2000)

Induction of $ß_1$ integrin (CD49/CD29) expression in polymorphonuclear leukocytes (PMN) has been shown to be associated with transendothelial migration recently. Yet, $ß_1$ integrin expression is relatively insensitive to cell activation with soluble agonists, such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLP). We hypothesized that $ß_2$ integrins (CD11/CD18), critically involved in PMN adhesion and extravasation, may play a role in regulating $ß_1$ integrin expression in PMN. Antibody cross-linking of CD18, mimicking adhesion-dependent engagement of $ß_2$ integrins, resulted in rapid, tyrosine kinase-dependent upregulation of $ß_1$ integrins. This response was potentiated by simultaneous chemoattractant (fMLP) stimulation of PMN. Moreover, upregulation of $ß_1$ integrins evoked by CD18 cross-linking was found to support adhesion of fMLP-stimulated PMN to matrix proteins and also was critical for the ability of PMN to migrate in collagen gels in response to a gradient of fMLP. Taken together, these data demonstrate that engagement of $ß_2$ integrins in human PMN induces $ß_1$ integrin expression in these cells of significance for their migration in the extravascular tissue. Thus, $ß_2$ integrins may serve the function to regulate PMN locomotion in extravascular tissue via receptor crosstalk with $ß_1$ integrins.

209. Role of complement and platelet-activating factor in the stimulation of phagocytosis and reactive oxygen species production during haemodialysis
Gastaldello, K., Husson, C., Wens, R., Vanherweghem, J-L. and Tielemans, C.
Nephrol. Dial. Transplant., 15, 1638-1646 (2000)

Background. Neutrophil phagocytic functions have been studied extensively in haemodialysis (HD) patients; however, results are contradictory and the mechanisms that modulate phagocytosis and oxidative burst during dialysis are not completely understood.
Methods. The present study investigated neutrophil functions in a selected population of patients before and during clinical dialysis with cuprophane, and polyacrylonitrile (AN69) membranes. We measured phagocytosis of *Escherichia coli* and intracellular hydrogen peroxide (H_2O_2) production by flow cytometry in whole blood.
Results. Before dialysis, neutrophils from HD patients showed normal phagocytic capability and H_2O_2 formation. Phagocytosis of FITC-*E. coli* was significantly stimulated in cuprophane but not AN69-treated patients. Spontaneous and stimulated H_2O_2 production was enhanced with both cuprophane and AN69 membranes. We then investigated *in vitro* the role of complement and platelet-activating factor (PAF) in the activation of neutrophils. Incubation of whole blood with C5a increased phagocytosis but not H_2O_2 production. On the contrary, the addition of synthetic PAF showed a markedly stimulated H_2O_2 production without increase in phagocytosis. Moreover, during dialysis with formaldehyde-reused cuprophane, complement activation was abolished and phagocytosis was no longer enhanced, while the stimulation of H_2O_2 production persisted. In addition, we also excluded a particular role of the membrane itself in the activation of neutrophils.
Conclusion. We demonstrated that in a selected population of HD patients, neutrophils exhibit normal phagocytic capability and normal intracellular H_2O_2 production. During dialysis, the stimulation of phagocytosis observed with cuprophane is complement dependent, whereas the enhanced H_2O_2 production observed with both cuprophane and AN69 membranes might be related to PAF production.

210. Decreased neutrophil adhesion to human cytomegalovirus-infected retinal pigment epithelial cells is mediated by virus-induced up-regulation of Fas ligand independent of neutrophil apoptosis
Cinatl, J. et al
J. Immunol., 165, 4405 (2000)

Human CMV (HCMV) retinitis frequently leads to blindness in iatrogenically immunosuppressed patients and in the end stage of AIDS. Despite the general proinflammatory potential of HCMV, virus infection is associated with a rather mild cellular inflammatory response in the retina. To investigate this phenomenon, the influence of HCMV (strains AD169 or Hi91) infection on C-X-C chemokine secretion, ICAM-1 expression, and neutrophil recruitment in cultured human retinal pigment epithelial (RPE) cells was studied. Supernatants from infected cultures contained enhanced levels of IL-8 and melanoma growth-stimulating activity/Gro α and induced neutrophil chemotaxis compared with supernatants from uninfected RPE cells. Despite HCMV-induced ICAM-1 expression on RPE cells, binding of activated neutrophils to HCMV-infected RPE cells and subsequent transepithelial penetration were significantly reduced. Reduced neutrophil adhesion to infected RPE cells correlated with HCMV-induced up-regulation of constitutive Fas ligand (FasL) expression. Functional blocking of FasL on RPE cells with the neutralizing mAbs NOK-1 and NOK-2 or of the Fas receptor on neutrophils with mAbB-D29 prevented the HCMV-induced impairment of neutrophil/RPE interactions. Fas-FasL-dependent impairment of neutrophil binding had occurred by 10 min after neutrophil/RPE coculture without apoptotic signs. Neutrophil apoptosis was first detected after 4 h. Treatment of neutrophils with a specific inhibitor of caspase-8 suppressed apoptosis, whereas it did not prevent impaired neutrophil binding to infected RPE. The current results suggest a novel role for FasL in the RPE regulation of neutrophil binding. This may be an important feature of virus escape mechanisms and for sustaining the immune-privileged character of the retina during HCMV ocular infection.

211. Fas activation reduces neutrophil adhesion to endothelial cells
Greenstein, S., Barnard, J., Zhou, K., Fong, M. and Hendey, B.

J. Leukoc. Biol., 68, 715 (2000)

Polymorphonuclear neutrophils (PMN) express apoptotic markers and lose effector functions including adhesion, chemotaxis, and phagocytosis when cultured overnight. Although the loss of function correlates with apoptosis, it is not clear if functions are lost before an early marker of apoptosis, the display of phosphatidylserine (PS), targets PMN for removal by phagocytic cells. To address this question, freshly isolated PMN were treated with Fas-activating antibodies to induce apoptosis rapidly. Early markers of apoptosis and PMA-stimulated adhesion to endothelial cells were measured. After 1 h of Fas exposure, only 16% PMN had externalized PS. In contrast, Fas activation reduced PMA-stimulated adhesion between 68 and 27% depending on PMA concentration. The loss of adhesion was accompanied by a reduction in ß2 integrin expression and receptor clustering. These results indicate that the Fas-induced loss of adhesion may precede PS externalization and could limit participation in the inflammatory response before PS externalization targets PMN for removal.

212. Transepithelial neutrophil migration is CXCR1 dependent in vitro and is defective in IL-8 receptor knockout mice
Godaly, G., Hang, L., Frendeus, B. and Svanborg, C.
J. Immunol., 165, 5287 (2000)

Neutrophil migration across infected mucosal surfaces is chemokine dependent, but the role of chemokine receptors has not been investigated. In this study, chemokine receptors were shown to be expressed by epithelial cells lining the urinary tract, and to play an essential role for neutrophil migration across the mucosal barrier. Uroepithelial CXCR1 and CXCR2 expression was detected in human urinary tract biopsies, and in vitro infection of human uroepithelial cell lines caused a dramatic increase in both receptors. As a consequence, there was higher binding of IL-8 to the cells and the IL-8-dependent neutrophil migration across the infected epithelial cell layers was enhanced. Abs to IL-8 or to the CXCR1 receptor inhibited this increase by 60% ($p < 0.004$), but anti-CXCR2 Abs had no effect, suggesting that CXCR1 was the more essential receptor in this process. Similar observations were made in the mouse urinary tract, where experimental infection stimulated epithelial expression of the murine IL-8 receptor, followed by a rapid flux of neutrophils into the lumen. IL-8 receptor knockout mice, in contrast, failed to express the receptor, their neutrophils were unable to cross the epithelial barrier, and accumulated in massive numbers in the tissues. These results demonstrate that epithelial cells express CXC receptors and that infection increases receptor expression. Furthermore, we show that CXCR1 is required for neutrophil migration across infected epithelial cell layers in vitro, and that the murine IL-8 receptor is needed for neutrophils to cross the infected mucosa of the urinary tract in vivo.

213. Inflammatory properties of IgG modified by oxygen radicals and peroxynitrite
Uesugi, M., Yoshida, K. and Jasin, H.E.
J. Immunol., 165, 6532 (2000)

In inflammatory arthritis, there is evidence indicating that the affected tissues produce large amounts of oxygen-free radicals and NO. Herein, we examine the biologic effects of exposure of IgG to hypochlorous acid (HOCl) and peroxynitrite (ONOO). The concentrations of IgG modified by chlorination and nitrosation were measured in synovial fluids from inflammatory and noninflammatory arthritis. Human IgG was exposed to increasing concentrations of HOCl and ONOO, and the resulting products were tested for complement component binding; binding to FcγRI; activation of polymorphonuclear neutrophils; effect on the Ab-combining site of Abs; and in vivo inflammatory activity in a rabbit model of acute arthritis. Rheumatoid synovial fluids contained significantly greater concentrations of nitrosated and chlorinated IgG compared with ostearthritic specimens. In vitro exposure of human IgG to HOCl and ONOO resulted in a concentration-dependent decrease in C3 and C1q fixation. The decrease in Fc domain-dependent biologic functions was confirmed by competitive binding studies to the FcγRI of U937 cells. HOCl-treated IgG monomer was 10 times less effective in competing for binding compared with native IgG, and ONOO-treated IgG was 2.5 times less effective. The modified IgGs were also ineffective in inducing synthesis of H_2O_2 by human PMN. The Ag-binding domains of IgG also showed a concentration-dependent decrease in binding to Ag. The ability of the modified IgGs to induce acute inflammation in rabbit knees decreased 20-fold as gauged by the intensity of the inflammatory cell exudates. These studies clarify the modulating role of biological oxidants in inflammatory processes in which Ag-autoantibody reactions and immune complex pathogenesis may play an important role.

214. Neutrophil function in pregnancy and rheumatoid arthritis
Crocker, I.P., Baker, P.N. and Fletcher, J.
Ann. Rheum. Dis., 59, 555 (2000)

BACKGROUND—Pregnancy exerts suppressive effects on rheumatoid arthritis (RA). An attenuation in neutrophil function in late pregnancy which may explain this amelioration has previously been reported.
OBJECTIVE—A longitudinal investigation of neutrophil activity in healthy pregnant women (n=9) and pregnant patients with RA (n=9), compared with age matched non-pregnant patients with RA (n=12) and healthy controls (n=22).
METHODS—Neutrophil activation was measured in response to the physiological receptor agonists, n-formyl-methionyl-leucyl-phenylalanine (fMLP) and zymosan activated serum (ZAS). Superoxide anion production (respiratory burst) was determined by lucigenin enhanced chemiluminescence (LUCL); secondary granule lactoferrin release by enzyme linked immunosorbent assay

(ELISA); and CD11b, CD18, and CD62L expression by flow cytometric analysis.
RESULTS—Stimulated neutrophil LUCL was significantly reduced in both pregnant women with RA and healthy pregnant women in the second (fMLP 43% and 69%, ZAS 43% and 59%, respectively) and third trimesters (fMLP 24% and 44%, ZAS 32% and 38%, respectively). Responses returned to normal within eight weeks of delivery and unstimulated levels remained unchanged throughout pregnancy. Basal and stimulated CD11b, CD18, and CD62L expression showed no variations throughout gestation for both pregnancy groups. Likewise, stimulated lactoferrin release and plasma lactoferrin remained unchanged. Certain morphological differences in RA neutrophils were highlighted by the flow cytometric analysis. Moreover, resting neutrophils and stimulated cells from patients with RA, including pregnant subjects, showed a marked increase in LUCL, but a reduction in CD11b, CD18, and CD62L. Low dose prednisolone and methylprednisolone had no effect on neutrophil parameters over the period of treatment with non-steroidal anti-inflammatory drugs.
CONCLUSION—The attenuation to neutrophil respiratory burst in both healthy and RA pregnancies may offer an explanation for the pregnancy induced remission of this inflammatory disorder.

215. Foreign DNA transmission by ICSI: injection of spermatozoa bound with exogenous DNA results in embryonic GFP expression and live Rhesus monkey births
Chan, A.W.S. et al
Mol. Hum. Reprod., 6, 26-33 (2000)

Exogenous DNA transfer, mediated by intracytoplasmic sperm injection (ICSI) with plasmid-bound spermatozoa, results in the production of transgene expressing embryos in rhesus macaques (*Macaca mulatta*, mean = 34.6%; $n = 81$). Rhodamine-tagged DNA encoding the green fluorescent protein (*GFP*) gene binds avidly to spermatozoa. The rhodamine signal, while lost at the egg surface during in-vitro fertilization (IVF), is traced by dynamic imaging during ICSI and remains as a brilliant marker on the microinjected spermatozoa within the oocyte cytoplasm. The transgene is expressed in preimplantation embryos produced by ICSI, but not IVF, as early as the 4-cell stage with the number of expressing cells and the percentage of expressing embryos increasing during embryogenesis to the blastocyst stage. The three offspring that resulted from seven embryo transfers (a set of anatomically normal twins, one male and one female, stillborn 35 days premature, and a healthy male born at term) demonstrate that primate spermatozoa with exogenously bound DNA retain their full reproductive capacity in ICSI, but raise the concern that, theoretically, ICSI could transmit infectious material as well.

216. Troglitazone Reduces Reactive Oxygen Species Generation by Leukocytes and Lipid Peroxidation and Improves Flow-Mediated Vasodilatation in Obese Subjects
Garg, R. et al
Hypertension, 36, 430 (2000)

Because troglitazone has been shown to have antioxidant properties, we investigated whether troglitazone administration to obese subjects causes a reduction in (1) reactive oxygen species (ROS) generation by polymorphonuclear leukocytes (**PMN**Ls) and mononuclear cells (MNCs) and (2) lipid peroxidation as reflected in the plasma concentrations of 9-hydroxyoctadecadienoic acid (9-HODE) and 13-hydroxyoctadecadienoic acid (13-HODE). Seven obese subjects were given 400 mg/d troglitazone for 4 weeks. Blood samples were obtained before troglitazone administration and at weekly intervals thereafter. Insulin concentrations fell significantly at week 1 and remained low at weeks 2 and 4 ($P<0.001$). ROS generation by **PMN**Ls fell to 77.6±25.1% of the basal at week 1 and 47.9±41.1% at week 4 ($P<0.001$). ROS generation by MNCs fell to 59.8±15.7% of the basal at week 1 and 35.1±17.6% at week 4 ($P<0.001$). 9-HODE and 13-HODE concentrations fell significantly from 787.4±52.4 and 713.1±44.7 pg/mL to 720.4±66.7 ($P<0.004$) and 675.2±65.0 pg/mL ($P<0.01$) after 4 weeks, respectively. Postischemic dilatation of the brachial artery was measured by ultrasonography. The mean percent dilatation after forearm ischemia before and after troglitazone was 5.5±3.01% and 8.75±3.37% ($P<0.02$), respectively. The percent increase in diameter after nitroglycerin was 17.08±1.18% before troglitazone, whereas it was 18.9±1.91% ($P<0.02$) after troglitazone. We conclude that troglitazone has a potent and rapid biological inhibitory effect on ROS generation by **PMN**Ls and MNCs and that it inhibits lipid peroxidation significantly. These changes are associated with a significant improvement in postischemic flow-mediated vasodilation in the brachial artery over a relatively short period of 4 weeks.

217. Hematopoietic-specific expression of MEFV, the gene mutated in familial Mediterranean fever, and subcellular localization of its corresponding protein, pyrin
Tidow, N. et al
Blood, 95, 1451-1455 (2000)

Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by recurrent, self-limited attacks of fever and serositis and by infiltration of affected tissues by large numbers of neutrophils. A candidate gene for FMF was identified by positional cloning and named "*MEFV*." The corresponding protein was named "pyrin." To elucidate the currently unknown function of pyrin, we characterized its tissue distribution, regulation of expression during hematopoietic differentiation, and subcellular localization. Reverse transcription-polymerase chain reaction analysis, followed by hybridization with an internal oligonucleotide, demonstrated expression of *MEFV* in different populations of peripheral blood cells. Among hematopoietic cell lines, *MEFV* was almost exclusively expressed in cells of the myeloid lineage. Furthermore, *MEFV* messenger RNA was strongly expressed within 24 hours of dimethyl sulfoxide-

induced granulocytic differentiation of HL-60 cells. Analysis of complementary DNA from human solid tumor-derived cell lines revealed expression of *MEFV* in several cell lines derived from colon and prostate cancers. Expression of *MEFV* fused to enhanced green fluorescent protein showed that pyrin localized in distinct patches in the cytoplasm, forming a perinuclear cap. Taken together, *MEFV* is predominantly expressed in myeloid cells and upregulated during myeloid differentiation, and the corresponding protein, pyrin, is expressed in the cytoplasm.

218. Determination of polymorphonuclear neutrophil adhesion receptors. Effect of pre-analytic factors
Latger-Cannard, V., Regnault, V., Dumas, D. and Nguyen, P.
J. Mal. Vasc., 25(3), 181-186 (2000)

Polymorphonuclear neutrophil (PMN) adherence receptors expression varies with leukocyte activation state. Their quantification need accurate and inter-laboratories reproducible methods, without artefactual activation. OBJECTIVE: The aim of this study was to study the influence of cell preparation on PMN adherence receptors expression. MATERIALS AND METHODS: It was proposed to quantify, using immunolabeling standard (QIFIKIT(R), Dako), surface expression of the main adherence receptors (L-selectin and B(2) integrins), from different preparations of PMN: total blood collected with EDTA, isolated PMN by density gradient Polymorphprep(TM) 1,113 (Nycomed Pharma) and formaldehyde fixed PMN. RESULTS: A decrease of all receptors was noted after isolation and fixation of PMN, in comparison with whole blood PMN analysis. These results differed from data previously reported since, in these studies, activated phenotypes (increased of B(2) integrins) were observed after isolation and fixation methods. CONCLUSION: The present study provides strong evidence that pre-analytical conditions are sources of biological variations and thus extreme care must be taken in the interpretation of results. It underlines the interest of consensual practices for these pre-analytic and analytic parameters in order to compare results in multicenter and longitudinal studies.

219. Expression of lactoferrin in the kidney: Implications for innate immunity and iron metabolism
Åbrink, M., Larsson, E., Gobl, A. and Hellman, L.
Kidney Int., 57, 2004-2010 (2000)

Background

Sequestering of free iron by lactoferrin (LF) is important in the defense against bacteria. In a screening for LF expression in various organs, high levels of LF mRNA were detected in human kidney. This indicated that LF is produced by the kidney and that it may participate in innate immunity of this organ.
Methods and Results

Antibody staining and in situ hybridization of paraffin-embedded kidney sections showed that LF is expressed in cells lining the distal collecting ducts of the medulla. High levels of both protein and mRNA were detected in these cells. However, a clear difference in the distribution of mRNA and protein within the tissue was observed. LF mRNA was detected along a relatively large portion of the tubuli, whereas LF antigen was found mainly in the very distal regions of the same tubuli. This indicates that LF is released by large regions of the tubuli and possibly reabsorbed in the most distal parts. Using enzyme-linked immunosorbent assay, only very low LF levels were detected in urine.
Conclusion

The present study shows that LF is produced by the kidney and that both LF mRNA and protein are distributed in a highly ordered fashion. This latter finding, together with the very low levels of LF detected in urine, indicates that LF may contribute to the immune defense in the kidney by reduction of available free iron in the urine. Other possibilities are that LF may play a role in the iron metabolism by recovering free iron from urine and making it available for metabolic use, and that LF may participate in the antioxidant defense systems protecting the kidney against nonmicrobial oxidative injury, that is, ischemia, reperfusion and inflammation.

220. Interaction of endothelial cells and neutrophils *in vitro*: kinetics of thrombomodulin, intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1 (VCAM-1): implications for the relevance as serological disease activity markers in vasculitides
Boehme, M.W.J., Raeth, U., Scherbaum, W.A., Galle, P.R. and Stremmel, W.
Clin. Exp. Immunol., 119(1), 250-254 (2000)

Recently markers of endothelial cell activation or injury gained increasing interest as serological parameters of disease activation in vasculitides. Among these, soluble serum thrombomodulin, ICAM-1, VCAM-1 and E-selectin are of particular interest. However, only thrombomodulin showed the expected close correlation. The objective of this study was to investigate *in vitro* the kinetics of these endothelial cell receptors after interaction of unstimulated or cytokine-activated polymorphonuclear neutrophils (PMN) and endothelial cells in order to find evidence explaining these different clinical findings. Over the time period of up to 48 h of incubation the kinetics of thrombomodulin, ICAM-1, E-selectin, and VCAM-1 levels in the supernatant of endothelial cells in co-culture with neutrophils were determined *in vitro* by ELISA under basal and partially cytokine-activated (tumour necrosis factor-alpha) conditions. Increased levels of

ICAM-1, E-selectin and VCAM-1 were already found due to cytokine activation of endothelial cells alone. This increase was augmented after coincubation with neutrophils. In contrast, a significant increase of thrombomodulin in the supernatant was only found due to cell injury after cell–cell interaction of cytokine-activated endothelial cells with neutrophils. In conclusion, this *in vitro* model of the kinetics of soluble endothelial cell receptors after cell–cell interaction of cytokine-activated PMN and endothelial cells underlines the advantage of thrombomodulin in contrast to the adhesion molecules as a marker of endothelial damage. Therefore, soluble thrombomodulin seems to be a promising, valuable serological disease activity marker in vasculitides.

221. Fibronectin synthesis by activated T lymphocytes: up‐regulation of asurface‐associated isoform with signalling function
Wagner, C. et al
Immunology, 99(4), 532-539 (2000)

Fibronectin (FN) is a major constituent of the extracellular matrix. We now provide evidence for a surface‐associated isoform of FN that is synthesized by T cells upon activation. The T‐cell‐derived FN has an unusual splice pattern: an additional domain, EDB, is produced whereas sequences within another domain, IIICS, are spliced out. CS1, the binding domain for very late antigen‐4 (VLA‐4), however, is still generated. To study the potential function of surface‐associated FN its synthesis was down‐regulated by an antisense oligonucleotide, then proliferation of T cells was induced by cross‐linked anti‐CD3. Proliferation was reduced as was expression of CD25. Moreover, when T cells were cultured in high density, the synthetic peptide QILDVPST, corresponding to CS1, inhibited proliferation, as did antibodies to VLA‐4. We propose that surface‐associated FN is a ligand for VLA‐4, which by binding to VLA‐4 on an adjacent cell, provides a costimulatory signal, thus sustaining T‐cell proliferation.

222. Plasminogen activator inhibitor 2 and urokinase-type plasminogen activator in plasma and leucocytes in patients with severe sepsis
Robbie, L.A., Dummer, S., Booth, N.A., Adey, G.D. and Bennett, B.
Br. J. Hematol., 109(2), 342-348 (2000)

Proteins influencing plasminogen activation to plasmin, namely plasminogen activators tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) and their principal inhibitors, plasminogen activator inhibitor 1 (PAI-1) and PAI-2, were measured in the plasma, the polymorph and mononuclear cell fractions taken from patients with major sepsis who were entering a general intensive care unit. The purpose of this study was to elucidate the factors favouring the persistence of fibrin in the microvasculature and thus contributing to multiple organ failure. Levels of u-PA antigen in plasma rose in sepsis and u-PA activity, not detectable in normal plasma, appeared. Levels of u-PA antigen in the cell fractions fell concomitantly. t-PA antigen in plasma and in the mononuclear cell fraction rose in sepsis, but t-PA activity was not detectable. Plasma PAI-1 antigen levels were strikingly raised in sepsis, presumably accounting for the complete neutralization of t-PA activity. PAI-2 antigen, not normally detected in plasma, appeared in the plasma of some patients, whereas it disappeared from the cellular fractions. Appearance of PAI-2 in plasma was associated with non-survival of the patient. The observations indicate that all the agents involved in plasminogen activation are released into the plasma in major sepsis. The levels of PAI-1 reached were quantitatively sufficient to suppress all activity of the released t-PA, but the inhibitors did not prevent expression of u-PA activity in the circulation. Circulating active u-PA and PAI-2 in the plasma of patients with severe sepsis may represent material originating from leucocytes. Leucocyte release of these agents within fibrin deposits may influence the persistence of fibrin and thus the development of multiple organ failure.

223. Thalidomide analogue CC-3052 reduces HIV+ neutrophil apoptosis in vitro
Guckian, M., Dransfield, I., Hay, P. and Dalgleish, A.G.
Clin. Exp. Immunol., 121(3), 472-479 (2000)

Thalidomide has significant immunomodulatory properties and has been used successfully in the treatment of oral ulcers and wasting in HIV patients. However, its use is limited by its poor bioavailability due to low solubility and short half life in solution, and teratogenic and neurotoxic side-effects. Recently, water-soluble analogues of thalidomide with significantly greater immunomodulatory activity and reduced side-effects have become available. We examined the effect of thalidomide and one analogue, CC-3052, on neutrophil apoptosis following culture for 20 h *in vitro*. Apoptosis was assessed by reduced CD16 expression and Annexin V binding using flow cytometry. Thalidomide or CC-3052 alone had no effect on neutrophil apoptosis when used at physiological levels. However, when used together with prostaglandin E_2 (10^{-7} M), a potent adenylate cyclase activator, CC-3052 but not thalidomide (both 10^{-5} M) reduced apoptosis in neutrophils from normal and HIV^+ donors. The reduced apoptosis could not be attributed to the ability of CC-3052 to reduce tumour necrosis factor-alpha (TNF-α) production, but may be due to its PDE4 inhibitor properties, as it increased $[cAMP]_i$, and mimicked the effect of increasing $[cAMP]_i$ using dibutryl cAMP, a membrane-permeable analogue of cAMP. The results suggest a role for thalidomide analogue CC-3052 in reducing persistent activation of the TNF-α system in HIV without markedly impairing neutrophil viability.

224. Long-term remission after intensive chemotherapy in advanced myelodysplastic syndromes is generally associated with restoration of polyclonal haemopoiesis
Aivado, M. et al

Br. J. Hematol., 110(4), 884-886 (2000)

The clonality of peripheral blood cells was assessed in eight female patients with myelodysplastic syndrome (MDS) by means of the human androgen receptor gene-based assay (HUMARA). The patients were in complete remission for a median follow-up time of 83 months after intensive chemotherapy. X-chromosome inactivation patterns (XCIPs) indicated polyclonal haemopoiesis in five patients. Two patients had skewed lyonization (i.e. unbalanced XCIPs in both granulocytes and T cells) and one patient presented monoclonal granulocytes together with polyclonal T cells. We conclude that long-term remission in MDS following intensive chemotherapy is usually associated with polyclonal haemopoiesis.

225. Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation
Radsak, M., Iking-Konert, C., Stegmaier, S., Andrassy, K. And Hänsch, G.M.
Immunology, 101(4), 521-530 (2000)

Polymophonuclear cells (PMN) of healthy donors do not express major histocompatibility complex (MHC) class II antigens or the T-cell costimulatory molecules CD80 or CD86. Expression of these receptors, however, is seen in patients with chronic inflammatory diseases. We now report that, by culturing PMN of healthy donors with autologous serum, interferon-γ (IFN-γ) and granulocyte–macrophage colony-stimulating factor (GM-CSF), *de novo* synthesis of MHC class II, CD80 and CD86 could be induced. MHC class II-positive PMN acquired the capacity to present staphylococcus enterotoxin to peripheral T cells, apparent as induction of interleukin-2 (IL-2) synthesis and proliferation of the T cells. Moreover, the PMN also processed tetanus toxoid (TT) and induced proliferation of TT-specific T cells in a MHC class II-restricted manner. Taken together, these data indicate that PMN can be activated to function as accessory cells for T-cell activation.

226. Comparison of Tc-99m-labelled antileukocyte fragment Fab' and Tc-99m-HMPAO leukocyte scintigraphy in the diagnosis of bone and joint infections: a prospective study
Devillers, A. et al
Nuclear Med. Comm., 21(8), 747-753 (2000)

Between January and July 1998, we conducted a prospective study to compare Tc-99m-labelled antigranulocyte monoclonal antibody fragment Fab' (LEUKOSCAN(R)) scintigraphy versus Tc-99m-hexamethylpropyleneamine oxime (Tc-99m-HMPAO)-labelled leukocyte scintigraphy (HMPAO-LS) for the diagnosis of unselected patients with bone and joint infection.
Twenty-three patients (16 men and 7 women; mean age, 67 years) with suspected bone infection were explored successively with bone scintigraphy, HMPAO-LS and LEUKOSCAN(R) scintigraphy. Thirty-two foci were studied (diabetic foot = 11, prosthetic material = 8, joint disease = 4, others = diagnosed in 18 cases, eight on the basis of bacteriological and histological examination of surgical or puncture specimens, with or without radiographic signs, and 10 on the basis of clinical course and radiographic findings. Overall sensitivity, specificity and accuracy were 86%, 72% and 78%, respectively, for LEUKOSCAN(R) scintigraphy (12 true positives (TP), 13 true negatives (TN), 5 false positives (FP), 2 false negatives (FN)), 93%, 100% and 96%, respectively, for HMPAO-LS (13TP, 18TN, 0FP, 1FN), and 100%, 17% and 53.3%, respectively, for bone scintigraphy.
In this small series, LEUKOSCAN(R) scintigraphy was found to be less specific for the diagnosis of osteomyelitis than HMPAO-LS. In addition, the interpretation of LEUKOSCAN(R) scintigraphy is more difficult than HMPAO-LS for the diagnosis of bone infection in the diabetic foot, and would appear to be less discriminating for differentiating soft tissue infection from osteitis in the case of plantar perforating ulcers.

227. Increased nuclear factor [kappa]B activation in critically ill patients who die.
Paterson, R.L., Galley, H., Dhillon, J.K. and Webster, N.R.
Crit. Care Med., 28(4), 1047-1051 (2000)

Objectives: To determine nuclear factor [kappa]B (NF[kappa]B) activation in mononuclear and neutrophils from critically ill patients and to compare NF[kappa]B activation with circulating concentrations of interleukin (IL)-6, IL-8, and soluble intercellular adhesion molecule (sICAM)-1.
Design: Observational study.
Setting: University Teaching Hospital, eight-bed intensive care unit in northeast Scotland.
Patients: Ten patients admitted to the intensive care unit who fulfilled the criteria for systemic inflammatory response syndrome were studied at 0, 24, 48, and 72 hrs. Six healthy volunteers were also studied.
Interventions: None.
Measurements and Main Results: NF[kappa]B activation was significantly higher in patients compared to healthy volunteers in both neutrophils (p = .001) and mononuclear leukocytes (p = .013). In the six patients who survived to 96 hrs, the level of NF[kappa]B activation in mononuclear cells remained constant (p = .9). However, in the four patients who died before 96 hrs, mononuclear cell NF[kappa]B activation increased markedly and was significantly higher before death than in those who survived to 96 hrs (p = .0105). NF[kappa]B activation in neutrophils similarly remained constant in patients who survived to 96 hrs (p = .4) but did not show the same

increase before death. Circulating concentrations of IL-6, IL-8, and sICAM-1 were elevated but were unrelated to leukocyte NF[kappa]B activation.
Conclusions: We found NF[kappa]B activation in mononuclear and neutrophils in patients with systemic inflammatory response syndrome, which increased markedly before death in mononuclear leukocytes and was not related to plasma IL-6, IL-8, and sICAM-1 concentrations. These data support the need for further study of the role of NF[kappa]B activation in mortality from systemic inflammatory response syndrome and sepsis.

228. Ketamine Modulates the Stimulated Adhesion Molecule Expression on Human Neutrophils In Vitro
Weigand, M.A. et al
Anesthesia & Analgesia, 90(1), 206 (2000)

Cytokine production, neutrophil adhesion to endothelial cells, and release of reactive oxygen species are thought to be critical events in sepsis or ischemia/reperfusion. Modulation of leukocyte responses by anesthetics may have an important role in limiting tissue injury under these conditions. Therefore, we investigated the effect of ketamine on the expression of CD18, CD62L, and oxygen radical production of human neutrophils in vitro and on interleukin-6 production in endotoxin-stimulated human whole blood. Ketamine inhibited both the N- formyl-methionyl-leucyl-phenylalanine- and phorbol 12-myristate 13-acetate-induced up-regulation of CD18 and shedding of CD62L, determined by flow cytometry, in a concentration-dependent manner. Ketamine also caused a significant suppression of oxygen radical generation of isolated human neutrophils. In addition, there was a significant decrease in endotoxin-stimulated interleukin-6 production in human whole blood. The inhibitory effects were similar for racemic ketamine and its isomers S(+)-ketamine and R(-)-ketamine, suggesting that the inhibition of stimulated neutrophil function is most likely not mediated through specific receptor interactions.
Implications: Modulation of leukocyte responses by anesthetics may have an important role in limiting tissue injury in sepsis or ischemia/reperfusion. Therefore, we examined the effect of ketamine on stimulated neutrophil functions in vitro. These neutrophil functions were significantly inhibited by ketamine, independent of whether the racemic mixture or isomers were tested.

229. Mediation by platelet-activating factor of 12-hydroxyeicosatetraenoic acid-induced cytosolic free calcium concentration elevation in neutrophils
Shibata, K. et al
Prostaglandins & Other Lipid Mediators, 62(4), 385-394 (2000)

12(R)-hydroxyeicosatetraenoic acid (HETE) shows biphasic increase in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in rabbit and human neutrophils; the initial transient phase and the continuous falling phase. 12(S)-HETE was less potent in both species. BN50739, a platelet-activating factor (PAF) receptor antagonist, inhibited both phases of 12(R)-HETE-induced $[Ca^{2+}]_i$ rise but did not affect leukotriene B_4 (LTB_4)-induced $[Ca^{2+}]_i$ rise. N-tosyl-L-phenylalanine chloromethyl ketone (TPCK), a PAF synthesis inhibitor, and manoalide, a phospholipase A_2 inhibitor, reduced 12(R)-HETE-induced $[Ca^{2+}]_i$ rise. These blockers inhibited the continuous phase of $[Ca^{2+}]_i$ rise induced by N-formyl-methionyl-leucyl-phenylalanine (FMLP) with little effect on the initial phase. It had no significant effect on LTB_4-induced $[Ca^{2+}]_i$ rise. SC-41930, a LTB_4-receptor antagonist, did not block 12-HETE-induced $[Ca^{2+}]_i$ rise. In 12(R)-HETE-, FMLP- and LTB_4-stimulated cells, accumulations of cell-associated PAF and released PAF were detected but not in unstimulated cells. BN50739 did not affect the accumulation of cell-associated PAF and release of PAF in 12(R)-HETE-stimulated cells. These results suggest that 12(R)-HETE-induced and partially, FMLP-induced, but not LTB_4-induced $[Ca^{2+}]_i$ rise are mediated by PAF, which is produced and released by stimulation of the cells by 12(R)-HETE and FMLP, respectively.

230. DNA breakage detection-fish (DBD-FISH): effect of unwinding time
Vazquez-Gundin, F., Gosalvez, J., de la Torre, J. and Fernandez, J.L.
Mutation Res./Fundamental Mol. Mechanisms of Mutagenesis, 453(1), 83-88 (2000)

DBD-FISH is a new procedure that allows detection and quantification of DNA breakage in situ within specific DNA target sites. Cells embedded in an agarose matrix on a slide are treated in an alkaline unwinding solution to transform DNA breaks into single-stranded DNA (ssDNA). After removal of proteins, DNA probes are hybridized and detected. DNA breaks increase the ssDNA and relax supercoiling of DNA loops, so more probe hybridizes, thereby increasing the surface area and fluorescence intensity of the FISH signal. The probe selects the chromatin area to be analysed.
In order to restrict the extension of unwound ssDNA to a region closer to the origin of the DNA break, human leukocytes were processed for DBD-FISH with a whole genome probe, after a 10 Gy dose of X-rays, for various unwinding times: 5, 2 min and 30 s. Two cell populations were detected after 30 s, but not with the 5 or 2 min unwinding times. One cell group had small to medium haloes corresponding to the relaxation of DNA supercoiling after DAPI staining, and strong DBD-FISH labelling of induced DNA breaks, whereas the other cell group showed big haloes of DNA loop unfolding and an absence of DBD-FISH labelling. The latter group was similar to cells processed by DBD-FISH without the unwinding step. Thus, they should correspond to cells unaffected by the alkaline unwinding solution, possibly because very brief unwinding times do not allow the diffusion of the alkali into the cells deep within the

gel, thus biasing the results. Taking this into account, 2 min seems to be the minimum unwinding time required for an accurate detection of a signal by DBD-FISH.

231. Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics
Varga, A., Hansson, P., Johnson, G. and Alling, C.
Clin. Chem. Acta, 299(1-2), 141-150 (2000)

Phosphatidylethanol (PEth) is an abnormal phospholipid which is formed in the presence of ethanol, via the action of phospholipase D (PLD). PEth in blood is a potential marker of alcohol abuse. The present study was made to determine the compartmentalization and the elimination rate of PEth in human whole blood. PEth was assayed by an improved HPLC technique, with evaporative light-scattering detection. Blood from six alcoholic males was separated into different blood cell fractions. The PEth concentration in whole blood was 2.5±0.9 and 1.9±1.1 μmol/l in erythrocytes. Only one subject had detectable PEth in the mononuclear cells. Fifteen patients (13 men, two women) with chronic alcoholism, were followed as inpatients, after admission to an alcohol detoxification clinic. PEth, carbohydrate-deficient transferrin (CDT) and γ-glutamyltransferase (GGT) were measured on days 1, 3, 5 and 7 after admission. Linear regression analysis of logarithmic PEth values in individuals, with measurable PEth at day 1, gave a good fit (P<0.001) with the one-compartment elimination model. The half-life was calculated as 4.0±0.7 days. A weak significance (P<0.05) was observed in the correlation of PEth at day 1 and half-life values of the same subjects.

232. Investigation of vascular endothelial growth factor effects on pulmonary endothelial monolayer permeability and neutrophil transmigration
Cullen, V.C., Mackarel, A.J., Hislip, S.J., O'Connor, C.M. and Keenan, A.K.
Gen. Pharmacol.: The Vascular System, 35(3), 149-157 (2000)

This study sought to determine whether vascular endothelial growth factor (VEGF)-induced permeabilisation of pulmonary endothelium to macromolecules could be related to a permissive role for neutrophil-derived VEGF in neutrophil transmigration. Treatment of human pulmonary artery endothelial cell (HPAEC) monolayers with 1, 10 or 100 ng/ml VEGF for 15 min or 1, 10 ng/ml for 90 min significantly increased endothelial permeability to trypan blue-labelled albumin (TB-BSA). These increases were correlated with changes in the cellular distribution of F-actin, as visualised by rhodamine–phalloidin staining: increased stress fibre formation, cellular elongation and formation of intercellular gaps after 15 min; at 90 min, there was also evidence of microspike formation and extension of spindle processes from the cell surface. Treatment of human neutrophil suspensions with 200 nM phorbol myristyl acetate (PMA), *n*-formyl-methionyl leucylphenylalanine (fMLP, 10 nM), interleukin-8 (IL-8, 10 nM) (but not with leukotriene B_4 (LTB_4) 100 nM), for 30 min caused significant extracellular release of neutrophil VEGF stores. A permissive role for neutrophil-derived VEGF in facilitating migration across HPAEC monolayers was assessed in experiments using a functional blocking antihuman VEGF antibody. In the presence of this antibody (10 μg/ml), neutrophil migration in response to fMLP (10 nM), IL-8 (10 nM) or LTB_4 (100 nM) was not significantly different to that in the absence of antibody. We conclude that neutrophil-derived VEGF does not play a functional role in facilitating neutrophil migration across pulmonary vascular endothelium, despite its ability to induce cytoskeletal changes and enhance endothelial macromolecular permeability.

233. Parasite Evasion Mechanism Altered by Zinc in Entamoeba histolytica
Vega-Robledo, G.B., Leandro, E. and Rico, G.
Arc. Med. Res., 31(4), Suppl. 1, S106-S107 (2000)

During the invasive process, amebas, like many other parasites, use mechanisms that alter or evade the host defense system [1]. We have previously reported that zinc inhibits replication and adherence of *Entamoeba histolytica* and diminishes hepatic abscess development in golden hamsters (*Mesocricetus aureatus*) [2 and 3]. As the effect of zinc might induce a loss of the deleterious action of *E. histolytica* on cells participating in the host immune response, the aim of this study was to evaluate the effect of the functional changes induced by zinc on *E. histolytica* in human polymorphonuclear cells (PMN).

234. Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents
Tan, A.S. and Berridge, M.V.
J. Immunol. Methods, 238(1-2), 59-68 (2000)

Activation of the respiratory burst of granulocytes and macrophages by invading microorganisms is a key first line cellular defence against infection. Failure to generate this response leads to persistent life-threatening infection unless appropriate antibiotic treatment is given. The respiratory burst of neutrophils is usually measured spectrophotometrically by following ferricytochrome c reduction, and histologically by using the tetrazolium salt, nitroblue tetrazolium, which is reduced intracellularly to an insoluble formazan. In both assays, reduction is mediated by superoxide generated via NADPH oxidase. Because ferricytochrome c has a high molecular mass and high background absorbance at 550 nm, the assay lacks sensitivity and is not ideally suited to microplate measurement. We have circumvented these limitations by using the cell-impermeable, sulfonated tetrazolium salt, WST-1, which exhibits very low background

absorbance and is efficiently reduced by superoxide to a stable water-soluble formazan with high molar absorptivity. This has permitted adaptation of the WST-1 assay to microplate format while retaining sensitivity. Reduction of WST-1 by activated human peripheral blood neutrophils correlated closely with ferricytochrome c reduction across a range of PMA concentrations and with time of activation by PMA and fMLP. Reduction of WST-1 was inhibited by 98% by superoxide dismutase (20 μg/ml) and by 88% by the NADPH oxidase inhibitor, diphenyleneiodinium (10 μM) but was resistant to catalase, azide and the NADH oxidase inhibitor, resiniferatoxin. WST-1 and ferricytochrome c reduction were also compared using xanthine/xanthine oxidase to generate superoxide. Under optimised assay conditions, both WST-1 and ferricytochrome c reduction were directly proportional to added xanthine. WST-1 generated approximately 2-fold greater increase in absorbance than ferricytochrome c at their respective wavelengths, and this translated into increased assay sensitivity. Addition of the intermediate electron acceptor, 1-methoxy phenazine methosulfate, increased the background of the neutrophil assay but did not affect the overall magnitude of the response. We have used the WST-1 assay to assess human neutrophil dysfunction and to compare anti-inflammatory activity.

235. Human tumour and dendritic cell hybrids generated by electrofusion: potential for cancer vaccines
Scott-Taylor, T.H. et al
Biochim. Biophys. Acta, 1500(3), 265-279 (2000)

Hybrid cells created by fusion of antigen presenting and tumour cells have been shown to induce potent protective and curative anti-tumour immunity in rodent cancer models. The application of hybrid cell vaccines for human tumour therapy and the timely intervention in disease control are limited by the requirement to derive sufficient autologous cells to preserve homologous tumour antigen presentation. In this study, the efficiency of various methods of electrofusion in generating hybrid human cells have been investigated with a variety of human haemopoietic, breast and prostate cell lines. Cell fusion using an electrical pulse is enhanced by a variety of stimuli to align cells electrically or bring cells into contact. Centrifugation of cells after an exponential pulse from a Gene Pulser electroporation apparatus provided the highest yield of mixed cell hybrids by FACS analysis. An extensive fusogenic condition generated in human cells after an electrical pulse contradicts the presumption that prior cell contact is necessary for cell fusion. Alignment of cells in a concurrent direct current charge and osmotic expansion of cells in polyethylene glycol also generated high levels of cell fusion. Waxing of one electrode of the electroporation cuvette served to polarise the fusion chamber and increase cell fusion 5-fold. Optimisation of a direct current charge in combination with a fusogenic pulse in which fusion of a range of human cells approached or exceeded 30% of the total pulsed cells. The yield of hybrid prostate and breast cancer cells with dendritic cells was similar to the homologous cell fusion efficiencies indicating that dendritic cells were highly amenable to fusion with human tumour cells under similar electrical parameters. Elimination of unfused cells by density gradient and culture is possible to further increase the quantity of hybrid cells. The generation and purification of quantities of hybrid cells sufficient for human vaccination raises the possibility of rapid, autologous tumour antigen presenting vaccines for trial with common human tumours.

236. Identification of lysophospholipid receptors in human platelets: the relation of two agonists, lysophosphatidic acid and sphingosine 1-phosphate
Motohashi, K., Shibata, S., Ozaki, Y., Yatomi, Y. and Igarashi, Y.
FEBS Lett., 468(2-3), 189-193 (2000)

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (Sph-1-P) are known as structurally related bio-active lipids activating platelets through their respective receptors. Although the receptors for LPA and Sph-1-P have been recently identified in various cells, the identification and characterization of ones in platelets have been reported only preliminarily. In this report, we first investigated the distinct modes of LPA and Sph-1-P actions in platelet activation and found that LPA functioned as a much stronger agonist than Sph-1-P, and high concentrations of Sph-1-P specifically desensitized LPA-induced intracellular Ca^{2+} mobilization. In order to identify the responsible receptors underlying these observations, we analyzed the LPA and Sph-1-P receptors which might be expressed in human platelets, by RT-PCR. We found for the first time that Edg2, 4, 6 and 7 mRNA are expressed in human platelets.

237. LTA4-derived 5-oxo-eicosatetraenoic acid: pH-dependent formation and interaction with the LTB4 receptor of human polymorphonuclear leukocytes
Falgueyret, J-P. and Riendeau, D.
Biochim. Biophys. Acta, 1484(1), 51-58 (2000)

5-Oxo-(7*E*,9*E*,11*Z*,14*Z*)-eicosatetraenoic acid (5-oxo-ETE) has been identified as a non-enzymatic hydrolysis product of leukotriene A_4 (LTA_4) in addition to 5,12-dihydroxy-(6*E*,8*E*,10*E*,14*Z*)-eicosatetraenoic acids (5,12-diHETEs) and 5,6-dihydroxy-(7*E*,9*E*,11*Z*,14*Z*)-eicosatetraenoic acids (5,6-diHETEs). The amount of 5-oxo-ETE detected in the mixture of the hydrolysis products of LTA_4 was found to be pH-dependent. After incubation of LTA_4 in aqueous medium, the ratio of 5-oxo-ETE to 5,12-diHETE was 1:6 at pH 7.5, and 1:1 at pH 9.5. 5-Oxo-ETE was isolated from the alkaline hydrolysis products of LTA_4 in order to evaluate its effects on human polymorphonuclear (PMN) leukocytes. 5-Oxo-ETE induced a rapid and dose-dependent mobilization of calcium in PMN leukocytes with an EC_{50} of 250 nM, as compared to values of 3.5 nM for leukotriene B_4 (LTB_4) and >500 nM for 5(*S*)-hydroxy-(6*E*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid (5-HETE). Pretreatment of the cells with LTB_4 totally abolished the calcium response induced by 5-oxo-ETE. In contrast, the preincubation with 5-oxo-ETE did not affect the calcium mobilization induced by LTB_4. The calcium response induced by

5-oxo-ETE was totally inhibited by the specific LTB_4 receptor antagonist LY223982. These data demonstrate that 5-oxo-ETE can induce calcium mobilization in PMN leukocyte via the LTB_4 receptor in contrast to the closely related analog 5-oxo-(6*E*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid which is known to activate human neutrophils by a mechanism independent of the receptor for LTB_4.

238. Superoxide possibly produced in endothelial cells mediates the neutrophil-induced lung injury
Tanita, T. et al
Ann. Thorac. Surg., 69(2), 402-407 (2000)

Background. The mechanism by which stimulated neutrophils (polymorphonuclear leukocytes [PMNs]) damage pulmonary vascular endothelium was investigated.
Methods. The ability of unstimulated and mechanically stimulated PMNs to adhere to pulmonary endothelial cells and, thereby, alter pulmonary vascular permeability was tested. Each series was conducted on 6 rats. To stimulate PMNs, they were agitated gently in a glass vial for 10 seconds.
Results. Perfusing lungs with the stimulated PMNs elicited a fivefold increase in permeability compared with lungs perfused with the unstimulated cells. This increase in permeability was blocked completely by preincubation of stimulated PMNs with CD18 monoclonal antibody. This increase in permeability was also blocked completely by superoxide dismutase (SOD) or the xanthine oxidase (XO) inhibitor allopurinol. Pulmonary vascular hemodynamics were unaffected by any treatment protocol. The accumulation of stimulated PMNs within the lungs was not inhibited by SOD but was partially blocked by allopurinol.
Conclusions. These findings suggest that stimulated PMN-induced increases in pulmonary vascular filtration resulted from endothelial cell injury caused by superoxide anion possibly generated by XO, exclusively present in the endothelial cells.

239. Cloning and quantitative determination of the human Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) isoforms in human beta cells
Rochlitz, H. et al
Diabetologia, 43(4), 465-473 (2000)

The Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) is highly expressed in pancreatic islets and associated with insulin secretion vesicles. The suppression of CaMK II disturbs insulin secretion and insulin gene expression. There are four isoforms of CaMK II, f to i, that are expressed from different genes in mammals. Our aim was to identify the isoforms of CaMK II expressed in human beta cells by molecular cloning from a human insulinoma cDNA library and to assess its distribution in humans.¶Methods. The previously unknown complete coding sequences of human CaMK IIg and the kinase domain of CaMK IIi were cloned from a human insulinoma cDNA library. Quantitative determination of CaMK II isoform mRNA was carried out in several tissues and beta cells purified by fluorescence activated cell sorting and compared to the housekeeping enzyme pyruvate dehydrogenase.¶Results. We found CaMK IIg occurred in three splice variants and was highly expressed in endocrine tissues such as adrenals, pituitary and beta cells. Liver showed moderate expression but adipose tissue or lymphocytes had very low levels of CaMK IIg-mRNA. In human beta cells CaMK IIg and i were expressed equally with pyruvate dehydrogenase whereas tenfold lower expression of CaMK IIn and no expression of CaMK IIf were found.¶Conclusion/interpretation. Although CaMK IIi is ubiquitously expressed, CaMK IIg shows preferential expression in neuroendocrine tissues. In comparison with the expression of a key regulatory enzyme in glucose oxidation, pyruvate dehydrogenase, two of the four CaM kinases investigated are expressed at equally high levels, which supports an important role in beta-cell physiology. These results provide the basis for exploring the pathophysiological relevance of CaMK IIg in human diabetes.

240. Tyrosine phosphorylation of cytoplasmic proteins in proliferating, differentiating, apoptotic HL-60 cells and blood neutrophils
Treigyte, G., Navakauskiene, R., Kulyte, A., Gineitis, A. and Magnusson, K-E.
Cell. Mol. Life Sci., 57(13-14), 1997-2008 (2000)

No abstract available

241. Differentiation of polymorphonuclear neutrophils in patients with systemic infections and chronic inflammatory diseases: evidence of prolonged life span and de novo synthesis of fibronectin
Wagner, C. et al
J. Mol. Med., 78(6), 337-345 (2000)

Polymorphonuclear neutrophils (PMN) are considered to be short-lived, terminally differentiated cells undergoing spontaneous apoptosis if not appropriately stimulated. In patients with systemic infections and inflammatory disease, however, PMN have an extended life span and acquire new surface receptors and functions. Expression of CD64, the high-affinity receptor for immunoglobulin, has been found, and functionally active elastase and surface-associated fibronectin as well. The latter is of particular interest since fibronectin is known as a multifunctional, multimodal extracellular matrix protein, participating in cell adherence, cell signaling, and cell cycle control. To study the surface-associated fibronectin further, PMN of healthy donors were cultivated to induce de novo synthesis of fibronectin. PMN produced fibronectin, which remained associated with the cell surface, where it was partially cleaved.

PMN derived fibronectin exhibited a rare splice pattern: predominantly fibronectin containing the extradomain B (EDB) was generated, but evidently no IIICS domain; the latter is known as a receptor for $\#_1$ integrins. How the presence of EDB affects the properties of fibronectin is not yet understood. Studies with recombinant EDB have failed to show a membrane-binding site or a direct participation of EDB in the adhesion process. The function of PMN-associated fibronectin is still under investigation. The rapid cleavage by surface-associated proteases suggests that fibronectin acts as a tightly regulated adhesion protein, and probably also as a precursor molecule for fibronectin-derived biologically active mediators.

242. Aberrant expression of the major sialoglycoprotein (CD43) on the monocytes of patients with myelodysplastic syndromes
Kyriakou, D et al
Ann. Hematol., 79(4), 198-205 (2000)

CD43, a sialylated glycoprotein expressed on the surface of most hematopoietic cells, has been implicated in cell adhesion and signaling. The reduced expression of this antigen in patients with WiscottAldrich syndrome, in which progressive immunodeficiency is a major problem, raised the question whether abnormal expression of this molecule could affect the susceptibility to infections in patients with myelodysplastic syndromes (MDS). We studied the expression of this antigen on the monocytes of ten patients with chronic myelomonocytic leukemia (CMML) and compared the results with 67 patients suffering from other MDS syndromes and with 18 healthy individuals. We chose this series as it plays an important role in MDS patients where in most cases the neutrophils are defective. We also examined the following antigens as indicative of activation and adhesion of the monocytes in these patients: CD11b, CD18, CD35, CD38, CD44, CD69. We found decreased expression of CD43 on the monocytes of the RA, RAS, RAEB, and RAEB-t patients compared with the CMML and controls. The other activation molecules studied were found to be upregulated, suggesting the existence of activated monocytes in these patients. The increased levels of soluble vascular cell adhesion molecule in these patients suggest vascular endothelial activation in the absence of infection. Further experiments are needed to investigate the significance of CD43 downregulation in these patients, its role in cell adherence and tissue migration, and the correlation of the phenomenon to the increased susceptibility to infections observed in these patients.

243. Role of platelets in tissue factor expression by monocytes in normal and hypercholesterolemic subjects. In vitro effect of cerivastatin
Puccetti, L. et al
Int. J. Clin. Lab. Res., 30(3), 147-156 (2000)

Thrombosis is a complication of atherosclerosis and monocytes play a determinant role either in the progression of atherosclerotic plaque or in blood coagulation by way of tissue factor expression. Platelets play a direct role in thrombosis and a hyperfunctional state has been described in hypercholesterolemic subjects. Moreover, platelets seem to be able to enhance monocyte activity. Cholesterol-lowering molecules (statins) are reported to reduce cardiovascular risk, either by decreasing the circulation level of cholesterol or by non-lipidic actions such as the reduction of monocyte and platelet activity. The aim of our study was to investigate the influence of platelets on the expression of tissue factor by monocytes and the effect induced by cerivastatin. We measured tissue factor levels by ELISA and the procoagulant activity of stimulated monocytes by a clotting assay on cellular preparations and whole blood in 40 hypercholesterolemic subjects (22 male, 18 female, mean age 52.7-12 years, total cholesterol 251.6-19.9 mg/dl) before and after cerivastatin addition. Tissue factor expression was enhanced in hypercholesterolemic subjects compared with normal subjects (31.6-7.6 vs. 23-5.8 pg/cells, $P<0.01$). The presence of platelets increased the amount of tissue factor (55.3-7.3 pg/cells, $P<0.001$) and cerivastatin reduced the expression of tissue factor in isolated monocytes, in the mixed cellular system, and in whole blood (19.4-4.1 pg/cells, $P<0.001$). In conclusion, tissue factor expression by monocytes is enhanced in hypercholesterolemic subjects compared with normal controls. Platelets enhance monocyte production of tissue factor, and cerivastatin is able to counteract this prothrombotic mechanism.

244. Effect of Granulocyte Colony-Stimulating Factor Treatment on *Ex Vivo* Neutrophil Functions in Nonneutropenic Surgical Intensive Care Patients
Gerber, A. et al
J. Interferon & Cytokine Res., 20(12), 1083-1090 (2000)

Granulocyte colony-stimulating factor (G-CSF) preferentially stimulates growth and differentiation of neutrophil precursors and activates neutrophil functions. The aim of the present study was to investigate the functional response of the neutrophil to exogenous recombinant human G-CSF (rHuG-CSF) in nonneutropenic patients. In 30 surgical intensive care unit patients with severely impaired wound healing, leukocyte differential count, plasma G-CSF level, and a broad spectrum of neutrophil functions were monitored before (day 0), throughout (days 1 and 5), and at days 1 and 5 after stopping G-CSF treatment. G-CSF application resulted in a 3.5-fold increase in peripheral blood granulocyte count at day 5 of treatment. The mean plasma G-CSF level rose from 48 to a maximum of 2314 pg/ml at day 1 of G-CSF therapy. Neutrophil chemotaxis and stimulated lysozyme release were decreased throughout G-CSF treatment, whereas respiratory burst activity, phagocytic activity, and intracellular calcium concentration were enhanced by G-CSF. Neutrophil membrane depolarization remained unaffected. The increased count and activation state of neutrophils were associated with clinical improvement in most of these patients. Thus, G-CSF may be a useful adjuvant treatment for nonneutropenic patients with severely impaired wound healing.

245. Altered airway surfactant phospholipid composition and reduced lung function in asthma
Wright, S.M. et al
J. Appl. Physiol., 89, 1283-1292 (2000)

Pulmonary surfactant in bronchoalveolar lavage fluid (BALF) and induced sputum from adults with stable asthma ($n = 36$) and healthy controls ($n = 12$) was analyzed for phospholipid and protein compositions and function. Asthmatic subjects were graded as mild, moderate, or severe. Phospholipid compositions of BALF and sputum from control subjects were similar and characteristic of surfactant. For asthmatic subjects, the proportion of dipalmitoyl phosphatidylcholine (16:0/16:0PC), the major phospholipid in surfactant, decreased in sputum ($P < 0.05$) but not in BALF.[1] In BALF, mole percent 16:0/16:0PC correlated with surfactant function measured in a capillary surfactometer, and sputum mole percent 16:0/16:0PC correlated with lung function (forced expiratory volume in 1 s). Neither surfactant protein A nor total protein concentration in either BALF or sputum was altered in asthma. These results suggest altered phospholipid composition and function of airway (sputum) but not alveolar (BALF) surfactant in stable asthma. Such underlying surfactant dysfunction may predispose asthmatic subjects to further surfactant inhibition by proteins or aeroallergens in acute asthma episodes and contribute to airway closure in asthma. Consequently, administration of an appropriate therapeutic surfactant could provide clinical benefit in asthma.

246. Comparison of azithromycin leukocyte disposition in healthy volunteers and volunteers with AIDS
McNAbb, J.C. et al
Int. J. Antimicrob. Agents., 16(1), 37-43 (2000)

Azithromycin, has been proved to be effective in the treatment and prophylaxis of a wide variety of infections. While the penetration of azithromycin into a number of types of mammalian cells has been well characterized, the influence of HIV infection on the intracellular disposition of this agent has not been studied. We therefore studied the disposition of azithromycin in polymorphonuclear (PMN) and mononuclear (MONO) leukocytes from six healthy volunteers and six volunteers with AIDS. After oral administration of a single 1200-mg dose of azithromycin (two 600-mg tablets), blood samples were collected over 6 days and intracellular azithromycin concentrations in MONOs and PMNs were measured. Analysis of the intracellular pharmacokinetics revealed an apparent difference in the MONO and PMN profile; this profile was similar for both groups. Intracellular concentrations of azithromycin remained high throughout the study period. Furthermore, no statistically significant differences in the intracellular area under the curve (11 309±2543 vs. 16 650±6254 for PMN; 14 180±3802 vs. 21 211±10 001 for MONO) were observed between the healthy and AIDS populations, respectively. Our data confirm the extensive uptake of azithromycin by white blood cells both in healthy volunteers and in AIDS patients.

247. Ceramides that Mediate Apoptosis Reduce Glucose Uptake and Transporter Affinity for Glucose in Human Leukaemic Cell Lines but Not in Neutrophils
Ahmed, N. and Berridge, M.V.
*Pharmacol. Toxicol., **86(3)**, 114-121 (2000)*

We have demonstrated that CD95-induced apoptosis in a human leukaemic T-cell line resulted in loss of glucose transporter function (Berridge et al. 1996). To determine whether ceramide, a mediator of CD95 and tumour necrosis factor-[alpha]-induced apoptosis, has similar effects on glucose transport, the human leukaemic cell lines, Jurkat and U937, and human peripheral blood neutrophils were treated with ceramide or sphingomyelinase and the effects on glucose transport determined by measuring [3H]-2-deoxyglucose uptake. We show that in U937 and Jurkat cells, the cell permeable ceramides, C2 (N-acetylsphingosine) and C6 (N-hexanoylsphingosine) inhibit glucose uptake within minutes of initiating ceramide treatment, 60-70% inhibition being observed within 2 hr. Loss of glucose transport correlated with loss of proliferative response, but metabolic activity as measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction, was affected to a much lesser extent. With Jurkat and U937 cells, the inhibitory effects of ceramides on glucose transport were associated with reduced affinity of glucose transporters for glucose (Km). Similar effects were observed with sphingomyelinase. With human peripheral blood neutrophils, C2 and C6-ceramides inhibited glucose uptake by 70-80% within 30 min. without affecting transporter affinity for glucose, but the maximum velocity of uptake (Vmax) was reduced. These results show that acute regulation of glucose transport is an early effector mechanism of cell death induced by ceramides in human leukaemic cell lines and peripheral blood neutrophils. This is the first study, which describes ceramide-induced early physiological/biochemical events leading to cell death in human cells.

248. Naive Monocytes Can Trigger Transendothelial Migration of Peripheral Blood Cells Through the Induction of Endothelial Tumour Necrosis Factor-[alpha]
Eissner, G. et al
*Scand. J. Immunol., **51(3)**, 251-261 (2000)*

In this manuscript we describe a potentially new mechanism by which unstimulated human monocytes activate endothelial cells (EC) through the secondary induction of endothelial tumour necrosis factor alpha (TNF-[alpha]). Serum free supernatants (SN) of peripheral blood mononuclear cells (PBMC) strongly induce the expression of intercellular adhesion molecule 1 (ICAM-1, CD54), vascular cell

adhesion molecule 1 (VCAM-1, CD106), and endothelial-leukocyte adhesion molecule 1 (ELAM-1, CD62E) on human EC 24 and 4 h post treatment, respectively. Further characterization of the responsible subpopulation revealed the CD14+ monocytes and a monocytic cell line (MM6) to produce an endothelial activating factor (EAF). The EAF also triggers an adhesion and a transendothelial migration (TEM) of peripheral blood cells. Using neutralization with an anti TNF-[alpha] MoAb MAK195, EAF is not identical with TNF-[alpha], but induces the expression of endothelial TNF-[alpha], since MAK195 blocked TEM only when coincubated with EC, not with monocytes. Furthermore, intracellular TNF-[alpha] was significantly upregulated in EC after treatment with SN-MM6. Another evidence for a secondary autocrine mechanism was provided by culturing the EC with a conditioned medium of SN-MM6 treated EC. This conditioned medium induces an adhesion molecule expression and TEM in a similar way to SN-MM6 and can completely be inactivated by anti TNF-[alpha]. Taken together, these data may have an impact for, e.g. transplantational settings that donor monocytes may trigger an inflammatory response in the absence of further activation signals by eliciting an endogenous TNF-[alpha] response in the host.

249. Construction of humanized anti-ganglioside monoclonal antibodies with potent immune effector functions
Nakamura, K., Tanaka, Y., Shitara, K. and Hanai, N.
Cancer Immunol. Immunother., 50(5), 275-284 (2001)

Gangliosides GD3, GD2 and GM2, which are the major gangliosides expressed on most human cancers of neuroectodermal and epithelial origin, have been focused on as effective targets for passive immunotherapy with monoclonal antibodies. We previously developed a chimeric anti-GD3 mAb, KM871, and a humanized anti-GM2 mAb, KM8969, which specifically bound to the respective antigen with high affinity and showed potent immune effector functions. Humanization of anti-ganglioside antibody is expected to enhance its use for human cancer therapy. In the present study, we generated a chimeric anti-GD2 mAb, KM1138, and further developed the humanized form of anti-GD2 and anti-GD3 mAbs by the complementarity-determining regions grafting method. The resultant humanized anti-GD2 mAb, KM8138, and anti-GD3 mAb, KM8871, showed binding affinity and specificity similar to those of their chimeric counterparts. In addition, both humanized mAbs had functional potency comparable to the chimeric mAbs in mediating the immune effector functions, consisting of antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. The production of these humanized anti-ganglioside mAbs, with potent effector functions and low immunogenicity, precedes the evaluation of the therapeutic value of anti-ganglioside mAbs in passive immunotherapy and the target validation for ganglioside-based vaccine therapy.

250. Transdifferentiation of polymorphonuclear neutrophils: acquisition of CD83 and other functional characteristics of dendritic cells
Iking-Koenert, C. Et al
J. Mol. Med., 79(8), 464-474 (2001)

Polymorphonuclear neutrophils (PMN) are in the first line of defense against bacterial infections. They are considered to be end-differentiated cells undergoing constitutive apoptosis within hours after release from the bone marrow. During pathological events, however, their life span is extended in conjunction with morphological and functional alterations indicative of a transdifferentiation of mature PMN. To further characterize differentiated PMN, the alterations seen in vivo were reproduced by cultivating PMN of healthy donors with either %-interferon, granulocyte/macrophage colony stimulating factor, or a combination thereof. Thus cultivated cells escaped from apoptosis, and protein synthesis was induced, notably of the major histocompatibility complex (MHC) class II antigens, CD80 and CD86. Moreover, CD83, thought to be specific for dendritic cells was synthesized, while typical markers of PMN, including CD66b, CD11a/CD11b/CD11c, CD15, CD18 were preserved. A profound alteration of both cellular morphology and of function was seen: the cultivated PMN lost their chemotactic activity but had acquired the ability to present to T-cells a peptide antigen in a MHC class II restricted manner. The data lead to the conclusion that mature PMN can differentiate further to cells with characteristics of DCs, thereby connecting PMN to the specific T-cell response.

251. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise
Miyazaki, H. et al
Eur. J. Appl. Physiol., 84(1-2), 1-6 (2001)

The aim of this study was to evaluate whether high-intensity endurance training would alleviate exercise-induced oxidative stress. Nine untrained male subjects (aged 19-21 years) participated in a 12-week training programme, and performed an acute period of exhausting exercise on a cycle ergometer before and after training. The training programme consisted of running at 80% maximal exercise heart rate for 60 min · day^{m1}, 5 days · week^{m1} for 12 weeks. Blood samples were collected at rest and immediately after exhausting exercise for measurements of indices of oxidative stress, and antioxidant enzyme activities [superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT)] in the erythrocytes. Maximal oxygen uptake ($V£O_{2max}$) increased significantly ($P < 0.001$) after training,

indicating an improvement in aerobic capacity. A period of exhausting exercise caused an increase ($P < 0.01$) in the ability to produce neutrophil superoxide anion ($O_2^{\cdot m}$) both before and after endurance training, but the magnitude of the increase was smaller after training ($P < 0.05$). There was a significant increase in lipid peroxidation in the erythrocyte membrane, but not in oxidative protein, after exhausting exercise, however training attenuated this effect. At rest, SOD and GPX activities were increased after training. However, there was no evidence that exhausting exercise enhanced the levels of any antioxidant enzyme activity. The CAT activity was unchanged either by training or by exhausting exercise. These results indicate that high-intensity endurance training can elevate antioxidant enzyme activities in erythrocytes, and decrease neutrophil $O_2^{\cdot m}$ production in response to exhausting exercise. Furthermore, this up-regulation in antioxidant defences was accompanied by a reduction in exercise-induced lipid peroxidation in erythrocyte membrane.

252. Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency in lung cancer patients and population controls
Hou, S-M. et al
Cancer Epidemiol. Biomarkers Prev., 10, 133 (2001)

We have studied the influence of *GSTM1* and *NAT2* genotypes on aromatic DNA adduct level (AL) and *HPRT* mutant frequency (MF) in smokers with newly diagnosed lung cancer and matched population controls. AL was analyzed in relation to genotypes in 170 cases and 144 controls (113 current/recent smokers and 201 former/never smokers), and MF in 157 cases and 152 controls (155 ever smokers and 154 never smokers). Both genotypes exhibited the *a priori* expected effects on AL and MF among controls only, especially among smoking controls [significantly lower pack-years (a pack-year is defined as 1 pack of cigarettes/day for 1 year) than among cases]. Among the 42 currently smoking controls, the *NAT2* slow genotype [odds ratio (OR), 7.5; 95% confidence interval (CI), 1.5–38.4], in particular in combination with the *GSTM1* null genotype (OR, 19.3, 95% CI, 1.1–338.6 for null/slow *versus* positive/rapid) was strongly associated with high AL. The null/slow combination was also significantly associated with high MF among ever smokers (cases and controls pooled) with lower pack-years (OR, 3.7; 95% CI, 1.3–10.7 *versus* all of the other genotypes; OR, 5.1; 95% CI, 1.2–22.4 *versus* positive/rapid). In contrast, an antagonistic gene-gene interaction was seen among smoking cases for both AL and MF. Only currently smoking cases with the combined *GSTM1* null and *NAT2* rapid genotype showed a positive correlation between lnAL and lnMF (r, 0.64; $P = 0.1$), and an increase of AL with both age and daily cigarette use. This genotype combination was also associated with high MF among ever-smoking cases (OR, 4.0; 95% CI, 0.9–17.7 *versus* positive/rapid). There was a significant interaction between *NAT2* genotype and pack-years of smoking among cases, so that the rapid genotype was associated with high MF among ever-smoking cases diagnosed at higher pack-years, whereas the slow genotype was associated with high MF at lower pack-years. These findings suggest that the influence of *NAT2* genotype on AL and MF and its interaction with *GSTM1* genotype may be dose dependent. The *NAT2* slow genotype, in particular when combined with the *GSTM1* null genotype, may confer increased susceptibility to adduct formation, gene mutation, and lung cancer when the smoking dose is low.

253. Integrin-mediated Adhesion Regulates Cell Polarity and Membrane Protrusion through the Rho Family of GTPases
Cox, E.A., Sastry, S.K. and Huttenlocher, A.
Mol. Biol. Cell, 12, 265 (2001)

Integrin-mediated adhesion is a critical regulator of cell migration. Here we demonstrate that integrin-mediated adhesion to high fibronectin concentrations induces a stop signal for cell migration by inhibiting cell polarization and protrusion. On fibronectin, the stop signal is generated through $\alpha_5\beta_1$ integrin-mediated signaling to the Rho family of GTPases. Specifically, Cdc42 and Rac1 activation exhibits a biphasic dependence on fibronectin concentration that parallels optimum cell polarization and protrusion. In contrast, RhoA activity increases with increasing substratum concentration. We find that cross talk between Cdc42 and Rac1 is required for substratum-stimulated protrusion, whereas RhoA activity is inhibitory. We also show that Cdc42 activity is inhibited by Rac1 activation, suggesting that Rac1 activity may down-regulate Cdc42 activity and promote the formation of stabilized rather than transient protrusion. Furthermore, expression of RhoA down-regulates Cdc42 and Rac1 activity, providing a mechanism whereby RhoA may inhibit cell polarization and protrusion. These findings implicate adhesion-dependent signaling as a mechanism to stop cell migration by regulating cell polarity and protrusion via the Rho family of GTPases.

254. Selective Inhibition of Inducible Nitric Oxide Synthase Exacerbates Erosive Joint Disease
McCartney-Francis, N.L., Song, X-y., Mizel, D.E. and Wahl, S.M.
J. Immunol., 166, 2734 (2001)

NO is an essential cytotoxic agent in host defense, yet can be autotoxic if overproduced, as evidenced in inflammatory lesions and tissue destruction in experimental arthritis models. Treatment of streptococcal cell wal1-induced arthritis in rats with N^G-monomethyl-L-arginine (L-NMMA), a competitive nonspecific inhibitor of both constitutive and inducible isoforms of NO synthase (NOS), prevents intraarticular accumulation of leukocytes, joint swelling, and bone erosion. Because increased inducible NOS (iNOS) expression and NO generation are associated with pathogenesis of chronic inflammation, we investigated whether a selective inhibitor of iNOS, *N*-iminoethyl-L-lysine (L-NIL), would have more directed anti-arthritic properties. Whereas both L-NMMA and L-NIL inhibited nitrite production by streptococcal cell wall-stimulated rat mononuclear cells in vitro and systemic treatment of arthritic rats with L-NMMA

ablated synovitis, surprisingly L-NIL did not mediate resolution of inflammatory joint lesions. On the contrary, daily administration of L-NIL failed to reduce the acute response and exacerbated the chronic inflammatory response, as reflected by profound tissue destruction and loss of bone and cartilage. Although the number of iNOS-positive cells within the synovium decreased after treatment with L-NIL, immunohistochemical analyses revealed a distinct pattern of endothelial and neuronal NOS expression in the arthritic synovium that was unaffected by the isoform-specific L-NIL treatment. These studies uncover a contribution of the constitutive isoforms of NOS to the evolution of acute and chronic inflammation pathology which may be important in the design of therapeutic agents.

255. Differential expression of Toll-like receptor 2 in human cells
Flo, T.H. et al
J. Leukoc. Biol., 69, 474 (2001)

Human Toll-like receptor 2 (TLR2) is a receptor for a variety of microbial products and mediates activation signals in cells of the innate immune system. We have investigated expression and regulation of the TLR2 protein in human blood cells and tissues by using two anti-TLR2 mAbs. Only myelomonocytic cell lines expressed surface TLR2. In tonsils, lymph nodes, and appendices, activated B-cells in germinal centers expressed TLR2. In human blood, $CD14^+$ monocytes expressed the highest level of TLR2 followed by $CD15^+$ granulocytes, and $CD19^+$ B-cells, $CD3^+$ T-cells, and $CD56^+$ NK cells did not express TLR2. The level of TLR2 on monocytes was after 20 h up-regulated by LPS, GM-CSF, IL-1, and IL-10 and down-regulated by IL-4, IFN-γ, and TNF. On purified granulocytes, LPS, GM-CSF, and TNF down-regulated, and IL-10 modestly increased TLR2 expression after 2 h. These data suggest that TLR2 protein expression in innate immune cells is differentially regulated by inflammatory mediators.

256.* Leukotriene B_4 Augments Neutrophil Phagocytosis of *Klebsiella pneumoniae
Mancuso, P., Nana-Sinkam, P. and Peters-Golden, M.
Infect. Immun., 69, 2011-2016 (2001)

Neutrophils play a critical role in the clearance of bacteria from the lung and other organs by their capacity for phagocytosis and killing. Previously, we identified an important role for the leukotrienes in rat alveolar macrophage phagocytosis of *Klebsiella pneumoniae*. In this report, we explored the possibility that the leukotrienes play an important role in phagocytosis by neutrophils as well. Inhibition of endogenous leukotriene synthesis by 5-lipoxygenase knockout in mice or by pharmacologic means in human peripheral blood neutrophils attenuated phagocytosis of opsonized *K. pneumoniae*. Reduced phagocytosis was also observed in human neutrophils pretreated with a leukotriene B_4 receptor but not a cysteinyl-leukotriene receptor antagonist. While leukotriene B_4 reconstituted defective phagocytosis in leukotriene-deficient neutrophils and enhanced phagocytosis in neutrophils capable of leukotriene synthesis, leukotriene C_4, leukotriene D_4, 5-hydroperoxyeicosatetraenoic acid, and 5-oxo-eicosatetraenoic acid were ineffective. To determine the opsonin dependence of the leukotriene B_4 augmentation of phagocytosis, we assessed the ability of leukotriene B_4 to modulate neutrophil phagocytosis and the adherence of sheep erythrocytes opsonized with immunoglobulin G or the complement fragment C3bi. While leukotriene B_4 augmented both Fc receptor- and complement receptor-mediated phagocytosis, increased adherence to leukotriene B_4-treated neutrophils was limited to complement opsonized targets. In conclusion, we have identified a novel role for leukotriene B_4 in the augmentation of neutrophil phagocytosis mediated by either the Fc or complement receptor.

257. Induction of Cell-Associated Chemokines after Endotoxin Administration to Healthy Humans
Olszyna, D., De Jonge, E., Dekkers, P.E.P., van Deventer, S.J.H. and van der Poll, T.
Infect. Immun., 69, 2736-2738 (2001)

Erythrocytes express the Duffy antigen receptor for chemokines. Endotoxin injection into humans induced high levels of interleukin-8 (IL-8), growth-related oncogene α, and monocyte chemoattractant protein 1 in circulating erythrocytes. IL-8 was also recovered from mononuclear and polymorphonuclear cells. Cell-associated chemokines may more accurately reflect their production than plasma concentrations.

258. Assessment of neutrophil N-formyl peptide receptors by using antibodies and fluorescent peptides
Loitto, V-M., Rasmusson, B. and Magnusson, K-E.
J. Leukoc. Biol., 69, 762, (2001)

Enrichment of chemoattractant receptors on the neutrophil surface has been difficult to assess, primarily because of limitations in sensitivity of visualization. Using an ultrasensitive, cooled charge-coupled device camera, we investigated spatial-temporal relationships between *N*-formyl peptide receptor distribution and directional motility of human neutrophils. Live cells were labeled with fluorescent receptor ligands, i.e., fluoresceinated *tert*-butyl-oxycarbonyl-Phe-(D)-Leu-Phe-(D)-Leu-Phe-OH (Boc-FLFLF) and formyl-Nle-Leu-Phe-Nle-Tyr-Lys (fnLLFnLYK), while fixed cells were labeled with either fluorescent peptides or monoclonal antibodies. Double labeling of receptors and filamentous actin (F-actin) was done to investigate possible colocalization. *N*-Formyl peptide receptors on unstimulated cells were randomly distributed. However, on polarized neutrophils, the receptors accumulated toward regions involved in motility and distributed nonuniformly. In fixed neutrophils, antibody-labeled receptors colocalized with the F-actin-rich leading edge whereas peptide-labeled receptors lagged behind this region. We suggest that neutrophils use an asymmetric receptor distribution for

directional sensing and sustained migration. A separation between receptors labeled with peptides and those labeled with antibodies reflects two functionally distinct receptor populations at the membrane of motile neutrophils.

259. The CGM1a (CEACAM3/CD66d)-mediated Phagocytic Pathway of Neisseria gonorrhoeae Expressing Opacity Proteins Is Also the Pathway to Cell Death
Chen, T. et al
J. Biol. Chem., 276, 17413-17419 (2001)

Phagocytosis of Opa^+ *Neisseria gonorrhoeae* (gonococcus, GC) by neutrophils is in part dependent on the interaction of Opa proteins with CGM1a (CEACAM3/CD66d) antigens, a neutrophil-specific receptor. However, the signaling pathways leading to phagocytosis have not been characterized. Here we show that interaction of OpaI bacteria with neutrophils or CGM1a-transfected DT40 cells induces calcium flux, which correlates with phagocytosis of bacteria. We identified an immunoreceptor tyrosine-based activation motif (ITAM) in CGM1a, and showed that the ability of CGM1a to transduce signals and mediate phagocytosis was abolished by mutation of the ITAM tyrosines. We also demonstrated that CGM1a-ITAM-mediated bacterial phagocytosis is dependent on Syk and phospholipase C activity in DT40 cells. Unexpectedly, the activation of the CGM1a-ITAM phagocytic pathway by Opa^+ GC results in induction of cell death.

260. Increasing the Affinity for Tumor Antigen Enhances Bispecific Antibody Cytotoxicity
McCall, A.M. et al
J. Immunol., 166, 6112 (2001)

We tested the hypothesis that bispecific Abs (Bsab) with increased binding affinity for tumor Ags augment retargeted antitumor cytotoxicity. We report that an increase in the affinity of Bsab for the HER2/*neu* Ag correlates with an increase in the ability of the Bsab to promote retargeted cytotoxicity against HER2/*neu*-positive cell lines. A series of anti-HER2/*neu* extracellular domain-directed single-chain Fv fragments (scFv), ranging in affinity for HER2/*neu* from 10^{-7} to 10^{-11} M, were fused to the phage display-derived NM3E2 human scFv. NM3E2 associates with the extracellular domain of human FcγRIII (CD16). The resulting series of Bsab promoted cytotoxicity of SKOV3 human ovarian carcinoma cells overexpressing HER2/*neu* by human PBMC preparations containing CD16-positive NK cells. The affinity for HER2/*neu* clearly influenced the ability of the Bsab to promote cytotoxicity of ^{51}Cr-labeled SKOV3 cells. Lysis was 6.5% with an anti-HER2/*neu* $K_D = 1.7 \times 10^{-7}$ M, 14.5% with $K_D = 5.7 \times 10^{-9}$ M, and 21.3% with $K_D = 1.7 \times 10^{-10}$ M at 50:1 E:T ratios. These scFv-based Bsab did not cross-link receptors and induce leukocyte calcium mobilization in the absence of tumor cell engagement. Thus, these novel Bsab structures should not induce the dose-limiting cytokine release syndromes that have been observed in clinical trials with intact IgG Bsab. Additional manipulations in Bsab structure that improve selective tumor retention or facilitate the ability of Bsab to selectively cross-link tumor and effector cells at tumor sites should further improve the utility of this therapeutic strategy.

261. Human Epidermal Growth Factor (EGF) Module-containing Mucin-like Hormone Receptor 3 Is a New Member of the EGF-TM7 Family That Recognizes a Ligand on Human Macrophages and Activated Neutrophil
Stacey, M, Lin, H-H., Hilyard, K.L., Gordon, S. and McKnight, A.J.
J. Biol. Chem., 276, 18863-18870 (2001)

The epidermal growth factor (EGF)-TM7 subgroup of G-protein-coupled receptors is composed predominantly of leukocyte-restricted glycoproteins defined by their unique hybrid structure, in which extracellular EGF-like domains are coupled to a seven-span transmembrane moiety via a mucin-like stalk. The EGF-TM7 group comprises mouse F4/80, human EGF module-containing mucin-like hormone receptor (EMR) 1, human EMR2, and human and mouse CD97, the genes for which map to human chromosome 19p13 and the syntenic regions of the mouse genome. In this study we describe the cloning and characterization of EMR3, a novel human EGF-TM7 molecule, and show the existence of its cellular ligand. The EMR3 gene maps closely to the existing members of the EGF-TM7 family on human chromosome 19p13.1 and, in common with other EGF-TM7 genes, is capable of generating different protein isoforms through alternative splicing. Two alternative splice forms have been isolated: one encoding a 652-amino acid cell surface protein consisting of two EGF-like domains, a mucin stalk, and a putative G-protein-coupled receptor domain and the other encoding a truncated soluble form containing only two EGF-like domains. As with other members of the EGF-TM7 family, EMR3 mRNA displays a predominantly leukocyte-restricted expression pattern, with highest levels in neutrophils, monocytes, and macrophages. Through the use of soluble EMR3 multivalent probes we have shown the presence of a ligand at the surface of monocyte-derived macrophages and activated human neutrophils. These interactions suggest a potential role for EMR3 in myeloid-myeloid interactions during immune and inflammatory responses.

262. **Immunocytochemical localization of peptidylarginine deiminase in human eosinophils and neutrophils**
Asaga, H., Nakashima, K., Senshu, T., Ishigama, A. and Yamada, M.
J. Leukoc. Biol., 70, 46 (2001)

Peptidylarginine deiminase, registered as PAD V in the DDBJ/GenBank/EMBL data banks, is expressed in HL-60 cells differentiated into granulocytes or monocytes. We analyzed PAD activities in density-fractionated human peripheral blood cell fractions. PAD activity with similar substrate specificity to that of PAD V was found in the eosinophil and neutrophil fractions, which showed single bands comigrating with authentic PAD V on immunoblotting with an anti-PAD V antibody. Both the biochemical and immunoblotting analyses showed marked enrichment of PAD V in the eosinophil fraction. Its immunoreactivity appeared to localize in eosinophilic granules at high density and in myeloperoxidase-negative cytoplasmic granules of neutrophils at low density, as determined by confocal laser-scanning microscopy. Possible roles of PAD V in myeloid differentiation and granulocyte function are discussed. In addition, we present evidence for the presence of PAD(s) that are antigenically different from PAD V in monocytes and lymphocytes.

263. Influence of Cellular Factors and Pharmacokinetics on the Formation of Platinum-DNA Adducts in Leukocytes of Children Receiving Cisplatin Therapy
Veal, G.J. et al
Clin. Cancer Res., 7, 2205 (2001)

The formation of platinum (Pt)-DNA adducts is thought to be crucial to the antitumor activity of cisplatin, and relationships between adduct formation in peripheral blood leukocytes (PBLs) and response to cisplatin therapy have been reported. The current study directly tests, for the first time, whether pharmacokinetic or other factors predominantly determine the drug-target interaction of cisplatin in a pediatric patient population.
Cisplatin pharmacokinetics and Pt-DNA adduct formation in PBLs were determined in 10 children in parallel with measurement of adduct levels after incubation of pretreatment blood samples with cisplatin *in vitro*. Total and unbound plasma Pt concentrations were determined by atomic absorption spectrophotometry and adduct measurements performed by competitive ELISA.
Pt-DNA adduct levels determined after cisplatin treatment showed considerable interindividual variation (peak levels at 24 h ranged from 0.15 to 1.31 nmol/g DNA) and correlated strongly with adduct levels determined after incubation of pretreatment whole blood with cisplatin ($r = 0.92$; $P = 0.0002$). No significant correlation was observed between *in vivo* adduct formation and either unbound or total cisplatin plasma concentrations ($r = 0.14$ and 0.18, respectively). A correlation was also observed between the degree of myelosuppression, as determined by WBC nadirs measured over a 14-day period after cisplatin treatment, and the extent of adduct formation, with greater WBC toxicity observed in patients with higher levels of Pt-DNA adducts ($P = 0.010$).
These preliminary results provide evidence that interpatient variation in formation of Pt-DNA adducts in PBLs of children is determined by host-specific factors other than cisplatin pharmacokinetics. These results imply that analysis of adducts in PBLs after incubation of pretreatment blood samples with cisplatin may be used to predict *in vivo* adduct levels, leukopenia, and, potentially, response to cisplatin therapy.

264. Protein Kinase C-δ Regulates Thrombin-Induced ICAM-1 Gene Expression in Endothelial Cells via Activation of p38 Mitogen-Activated Protein Kinase
Rahman, A. et al
Mol. Cell Biol., 21, 5554-5565 (2001)

The procoagulant thrombin promotes the adhesion of polymorphonuclear leukocytes to endothelial cells by a mechanism involving expression of intercellular adhesion molecule 1 (ICAM-1) via an NF-κB-dependent pathway. We now provide evidence that protein kinase C-δ (PKC-δ) and the p38 mitogen-activated protein (MAP) kinase pathway play a critical role in the mechanism of thrombin-induced ICAM-1 gene expression in endothelial cells. We observed the phosphorylation of PKC-δ and p38 MAP kinase within 1 min after thrombin challenge of human umbilical vein endothelial cells. Pretreatment of these cells with the PKC-δ inhibitor rottlerin prevented the thrombin-induced phosphorylation of p38 MAP kinase, suggesting that p38 MAP kinase signals downstream of PKC-δ. Inhibition of PKC-δ or p38 MAP kinase by pharmacological and genetic approaches markedly decreased the thrombin-induced NF-κB activity and resultant ICAM-1 expression. The effects of PKC-δ inhibition were secondary to inhibition of IKKβ activation and of subsequent NF-κB binding to the ICAM-1 promoter. The effects of p38 MAP kinase inhibition occurred downstream of IκBα degradation without affecting the DNA binding function of nuclear NF-κB. Thus, PKC-δ signals thrombin-induced ICAM-1 gene transcription by a dual mechanism involving activation of IKKβ, which mediates NF-κB binding to the ICAM-1 promoter, and p38 MAP kinase, which enhances transactivation potential of the bound NF-κB p65 (RelA).

265. **CD18 Dependency of Transendothelial Neutrophil Migration Differs During Acute Pulmonary Inflammation**
Mackarel, A.J. et al
J. Immunol., 167, 2839 (2001)

Neutrophil extravasation during inflammation can occur either by a mechanism that requires the neutrophil integrin complex, CD18, or by an alternative CD18-independent route. Which of the two pathways is used has been shown to depend on the site and nature of the inflammatory insult. More recent evidence suggests that selection may also depend on whether inflammation is chronic or acute, but why this is the case remains unknown. Using an in vitro model that supports both migratory mechanisms, we examined the CD18 dependency of migration of neutrophils isolated from patients with either chronic or acute pulmonary infection. Chronic neutrophils were found to behave like normal neutrophils by migrating to IL-8 and leukotriene B_4 using the CD18-independent pathway, but to the

bacterial product, FMLP, using the CD18-dependent route. In contrast, migration of acute neutrophils to all of these stimuli was CD18 dependent. Normal neutrophils could be manipulated to resemble acute neutrophils by exposing them to FMLP before migration, which resulted in a "switch" from the CD18-independent to -dependent mechanism during migration to IL-8 or leukotriene B_4. Although treatment of normal neutrophils with FMLP caused selective down-regulation of the IL-8 receptor, CXCR2, and acute neutrophils were found to have less CXCR2 than normal, a functional relationship between decreased CXCR2 and selection of CD18-dependent migration was not demonstrated. Results indicate that selection of the CD18-dependent or -independent migration mechanism can be controlled by the neutrophil and suggest that the altered CD18 requirements of acute neutrophils may be due to priming in the circulation during acute infection.

266. Cytoskeleton-dependent membrane domain segregation during neutrophil polarization
Seveau, S., Eddy, R.J., Maxfield, F.R. and Pierini, L.M.,
Mol. Biol. Cell, 12, 3550 (2001)

On treatment with chemoattractant, the neutrophil plasma membrane becomes organized into detergent-resistant membrane domains (DRMs), the distribution of which is intimately correlated with cell polarization. Plasma membrane at the front of polarized cells is susceptible to extraction by cold Triton X-100, whereas membrane at the rear is resistant to extraction. After cold Triton X-100 extraction, DRM components, including the transmembrane proteins CD44 and CD43, the GPI-linked CD16, and the lipid analog, $DiIC_{16}$, are retained within uropods and cell bodies. Furthermore, CD44 and CD43 interact concomitantly with DRMs and with the F-actin cytoskeleton, suggesting a mechanism for the formation and stabilization of DRMs. By tracking the distribution of DRMs during polarization, we demonstrate that DRMs progress from a uniform distribution in unstimulated cells to small, discrete patches immediately after activation. Within 1 min, DRMs form a large cap comprising the cell body and uropod. This process is dependent on myosin in that an inhibitor of myosin light chain kinase can arrest DRM reorganization and cell polarization. Colabeling DRMs and F-actin revealed a correlation between DRM distribution and F-actin remodeling, suggesting that plasma membrane organization may orient signaling events that control cytoskeletal rearrangements and, consequently, cell polarity.

267. Immature Human Dendritic Cells Express Asialoglycoprotein Receptor Isoforms for Efficient Receptor-Mediated Endocytosis
Valladeau, J. et al
J. Immunol., 167, 5767 (2001)

In a search for genes expressed by dendritic cells (DC), we have cloned cDNAs encoding different forms of an asialoglycoprotein receptor (ASGPR). The DC-ASGPR represents long and short isoforms of human macrophage lectin, a Ca^{2+}-dependent type II transmembrane lectin displaying considerable homology with the H1 and H2 subunits of the hepatic ASGPR. Immunoprecipitation from DC using an anti-DC-ASGPR mAb yielded a major 40-kDa protein with an isoelectric point of 8.2. DC-ASGPR mRNA was observed predominantly in immune tissues. Both isoforms were detected in DC and granulocytes, but not in T, B, or NK cells, or monocytes. DC-ASGPR species were restricted to the CD14-derived DC obtained from $CD34^+$ progenitors, while absent from the CD1a-derived subset. Accordingly, both monocyte-derived DC and tonsillar interstitial-type DC expressed DC-ASGPR protein, while Langerhans-type cells did not. Furthermore, DC-ASGPR is a feature of immaturity, as expression was lost upon CD40 activation. In agreement with the presence of tyrosine-based and dileucine motifs in the intracytoplasmic domain, mAb against DC-ASGPR was rapidly internalized by DC at 37°C. Finally, intracellular DC-ASGPR was localized to early endosomes, suggesting that the receptor recycles to the cell surface following internalization of ligand. Our findings identify DC-ASGPR/human macrophage lectin as a feature of immature DC, and as another lectin important for the specialized Ag-capture function of DC.

268. Serum and WBC pharmacokinetics of 1500 mg of azithromycin when given either as a single dose or over a 3 day period in healthy volunteers
Amsden, G.W. and Gray C.L.
J. Antimicrob. Chemother., 47, 61-66 (2001)

Owing to azithromycin's prolonged half-life, shorter and shorter dosage regimens are being studied for treatment of respiratory tract infections. Previous studies have concluded that the 3 and 5 day (1.5 g total) regimens not only provide at least equal serum and WBC exposures but also equal efficacy rates. An earlier clinical study using the entire 1.5 g dose at once or the current 3 day regimen in patients with atypical pneumonia noted equal efficacy. Similar trials are currently underway in both adult and paediatric populations. The goal of the present study was to investigate whether there were equal serum and WBC exposures when azithromycin was dosed as the current 3 day regimen or as a single large dose. Equal exposures would help validate future clinical trials of single dose regimens. Twelve healthy volunteers received both azithromycin regimens (1.5 g single dose and 500 mg/day for 3 days) in random order. Serum and WBC samples were collected at baseline and repeatedly for 10 days following the first dose of each regimen. Serum samples were assayed via HPLC (CV% < 10) and WBC samples via liquid chromatography/mass spectrometry (CV% < 10). Data were modelled using noncompartmental methods. Statistics were via ANOVA with significance defined as $P < 0.05$. All subjects completed both regimens with minimal incidence of adverse effects. Serum data [mean (range)] demonstrated no significant difference in exposure between the two regimens [single 13.1 (3.02–20.6) mg•h/L versus 3 day 11.2 (2.98–24.5) mg•h/L: $P = 0.12$], although it favoured the shorter regimen. WBC results demonstrated much higher exposures than seen with serum, but no significant difference between the two

regimens was identified. These results suggest that a single oral 1.5 g regimen of azithromycin for respiratory tract infections should provide exposure at least equal to currently approved treatment regimens.

269. The Suppressive Effect of Dietary Restriction and Weight Loss in the Obese on the Generation of Reactive Oxygen Species by Leukocytes, Lipid Peroxidation, and Protein Carbonylation
Dandona, P. et al
J. Clin. Endocrinol. Metab., 86, 355-362 (2001)

Increased reactive oxygen species generation by the leukocytes of the obese may be responsible for increased oxidative injury to lipids and proteins and, hence, atherosclerosis. We have investigated whether reactive oxygen species generation by leukocytes and other indexes of oxidative damage in the body fall with short-term dietary restriction and weight loss. Nine nondiabetic obese subjects (body mass index, 32.5–64.4 kg/m^2), not taking any antioxidants, were put on a 1000-Cal diet. Fasting blood samples were taken at 0, 1, 2, 3, and 4 weeks and at 12 weeks after the cessation of dietary restriction. Blood samples were also obtained at 1 and 2 h after administration of 75 g oral glucose at 0 and 4 weeks. Mononuclear cells (MNC) and polymorphonuclear leukocytes (**PMN**) were isolated, and reactive oxygen species generation was measured. Plasma concentrations of thiobarbituric acid-reactive species (TBARS), 13-hydroxyoctadecadienoic acid (13-HODE), 9-hydroxyoctadecadienoic acid (9-HODE), carbonylated proteins, *o*-tyrosine, and *m*-tyrosine as indexes of oxidative damage to lipids, proteins and amino acids, respectively, were measured. Antioxidant vitamins were measured as indexes of antioxidant reserves. Plasma tumor necrosis factor-α concentrations were also measured. Mean weight loss was 2.4 ± 0.6 kg at week 1, 2.5 ± 1.7 kg at week 2, 3.9 ± 0.8 kg at week 3, and 4.5 ± 2.8 kg at week 4 ($P < 0.05$). Reactive oxygen species generation by **PMN** fell from 236.4 ± 95.8 to 150.9 ± 69.0, 125.9 ± 24.3, 96.0 ± 39.9, and 103.1 ± 35.7 mV at weeks 1, 2, 3, and 4, respectively ($P < 0.001$). It increased 3 months after the cessation of dietary restriction to 270.0 ± 274.3 mV. Reactive oxygen species generation by MNC fell from 187.8 ± 75.0 to 101.7 ± 64.5, 86.9 ± 42.8, 63.8 ± 14.3, and 75.1 ± 32.2 mV and increased thereafter to 302.0 ± 175.5 mV at 1, 2, 3, 4, and 16 weeks, respectively ($P < 0.005$). Reactive oxygen species generation by **PMN** and MNC increased in response to glucose; the relative increase was greater at 4 weeks than that at week 0 due to a fall in the basal levels of reactive oxygen species generation. Consistent with the fall in reactive oxygen species generation, there was a reduction in plasma TBARS from 1.68 ± 0.17 μmol/L at week 0 to 1.47 μmol/L at 4 weeks ($P < 0.05$). The 13-HODE to linoleic acid ratio fell from a baseline of 100% to 56.4 ± 36.1% at 4 weeks ($P < 0.05$), and the 9-HODE to linoleic acid ratio fell from a baseline of 100% to 60.5 ± 37.7% at 4 weeks ($P < 0.05$). Carbonylated proteins fell from 1.39 ± 0.27 μg/mg protein at week 0 to 1.17 ± 0.12 μg/mg protein at week 4 ($P < 0.05$); *o*-tyrosine fell from 0.42 ± 0.03 mmol/mol phenylalanine at week 0 to 0.36 ± 0.02 mmol/mol phenylalanine at 4 weeks ($P < 0.005$), and *m*-tyrosine fell from 0.45 ± 0.04 mmol/mol phenylalanine at week 0 to 0.40 ± 0.03 mmol/mol phenylalanine at 4 weeks ($P < 0.05$). The basal concentrations of TBARS, 9-HODE, 13-HODE, carbonylated proteins, *o*-tyrosine, and *m*-tyrosine in the obese were significantly greater than those in normal subjects. On the other hand, tumor necrosis factor-α concentrations did not change during this 4-week period, nor was there any change in antioxidant vitamins. This is the first demonstration of 1) an increase in reactive oxygen species-induced damage in lipids, proteins, and amino acids in the obese compared with normal subjects; and 2) a decrease in reactive oxygen species generation by leukocytes and oxidative damage to lipids, proteins, and amino acids after dietary restriction and weight loss in the obese over a short period.

270. PAF-mediated Ca2+ influx in human neutrophils occurs via store-operated mechanisms
Hauser, C.J. et al
J. Leukoc. Biol., 69, 63 (2001)

Many inflammatory mediators activate neutrophils (**PMN**) partly by increasing cytosolic calcium concentration ($[Ca^{2+}]_i$). Modulation of **PMN** $[Ca^{2+}]_i$ might therefore be useful in regulating inflammation after shock or sepsis. The hemodynamic effects of traditional Ca^{2+} channel blockade, however, could endanger unstable patients. Store-operated calcium influx (SOCI) is known now to contribute to Ca^{2+} flux in "nonexcitable" cells. Therefore, we studied the role of SOCI in human **PMN** responses to the proinflammatory ligand PAF. **PMN** $[Ca^{2+}]_i$ was studied by spectrofluorometry with and without external calcium. We studied the effects of PAF on Mn^{2+} entry into and on Ca^{2+} efflux from thapsigargin (Tg)-treated cells. Influx was assessed in the presence and absence of the blockers SKF-96365 (SKF), TMB-8, and 2-APB. Half of PAF $[Ca^{2+}]_i$ mobilization occurs via calcium influx. The kinetics of calcium entry were typical of SOCI rather than receptor-mediated calcium entry (RMCE). SKF had multiple nonspecific effects on $[Ca^{2+}]_i$. Inhibition of store emptying by TMB-8 and 2-APB blocked all calcium entry, demonstrating influx was store depletion-dependent. PAF has no direct effect on calcium efflux. Where SOCI is maximal, PAF has no further effect on calcium-channel traffic. PAF-induced calcium signals are highly dependent on SOCI and independent of RMCE. SOCI-specific blockade might modulate **PMN**-mediated inflammation and spare cardiovascular function in shock and sepsis.

271. Insulin Inhibits Intranuclear Nuclear Factor κB and Stimulates IκB in Mononuclear Cells in Obese Subjects: Evidence for an Anti-inflammatory Effect?
Dandona, P. et al
J. Clin. Endocrinol. Metab., 86, 3257-3265 (2001)

In view of the fact that insulin resistance is associated with atherogenesis and that troglitazone, an insulin sensitizer, has anti-inflammatory effects, which may be potentially antiatherogenic in the long term, we have now investigated whether insulin has potential anti-inflammatory effects. We infused 2.0 to 2.5 IU/h in 5% dextrose (100 mL/h) iv into 10 obese subjects for 4 h followed by 5% dextrose alone for 2 h. The rate of insulin infusion was varied to maintain glucose concentrations as close to the baseline as possible. Blood samples were obtained before and at 2, 4, and 6 h. Subjects were also infused with 5% dextrose without insulin and with saline on separate occasions. Intranuclear nuclear factor κB (NFκB) in mononuclear cells fell at 2 and further at 4 h, reverting toward the baseline at 6 h ($P < 0.05$). IκB increased significantly at 2 h, increasing further at 4 h and remaining elevated at 6 h ($P < 0.001$). Reactive oxygen species (ROS) generation by mononuclear cells fell significantly at 2 h and fell further at 4 h; it partially reverted to baseline at 6 h ($P < 0.005$). $p47^{phox}$ subunit, the key protein of nicotinamide adenine dinucleotide phosphate oxidase also fell at 2 h and 4 h, reverting toward the baseline at 6 h ($P < 0.05$). In addition, soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1) fell significantly following insulin infusion. Glucose or saline infusions without insulin caused no alteration in NFκB, IκB, ROS generation, $p47^{phox}$ subunit, sICAM-1, MCP-1, or PAI-1.

272. Troglitazone Reduces the Expression of PPARγ While Stimulating That of PPARα in Mononuclear Cells in Obese Subjects
Aljada, A. et al
J. Clin. Endocrinol. Metab., 86, 3130-3133 (2001)

We have recently demonstrated that troglitazone exerts an anti-inflammatory effect in the insulin resistant obese *in vivo* in parallel with its insulin-sensitizing effect. Because these effects are thought to be mediated through peroxisome proliferator-activated receptors α and γ (PPARα and PPARγ), we have now examined the possibility that troglitazone may modulate the expression of PPARα and PPARγ. Seven obese hyperinsulinemic subjects were administered 400 mg troglitazone daily for 4 weeks. Fasting blood samples were obtained before and during troglitazone therapy at 1, 2, and 4 weeks. Fasting insulin concentrations fell at week 1 and persisted at lower levels till 4 weeks. PPARγ expression fell significantly at week 1 and fell further at weeks 2 and 4. In contrast, PPARα expression increased significantly at week 2 and further at week 4. 9- and 13-hydroxyoctadecanoic acid, products of linoleic acid peroxidation and agonists of PPARγ, decreased during troglitazone therapy. We conclude that troglitazone, an agonist for both PPARα and PPARγ, has significant but dramatically opposite effects on PPARα and PPARγ. These effects may be relevant to its insulin sensitizing and anti-inflammatory effects.

273. Nuclear Factor-κB Suppressive and Inhibitor-κB Stimulatory Effects of Troglitazone in Obese Patients with Type 2 Diabetes: Evidence of an Antiinflammatory Action?
Aljada, A. et al
J. Clin. Endocrinol. Metab., 86, 3250-3256 (2001)
It has been shown recently that troglitazone exerts an anti-inflammatory effect, *in vitro*, and in experimental animals. To test these properties in humans, we investigated the effect of troglitazone on the proinflammatory transcription factor nuclear factor-κB and its inhibitory protein IκB in mononuclear cells (MNC) and plasma soluble intracellular adhesion molecule-1, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and C-reactive protein. We also examined the effect of troglitazone on reactive oxygen species generation, $p47^{phox}$ subunit expression, 9-hydroxyoctadecadienoic acid (9-HODE), 13-HODE, *o*-tyrosine, and *m*-tyrosine in obese patients with type 2 diabetes. Seven obese patients with type 2 diabetes were treated with troglitazone (400 mg/day) for 4 weeks. Blood samples were obtained at weekly intervals. Nuclear factor-κB binding activity in MNC nuclear extracts was significantly inhibited after troglitazone treatment at week 1 and continued to be inhibited up to week 4. On the other hand, IκB protein levels increased significantly after troglitazone treatment at week 1, and this increase persisted throughout the study. Plasma monocyte chemoattractant protein-1 and soluble intracellular adhesion molecule-1 concentrations did not decrease significantly after troglitazone treatment, although there was a trend toward inhibition. Reactive oxygen species generation by polymorphonuclear cells and MNC, $p47^{phox}$ subunit protein quantities, plasminogen activator inhibitor-1, and C-reactive protein levels decreased significantly after troglitazone intake. 13-HODE/linoleic acid and 9-HODE/linoleic acid ratios also decreased after troglitazone intake. However, *o*-tyrosine/phenylalanine and *m*-tyrosine/phenylalanine ratios did not change significantly. These data show that troglitazone has profound antiinflammatory effects in addition to antioxidant effects in obese type 2 diabetics; these effects may be relevant to the recently described beneficial antiatherosclerotic effects of troglitazone at the vascular level.

274. Cutting Edge: IFN-Inducible ELR- CXC Chemokines Display Defensin-Like Antimicrobial Activity
Cole, A.M. et al
J. Immunol., 167, 623 (2001)

Recent reports highlighted the chemotactic activities of antimicrobial peptide defensins whose structure, charge, and size resemble chemokines. By assaying representative members of the four known families of chemokines we explored the obverse: whether some chemokines exert antimicrobial activity. In a radial diffusion assay, only recombinant monokine induced by IFN-γ (MIG/CXCL9), IFN-γ-inducible protein of 10 kDa (IP-10/CXCL10), and IFN-inducible T cell α chemoattractant (I-TAC/CXCL11), members of the IFN-γ-inducible tripeptide motif Glu-Leu-Arg (ELR)$^-$ CXC chemokines, were antimicrobial against *Escherichia coli* and *Listeria monocytogenes*. Similar to human defensins, antimicrobial activities of the chemokines were inhibited by 50 and 100 mM NaCl. The concentration of MIG/CXCL9 and IP-10/CXCL10 released from IFN-γ-stimulated PBMC in 24 h were, respectively, 35- and 28-fold

higher than from unstimulated cells. Additionally, the amounts of chemokines released per monocyte suggest that, in tissues with mononuclear cell infiltration, IFN-γ-inducible chemokines may reach concentrations necessary for microbicidal activity. IFN-γ-inducible chemokines may directly inactivate microbes before attracting other host defense cells to the area of infection.

275. Hydrodynamic Shear Regulates the Kinetics and Receptor Specificity of Polymorphonuclear Leukocyte-Colon Carcinoma Cell Adhesive Interactions
Jadhav, S., Bochner, B.S. and Konstantopoulos, K.
J. Immunol., 167, 5986 (2001)

The ability of tumor cells to metastasize hematogenously is regulated by their interactions with polymorphonuclear leukocytes (**PMN**s). However, the mechanisms mediating **PMN** binding to tumor cells under physiological shear forces remain largely unknown. This study was designed to characterize the molecular interactions between **PMN**s and tumor cells as a function of the dynamic shear environment, using two human colon adenocarcinoma cell lines (LS174T and HCT-8) as models. **PMN** and colon carcinoma cell suspensions, labeled with distinct fluorophores, were sheared in a cone-and-plate rheometer in the presence of the **PMN** activator fMLP. The size distribution and cellular composition of formed aggregates were determined by flow cytometry. **PMN** binding to LS174T cells was maximal at 100 s^{-1} and decreased with increasing shear. At low shear (100 s^{-1}) **PMN** CD11b alone mediates **PMN**-LS174T heteroaggregation. However, L-selectin, CD11a, and CD11b are all required for **PMN** binding to sialyl $Lewis^x$-bearing LS174T cells at high shear (800 s^{-1}). In contrast, sialyl $Lewis^x$-low HCT-8 cells fail to aggregate with **PMN**s at high shear conditions, despite extensive adhesive interactions at low shear. Taken together, our data suggest that **PMN** L-selectin initiates LS174T cell tethering at high shear by binding to sialylated moieties on the carcinoma cell surface, whereas the subsequent involvement of CD11a and CD11b converts these transient tethers into stable adhesion. This study demonstrates that the shear environment of the vasculature modulates the dynamics and molecular constituents mediating **PMN**-tumor cell adhesion.

276. **Effect on Polymorphonuclear Cell Function of a Human-Specific Cytotoxin, Intermedilysin, Expressed by *Streptococcus intermedius***
Macey, M.G., Whiley, R.A., Miller, L and Nagamune, H.
Infect. Immun., 69, 6102-6109 (2001)

Streptococcus intermedius is a member of the normal flora of the mouth but is also an opportunistic pathogen associated with purulent infections at oral and nonoral sites. Intermedilysin (ILY) has been shown to be a cytolysin capable of generating pores in the cell membrane of erythrocytes demonstrable by electron microscopy. This effect has been shown to be specific for human cells. Since polymorphonuclear cells (PMNs) are the main cell involved in innate immunity we investigated the effect of purified intermedilysin from *Streptococcus intermedius* on PMN function. Active ILY at a concentration of 40 ng/µl caused a significant decrease in the number of intact PMNs after 60 min. The active cytolysin, when compared with heat-inactivated ILY, did not appear to be chemotactic for the PMNs but did cause an increase in intracellular calcium, with increased cell surface CD11b expression, metabolic burst, and phagocytosis of *Staphylococcus aureus*. These findings may have implications for the role of ILY in deep-seated abscesses.

277. Role of platelets and the arachidonic acid pathway in the regulation of neutrophil oxidase activity
Herbertsson, H. and Bengtsson, T.
Scand. J. Clin. Lab. Invest., 61(8), 641-649 (2001)

The intercellular mechanisms involved in platelet-mediated regulation of neutrophil function remain incompletely understood. This study investigated the role of the arachidonic acid pathway in the modulation of chemoattractant-induced production of oxygen metabolites, measured as luminol-amplified chemiluminescence (CL). We demonstrate that platelets dose-dependently inhibit the CL response in neutrophils stimulated with N-formyl-methionyl-leucyl-phenylalanine (fMLP). Incubation with eicosatetrayonic acid (ETYA), a combined cyclooxygenase and lipooxygenase inhibitor, dramatically decreased the fMLP-induced CL response in neutrophils, an effect that was further enhanced in the presence of platelets. The separate effects of eicosatriyonic acid (ETI) and indomethacin, specific inhibitors of lipoxygenase and cyclooxygenase, respectively, were significantly lower compared to the action of ETYA. On the contrary, impediment of arachidonic acid release with the phospholipase A_2 inhibitor arachidonyl trifluoromethyl ketone (ATK) markedly increased the production of oxygen radicals triggered by fMLP. The addition of exogenous arachidonic acid clearly decreased the fMLP-induced CL response in neutrophils, which further strengthens a downregulating effect of arachidonic acid on oxidase activity. This inhibitory action of arachidonic acid, however, was reversed upon co-incubation with platelets. In conclusion, this study suggests that an accumulation of arachidonic acid, following chemotactic peptide stimulation, turns off neutrophil oxidase activity. Furthermore, platelets may support the synthesis of reactive arachidonic acid metabolites, which modulate oxygen radical production in neutrophils.

278. The pan-chemokine inhibitor NR58-3.14.3 abolishes tumour necrosis factor-α accumulation and leucocyte recruitment induced by lipopolysaccharide *in vivo*
Reckless, J., Tatalick, L.M. and Grainger, D.J.
Immunology, 103(2), 244-254 (2001)

Chemokines participate in the regulation of leucocyte recruitment in a wide variety of inflammatory processes, including host defence and diseases such as asthma, atherosclerosis and autoimmune disorders. We have previously described the properties of Peptide 3, the first broad-specificity chemokine inhibitor *in vitro*. Here, we report the properties of NR58-3.14.3, a retroinverso analogue of Peptide 3. NR58-3.14.3 inhibited leucocyte migration induced by a range of chemokines, including monocyte chemoattractant protein-1 (MCP-1) (2·5 nM), macrophage inflammatory protein-1α (MIP-1α) (5 nM), regulated on activation, normal T-cell expressed and presumably secreted (RANTES) (20 nM), stromal cell-derived factor-1α (SDF-1α) (25 nM) and interleukin-8 (IL-8) (30 nM), but did not affect migration induced by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) or complement C5a (> 100 μM). NR58-3.14.3 is therefore ≈ 1000-fold more potent than Peptide 3 but retains the broad-spectrum chemokine inhibitory activity of the parent peptide. *In vivo*, pretreatment with a systemic dose of 10 mg of NR58-3.14.3, but not the inactive derivative NR58-3.14.4, abolished leucocyte recruitment in response to intradermal injection of 500 ng of MCP-1 into rat skin. This suggests that NR58-3.14.3 is a functional chemokine inhibitor *in vivo* as well as *in vitro*. We utilized NR58-3.14.3 as a tool to investigate the role of chemokine activity during leucocyte recruitment in response to lipopolysaccharide (LPS) *in vivo*. NR58-3.14.3, but not NR58-3.14.4, abolished leucocyte recruitment in response to intradermal injection of 50 ng of LPS into rat skin. Furthermore, NR58-3.14.3 completely inhibited LPS-induced accumulation of tumour necrosis factor-α (TNF-α). This data is consistent with a model in which multiple chemokines act in parallel upstream of TNF-α. NR58-3.14.3 is therefore a powerful anti-inflammatory agent *in vivo*, suppressing proinflammatory cytokine production and leucocyte recruitment in response to endotoxin stimulus in rat skin.

279. Nitric oxide generation from hydroxylamine in the presence of neutrophils and in the cell-free system
Klink, M., Swerzko, A. and Sulowska, Z.
APMIS, 109(7-8), 493-499 (2001)

Conversion of hydroxylamine (HA) to nitric oxide (NO) has been studied in the presence or absence of human neutrophils with or without myristate acetate phorbol (PMA), catalase (CAT), hydrogen peroxide (H_2O_2), and superoxide dismutase (SOD) and nitric oxide synthase (NOS) inhibitors. The generation of NO from HA in the presence of neutrophils was higher than in the cell-free system. We found that catalase did not influence the nitrite generation from HA in the cell-free system and in the presence of neutrophils. The H_2O_2 enhanced the NO generation from HA in the presence of neutrophils only. When catalase and H_2O_2 were added together, a high increase of NO generation from HA in both systems was observed. The addition of SOD decreased whereas addition of PMA enhanced the NO generation from HA in the presence of neutrophils. The presented data show the possible role of oxygen radicals in the decomposition of HA to NO. The addition of NOS inhibitors to the culture of neutrophils decreased the generation of nitrite from HA. Our results suggest that NO generation from HA, which is an intermediate in NO production from L-arginine, may be supported by an enzymatic pathway in which cellular NO synthase is involved.

280. Effect of interleukin 10 on the release of the CXC chemokines growth related oncogene GRO-α and epithelial cell-derived neutrophil activating peptide (ENA)-78 during human endotoxemia
Olszyna, D.P., Pajkrt, D., van Deventer, S.J.H. and van der Poll, T.
FEBS Lett., 78(1), 41-44 (2001)

Pretreatment with interleukin (IL)-10 inhibited the release of growth-related oncogene GRO-α but not of epithelial-cell derived neutrophil activating protein (ENA)-78, after injection of lipopolysaccharide (LPS) into healthy humans. In vitro, IL-10 dose-dependently attenuated LPS-induced release of both GRO-α and ENA-78 in whole blood and in cultures of isolated polymorphonuclear and mononuclear cells.

281. Determination, activity and biological role of adenylosuccinate lyase in blood cells
Tabucchi, A., Carlucci, F., Rosi, F., Guerranti, R.R. and Marinello, E.
Biomed. Pharmacother., 55(5), 277-283 (2001)

Adenylosuccinate lyase deficiency, which is associated with severe mental retardation and autistic features, was discovered in 1984. Since then this enzyme has been analyzed in many human tissues and it is now generally agreed that screening for this enzyme defect should be performed in all unexplained neurological diseases. The aim of the present study was to analyze adenylosuccinate lyase activity in blood cells by a fast simple method adaptable to screening purposes. The activity was also analyzed in B-lymphocytes from patients with B-cell chronic lymphocytic leukemia. The biological role of adenylosuccinate lyase and its importance in regulating cellular levels of AMP is discussed.

282. Tumour necrosis factor-[alpha] potentiates CR3-induced respiratory burst by activating p38 MAP kinase in human neutrophils
Forsberg, M., Lofgren, R., Zheng, L. and Stendahl, O.
*Immunology, **103(4)**, 465-472 (2001)*

CR3 and Fc[gamma]Rs are the main receptors involved in the phagocytic process leading to engulfment and killing of microbes by production of reactive oxygen intermediates (ROI) and degranulation. Various inflammatory mediators, such as tumour necrosis factor-

[alpha] (TNF-[alpha]) and lipopolysaccharide (LPS), are known to prime neutrophils leading to increased bactericidal responses, but the underlying mechanism of priming has only been partially elucidated. The purpose of this study was to investigate how TNF-[alpha] primes neutrophils for subsequent stimuli via either CR3 or Fc[gamma]R. The receptors were specifically activated with pansorbins (protein-A-positive Staphylococcus aureus) coated with anti-CR3, anti-Fc[gamma]RIIa, or anti-Fc[gamma]RIIIb monoclonal antibody. Activation of neutrophils with these particles resulted in ROI production as measured by chemiluminescence. Anti-CR3 pansorbins induced the most prominent ROI production in neutrophils. TNF-[alpha] potentiated the CR3-mediated respiratory burst but had little effect on that mediated by Fc[gamma]Rs. The priming effect of TNF-[alpha] on CR3-mediated ROI production is associated with an increased activation of p38 MAPK as well as tyrosine phosphorylation of p72syk. Pretreatment of neutrophils with the inhibitors for p38 MAPK and p72syk markedly suppressed the respiratory burst induced by CR3. Furthermore, TNF-[alpha] induced about a three-fold increase in the expression of CR3 in neutrophils, an effect which is blocked by the p38 MAPK inhibitor. Taken together, these results showed that TNF-[alpha] potentiates the CR3-mediated respiratory burst in neutrophils not only by triggering a p38 MAPK-dependent up-regulation of CD11b/CD18 but also by modulating the signalling pathways.

283. Polymorphonuclear neutrophils in Wegener's granulomatosis acquire characteristics of antigen presenting cells
Iking-Konert, C. Et al
*Kidney Int., **60(6)**, 2247-2262 (2001)*

Background. Constitutive expression of major histocompatibility complex (MHC) class II antigens and of the co-stimulatory receptors CD80 and CD86 is restricted to professional antigen presenting cells. Polymorphonuclear neutrophils (PMN) of healthy donors are negative for those antigens. Our recent study, however, found that PMN of patients with active Wegener's granulomatosis acquired MHC class II antigens.
Methods. To continue and extend the previous study results, PMN and monocytes of 60 patients with Wegener's granulomatosis, 24 patients with microscopic polyangiitis (MPA), 20 patients with acute bacterial infection, and 53 healthy donors were analyzed for the expression of MHC class II antigens as well as of CD80 and CD86. Moreover, induction on PMN of MHC class II expression was studied, as was antigen presentation as a possible functional consequence.
Results. PMN of patients with acute, active Wegener's granulomatosis expressed MHC class II antigens, CD80 and CD86; on monocytes up-regulation of MHC class II was seen. In contrast, PMN of patients with inactive disease, or with relapse, patients with microscopic polyangiitis or with bacterial infections expressed neither MHC class II, nor CD80 or CD86. PMN of healthy donors acquired these antigens when cultured in the presence of T cells or T cell-derived cytokines. The PMN were then able to present to T cell antigens in a MHC-class II restricted manner.
Conclusion. During active disease, the PMN of patients with Wegener's granulomatosis acquire characteristics of antigen presenting cells, whereas the PMN of patients with MPA or bacterial infection do not. The finding reflects differences in the pattern of the respective inflammatory response and suggests new effector functions of PMN. Moreover, MHC class II expression on PMN could serve as a novel marker for active Wegener's granulomatosis.

284. Neutrophil leukocyte motility requires directed water influx
Loitto, V-M., Forslund, T., Sundqvist, T., Magnusson, K-E. and Gustavsson, M.
J. Leukoc. Biol., 71, 212 (2002)

The ability of neutrophils to sense and move to sites of infection is essential for our defense against pathogens. For motility, lamellipodium extension and stabilization are prerequisites, but how cells form such membrane protrusions is still obscure. Using contrast-enhanced video microscopy and Transwell® assays, we show that water-selective aquaporin channels regulate lamellipodium formation and neutrophil motility. Addition of anti-aquaporin-9 antibodies, $HgCl_2$, or tetraethyl ammonium inhibited the function(s) of the channels and blocked motility-related shape changes. On human neutrophils, aquaporin-9 preferentially localized to the cell edges, where *N*-formyl peptide receptors also accumulated, as assessed with fluorescence microscopy. To directly visualize water fluxes at cell edges, cells were loaded with high dilution-sensitive, self-quenching concentrations of fluorophore. In these cells, motile regions always displayed increased fluorescence compared with perinuclear regions. Our observations provide the first experimental support for motility models where water fluxes play a pivotal role in cell-volume increases accompanying membrane extensions.

285. Clinical significance of telomerase activity in peripheral blood of patients with esophageal squamous cell carcinoma
Koyanagi, K. et al
Ann. Thorac. Surg., 73, 927 (2002)

Background. The presence of tumor cells in the blood stream is considered evidence of a high risk of distant organ metastasis. We examined the usefulness of telomerase activity in peripheral blood polymorphonuclear cells as an indicator of distant metastasis in patients with esophageal squamous cell carcinoma.
Methods. Telomerase activity was measured in the peripheral blood mononuclear cell and polymorphonuclear cell fractions obtained from blood samples of healthy volunteers mixed with squamous cell carcinoma cell lines, and cell distribution was analyzed by flow cytometry. Then telomerase activity of forty-two polymorphonuclear cell fractions obtained from esophageal squamous cell carcinoma patients was measured.
Results. Telomerase activity was detected in polymorphonuclear cell fractions and cell distribution analysis revealed the presence of esophageal squamous cell carcinoma cells. Organ metastasis was detected in 7 (78%) of the 9 patients with telomerase-positive polymorphonuclear cell fractions as opposed to only five (15%) of the 33 with telomerase-negative cases, and there was a significant positive correlation between telomerase activity and organ metastasis ($p < 0.0008$).
Conclusions. Measurement of telomerase activity in the polymorphonuclear cell fractions is useful for identifying a high risk group for distant organ metastasis in patients with esophageal squamous cell carcinoma.

286. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk
Hou, S-M. et al
Carcinogenesis, 23, 599-603 (2002)

The DNA repair protein xeroderma pigmentosum complementation group D (XPD) is involved in the nucleotide excision repair of DNA lesions induced by many tobacco and environmental carcinogens. In order to study the functional impact of the common polymorphisms in *XPD* exon 10 (G > A, Asp312Asn) and exon 23 (A > C, Lys751Gln), we have genotyped 185 Swedish lung cancer cases (97 smokers and 88 never-smokers) and 162 matched population controls (83 smokers and 79 never-smokers). Presence of one or two variant alleles was associated with increased risk for lung cancer among never-smokers only, in particular younger (<70 years) never-smokers [odds ratio (OR) = 2.6, 95% confidence interval (CI) = 1.1–6.5 for exon 10; OR = 3.2, 95% CI = 1.3–8.0 for exon 23, adjusted for age, gender and environmental tobacco smoke]. Aromatic DNA adduct level (AL) in peripheral lymphocytes was found to be similar between cases and controls, but significantly increased by current or recent smoking. Overall, there was a significant trend for increasing AL with increasing number of variant alleles in exon 10 ($P = 0.02$) or in exon 23 ($P = 0.001$). In addition, subjects with the combined exon 10 AA and exon 23 CC genotype showed a significantly higher AL compared with all those with any of the other genotypes ($P = 0.02$). We conclude that the *XPD* variant alleles may be associated with reduced repair of aromatic DNA adducts in general and increased lung cancer risk among never-smokers.

287. Inhibitory actions of glucosamine, a therapeutic agent for osteoarthritis, on the functions of neutrophils
Hua, J., Sakamoto, K. and Nagaoka, I.
J. Leukoc. Biol., 71, 632 (2002)

Glucosamine, an amino monosaccharide naturally occurring in the connective and cartilage tissues, contributes to maintaining the strength, flexibility, and elasticity of these tissues. In recent years, glucosamine has been used widely to treat osteoarthritis in humans and animal models. Neutrophils, which usually function as the primary defenders in bacterial infections, are also implicated in the destructive, inflammatory responses in arthritis. In this study, we have evaluated the effects of glucosamine on neutrophil functions using human peripheral blood neutrophils. Glucosamine (0.01–1 mM) dose-dependently suppressed the superoxide anion generation induced by formyl-Met-Leu-Phe (fMLP) or complement-opsonized zymosan and inhibited the phagocytosis of complement-opsonized zymosan or IgG-opsonized latex particles. Furthermore, glucosamine inhibited the release of granule enzyme lysozyme from phagocytosing neutrophils and suppressed neutrophil chemotaxis toward zymosan-activated serum. In addition, glucosamine inhibited fMLP-induced up-regulation of CD11b significantly, polymerization of actin, and phosphorylation of p38 mitogen-activated protein kinase (MAPK). In contrast, *N*-acetyl-glucosamine, an analogue of glucosamine, did not affect these neutrophil functions (superoxide generation, phagocytosis, granule enzyme release, chemotaxis, CD11b expression, actin polymerization, and p38 MAPK phosphorylation) at the concentrations examined (1–10 mM). Together these observations likely suggest that glucosamine suppresses the neutrophil functions, thereby possibly exhibiting anti-inflammatory actions in arthritis.

288. IL-9 Inhibits Oxidative Burst and TNF-α Release in Lipopolysaccharide-Stimulated Human Monocytes Through TGF-β
Pilette, C. et al
J. Immunol., 168, 4103 (2002)

IL-9 is a Th2 cytokine that exerts pleiotropic activities on T cells, B cells, mast cells, hematopoietic progenitors, and lung epithelial cells, but no effect of this cytokine has been reported so far on mononuclear phagocytes. Human blood monocytes preincubated with IL-9 for 24 h before LPS or PMA stimulation exhibited a decreased oxidative burst, even in the presence of IFN-γ. The inhibitory effect of IL-9 was specifically abolished by anti-hIL-9R mAb, and the presence of IL-9 receptors was demonstrated on human blood monocytes by FACS. IL-9 also down-regulated TNF-α and IL-10 release by LPS-stimulated monocytes. In addition, IL-9 strongly up-regulated the production of TGF-β1 by LPS-stimulated monocytes. The suppressive effect of IL-9 on the respiratory burst and TNF-α production in LPS-stimulated monocytes was significantly inhibited by anti-TGF-β1, but not by anti-IL-10Rβ mAb. Furthermore, IL-9 inhibited LPS-

induced activation of extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases in monocytes through a TGF-β-mediated induction of protein phosphatase activity. In contrast, IL-4, which exerts a similar inhibitory effect on the oxidative burst and TNF-α release by monocytes, acts primarily through a down-regulation of LPS receptors. Thus, IL-9 deactivates LPS-stimulated blood mononuclear phagocytes, and the mechanism of inhibition involves the potentiation of TGF-β1 production and extracellular signal-regulated kinase inhibition. These findings highlight a new target cell for IL-9 and may account for the beneficial activity of IL-9 in animal models of exaggerated inflammatory response.

289. Fas activation opposes PMA-stimulated changes in the localization of PKCδ: a mechansim for reducing neutrophil adhesion to endothelial cells
Hendey, B., Zhu, C.L. and Greenstein, S.
J. Leukoc. Biol., 71, 863 (2002)

We have shown previously that Fas activation results in a partial reduction of phorbol 12-myristate 13-acetate (PMA)-stimulated neutrophil adhesion to endothelial cells. The reduction in adhesion precedes early membrane markers of apoptosis and is not associated with any loss of membrane integrity. Rather, Fas activation reduces the PMA-stimulated expression and aggregation of ß2 integrins responsible for endothelial adhesion. A possible signaling mechanism for Fas effects on adhesion is the localization of protein kinase C δ (PKCδ). Western blot and immunofluorescence studies indicated that 1 h of Fas activation is required to reduce PMA-stimulated translocation of PKCδ to the membrane and adhesion. Rottlerin, a PKCδ inhibitor, also reduced PMA-induced PKCδ translocation and adhesion. In contrast, Gö6976, an inhibitor of conventional PKC isotypes, did not affect PMA-stimulated PKCδ translocation or reduce adhesion. There was no additive effect of Fas activation and rottlerin on reducing adhesion, suggesting that both agents were using a common pathway.

290. Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils
Iwabuchi, K. and Nagaoka, I.
Blood, 100, 1454-1464 (2002)

This study is focused on the functional significance of neutrophil lactosylceramide (LacCer)-enriched microdomains, which are involved in the initiation of a signal transduction pathway leading to superoxide generation. Treatment of neutrophils with anti-LacCer antibody, T5A7 or Huly-m13, induced superoxide generation from the cells, which was blocked by PP1, a Src kinase inhibitor; wortmannin, a phosphatidylinositol-3 kinase inhibitor; SB203580, a p38 mitogen-activated protein kinase (MAPK) inhibitor; and H7, an inhibitor for protein kinase C. When promyelocytic leukemia HL-60 cells were differentiated into neutrophilic lineage by dimethyl sulfoxide (DMSO) treatment, they acquired superoxide-generating activity but did not respond to anti-LacCer antibodies. Density gradient centrifugation revealed that LacCer and Lyn were recovered in detergent-insoluble membrane (DIM) of neutrophils and DMSO-treated HL-60 cells. However, immunoprecipitation experiments indicated that LacCer was associated with Lyn in neutrophils but not in DMSO-treated HL-60 cells. Interestingly, T5A7 induced the phosphorylation of Lyn in neutrophils but not in DMSO-treated HL-60 cells. Moreover, T5A7 induced the phosphorylation of p38 MAPK in neutrophils. T5A7-induced Lyn phosphorylation in neutrophil DIM fraction was significantly enhanced by cholesterol depletion or sequestration with methyl-β-cyclodextrin or nystatin. Collectively, these data suggest that neutrophils are characterized by the presence of cell surface LacCer-enriched glycosphingolipid signaling domain coupled with Lyn and that the ligand binding to LacCer induces the activation of Lyn, which may be suppressibly regulated by cholesterol, leading to superoxide generation through the phosphatidylinositol-3 kinase-, p38 MAPK-, and protein kinase C-dependent signal transduction pathway.

291. Neutrophil Membrane Expression of Proteinase 3 (PR3) Is Related to Relapse in PR3-ANCA-Associated Vasculitis
Rarok, A.A., Stegeman, C.A., Limburg, P.C. and Kallenberg, C.G.M.
J. Am. Soc. Nephrol., 13, 2232 (2002)

Wegener granulomatosis (WG) is strongly associated with the presence of antineutrophil cytoplasm autoantibodies (ANCA) with specificity for proteinase 3 (PR3). Relapses of WG are frequently preceded by a rise of autoantibody titer and PR3-ANCA are able to activate primed neutrophils *in vitro*. Except being stored intracellularly and translocated to the cell surface upon neutrophil stimulation, PR3 can also be detected on the surface of non-stimulated neutrophils (membrane PR3 or mPR3), with an interindividual variability in percentages of $mPR3^-$-positive cells and level of mPR3 expression. This study began with the hypothesis that the presence of PR3 on the surface of non-stimulated neutrophils enables interaction with PR3-ANCA and influences clinical manifestations of the disease. It analyzed mPR3 expression on neutrophils of 89 WG patients in complete remission and 72 healthy controls to evaluate whether the presence of PR3 on the surface of resting neutrophils is related to clinical manifestations of WG and/or to the susceptibility to develop relapses. The number of patients with a bimodal mPR3 expression on resting neutrophils did not differ between patients and controls. However, in WG patients, an increased percentage of $mPR3^+$ neutrophils and an elevated level of mPR3 expression compared with healthy individuals ($P = 0.037$) were found. Within the group of WG patients, an elevated level of mPR3 expression was significantly associated with an increased risk for relapse ($P = 0.021$) and with an increased relapse rate ($P = 0.011$), but not with the disease extent or particular manifestations at diagnosis or at relapse. These data support the hypothesis that PR3 expression on the membrane of neutrophils plays a role in the pathophysiology of PR3-ANCA associated vasculitis.

292. Polymorphonuclear Leukocytes from Individuals Carrying the G329A Mutation in the α1,3-Fucosyltransferase VII Gene (FUT7) Roll on E- and P-Selectins

Bengtson, P., Lundblad, A., Larson, G. and Påhlsson, P.

J. Immunol., 169, 3940 (2002)

We recently identified several individuals carrying a missense mutation (G329A; Arg^{110}-Gln) in the *FUT7* gene encoding fucosyltransferase VII. This enzyme is involved in the biosynthesis of the sialyl Lewis x (Le^x) epitope on human leukocytes, which has been identified as an important component of leukocyte ligands for E- and P-selectin. No enzyme activity was measurable in expression studies in COS-7 cells using the mutated *FUT7* construct. One of the identified individuals carried this mutation homozygously. Flow cytometry analysis of polymorphonuclear leukocytes (PMN) from this individual showed a nearly complete absence of staining with mAbs directed against sialyl Le^x and a diminished staining with an E-selectin IgG chimera. However, staining with P-selectin IgG chimera and Abs directed against P-selectin glycoprotein ligand-1 was not affected by the mutation. PMN from the homozygously mutated individual was further analyzed in an in vitro flow chamber assay. The number of rolling PMN and the rolling velocities on both E- and P-selectin were in the range of PMN from nonmutated individuals. *FUT4* and *FUT7* mRNA was quantified in PMN isolated from individuals carrying the *FUT7* mutation. It was found that PMN from both *FUT7* homozygously and heterozygously mutated individuals exhibited an elevated expression of *FUT4* mRNA compared with PMN from *FUT7* nonmutated individuals. The elevated expression of fucosyltransferase IV was reflected as an increased expression of the Le^x and CD65s Ags on PMN from these individuals. The significance of the mutation was supported by transfection of BJAB cells.

293. Discriminating between the Activities of Human Neutrophil Elastase and Proteinase 3 Using Serpin-derived Fluorogenic Substrates

Korkmaz, B. et al

J. Biol. Chem., 277, 39074-39081 (2002)

Human neutrophil elastase (HNE) has long been linked to the pathology of a variety of inflammatory diseases and therefore is a potential target for therapeutic intervention. At least two other serine proteases, proteinase 3 (Pr3) and cathepsin G, are stored within the same neutrophil primary granules as HNE and are released from the cell at the same time at inflammatory sites. HNE and Pr3 are structurally and functionally very similar, and no substrate is currently available that is preferentially cleaved by Pr3 rather than HNE. Discrimination between these two proteases is the first step in elucidating their relative contributions to the development and spread of inflammatory diseases. Therefore, we have prepared new fluorescent peptidyl substrates derived from natural target proteins of the serpin family. This was done because serpins are rapidly cleaved within their reactive site loop whether they act as protease substrates or inhibitors. The hydrolysis of peptide substrates reflects the specificity of the parent serpin including those from α-1-protease inhibitor and monocyte neutrophil elastase inhibitor, two potent inhibitors of elastase and Pr3. More specific substrates for these proteases were derived from the reactive site loop of plasminogen activator inhibitor 1, proteinase inhibitors 6 and 9, and from the related viral cytokine response modifier A (CrmA). This improved specificity was obtained by using a cysteinyl residue at P1 for Pr3 and an Ile residue for HNE and because of occupation of protease S' subsites. These substrates enabled us to quantify nanomolar concentrations of HNE and Pr3 that were free in solution or bound at the neutrophil surface. As membrane-bound proteases resist inhibition by endogenous inhibitors, measuring their activity at the surface of neutrophils may be a great help in understanding their role during inflammation.

294. Virulence factor expression by Gram-positive cocci exposed to subinhibitory concentrations of linezolid

Gemmell, C.G. and Ford, C.W.

J. Antimicrob. Chemother., 50, 665-672 (2002)

Linezolid is a new oxazolidinone with potent antibacterial activity against Gram-positive cocci; it uniquely inhibits bacterial translation through inhibition of 70S initiation complex formation. The effects of sub-growth-inhibitory concentrations of linezolid on the expression of various structural and soluble virulence factors of *Staphylococcus aureus* and *Streptococcus pyogenes* were examined. For *S. aureus*, strains Wood 46 and Cowan 1 (NCTC 8532) were used to measure protein A, coagulase, α-haemolysin (hla) and δ-haemolysin (hld). For *S. pyogenes*, strain NCTC 9994 was used to measure M protein, streptolysin O (SLO) and DNase. Coagulase was assayed by clotting of citrated rabbit plasma, and hla, hld and SLO by lysis of rabbit, human and horse erythrocytes, respectively. Protein A and M protein were measured indirectly using bacterial susceptibility to phagocytic ingestion of radiolabelled bacteria by human neutrophils. When *S. aureus* was grown in 1/2, 1/4 and 1/8 MIC, linezolid, coagulase, hla and hld production were impaired. Susceptibility to phagocytosis was changed by growth in the presence of 1/2 MIC linezolid compared with that in its absence (50.8 ± 4.1% versus 38.9 ± 2.9%; $P \leq 0.05$). When *S. pyogenes* was grown in 1/2, 1/4 and 1/8 MIC linezolid, SLO and DNase production were impaired compared with that of bacteria grown in the absence of the drug; its susceptibility to phagocytosis was also increased (52.8% bacteria ingested versus 37.5%; $P \leq 0.05$). A reduction in virulence factor expression at sub-MIC linezolid concentrations may be of benefit in the treatment of Gram-positive infections.

295. Chemoattractant-Stimulated Rac Activation in Wild-Type and Rac2-Deficient Murine Neutrophils: Preferential Activation of Rac2 and Rac2 Gene Dosage Effect on Neutrophil Functions
Li, S. et al
J. Immunol., 169, 5043 (2002)

The hemopoietic-specific Rho family GTPase Rac2 shares 92% amino acid identity with ubiquitously expressed Rac1. Neutrophils from *rac2*$^{-/-}$ mice have multiple defects, including chemoattractant-stimulated NADPH oxidase activity and chemotaxis, which may result from an overall reduction in cellular Rac or mechanisms that discriminate Rac1 and Rac2. We show that murine neutrophils have similar amounts of Rac1 and Rac2, unlike human neutrophils, which express predominantly Rac2. An affinity precipitation assay for Rac-GTP showed that although FMLP-induced activation of both isoforms in wild-type neutrophils, ≈4-fold more Rac2-GTP was detected than Rac1-GTP. Wild-type and Rac2-deficient neutrophils have similar levels of total Rac1. FMLP-induced Rac1-GTP in *rac2*$^{-/-}$ neutrophils was ≈3-fold greater than in wild-type cells, which have similar levels of total Rac1, yet FMLP-stimulated F-actin, chemotaxis, and superoxide production are markedly impaired in *rac2*$^{-/-}$ neutrophils. Heterozygous *rac2*$^{+/-}$ neutrophils, which had intermediate levels of total and FMLP-induced activated Rac2, exhibited intermediate functional responses to FMLP, suggesting that Rac2 was rate limiting for these functions. Thus, phenotypic defects in FMLP-stimulated Rac2-deficient neutrophils appear to reflect distinct activation and signaling profiles of Rac 1 and Rac2, rather than a reduction in the total cellular level of Rac.

296. Cleavage of p21waf1 by Proteinase-3, a Myeloid-specific Serine Protease, Potentiates Cell Proliferation
Witko-Sarsat, V. et al
J. Biol. Chem., 277, 47338-47347 (2002)

In this study, we present evidence for the critical role of proteinase-3 (PR3) in the proliferation of myeloid cells via the proteolytic regulation of the cyclin-dependent kinase inhibitor p21^{waf1}. Expression of recombinant PR3 in rat (RBL) or human (HMC1) mast cell lines increased bromodeoxyuridine incorporation and CDK2 activity compared with RBL and HMC1 cells transfected with an enzymatically inactive PR3 mutant (PR3(S203A)) or with human neutrophil elastase. Western blot analysis of p21^{waf1} showed an absence of detectable protein, despite normal levels of p21 mRNA. Ectopic overexpression of p21 restored normal levels of p21 in the RBL/PR3/p21 double transfectants and reverted the proliferative effect of PR3. Inhibition of the 26 S proteasome by lactacystin or of caspases by benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone did not inhibit p21 proteolysis. p21 cleavage correlated with PR3 expression in HMC1 cells infected with recombinant adenoviral vector Ad/PR3. During *in vitro* studies, purified p21 was cleaved by PR3, resulting in a 10-kDa p21 fragment. Employing double immunofluorescence confocal microscopy, subcellular fractionation, and co-immunoprecipitation, we found that PR3 and p21 colocalized in the cytosol. In human neutrophils treated with tumor necrosis factor-α, which induces PR3 re-expression, we observed that p21 disappeared and was reversed by Pefabloc, a serine proteinase inhibitor. The physiopathological implications of the cleavage of p21 by PR3 have to be determined.

297. Characterization of Human Alpha-Dystrobrevin Isoforms in HL-60 Human Promyelocytic Leukemia Cells Undergoing Granulocytic Differentiation
Kulyte, A., Navakauskiene, R., Treigyte, G., Gineitis, A., Bergman, T. And Magnusson, K-E.
Mol. Biol. Cell, 13, 4195 (2002)

The biochemical properties and spatial localization of the protein alpha-dystrobrevin and other isoforms were investigated in cells of the human promyelocytic leukemia line HL-60 granulocytic differentiation as induced by retinoic acid (RA). Alpha-dystrobrevin was detected both in the cytosol and the nuclei of these cells, and a short isoform (gamma-dystrobrevin) was modified by tyrosine phosphorylation soon after the onset of the RA-triggered differentiation. Varying patterns of distribution of alpha-dystrobrevin and its isoforms could be discerned in HL-60 promyelocytes, RA-differentiated mature granulocytes, and human neutrophils. Moreover, the gamma-dystrobrevin isoform was found in association with actin and myosin light chain. The results provide new information about potential involvement of alpha-dystrobrevin and its splice isoforms in signal transduction in myeloid cells during induction of granulocytic differentiation and/or at the commitment stage of differentiation or phagocytic cells.

298. Phospholipase Cγ2 Is Essential for Specific Functions of FcεR and FcγR
Wen, R., Jou, S-T., Chen, Y., Hoffmeyer, A. and Wang, D.
J. Immunol., 169, 6743 (2002)

Phospholipase Cγ2 (PLCγ2) plays a critical role in the functions of the B cell receptor in B cells and of the FcRγ chain-containing collagen receptor in platelets. Here we report that PLCγ2 is also expressed in mast cells and monocytes/macrophages and is activated by cross-linking of FcεR and FcγR. Although PLCγ2-deficient mice have normal development and numbers of mast cells and monocytes/macrophages, we demonstrate that PLCγ2 is essential for specific functions of FcεR and FcγR. While PLCγ2-deficient mast cells have normal mitogen-activated protein kinase activation and cytokine production at mRNA levels, the mutant cells have impaired FcεR-mediated Ca^{2+} flux and inositol 1,4,5-trisphosphate production, degranulation, and cytokine secretion. As a physiological consequence of the effect of PLCγ2 deficiency, the mutant mice are resistant to IgE-mediated cutaneous inflammatory skin reaction.

Macrophages from PLCγ2-deficient mice have no detectable FcγR-mediated Ca^{2+} flux; however, the mutant cells have normal FcγR-mediated phagocytosis. Moreover, PLCγ2 plays a nonredundant role in FcγR-mediated inflammatory skin reaction.

299. Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells
Mohanty, P. et al
Am. J. Clin. Nutrition, 75, 767 (2002)

Background: It was recently shown that glucose challenge leads to increased generation of reactive oxygen species (ROS) by polymorphonuclear leukocytes (**PMN**s) and mononuclear cells (MNCs).
Objective: To further elucidate the relation between nutrition and ROS generation, we investigated the effect of lipid and protein challenges on ROS generation by leukocytes.
Design: After having fasted overnight, one group of healthy subjects consumed a carbohydrate- and protein-free cream preparation (1257 kJ) and another group of healthy subjects consumed an equienergetic pure preparation of casein. Sequential blood samples were obtained after the intake of cream and casein. ROS were measured by chemiluminescence after stimulation by *N*-formyl-methionyl-leucinyl-phenylalanine. Lipid peroxidation was measured as thiobarbituric acid–reactive substances (TBARS) and α-tocopherol was measured by HPLC.
Results: ROS generation by MNCs and **PMN**s increased significantly 1, 2, and 3 h after cream intake and 1 h after protein intake. Cholesterol concentrations did not change significantly, whereas triacylglycerol concentrations increased significantly 2 h after cream intake. Total TBARS concentrations increased 1 h after cream intake and remained elevated 3 h after intake, but the increase was not significant when corrected for changes in triacylglycerol. After casein intake, total cholesterol, triacylglycerol, and TBARS concentrations did not change significantly. α-Tocopherol concentrations did not change significantly after either cream or casein intake.
Conclusions: Both fat and protein intakes stimulate ROS generation. The increase in ROS generation lasted 3 h after cream intake and 1 h after protein intake. Cream intake also caused a significant and prolonged increase in lipid peroxidation. These data are important because increased ROS generation and lipid peroxidation are key events in atherogenesis.

300. Store-Operated Calcium Entry in Human Neutrophils Reflects Multiple Contributions from Independently Regulated Pathways
Itagaki, K. et al
J. Immunol., 168m 4063 (2002)

Human polymorphonuclear neutrophil (**PMN**) responses to G protein-coupled chemoattractants are highly dependent upon store-operated Ca^{2+} entry (SOCE). Recent research suggests that SOCE currents can be mediated by a variety of related channel proteins of the transient receptor potential superfamily. SOCE has been regarded as a specific response to depletion of cell calcium stores. We hypothesized that net SOCE might reflect the contributions of more than one calcium entry pathway. SOCE was studied in normal human **PMN** using Ca^{2+} and Sr^{2+} ions. We found that **PMN** SOCE depends on at least two divalent cation influx pathways. One of these was nonspecific and Sr^{2+} permeable; the other was Ca^{2+} specific. The two pathways show different degrees of dependence on store depletion by thapsigargin and ionomycin, and differential sensitivity to inhibition by 2-aminoethyoxydiphenyl borane and gadolinium. The inflammatory G protein-coupled chemoattractants fMLP, platelet-activating factor, and IL-8 elicit unique patterns of Sr^{2+} and Ca^{2+} influx channel activation, and SOCE responses to these agonists displayed differing degrees of linkage to prior Ca^{2+} store depletion. The mechanisms of **PMN** SOCE responses to G protein-coupled chemoattractants are physiologically diverse. They appear to reflect Ca^{2+} transport through a variety of channels that are independently regulated to varying degrees by store depletion and by G protein-coupled receptor activation.

301. Dynamic Regulation of LFA-1 Activation and Neutrophil Arrest on Intercellular Adhesion Molecule 1 (ICAM-1) in Shear Flow
Lum, A.F.H., Green, C.E., Lee, G.R., Staunton, D.E. and Simon, S.I.
J. Biol. Chem., 277, 20660-20670 (2002)

Neutrophil recruitment during acute inflammation is triggered by G-protein-linked chemotactic receptors that in turn activate β_2 integrin (CD18), deemed a critical step in facilitating cell capture and arrest under the shear force of blood flow. A conformational switch in the I domain allosteric site (IDAS) and in CD18 regulates LFA-1 affinity for endothelial ligands including intercellular adhesion molecule 1 (ICAM-1). We examined the dynamics of CD18 activation in terms of the efficiency of neutrophil capture of ICAM-1, and we correlated this with the membrane topography of 327C, an antibody that recognizes the active conformation of CD18 I-like domain. Adhesion increased in direct proportion to chemotactic stimulus rising 7-fold over a log range of interleukin-8 (IL-8). A threshold dose of ~75 pM IL-8, corresponding to ligation of only ~10-100 receptors, was sufficient to activate ~20,000 CD18 and a rapid boost in the capture efficiency on ICAM-1. This was accompanied by a rapid redistribution of active LFA-1, but not Mac-1, into membrane patches, a necessary component for optimum adhesion efficiency. Shear-resistant arrest on a monolayer of ICAM-1 was reversed within minutes of chemotactic stimulation correlating with a shift from high to low affinity CD18 and dispersal of patches of active CD18. Mobility of active CD18 into high avidity patches was dependent on phosphatidylinositol 3-kinase activity and not F-actin polymerization. The data reveal that the number of chemotactic receptors bound and the topography and lifetime of high affinity LFA-1 tightly regulate the efficiency of neutrophil capture on ICAM-1.

302. Fluid Shear Regulates the Kinetics and Molecular Mechanisms of Activation-Dependent Platelet Binding to Colon Carcinoma Cells

McCarty, O.J.T., Jadhav, S., Burdick, M.M., Bell, W.R. and Konstantopoulos, K.
Biophys. J., 83, 836 (2002)

This study was undertaken to investigate the kinetics and molecular requirements of platelet binding to tumor cells in bulk suspensions subjected to a uniform linear shear field, using a human colon adenocarcinoma cell line (LS174T) as a model. The effects of shear rate (20-1000 s^{-1}), shear exposure time (30-300 s), shear stress (at constant shear rate by adjusting the viscosity of the medium from 1.3-2.6 cP), cell concentration, and platelet activation on platelet-LS174T heteroaggregation were assessed. The results indicate that hydrodynamic shear-induced collisions augment platelet-LS174T binding, which is further potentiated by thrombin/GPRP-NH_2. Peak adhesion efficiency occurs at low shear and decreases with increasing shear. Intercellular contact duration is the predominant factor limiting heteroaggregation at shear rates up to 200 s^{-1}, whereas these interactions become shear stress-sensitive at $\geq$400 s^{-1}. Heteroaggregation increases with platelet concentration due to an elevation of the intercellular collision frequency, whereas adhesion efficiency remains nearly constant. Moreover, hydrodynamic shear affects the receptor specificity of activation-dependent platelet binding to LS174T cells, as evidenced by the transition from a P-selectin-independent/Arg-Gly-Asp (RGD)-dependent process at 100 s^{-1} to a P-selectin/$\alpha_{IIb}\beta_3$-dependent interaction at 800 s^{-1}. This study demonstrates that platelet activation and a fluid-mechanical environment representative of the vasculature affect platelet-tumor cell adhesive interactions pertinent to the process of blood-borne metastasis.

303. IL-17 Expression in Human Herpetic Stromal Keratitis: Modulatory Effects on Chemokine Production by Corneal Fibroblasts

Maertzdorf, J., Osterhaus, A.D.M.E., Verjans, G.M.G.M.
J. Immunol., 169, 5897 (2002)

Herpetic stromal keratitis (HSK) is an immunopathologic disease triggered by infection of the cornea with HSV. Key events in HSK involve the interaction between cornea-infiltrating inflammatory cells and resident cells. This interaction, in which macrophages, producing IL-1 and TNF-α, and IFN-γ-producing Th1 cells play a crucial role, results in the local secretion of immune-modulatory factors and a major influx of neutrophils causing corneal lesions and blindness. The Th1-derived cytokine IL-17 has been shown to play an important role in several inflammatory diseases characterized by a massive infiltration of neutrophils into inflamed tissue. Here we show that IL-17 is expressed in corneas from patients with HSK and that the IL-17R is constitutively expressed by human corneal fibroblasts (HCF). IL-17 exhibited a strong synergistic effect with TNF-α on the induction of IL-6 and IL-8 secretion by cultured HCF. Secreted IL-8 in these cultures had a strong chemotactic effect on neutrophils. IL-17 also enhanced TNF-α- and IFN-γ-induced secretion of macrophage-inflammatory proteins 1α and 3α, while inhibiting the induced secretion of RANTES. Furthermore, considerable levels of IFN-γ-inducible protein 10 and matrix metalloproteinase 1 were measured in stimulated HCF cultures, while the constitutive secretion of monocyte chemotactic protein 1 remained unaffected. The data presented suggest that IL-17 may play an important role in the induction and/or perpetuation of the immunopathologic processes in human HSK by modulating the secretion of proinflammatory and neutrophil chemotactic factors by corneal resident fibroblasts.

304. IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G

Von Pawel-Rammingen, U., Johansson, B.P. and Björk, L.
EMBO J., 21, 1607-1615 (2002)

Recent work from several laboratories has demonstrated that proteolytic mechanisms significantly contribute to the molecular interplay between Streptococcus pyogenes, an important human pathogen, and its host. Here we describe the identification, purification and characterization of a novel extracellular cysteine proteinase produced by S.pyogenes. This enzyme, designated IdeS for Immunoglobulin G-degrading enzyme of S.pyogenes, is distinct from the well-characterized streptococcal cysteine proteinase, SpeB, and cleaves human IgG in the hinge region with a high degree of specificity. Thus, other human proteins, including immunoglobulins M, A, D and E, are not degraded by IdeS. The enzyme efficiently cleaves IgG antibodies bound to streptococcal surface structures, thereby inhibiting the killing of S.pyogenes by phagocytic cells. This and additional observations on the distribution and expression of the ideS gene indicate that IdeS represents a novel and significant bacterial virulence determinant, and a potential therapeutic target.

305. Increased IκB kinase activity is associated with activated NF-κB in acute myeloid blasts

Baumgartner, B. et al
Leukemia, 16, 2062-2071 (2002)

NF-κB/Rel transcription factors are modulators of immune and inflammatory processes and are also involved in malignancy. Phosphorylation of the IκB inhibitors by the IκB kinase (IKK) complex leads to their proteasomal degradation, resulting in activated NF-κB. Here, we investigated the activation status of NF-κB and the IKK complex in acute myeloid leukemia (AML). Gelshift assays revealed an increased level of activated nuclear NF-κB in myeloid blasts. Both bone marrow and peripheral blood blasts from AML patients showed enhanced IKK activity relative to controls, whereas the IKK protein concentrations were comparable. In addition, an

increased level of IκB-α was detected in AML blast cells, although this appeared to be insufficient to block nuclear translocation of NF-κB, also confirmed by immunofluorescence. In subtype M4 and M5 AML cells a more extensive NF-κB activation and higher IKK activity was found than in M1/M2 specimens. Isolated AML blasts cultured *ex vivo*responded to external stimulation (TNF, LPS) by further IKK activation, IκB degradation and NF-κB activation. Preincubation with the proteasome inhibitor PSI inhibited the NF-κB system in isolated AML blasts. This study established for the first time a dysregulation of IKK signaling in AML leading to increased NF-κB activity suggesting potential therapeutic avenues.

306. Favourable response to antithymocyte or antilymphocyte globulin in low-risk myelodysplastic syndrome patients with a 'non-clonal' pattern of X-chromosome inactivation in bone marrow cells
Aivado, M. et al
Eur. J. Hematol., 68(4), 210-216 (2002)

Objective: Antithymocyte and antilymphocyte globulin (ATG/ALG) have a therapeutic effect in about 30% of patients with myelodysplastic syndromes (MDS). We were interested to know whether responding patients achieve clonal or polyclonal remissions. Patients: Ten women with low-risk MDS received either ALG or ATG. Before treatment and 3, 6, and 12 months later, X-chromosome inactivation patterns of peripheral blood T lymphocytes were compared with those of peripheral blood granulocytes or bone marrow cells, using the human androgen receptor gene assay and the phosphoglycerate kinase-1 assay. Results: Six women did not respond to therapy. Prior to treatment, four of them had a monoclonal, one had an oligoclonal, and one had a skewed X-chromosome inactivation pattern (XCIP). Four patients responded to ATG/ALG. Three of them were informative in our X-inactivation assays, and showed a non-clonal XCIP which did not change significantly after treatment with ATG/ALG. *Conclusion*: A non-clonal XCIP in the bone marrow was associated with a response to ATG/ALG. Non-clonal XCIPs do not necessarily imply that there is no pathological clone. By definition, they just indicate that there is no evidence of a clone contributing more than 50% of cells in a sample. Non-clonal XCIPs may therefore be attributable to incomplete clonal expansion. This, in turn, might be explained by a vigorous immune attack against the MDS clone, which simultaneously causes collateral damage in the remaining normal haemopoiesis. In such patients, ATG/ALG may improve normal haemopoiesis by relieving the immunological pressure on the innocent bystanders.

307. Characterization and functional analysis of granulocyte concentrates collected from donors after repeated G–CSF stimulation
Joos, K., Herzog, R., Einsele, H., Northoff, H. and Neumeister, B.
Transfusion, 42(5), 603-611 (2002)

BACKGROUND: Neutropenic patients often develop bacterial or fungal infections not responding to broad-spectrum antibacterial or antifungal agents. Clinical efforts were made with transfusion of granulocyte concentrates; however, functions of granulocytes after multiple G–CSF stimulations and after apheresis are not yet investigated and described sufficiently.
STUDY DESIGN AND METHODS: The aim of this study was to characterize functional and immunologic variables of granulocytes in blood samples drawn from donors before and after each stimulation episode with G–CSF, in the resulting granulocyte concentrates and in the patients 8 hours after transfusion.
RESULTS: Chemotaxis was not influenced, neither by G–CSF application nor by apheresis. Multiple G–CSF stimulations enhanced oxidative burst and phagocytosis of *Escherichia coli* in donor granulocytes. These values returned to basal levels in granulocyte concentrates. Expression of granulocytic surface antigens was downregulated after application of G–CSF but returned to normal and in part enhanced values in concentrates. A clinically relevant increase of proinflammatory cytokines could not be detected. Leukotriene B_4 production was reduced after the fourth G–CSF stimulation in the donor blood and enhanced in the granulocyte concentrate after apheresis. Results in recipients indicate that changes of granulocyte function noted in concentrates were only transient.
CONCLUSION: Stimulation of healthy donors with repeated G–CSF injections and subsequent granulocyte apheresis does not dramatically change decisive functions of granulocytes.

308. Characterisation of a resource population of pigs screened for resistance to salmonellosis
Van Diemen, P.M., Kreukniet, M.B., Galina, L., Bumstead, N. And Wallis, T.S.
Vet. Immunol. Immunopathol., 88(3-4), 183-196 (2002)

The degree of resistance to *Salmonella choleraesuis* infection in a reference family purposely bred to map resistance genes was assessed. Aspects of the innate and specific immune system were studied to find a parameter that might predict the resistance of pigs to salmonellosis. The family was bred from commercial full-sister pairs of F1-gilts and four boars. One boar (G398) was identified as breeding susceptible offspring, and one boar (G402) as breeding resistant offspring on the basis of pyrexial responses and numbers of *Salmonella* in liver and spleen post mortem. The other two boars were classified as ‘possible resistant’ (Y2008) and ‘unknown’ (Y6101) respectively. Functional differences in immune cells (neutrophils and lymphocytes) between the offspring of G398 and G402 were detected. The most resistant piglets had a higher number of circulating neutrophils and better polymorphonuclear neutrophils (PMNs) function, but a lower mitogenic response of lymphocytes both pre- and post-infection and a lower antibody response. Between the offspring groups of Y2008 and Y6101 no differences were found in the number of viable *Salmonella* in liver and spleen at post mortem or in immune cell function, however, the survival rate of these offspring groups was clearly different. Twenty three percent of the Y2008-offspring and 33% of the Y6101-offspring reached the predetermined humane clinical endpoint before the end of the

experiment. Our findings suggest a role for several inherited immunological traits, including PMN function and lecithin-induced mitogenic proliferation, which appear to influence resistance to salmonellosis.

309. Synaptotagmin II could confer Ca2+ sensitivity to phagocytosis in human neutrophils
Lindmark, I.M. et al
Biochim. Biophys. Acta, 1590(1-3), 159-166 (2002)

Phagolysosome fusion and granule exocytosis in neutrophils are calcium-dependent processes. The calcium requirements vary between granule types, suggesting the presence of different calcium sensors. The synaptotagmins, a family of calcium-binding proteins, previously shown to participate in vesicle fusion and vesicle recycling in excitable cells, are putative calcium-sensors of exocytosis in excitable cells. In this study, we show that synaptotagmin II is present in human neutrophils and may participate in phagocytic and in exocytotic processes. In protein extracts from human neutrophils, we identified synaptotagmin II by Western blot as an 80 kDa protein. Subcellular fractionation revealed that synaptotagmin II was associated with the specific granules. In fMLP-stimulated cells, synaptotagmin II translocated to the plasma membrane. This correlated with the upregulation of complement receptor 3 (CR 3), reflecting the translocation of specific granules to the cell surface. Synaptotagmin II also translocated to the phagosome after complement-mediated phagocytosis in the presence of calcium. LAMP-1 translocated in parallel but probably was located to another subcellular compartment than synaptotagmin II. Under calcium-reduced conditions, neither synaptotagmin II nor LAMP-1 translocated to the phagosome. We therefore suggest a role for synaptotagmin II as calcium-sensor during phagocytosis and secretion in neutrophils.

310. Effects of gatifloxacin on phagocytosis, intracellular killing and oxidant radical production by human polymorphonuclear neutrophils
Braga, P.C., Dal Sasso, M., Bovio, C., Zavaroni, E. and Fonti, E.
Int. J. Antimicrobial Agents, 19(3), 183-187 (2002)

The ingestion and killing of bacteria by phagocytic cells is an important step in the sequence of interactions between invading microorganisms and host defense systems and may be affected by antibiotics. We investigated the effects of gatifloxacin on the phagocytosis, killing and oxidative bursts of human polymorphonuclear neutrophils (PMNs). The percentage phagocytosis and the phagocytosis index were unaffected by exposure of *Escherichia coli* strains to sub-MICs of gatifloxacin to a 1/64 dilution. However a significant increase in percentage intraphagocytic killing and the killing index occurred in one *E. coli* strain at 1/32 MIC and in two strains at 1/16 MIC. The incubation of PMNs with sub-MICs and supra-MICs of gatifloxacin (to 32 MIC) did not affect the oxidative bursts.

311. Clinical significance of telomerase activity in peripheral blood of patients with esophageal squamous cell carcinoma
Koyanagi, K.et al
Ann. Thorac. Surg., 73(3), 927-932 (2002)

Background. The presence of tumor cells in the blood stream is considered evidence of a high risk of distant organ metastasis. We examined the usefulness of telomerase activity in peripheral blood polymorphonuclear cells as an indicator of distant metastasis in patients with esophageal squamous cell carcinoma.
Methods. Telomerase activity was measured in the peripheral blood mononuclear cell and polymorphonuclear cell fractions obtained from blood samples of healthy volunteers mixed with squamous cell carcinoma cell lines, and cell distribution was analyzed by flow cytometry. Then telomerase activity of forty-two polymorphonuclear cell fractions obtained from esophageal squamous cell carcinoma patients was measured.
Results. Telomerase activity was detected in polymorphonuclear cell fractions and cell distribution analysis revealed the presence of esophageal squamous cell carcinoma cells. Organ metastasis was detected in 7 (78%) of the 9 patients with telomerase-positive polymorphonuclear cell fractions as opposed to only five (15%) of the 33 with telomerase-negative cases, and there was a significant positive correlation between telomerase activity and organ metastasis ($\boldsymbol{p} < 0.0008$).
Conclusions. Measurement of telomerase activity in the polymorphonuclear cell fractions is useful for identifying a high risk group for distant organ metastasis in patients with esophageal squamous cell carcinoma.

312. Leucocyte versus erythrocyte thioguanine nucleotide concentrations in children taking thiopurines for acute lymphoblastic leukaemia
Lancaster, D., Patel, N., Lennard, L. and Lilleyman, J.
Cancer Chemother. Pharmacol., 50(1), 33-36 (2002)

Purpose. The aim of this study was to compare leucocyte and erythrocyte thioguanine nucleotide (TGN) cytotoxic metabolite concentrations in children with lymphoblastic leukaemia taking mercaptopurine (MP) or thioguanine (TG) as part of their long-term remission maintenance chemotherapy.

Methods. Ten consecutive children treated on the MRC ALL97 protocol were studied. Six were randomized to TG and four to MP. Leucocyte and erythrocyte thiopurine nucleotide metabolites were measured after the children had been titrated to the standard thiopurine protocol dose, or higher.
Results. Children taking TG accumulated significantly higher erythrocyte TGN concentrations than those taking MP (median difference 1171 pmol/8210^8 erythrocytes, 95% CI 766 to 2169, P<0.02), but there was no significant difference in the concentration range of leucocyte TGNs generated from TG or MP. In those children taking TG, median TGN concentrations were 5142 pmol/8210^8 leucocytes and 1472 pmol/8210^8 erythrocytes (3.5-fold difference, median difference 3390 pmol/8210^8 cells, 95% CI 1559 to 7695, P=0.005), compared to 5422 pmol/8210^8 leucocytes and 261 pmol/8210^8 erythrocytes (20-fold difference, median difference 5054 pmol/8210^8 cells, 95% CI 2281 to 6328, P=0.03) in those taking MP.
Conclusions. Despite the accumulation of significantly higher erythrocyte TGN concentrations for TG compared with MP, the accumulation of leucocyte TGNs in children taking TG was similar to the range of leucocyte TGNs in children taking MP. Therefore, when correlating intracellular TGNs to clinical effect, the range of erythrocyte TGN metabolites will be higher for those children taking TG than in those taking MP.

313. Modulatory Effects of Plasma and Colonic Milieu of Patients with Ulcerative Colitis on Neutrophil Reactive Oxygen Species Production in Presence of a Novel Antioxidant, Rebamipide
Farhadi, A. et al
Digest. Dis. Sci., 47(6), 1342-1348 (2002)

Rebamipide protects gastrouintestinal mucosal integrity against reactive oxygen species (ROS). The effect of rebamipide on the capability of PMNs to produce ROS in the presence of plasma and rectal dialysates (RD) of control and ulcerative colitis (UC) subjects was evaluated. We recruited six healthy volunteers for obtaining PMNs, control plasma, and control RD and six patients with inactive UC for obtaining plasma and RD. PMNs were activated using fMLP, and ROS was measured by fluorescent microplate assay (DCFD). Rebamipide significantly inhibited the neutrophil respiratory burst by 45%. Plasma from both control subjects and UC patients significantly blunted the fMLP-induced respiratory burst. However, the plasma of the UC patients was significantly less inhibitory than the plasma of control subjects. RD from control subjects significantly blunted the fMLP-induced respiratory burst while, RD from patients with UC did not. Rebamipide maintained its antioxidant effects in the presence of plasma or RD obtained from both controls and UC patients. In conclusion, partial loss of the inhibitory effects of plasma and RD in patients with UC may contribute to oxidative-induced tissue damage in UC and rebamipide antioxidant properties were not hampered by the biological milieu of patients with UC.

314. Tissue-Specific ICAM-1 Expression and Neutrophil Transmigration in the Copper-Deficient Rat
Schuschke, D.A., Percival, S.S., lominadze, D., Saari, J.T. and Lentsch, A.B.
Inflammation, 26(6), 297-303 (2002)

Dietary copper deficiency promotes neutrophil accumulation in rat lungs. We have now investigated the potential mechanisms of this effect. Male weanling rats were fed a Cu-adequate (6.0 mg diet) or Cu-deficient diet (0.30 mg) for 4 wks. Endothelial intercellular adhesion molecule-1 (ICAM-1) expression was measured *in vivo* and *in vitro* using a radiolabeled monoclonal antibody to rat ICAM-1. Tissue neutrophil accumulation was measured by myeloperoxidase (MPO) content and neutrophil transendothelial migration was assessed *in vitro*. Dietary copper deficiency had no effects on the expression of ICAM-1 in lung, liver, heart, kidney, or cremaster. However, MPO content was significantly greater in the lungs of copper-deficient rats. Endotoxin-induced ICAM-1 expression was greater in the lungs and hearts of copper-deficient rats. Similarly, cultured rat endothelial cells that were Cu-chelated expressed more ICAM-1 after endotoxin. This correlated with the significant increase in MPO in lungs of copper-deficient rats treated with endotoxin. The results suggest a tissue-specific difference in ICAM-1 expression and neutrophil accumulation during inflammation in copper-deficient rats. The findings suggest that lung inflammatory mechanisms are particularly sensitive to copper deficiency.

315. Polyclonal human antibodies reduce bacterial attachment to soft contact lens and corneal cell surfaces
Rediske, A.M. et al
Biomaterials, 23(23), 4565-4572 (2002)

Bacterial keratitis due to *Pseudomonas aeruginosa* is a potentially serious complication of extended-wear contact lens use. Adhesion of *P. aeruginosa* to soft contact lens materials or corneal endothelial cells in the presence of pooled human immunoglobulins and/or neutrophils in artificial tear fluid was studied in vitro as a potential method to treat contact lens-associated infection. Soft hydrophilic contact lens materials equilibrated in sterile saline were soaked in artificial tear fluid for 18 h prior to use. *P. aeruginosa* IFO 3455 was added to groups of lenses or confluent cultured bovine corneal endothelial cells with varying amounts of human polyclonal immunoglobulin (IgG) and human blood neutrophils or serum albumin as a control. After 2 or 4 h incubation, adherent viable bacteria on lenses were quantified. Fluorescence microscopy was used to assess bacterial adherence to bovine corneal endothelial cells in the presence and absence of IgG and neutrophils. Various concentrations of albumin had no effect on adhesion. Human immunoglobulin solutions (25 mg/ml) reduced *P. aeruginosa* adhesion by nearly 1 log and 2 logs after 2 and 4 h incubations, respectively. Neutrophils in combination with 25 mg/ml IgG reduced bacterial adhesion approximately 1 log over reduction in adhesion by neutrophils alone. Diluted human IgG (10 mg/ml) did not significantly decrease bacterial adhesion after 2 or 4 h, but did reduce adhesion in combination

with human neutrophils at both time points. Similar reductions in amounts of fluorescently labeled bacteria adhered to cultured monolayers of corneal endothelial cells under these conditions were qualitatively observed.

316. Fluid shear- and time-dependent modulation of molecular interactions between PMNs and colon carcinomas
Jadhav, S. and Konstantopoulos, K.
Am. J. Physiol. Cell Physiol., 283, C1133.C1143 (2002)

This study compares the effects of fluid shear on the kinetics, adhesion efficiency, stability, and molecular requirements of polymorphonuclear leukocyte (**PMN**) binding to two colon adenocarcinoma cell-lines, the CD54-negative/sLe^x-bearing LS174T cells and the CD54-expressing/sLe^x-low HCT-8 cells. The efficiency of **PMN**-colon carcinoma heteroaggregation decreases with increasing shear, with **PMN**s binding HCT-8 more efficiently than LS174T cells at low shear (50-200 s^{-1}). In the low shear regime, CD11b is sufficient to mediate **PMN** binding to LS174T cells. In contrast, both CD11a and CD11b contribute to **PMN**-HCT-8 heteroaggregation, with CD54 on HCT-8 cells acting as a CD11a ligand at early time points. At high shear, only **PMN**-LS174T heteroaggregation occurs, which is initiated by **PMN** L-selectin binding to a sialylated, O-linked, protease-sensitive ligand on LS174T cells. **PMN**-LS174T heteroaggregation is primarily dependent on the intercellular contact duration (or shear rate), whereas **PMN**-HCT-8 binding is a function of both the intercellular contact duration and the applied force (or shear stress). Cumulatively, these studies suggest that fluid shear modulates the kinetics and molecular mechanisms of **PMN**-colon carcinoma cell aggregation.

317. Measurement of free and membrane-bound cathepsin G in human neutrophils using new sensitive fluorogenic substrates
Attucci, S. et al
*Biochem. J., **366**, 965-970 (2002)*

Activated human polymorphonuclear neutrophils at inflammatory sites release the chymotrypsin-like protease cathepsin G, together with elastase and proteinase 3 (myeloblastin), from their azurophil granules. The low activity of cathepsin G on synthetic substrates seriously impairs studies designed to clarify its role in tissue inflammation. We have solved this problem by producing new peptide substrates with intramolecularly quenched fluorescence. These substrates were deduced from the sequence of putative protein targets of cathepsin G, including the reactive loop sequence of serpin inhibitors and the N-terminal domain of the protease-activated receptor of thrombin, PAR-1. Two substrates were selected, Abz-TPFSGQ-EDDnp and Abz-EPFWEDQ-EDDnp, that are cleaved very efficiently by cathepsin G but not by neutrophil elastase or proteinase 3, with specificity constants (k_{cat}/K_m) in the $10^5 M^{-1}\cdot s^{-1}$ range. They can be used to measure subnanomolar concentrations of free enzyme *in vitro* and at the surface of neutrophils purified from fresh human blood. Purified neutrophils express 0.02–0.7pg of cathepsin G/cell (n = 15) at their surface. This means that about 10^4 purified cells may be enough to record cathepsin G activity within minutes. This may be most important for investigating the role of cathepsin G as an inflammatory agent, especially in bronchoalveolar lavage fluids from patients with pulmonary inflammatory disorders.

318. Up-regulation of the dendritic cell marker CD83 on polymorphonuclear neutrophils (PMN): divergent expression in acute bacterial infections and chronic inflammatory disease
Iking-Konert, C. Et al
*Clin. Exp. Immunol., **130(3)**, 501-508 (2002)*

Upon cultivation with interferon-[gamma] (IFN-[gamma]) and granulocyte/macrophage-colony stimulating factor (GM-CSF) polymorphonuclear neutrophils (PMN) acquire characteristics of dendritic cells, including expression of major histocompatibility complex (MHC) class II antigens, of the co-stimulatory antigens CD80, CD86 and of CD83, the latter considered to be specific for dendritic cells. Dendritic-like PMN were also able to present to T cells antigens in a MHC class II-restricted manner. To assess whether dendritic-like PMN are also generated in vivo, cells of patients with acute bacterial infections and of patients with chronic inflammatory diseases (primary vasculitis) were tested. During acute infection up to 80% of PMN acquired CD83, but remained negative for MHC class II, CD80 or CD86. PMN of patients with primary vasculitis expressed MHC class II antigens, CD80 and CD86, but not CD83, indicating that up-regulation of MHC class II and of CD83 are not necessarily linked to each other. Indeed, parallel studies with PMN of healthy donors showed that while IFN-[gamma] and granulocyte/macrophage colony stimulating factor (GM-CSF) induced both, MHC class II and CD83, tumour necrosis factor (TNF)-[alpha] selectively induced de novo synthesis of CD83. The function of CD83 on PMN is still elusive. A participation in the MHC class II-restricted antigen presentation could be ruled out, consistent with the segregation of MHC class II and CD83 expression. Regardless, however, of its function, CD83 expression could serve as a marker to differentiate between acute and chronic inflammation.

319. Perioperative elastase activity in cardiac surgery and its role in endothelial leakage
Scholz, M. et al
Inflammation Res., 52(10), 433-438 (2003)

Objective and Design: The functional activity and pathophysiological effects of polymorphonuclear elastase (PMNE) in cardiac surgery patients are unknown. This in vitro study was done to evaluate whether PMNE activity in patient blood samples may be correlated with decreased endothelial wall integrity.
Methods and Subjects: PMNE was serially analyzed by PMNE activity in plasma samples from 40 high risk cardiac surgery patients. Endothelial cell cultures were used to study the influence of patient serum on the intercellular integrity.
Results: Ex vivo, samples with high PMNE activity (>1.0 mg/ ml), found in 14 patients (35%), neither induced hyperpermeability in cultured endothelial cells nor resulted in intracellular redistribution of the junction molecules cadherin-5 or β-catenin. However, pretreatment of endothelial cells with these samples but not with low activity (<0.5 mg/ml) samples augmented neutrophil transendothelial migration (>20-fold) in conjunction with formation of intercellular gaps and irregular membrane-associated β-catenin staining. Neutrophil transmigration was inhibited by blocking neutrophil β1 integrin but not by the proteinase inhibitor methoxysuccinyl-Ala-Ala-Pro-Ala.
Conclusions: Augmented PMNE activity in cardiac surgery patients does not directly induce endothelial leakage but may indirectly promote neutrophil extravasation and thus perioperative endothelial hyperpermeability.

320. Type 4A cAMP-specific phosphodiesterase is stored in granules of human neutrophils and eosinophils
Pryzwansky, K.B. and Madden, V.J.
Cell and Tissue Res., 312(3), 301-311 (2003)

Persistent elevations of cAMP levels are generally accompanied by an inhibition of granulocyte functions. Phosphodiesterases play a critical role in regulating intracellular levels of cAMP. The expression of three isoforms of type 4 cAMP-specific phosphodiesterase (PDE4) in neutrophils suggests diversity of isoform localization and targeting in regulating cell function. The sites of cAMP regulation in granulocytes by the PDE4A isoform were investigated by immunoelectron microscopy. PDE4A was localized uniformly in all granule classes of eosinophils, but was restricted in neutrophils to a subset of myeloperoxidase (MPO)-containing granules that were round or elongated with a central crystalloid core. Granulocytes were stimulated with fMLP to investigate the sites of PDE4A targeting during cell activation. In neutrophils, fMLP induced a rapid (1 min) translocation of granules containing PDE4A to the plasmalemma, where some PDE4A and MPO were exocytosed. In these cells, PDE4A labeling within granules was focal and no longer homogeneous. While immunogold labeling of PDE4A was reduced after fMLP stimulation, staining of MPO-containing granules remained high. Extracellular release of PDE4A was also observed in eosinophils stimulated with fMLP. Morphometry revealed that Au labeling was significantly reduced within 1 min, and that there was a shift in PDE4A localization within eosinophil granules from the crystalline core to the matrix. Fluctuations of cAMP levels and ectoprotein kinase activity with PKA properties occur in blood under normal and pathological conditions. The exclusive localization of PDE4A within granules of neutrophils and eosinophils suggests that PDE4A may function to downregulate cAMP signaling at the cell membrane and/or in the extracellular space at the time of granule release.

321. Critical Role of the Carboxyl Terminus of Proline-rich Tyrosine Kinase (Pyk2) in the Activation of Human Neutrophils by Tumor Necrosis Factor: Separation of Signals for the Respiratory Burst and Degranulation
Han, H., Fuortes, M. and Nathan, C.
J. Exp. Med., 197, 63, (2003)

Transduction of Tat-tagged fusion proteins confirmed a hypothesis based on pharmacologic inhibitors (Fuortes, M., M. Melchior, H. Han, G.J. Lyon, and C. Nathan. 1999. *J. Clin. Invest.* 104:327–335) that proline-rich tyrosine kinase (Pyk2) plays a critical role in the activation of adherent human neutrophils, and allowed an analysis of individual Pyk2 domains not possible with chemical inhibitors. Acting as a dominant negative, the COOH terminus of Pyk2 fused to a Tat peptide (Tat-CT), but not other regions of Pyk2, specifically inhibited the respiratory burst of cells responding to tumor necrosis factor (TNF), *Salmonella*, or *Listeria*, while sparing responses induced by phorbol ester. Tat-CT suppressed TNF-triggered cell spreading and the phosphorylation of endogenous Pyk2 and the associated tyrosine kinase Syk without blocking the ability of neutrophils to degranulate and kill bacteria. Thus, separate signals control the respiratory burst and degranulation, and a normal rate of killing of some bacteria can be sustained by granule products in conjunction with a minimal residual respiratory burst. Inhibition of select inflammatory functions without impairment of antibacterial activity may commend the Pyk2 pathway as a potential target for antiinflammatory therapy.

322. The Absence of Fucose but Not the Presence of Galactose or Bisecting N-Acetylglucosamine of Human IgG1 Complex-type Oligosaccharides Shows the Critical Role of Enhancing Antibody-dependent Cellular Cytotoxicity
Shinkawa, T. et al
J. Biol. Chem., 278, 3466-3473 (2003)

An anti-human interleukin 5 receptor (hIL-5R) humanized immunoglobulin G1 (IgG1) and an anti-CD20 chimeric IgG1 produced by rat hybridoma YB2/0 cell lines showed more than 50-fold higher antibody-dependent cellular cytotoxicity (ADCC) using purified human

peripheral blood mononuclear cells as effector than those produced by Chinese hamster ovary (CHO) cell lines. Monosaccharide composition and oligosaccharide profiling analysis showed that low fucose (Fuc) content of complex-type oligosaccharides was characteristic in YB2/0-produced IgG1s compared with high Fuc content of CHO-produced IgG1s. YB2/0-produced anti-hIL-5R IgG1 was subjected to *Lens culinaris* agglutin affinity column and fractionated based on the contents of Fuc. The lower Fuc IgG1 had higher ADCC than the IgG1 before separation. In contrast, the content of bisecting GlcNAc of the IgG1 affected ADCC much less than that of Fuc. In addition, the correlation between Gal and ADCC was not observed. When the combined effect of Fuc and bisecting GlcNAc was examined in anti-CD20 IgG1, only a severalfold increase of ADCC was observed by the addition of GlcNAc to highly fucosylated IgG1. Quantitative PCR analysis indicated that YB2/0 cells had lower expression level of FUT8 mRNA, which codes α1,6-fucosyltransferase, than CHO cells. Overexpression of FUT8 mRNA in YB2/0 cells led to an increase of fucosylated oligosaccharides and decrease of ADCC of the IgG1. These results indicate that the lack of fucosylation of IgG1 has the most critical role in enhancement of ADCC, although several reports have suggested the importance of Gal or bisecting GlcNAc and provide important information to produce the effective therapeutic antibody.

323. Membrane Lipid Organization Is Critical for Human Neutrophil Polarization
Pierini, L.M. et al
J. Biol. Chem., 278, 10831-10841 (2003)

In response to chemoattractants neutrophils extend an actin-rich pseudopod, which imparts morphological polarity and is required for migration. Even when stimulated by an isotropic bath of chemoattractant, neutrophils exhibit persistent polarization and continued lamellipod formation at the front, suggesting that the cells establish an internal polarity. In this report, we show that perturbing lipid organization by depleting plasma membrane cholesterol levels reversibly inhibits cell polarization and migration. Among other receptor-mediated responses, β_2 integrin up-regulation was unaffected, and initial calcium mobilization was only partially reduced by cholesterol depletion, indicating that this treatment did not abrogate initial receptor-mediated signal transduction. Interestingly, cholesterol depletion did not prevent initial activation of the GTPase Rac or an initial burst of actin polymerization, but rather it inhibited prolonged activation of Rac and sustained actin polymerization. Collectively, these findings support a model in which the plasma membrane is organized into domains that aid in amplifying the chemoattractant gradient and maintaining cell polarization.

324. Heparan sulfate on endothelial cells mediates the binding of Plasmodium falciparum-infected erythrocytes via the DBL1α domain of PfEMP1
Vogt, A.M. et al
Blood, 101, 2405-2411 (2003)

Plasmodium falciparum may cause severe forms of malaria when excessive sequestration of infected and uninfected erythrocytes occurs in vital organs. The capacity of wild-type isolates of *P falciparum*-infected erythrocytes (parasitized red blood cells [pRBCs]) to bind glycosaminoglycans (GAGs) such as heparin has been identified as a marker for severe disease. Here we report that pRBCs of the parasite FCR3S1.2 and wild-type clinical isolates from Uganda adhere to heparan sulfate (HS) on endothelial cells. Binding to human umbilical vein endothelial cells (HUVECs) and to human lung endothelial cells (HLECs) was found to be inhibited by HS/heparin or enzymes that remove HS from cell surfaces. ^{35}S-labeled HS extracted from HUVECs bound directly to the pRBCs' membrane. Using recombinant proteins corresponding to the different domains of *P falciparum* erythrocyte membrane protein 1 (PfEMP1), we identified Duffy-binding-like domain-1α (DBL1α) as the ligand for HS. DBL1α bound in an HS-dependent way to endothelial cells and blocked the adherence of pRBCs in a dose-dependent manner. ^{35}S-labeled HS bound to DBL1α-columns and eluted as a distinct peak at 0.4 mM NaCl. ^{35}S-labeled chondroitin sulfate (CS) of HUVECs did not bind to PfEMP1 or to the pRBCs' membrane. Adhesion of pRBCs of FCR3S1.2 to platelet endothelial cell adhesion molecule-1 (PECAM-1)/CD31, mediated by the cysteine-rich interdomain region 1α (CIDR1α), was found be operative with, but independent of, the binding to HS. HS and the previously identified HS-like GAG on uninfected erythrocytes may act as coreceptors in endothelial and erythrocyte binding of rosetting parasites, causing excessive sequestration of both pRBCs and RBCs.

325. Secretory Component Is Cleaved by Neutrophil Serine Proteinases but its Epithelial Production Is Increased by Neutrophils through NF-κB– and p38 Mitogen-Activated Protein Kinase–Dependent Mechanisms
Pilette, C., Quadrhiri, Y., Dimanche, F., Vaerman, J-P. and Sibille, Y.
Am. J. Respir, Cell Mol. Biol., 28, 485 (2003)

We previously showed that expression of polymeric immunoglobulin receptor (pIgR)/secretory component (SC), the epithelial receptor assuming transport of polymeric IgA in mucosal secretions, is strongly decreased in severe chronic obstructive pulmonary disease. Here, we evaluated *in vitro* the effects of polymorphonuclear neutrophil (PMN) mediators on pIgR/SC. On polyacrylamide gel electrophoresis analysis, soluble SC was rapidly cleaved by supernatants from phorbol-myristate-acetate-activated PMN, through a serine proteinase activity. Moreover, purified PMN serine proteinases also cleaved SC. Similarly, polymeric IgA was rapidly cleaved in monomers by neutrophil elastase, whereas secretory immunoglobulin A was relatively resistant to neutrophil elastase. Surface pIgR on human bronchial epithelial cells was also cleaved by serine proteinases, as shown by immunofluorescence. In contrast, pIgR/SC production by cultured epithelial cells (quantified by enzyme-linked immunosorbent assay) was significantly increased by supernatants from

interleukin-8/formylmethionylleucylphenylalanine-activated PMN (122.6 ± 17.3 versus 70.9 ± 9 ng/mg protein, $P < 0.01$). Upregulation of pIgR/SC production by bronchial epithelial cells was abolished by nuclear factor κB- and p38 mitogen-activated protein kinase (MAPK) inhibitors. Moreover, supernatants from interleukin-8/formylmethionylleucylphenylalanine-activated PMN induced the phosphorylation of IκB-α and p38 MAPK in epithelial cells, independently of serine proteinases. Thus, PMN serine proteinases cleave pIgR/SC, whereas activated PMN induce an increased pIgR/SC expression through epithelial activation of nuclear factor κB and p38 MAPK pathways.

326. The heat shock protein Gp96 binds to human neutrophils and monocytes and stimulates effector functions
Radsak, M.P. et al
Blood, 101, 2810-2815 (2003)

The endoplasmic reticulum (ER)-resident heat shock protein Gp96 is involved in protein folding and is released into the extracellular space after necrotic cell death. In this context, Gp96 has immunostimulatory properties: it activates dendritic cells or macrophages and delivers associated peptides into the antigen presentation pathway, resulting in the induction of specific T-cell responses. The inflammatory response after necrotic tissue damage leads to the recruitment of polymorphonuclear neutrophils (PMNs) and monocytes, allowing them to make their first encounter with Gp96. We therefore investigated whether PMNs and monocytes interact with Gp96. We were able to show that PMNs and monocytes specifically bind fluorescein isothiocyanate (FITC)-conjugated Gp96. The binding of Gp96-FITC was competed by lipopolysaccharide (LPS) or fucoidan, a known inhibitor of scavenger receptors. Interestingly, the binding of LPS-FITC was also competed not only by fucoidan, but by Gp96, suggesting that LPS and Gp96 share a common receptor on PMNs. One important effector function of PMNs is the clearance of an inflammatory site by phagocytosis. We therefore assessed the influence of Gp96 on phagocytic activity using fluorochrome-labeled polystyrene beads. We found a marked enhancement of phagocytosis in the presence of Gp96 and concluded that PMNs not only bind Gp96, but are also activated by it. Additionally, Gp96-stimulated PMNs and especially monocytes release large amounts of interleukin-8, a potent neutrophil-attracting chemokine. In conclusion, we demonstrate that Gp96 specifically binds to and activates PMNs and monocytes, extending the function of Gp96 as a danger signal to additional members of the innate immune system.

327. Dietary fruit and vegetables protect against somatic mutation in vivo, but low or high intake of carotenoids does not
Nyberg, F., Hou, S-M., Pershagen, G. and Lambert, B.
Carcinogenenesis, 24, 689-696 (2003)

Epidemiological studies have demonstrated protective effects of vegetables and fruit on risk of cancer, but underlying mechanisms remain unclear. Intervention studies have in some cases contradicted previous epidemiological evidence, e.g. for beta-carotene supplementation and lung cancer, emphasizing the need for mechanistic data. We assessed *in vivo* mutagenic effects of several dietary items using the *HPRT* (hypoxanthine-guanine phosphoribosyl transferase) gene assay with T-lymphocytes from 312 individuals (158 lung cancer cases, 154 population controls), who provided information on diet and smoking habits. *HPRT* mutant frequency (MF) was significantly decreased in relation to intake of vegetables, citrus fruits and berries, respectively, as well as calculated vitamin C intake from diet. There was a significant U-shaped association with dietary carotenoid intake, with lowest MF near population average carotenoid intakes and higher mutation frequencies both at low and high intakes, and a similar borderline significant association was observed for beta-carotene. Our study is consistent with known diet–cancer associations and provides novel human *in vivo* mechanistic support for a cancer-protective effect of vegetables and fruit by modulation of somatic mutagenesis. Our results also provide support for the increase in lung cancer risk observed particularly in smokers in studies of beta-carotene supplementation.

328. Concurrent evolution and resolution in an acute inflammatory model of rat carrageenin-induced pleurisy
Murai, N. et al
J. Leukoc. Biol., 73, 456 (2003)

Granulocyte apoptosis and subsequent clearance by phagocytes are critical for the resolution of inflammation. However, no studies have addressed how the resolution proceeds in the inflammatory site. We studied the time course of neutrophil apoptosis and the following ingestion by mononuclear leukocytes in rat carrageenin-induced pleurisy, detecting DNA fragmentation by the deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) method, by acridine orange staining, and from the DNA ladder pattern on electrophoresis. Neutrophil accumulation started 3–5 h after carrageenin injection and then maintained a plateau until 24 h. Neutrophils decreased steeply between days 1 and 3. Mononuclear leukocytes started to accumulate at 5 h and reached a peak at day 2. TUNEL-positive bodies and acridine orange-positive bodies first became detectable in the cytoplasm of the mononuclear leukocytes from 24 h and 9 h, respectively. Both methods indicated that mononuclear leukocytes containing fragmented DNA increased rapidly on days 1 and 2 and reached a peak at day 3. The characteristic ladder pattern of neutrophil DNA was observed from 5 h. Tumor necrosis factor-α was detectable on the start, and the levels of interleukin-10 and transforming growth factor-ß1 rose together with signs of neutrophil apoptosis and the following ingestion by mononuclear leukocytes. These results indicate that neutrophils start to undergo apoptosis just after the beginning of their accumulation in the inflammation site. Thus, evolution and resolution processes may proceed concurrently in acute inflammation.

329. Involvement of nitric oxide donor compounds in the bactericidal activity of human neutrophils in vitro
Klink, M. Cedzynski, M., St Swierzko, A., Tchorzewski, H. and Sulowska, Z.
J. Med. Microbiol., 52, 303 (2003)

The bactericidal activity of human neutrophils against extracellular and facultatively intracellular bacteria was studied in the presence of the nitric oxide (NO) donors sodium nitroprusside (SNP) and 3-morpholinosydnonimine (SIN-1), a molsidomine metabolite. SNP and molsidomine are drugs commonly used as nitrovasodilators in coronary heart disease. It is demonstrated here that the NO donor compounds themselves did not affect the viability and survival of the bacterial strains tested. Neither SNP nor SIN-1 had any effect on the process of bacteria ingestion. In contrast, NO donors enhanced the ability of neutrophils to kill *Escherichia coli*, *Proteus vulgaris* and *Salmonella* Anatum. However, strains differed in their susceptibility to SNP- and SIN-1-mediated killing by neutrophils. Removal of the superoxide anion reduced the bactericidal activity of SNP- and SIN-1-treated neutrophils against *E. coli* and *S.* Anatum. This suggests that the NO derivatives formed in the reaction of NO generated from donors with the reactive oxygen species released by phagocytosed neutrophils potentiate the bactericidal activity of human neutrophils *in vitro*. The above original observation discussed here suggests clinical significance for the treatment of patients with nitrovasodilators in the course of coronary heart disease therapy.

330. Oxidative stress-induced cell death of human oral neutrophils
Sato, E.F. et al
Am. J. Physiol. Cell Physiol., 284, C1048-C1053 (2003)

Polymorphonuclear leukocytes (PMN) play crucial roles in protecting hosts against invading microbes and in the pathogenesis of inflammatory tissue injury. Although PMN migrate into mucosal layers of digestive and respiratory tracts, only limited information is available of their fate and function in situ. We previously reported that, unlike circulating PMN (CPMN), PMN in the oral cavity spontaneously generate superoxide radical and nitric oxide (NO) in the absence of any stimuli. When cultured for 12 h under physiological conditions, oral PMN (OPMN) showed morphological changes that are characteristic of those of apoptosis. Upon agarose gel electrophoresis, nuclear DNA samples isolated from OPMN revealed ladder-like profiles characteristic of nucleosomal fragmentation. L-cysteine, reduced glutathione (GSH), and herbimycin A, a protein tyrosine kinase inhibitor, suppressed the activation of caspase-3 and apoptosis of OPMN. Neither thiourea, superoxide dismutase (SOD), nor catalase inhibited the activation of caspase-3 and apoptosis. Moreover, *N*-acetyl-Asp-Glu-Val-Asp-aldehyde (Ac-DEVD-CHO), inhibitor for caspase-3, inhibited the fragmentation of DNA. These results suggested that oxidative stress and/or tyrosine-kinase-dependent pathway(s) activated caspase-3 in OPMN, thereby inducing their apoptosis.

331. Identification and Characterization of Novel Antagonists of the CCR3 Receptor
Warrior, U. et al
J. Biomol. Screen., 8, 324-331 (2003)

Eotaxin, an inducer of eosinophil migration and activation, exerts its activity by binding to CCR3, the C-C chemokine receptor 3. An inhibitor of the eotaxin-CCR3 binding interaction may have potential as an anti-inflammatory drug for treatment of asthma, parasitic infections, and allergic disorders. A radioligand binding assay was developed using HEK cells transfected with CCR3, with ^{125}I eotaxin as the ligand. Whole cells grown on polylysine-coated plates were used as the receptor source for the screen. Screening of more than 200,000 compounds with this assay yielded a number of screening hits, and of these, 2 active novel antagonists were identified. These compounds showed inhibitory effects on eosinophil chemotaxis in both in vitro and in vivo assays.

332. Evidence for a role for Gαi1 in mediating weak agonist-induced platelet aggregation in human platelets: reduced Gαi1 expression and defective Gi signaling in the platelets of a patient with a chronic bleeding disorder
Patel, Y.M. et al
Blood, 101, 4828-4835 (2003)

We have examined platelet functional responses and characterized a novel signaling defect in the platelets of a patient suffering from a chronic bleeding disorder. Platelet aggregation responses stimulated by weak agonists such as adenosine diphosphate (ADP) and adrenaline were severely impaired. In comparison, both aggregation and dense granule secretion were normal following activation with high doses of collagen, thrombin, or phorbol-12 myristate-13 acetate (PMA). ADP, thrombin, or thromboxane A_2 (TxA_2) signaling through their respective G_q-coupled receptors was normal as assessed by measuring either mobilization of intracellular calcium, diacylglycerol (DAG) generation, or pleckstrin phosphorylation. In comparison, G_i-mediated signaling induced by either thrombin, ADP, or adrenaline, examined by suppression of forskolin-stimulated rise in cyclic AMP (cAMP) was impaired, indicating dysfunctional $G\alpha_i$ signaling. Immunoblot analysis of platelet membranes with specific antiserum against different $G\alpha$ subunits indicated normal levels of $G\alpha_{i2}$,$G\alpha_{i3}$,$G\alpha_z$, and $G\alpha_q$ in patient platelets. However, the $G\alpha_{i1}$ level was reduced to 25% of that found in normal platelets. Analysis of platelet cDNA and gDNA revealed no abnormality in either the $G\alpha_{i1}$ or $G\alpha_{i2}$ gene sequences. Our studies implicate the minor expressed $G\alpha_i$ subtype $G\alpha_{i1}$ as having an important role in regulating signaling pathways associated with the activation of $\alpha_{IIb}\beta_3$ and subsequent platelet aggregation by weak agonists.

333. Activation of Rac2 and Cdc42 on Fc and complement receptor ligation in human neutrophils
Forsberg, M., Druid, P., Zheng, L., Stendahl, O. and Särndahl, E.
J. Leukoc. Biol., 74, 611 (2003)

Phagocytosis is a complex process engaging a concerted action of signal-transduction cascades that leads to ingestion, subsequent phagolysosome fusion, and oxidative activation. We have previously shown that in human neutrophils, C3bi-mediated phagocytosis elicits a significant oxidative response, suggesting that activation of the small GTPase Rac is involved in this process. This is contradictory to macrophages, where only Fc receptor for immunoglobulin G (FcγR)-mediated activation is Rac-dependent. The present study shows that engagement of the complement receptor 3 (CR3) and FcγR and CR3- and FcγR-mediated phagocytosis activates Rac, as well as Cdc42. Furthermore, following receptor-engagement of the CR3 or FcγRs, a downstream target of these small GTPases, p21-activated kinase, becomes phosphorylated, and Rac2 is translocated to the membrane fraction. Using the methyltransferase inhibitors N-acetyl-S-farnesyl-L-cysteine and N-acetyl-S-geranylgeranyl-L-cysteine, we found that the phagocytic uptake of bacteria was not Rac2- or Cdc42-dependent, whereas the oxidative activation was decreased. In conclusion, our results indicate that in neutrophils, Rac2 and Cdc42 are involved in FcR- and CR3-induced activation and for properly functioning signal transduction involved in the generation of oxygen radicals.

334. Murine neutrophils require α1,3-fucosylation but not PSGL-1 for productive infection with Anaplasma phagocytophilum
Carlyon, J.A. et al
Blood, 102, 3387-3395 (2003)

Anaplasma phagocytophilum causes human granulocytic ehrlichiosis, the second most common tick-borne disease in the United States. Mice are natural reservoirs for this bacterium and man is an inadvertent host. *A phagocytophilum's* tropism for human neutrophils is linked to neutrophil expression of P-selectin glycoprotein ligand-1 (PSGL-1), as well as sialylated and α1,3-fucosylated glycans. To determine whether *A phagocytophilum* uses similar molecular features to infect murine neutrophils, we assessed in vitro bacterial binding to neutrophils from and infection burden in wild-type mice; mice lacking α1,3-fucosyltransferases Fuc-TIV and Fuc-TVII; or mice lacking PSGL-1. Binding to Fuc-TIV$^{-/-}$/Fuc-TVII$^{-/-}$ neutrophils and infection of Fuc-TIV$^{-/-}$/Fuc-TVII$^{-/-}$ mice were significantly reduced relative to wild-type mice. *A phagocytophilum* binding to PSGL-1$^{-/-}$ neutrophils was modestly reduced, whereas sialidase treatment significantly decreased binding to both wild-type and PSGL-1$^{-/-}$ neutrophils. *A phagocytophilum* similarly infected PSGL-1$^{-/-}$ and wild-type mice in vivo. *A phagocytophilum* induced comparable levels of chemokines from wild-type and PSGL-1$^{-/-}$ neutrophils in vitro, while those induced from Fuc-TIV$^{-/-}$/Fuc-TVII$^{-/-}$ neutrophils were appreciably reduced. Therefore, *A phagocytophilum* infection in mice, as in humans, requires sialylation and α1,3-fucosylation of neutrophils. However, murine infection does not require neutrophil PSGL-1 expression, which has important implications for understanding how *A phagocytophilum* binds and infects neutrophils.

335. Human Neutrophil Chemotaxis Is Modulated by Capsule and O Antigen from an Extraintestinal Pathogenic Escherichia coli Strain
Russo, T.A. et al
Infect. Immun., 71, 6435-6445 (2003)

Gram-negative enteric bacilli, such as *Escherichia coli*, are common causes of nosocomial pneumonia. The interaction between pulmonary neutrophils and the infecting pathogen is a critical step in determining the outcome. Previous studies from our laboratory, for which a rat model of pneumonia was used, established that pulmonary neutrophil recruitment was modulated by the *E. coli* virulence factors capsule and O-specific antigen. To begin to understand the mechanism by which this recruitment occurs, we conducted in vitro and ex vivo chemotaxis assays, for which we used a clinically relevant *E. coli* isolate (CP9) and isogenic derivatives that were deficient in only the O antigen (CP921) or capsule (CP9.137) as chemoattractants with or without the high-affinity N-formylmethionyl-leucyl-phenylalanine receptor antagonist *N-tert*-butoxycarbonyl-methionine-leucine-phenylalanine (*N*-t-BOC). Given that only live *E. coli* was used for the initial in vitro chemotaxis assays, it was predicted that only *N*-t-BOC-sensitive chemotaxis would occur. However, both *N*-t-BOC-sensitive and -insensitive chemotaxis was observed. *N*-t-BOC-insensitive chemotaxis was mediated in part by interleukin 8, which was produced by neutrophils that had migrated toward *E. coli*. *N*-t-BOC-insensitive chemotaxis was only observed when live *E. coli* bacteria, not cell-free *E. coli* culture supernatants, were used as chemoattractants, suggesting that a direct *E. coli*-neutrophil interaction was necessary. The presence of both capsule and O antigen diminished total, *N*-t-BOC-sensitive, and *N*-t-BOC-insensitive neutrophil chemotaxis in vitro. The presence of capsule significantly decreased total, *N*-t-BOC-sensitive, and *N*-t-BOC-insensitive neutrophil chemotaxis ex vivo when cell-free bronchoalveolar lavage fluid from infected rats was used as the source of chemotactic factors. These effects of *E. coli* capsule and O antigen on neutrophil chemotaxis are novel, and they expand our understanding of the mechanisms by which these virulence traits contribute to the pathogenesis of gram-negative pneumonia and other extraintestinal infections.

336. Chemokines Accumulate in the Lungs of Rats with Severe Pulmonary Embolism Induced by Polystyrene Microspheres
Zagorski, J., Debelak, J., Gellar, M., Watts, J.A. and Kline, J.A.
J. Immunol., 171, 5529 (2003)

Pulmonary thromboembolism (PEm) is a serious and life threatening disease and the most common cause of acute pulmonary vascular occlusion. Even following successful treatment of PEm, many patients experience long-term disability due to diminished heart and lung function. Considerable damage to the lungs presumably occurs due to reperfusion injury following anti-occlusive treatments for PEm and the resulting chronic inflammatory state in the lung vasculature. We have used a rat model of irreversible PEm to ask whether pulmonary vascular occlusion in the absence of reperfusion is itself sufficient to induce an inflammatory response in lungs. By adjusting the severity of the vascular occlusion, we were able to generate hypertensive and nonhypertensive PEm, and then examine lung tissue for expression of CXC and C-C chemokine genes and bronchoalveolar lavage (BAL) fluid for the presence of chemokine proteins. Hypertensive and nonhypertensive PEm resulted in increased expression of both CXC and C-C chemokines genes in lung tissues. Hypertensive PEm was also associated with a 50–100-fold increase in protein content in lung BAL fluid, which included the CXC chemokines cytokine-induced neutrophil chemoattractant and macrophage-inflammatory protein 2. The presence of chemokines in BALs was reflected by a potent neutrophil chemotactic activity in in vitro chemotaxis assays. Abs to cytokine-induced neutrophil chemoattractant blocked the in vitro neutrophil chemotactic activity of BAL by 44%. Our results indicate that the ischemia and hypertension associated with PEm are sufficient to induce expression of proinflammatory mediators such as chemokines, and establish a proinflammatory environment in the ischemic lung even before reperfusion.

337. The laminin receptor modulates granulocyte–macrophage colony-stimulating factor receptor complex formation and modulates its signaling
Chen, J. et al
PNAS, 100(24), 14000-14005 (2003)

Basement membrane matrix proteins are known to up-regulate granulocyte–macrophage colony-stimulating factor (GM-CSF) signaling in neutrophils and mononuclear phagocytes, but the mechanisms involved are poorly understood. We used the intracellular portion of the α subunit of the GM-CSF receptor (αGMR) to search for interacting proteins and identified the 67-kDa laminin receptor (LR), a nonintegrin matrix protein receptor expressed in several types of host defense cells and certain tumors, as a binding partner. LR was found to interact with the β subunit of the GMR (βGMR) as well. Whereas GM-CSF functions by engaging the αGMR and βGMR into receptor complexes, LR inhibited GM-CSF-induced receptor complex formation. Laminin and fibronectin binding to LR was found to prevent the binding of βGMR to LR and relieved the LR inhibition of GMR. These findings provide a mechanistic basis for enhancing host defense cell responsiveness to GM-CSF at transendothelial migration sites while suppressing it in circulation.

338. Exogenous eosinophil activation converts PSGL-1-dependent binding to CD18-dependent stable adhesion to platelets in shear flow
McCarthy, O.J.T., Tien, N., Bochner, B.S. Konstantopoulos, K.
Am. J. Physiol. Cell Physiol., 284, C1223-C1234 (2003)

This study examined the binding kinetics and molecular requirements of eosinophil adhesion to surface-anchored platelets in shear flow. P-selectin glycoprotein ligand-1 (PSGL-1) binding to platelet P-selectin initiates tethering and rolling of eosinophils to platelets under flow. These primary interacting cells assist in the capture of free-flowing eosinophils through homotypic tethering (secondary interactions) mediated via L-selectin-PSGL-1 interactions. Differences between eosinophils and neutrophils in PSGL-1 and L-selectin expression levels predict the pattern and relative extent of their adhesive interactions with immobilized platelets under shear, as well as the relative magnitude of their average rolling velocities. The majority of tethered eosinophils become rapidly stationary on the platelet layer, a process that is predominantly mediated via eosinophil PSGL-1 binding to platelet P-selectin and has an absolute requirement for intact cytoskeleton. Only a small fraction of these stationary eosinophils develop shear-resistant attachments mediated by CD18 integrins. However, stimulation of eosinophils with eotaxin-2 converts PSGL-1-P-selectin-dependent stationary adhesion to CD18-mediated shear-resistant stable attachment. These studies provide insights for designing strategies based on blocking of eosinophil-platelet interactions to combat thrombotic disorders in hypereosinophilic patients.

339. Angiotensin II Receptor Blocker Valsartan Suppresses Reactive Oxygen Species Generation in Leukocytes, Nuclear Factor-κB, in Mononuclear Cells of Normal Subjects: Evidence of an Antiinflammatory Action
Dandona, P. et al
J. Clin. Endocrinol. Metab., 88, 4496-4501 (2003)

view of the pro-oxidant and proinflammatory effects of angiotensin II, we have tested the hypothesis that valsartan, an angiotensin receptor blocker, may exert a suppressive action on reactive oxygen species (ROS) generation, nuclear factor κB (NF-κB) in mononuclear cells. Four groups of eight normal subjects were given 1) 160 mg daily of valsartan, 2) 80 mg daily of simvastatin, 3) 40 mg quinapril, or 4) no treatment. Fasting blood samples were obtained before treatment and at d 1, 8, and 14 (7 d after the cessation of the drug). After valsartan, ROS generation by polymorphonuclear cells and mononuclear cells fell significantly by more than 40% ($P < 0.01$). NF-κB binding activity and the expression of total cellular p65, a protein component of NF-κB, fell significantly ($P < 0.01$). The expression of inhibitor κB (IκB) increased significantly ($P < 0.05$). Plasma C-reactive protein (CRP) concentration fell significantly ($P < 0.01$). All indices, except IκB, reverted toward baseline, 7 d after the cessation of the drug. IκB persisted in an elevated state. Neither quinapril nor simvastatin given for 7 d produced a suppression of ROS generation, intranuclear NF-κB, p65, or CRP, and these two

agents did not alter cellular IκB either. The untreated controls also did not demonstrate a change in their ROS generation or NF-κB binding activity or plasma CRP concentration. We conclude that valsartan at a modest dose exerts a profound and rapid ROS and inflammation-suppressive effect that may be relevant to its potential beneficial effects in atherosclerosis, diabetes, and congestive cardiac failure. In contrast, quinapril and simvastatin produced no similar effect over the period of 1 wk. Our observations may also have implications to clinical situations in which a rapid antiinflammatory effect is required.

340. Elevation of Free Fatty Acids Induces Inflammation and Impairs Vascular Reactivity in Healthy Subjects
Tripathy, D. et al
Diabetes, 52, 2882 (2003)

To test the possible acute proinflammatory effects of fatty acids, we induced an increase in plasma free fatty acid (FFA) concentrations after a lipid and heparin infusion for 4 h in 10 healthy subjects. We determined the nuclear factor-κB (NF-κB) binding activity in mononuclear cells (MNCs), the p65 subunit of NF-κB, reactive oxygen species (ROS) generation by MNC, and polymorphonuclear leukocytes (**PMN**). Brachial artery reactivity, using postischemic flow-mediated dilation, was also measured. NF-κB binding activity in the MNC nuclear extracts increased to 163 ± 17% and 144 ± 14% as compared with basal levels at 2 and 4 h ($P < 0.005$) and remained elevated ($P < 0.05$) at 6 h (2 h after cessation of lipid infusion). NF-κB p65 subunit protein expression in MNC homogenates also increased at 2, 4, and 6 h ($P < 0.05$). ROS generation by **PMN**s increased significantly at 2 and 4 h ($P < 0.005$), whereas that by MNCs increased at 4 h ($P < 0.05$). Plasma macrophage migration inhibitory factor increased at 2 ($P < 0.05$) and 4 h ($P < 0.005$), respectively, and declined to baseline at 6 h. The postischemic flow-mediated dilation of brachial artery decreased from 6.3 ± 1.1% at baseline to 4.3 ± 1.9% and 2.7 ± 2.1% ($P < 0.01$) at 2, 4, and 6 h, respectively. We conclude that an increase in FFA concentration induces oxidative stress and has a proinflammatory effect; it also impairs postischemic flow-mediated vasodilation of the brachial artery.

341. Inflammatory Chemoreceptor Cross-Talk Suppresses Leukotriene B4 Receptor 1-Mediated Neutrophil Calcium Mobilization and Chemotaxis After Trauma
Tarlowe, M.H. et al
J. Immunol., 171, 2066 (2003)

G protein-coupled chemoattractants recruit neutrophils (**PMN**) to sites of injury and infection. The leukotrienes (LT) and CXC chemokines (CXC) and their receptors (BLT1/BLT2 and CXCR1/CXCR2) are all known to play roles in these responses. Each system has been studied separately in vitro, but in vivo they act concurrently, and the clinical interactions between the two systems are unstudied. We prospectively studied calcium mobilization and chemotactic responses to LTB_4 in **PMN** from major trauma patients. The responses of the high affinity BLT1 receptor were suppressed at the 3-day postinjury time point, but recovered by 1 wk. Trauma patients had transient elevations of plasma LT and CXC levels. Functional deficits identical with those in trauma **PMN** were reproduced in vitro by exposing healthy **PMN** to CXCs at the elevated plasma concentrations found. Functional responses to LTB_4 were suppressed by cross-talk with CXC and BLT2 receptors that desensitize BLT1. Since the suppression of intracellular calcium mobilization was prominent, we also studied the role of suppressed cell calcium mobilization in the defective chemotactic responses to LTB_4. We noted that **PMN** chemotaxis to LTB_4 showed far more dependence on store-operated calcium entry than on the release of cellular calcium stores, and that store-operated calcium responses to BLT1 activation were markedly inhibited during the same time period as was chemotaxis. The intermittent release of inflammatory mediators after injury can blunt **PMN** responses to LTs by suppressing BLT1 as well as downstream calcium entry. Diminished LT receptor activity due to cross-talk with CXC receptors can inhibit **PMN** recruitment to infective sites. This may predispose injured patients to septic complications.

342. Cigarette Smoking Increases Neutrophil Formyl Methionyl Leucyl Phenylalanine Receptor Numbers
Matheson, M., Rynell, A-C., McClean, M. and Berend, N.
Chest, 123, 1642 (2003)

Study objectives: The purpose of this study was to explore the relationship between cigarette smoking and COPD on the number of formyl methionyl leucyl phenylalanine (FMLP) receptors on peripheral neutrophils.
Design and participants: Three groups of subjects were studied: subjects with COPD (n = 13), healthy smokers (n = 6), and healthy nonsmokers (n = 6).
Interventions: Fifty milliliters of venous blood were collected from each subject, and neutrophils were isolated. Neutrophil FMLP receptor numbers were determined by incubating with tritiated FMLP at six doubling concentrations from 1.4 to 45 pmol. Three of the subjects from group 1 (the COPD group) were current smokers, and we elected to analyze these subjects as a separate group.
Measurements and results: The analysis of variance looking at the three factors—FMLP, COPD and smoking—showed significant differences among levels of FMLP (p = 0.0001), as would be expected, and also overall smoking vs nonsmoking (p = 0001) and COPD vs non-COPD (p = 0.02). Within each level of FMLP, there was only one instance of a significant difference between COPD nonsmokers and normal nonsmokers, and no instance of a significant difference between COPD smokers and normal smokers. At five of the six concentrations of tritiated FMLP, smoking was a significant factor.
Conclusions: This study suggests that the overriding influence on peripheral neutrophil FMLP receptor numbers is current smoking rather than the presence of COPD.

343. Tumor Necrosis Factor-α Induces Early-Onset Endothelial Adhesivity by Protein Kinase Cζ–Dependent Activation of Intercellular Adhesion Molecule-1
Javaid, K. et al
Circ. Res., 92, 1089 (2003)

We tested the hypothesis that TNF-α induces early-onset endothelial adhesivity toward PMN by activating the constitutive endothelial cell surface ICAM-1, the ß$_2$-integrin (CD11/CD18) counter-receptor. Stimulation of human pulmonary artery endothelial cells with TNF-α resulted in phosphorylation of ICAM-1 within 1 minute, a response that was sustained up to 15 minutes after TNF-α challenge. We observed that TNF-α induced 10-fold increase in PMN adhesion to endothelial cells in an ICAM-1–dependent manner and that this response paralleled the rapid time course of ICAM-1 phosphorylation. We also observed that the early-onset TNF-α–induced endothelial adhesivity was protein synthesis–independent and associated with cell surface ICAM-1 clustering. Pretreatment of cells with the pan-PKC inhibitor, chelerythrine, prevented the activation of endothelial adhesivity. As PKCζ, an atypical PKC isoform abundantly expressed in endothelial cells, is implicated in signaling TNF-α–induced ICAM-1 gene transcription, we determined the possibility that PKCζ was involved in mediating endothelial adhesivity through ICAM-1 expression. We observed that TNF-α stimulation of endothelial cells induced PKCζ activation and its association with ICAM-1. Inhibition of PKCζ by pharmacological and genetic approaches prevented the TNF-α–induced phosphorylation and the clustering of the cell surface ICAM-1 as well as activation of endothelial adhesivity. Thus, TNF-α induces early-onset, protein synthesis–independent expression of endothelial adhesivity by PKCζ-dependent phosphorylation of cell surface ICAM-1 that precedes the de novo ICAM-1 synthesis. The rapid ICAM-1 expression represents a novel mechanism for promoting the stable adhesion of PMN to endothelial cells that is needed to facilitate the early-onset transendothelial migration of PMN.

344. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors
Fan, J. and Malik, A.B.
Nature Med., 9, 315-321 (2003)

Polymorphonuclear leukocytes (PMNs) are critical effector cells of the innate immune system that protect the host by migrating to inflammatory sites and killing pathogenic microbes. We addressed the role of chemokine receptor desensitization induced by G-protein-coupled receptor kinases (GRKs) in the feedback control of PMN migration. We show that the chemokine macrophage inflammatory protein-2 (MIP-2) induces GRK2 and GRK5 expression in PMNs through phosphoinositide-3-kinase (PI3K)-γ signaling. We also show that lipopolysaccharide (LPS)-activated signaling through the Toll-like receptor (TLR)-4 pathway transcriptionally downregulates the expression of GRK2 and GRK5 in response to MIP-2. The reduced expression of GRKs lowers chemokine receptor desensitization and markedly augments the PMN migratory response. These data indicate that TLR4 modulation of PMN surface chemokine receptor expression subsequent to the downregulation of GRK2 and GRK5 expression is a critical determinant of PMN migration.

345. Ultrasound-enhanced latex immunoagglutination test (USELAT) for detection of capsular polysaccharide antigen of Neisseria meningitidis from CSF and plasma
Porritt, R.J., Mercer, J.L. and Munro, R.
Pathology, 35(1), 61-64 (2003)

Aims: An ultrasonic instrument, the Immunosonic, was used to evaluate ultrasound-enhanced latex immunoagglutination testing (USELAT) for detection and serogroup determination of *Neisseria meningitidis* in clinical specimens.
Methods: Eighty-two CSF and EDTA blood specimens from patients with suspected meningococcal disease (MD) were tested by USELAT. Results were compared with routine laboratory tests for confirmation of MD and discrepant results were resolved by analysis of further laboratory and clinical data.
Results: Using the Wellcogen Bacterial Antigen Kit, USELAT was positive in 20 (24%) specimens. The resolved sensitivity of USELAT was 49% compared with 67% for PCR. There were no discrepancies between serogroups indicated by USELAT and serogroups confirmed by PCR or culture grouping.
Conclusions: Although USELAT could be performed in laboratories without facilities for PCR testing, a specific ultrasonic instrument is necessary and some experience is required in interpreting results. The lower resolved sensitivity makes USELAT unsuitable as a stand-alone rapid test, and it added little value to standard laboratory culture with PCR testing.

346. Ex vivo myeloid differentiation of cord blood CD34+ cells: comparison of four serum-free media containing bovine or human albumin
De Bruyn, C., Delforge, A. and Bron, D.
Cytotherapy, 5(2), 153-160 (2003)

Background

Infusion of ex vivo generated myeloid post-progenitor cells associated with unmanipulated cells appears to be a promising approach to reduce neutropenia following cord blood (CB) transplant. We compared four commercially available serum-free media, two containing BSA and two containing human albumin, on the in vitro differentiation of CB $CD34^+$cells into post-myeloid progenitor cells. Methods CB $CD34^+$ cells were cultured for 7 days in CTM-H00 (Mabio-International), StemSpanH2000 (StemCell Technologies), RM-B00 (Mabio-International) and StemαA (Stem Alpha). The cells were stimulated by SCF, G-CSF, IL3 and Flt3-ligand (FL) added once at Day 0. Expansion was evaluated as the increase of leucocytes, $CD34^+$ cells and $CD13^+$ cells. Maturation of cells into the myeloid lineage was evaluated by expression of CD15, CD11b and CD16 Ags and by the presence of primary (myeloperoxydase, MPO) and secondary granules (lactoferrin, LF). The capacity of cells to phagocyte latex beads was evaluated to assess their functionality. Results We observed that: a) the mononuclear cell and $CD34^+$ cell expansions were significantly different according to the medium tested (respectively 61.5±7.7 and 15.5±3.4 for RM-B00, 37.3±5.4 and 10.3±1.6 for CTM-H00, 23.2±6.5 and 5.8±0.9 for StemSpanH2000 and 16.6±2.4 and 3.9±0.7 for StemαA); b) the expansion of myeloid precursors is higher with RM-B00, similar with CTM-H00 and StemSpanH2000, and lower with StemαA. This difference is essentially due to total leucocyte expansion, rather than to a selective expansion of myeloid cells, except for StemalphaA, for which the percentages of neutrophil precursor cells [promyelocytes ($CD15^+CD11b^+$), myelocytes ($CD11b^+CD16^-$) and mature cells ($CD11b^+CD16^+$)] are significantly decreased. *Discussion*
It appears that during *ex vivo* differentiation into myeloid lineages, the medium is critical and should be systematically screened before use in preclinical protocols. ((**Author query**))
The use of human rather than bovine albumin, seems to have neither a negative, nor positive impact on the effectiveness of the medium.

347. Ex vivo expansion of neutrophil precursor cells from fresh and cryopreserved cord blood cells
De Bruyn, C., Delforge, A. and Bernier, M.
Cytotherapy, 5(1), 87-98 (2003)

Background
Neutropenia following cord blood (CB) transplantation may be abrogated by infusion of granulopoietic progenitor cells. The purpose of this study was to determine whether myeloid progenitors can be obtained by ex vivo expansion of cryopreserved cord blood aliquots, and whether these progenitors present the morphologic, biologic and functional properties of myeloid progenitors at various stages of differentiation.
Methods
The cells, plated for 7 days in serum-free medium with SCF, IL-3, G-CSF, Flt3-ligand and thrombopoietin in various combinations, were assessed for the expression of CD34, CD38 and CD13. Maturation of cells into the myeloid lineage was evaluated by the expression of CD15, CD11b and CD16 and by the presence of primary (myeloperoxidase) and secondary granules (lactoferrin). The capacity of cells to phagocyte latex beads was evaluated to assess their functionality.
Results
We have shown that a) $CD34^+$ cells isolated from thawed samples were able to produce expansions similar to fresh samples. b) The best combination for the expansion of neutrophil precursor cells was S3FG; c) in these conditions, all stages of myeloid progenitors were represented, but few mature cells were observed. d) However, when the cells were plated on a BM stroma to try to reproduce conditions occurring during transplant, they acquired rapidly the characteristics of mature segmented cells. e) The ex vivo generated granulocytes were able to phagocyte latex beads.
Discussion
In conclusion, it seems reasonable to systematically aliquot CB samples before cryopreservation. Some aliquots can then be thawed, enriched in $CD34^+$ cells and ex vivo differentiated into myeloid lineage, while the other aliquots are conserved to be infused without manipulation.

348. Human Polymorphonuclear Cell Death after Exposure to Resuscitation Fluids In Vitro: Apoptosis versus Necrosis
Stanton, K. et al
J. Trauma-Injury Infect. Crit. Care, 54(6), 1065—1076 (2003)

Background : Resuscitation fluids can have variable effects on key functions of circulating polymorphonuclear neutrophils (PMNs) such as oxidative burst, chemotaxis, and bacterial killing. We hypothesized that choice of resuscitation fluids will also affect the rate of PMN apoptosis. To test this, we studied cellular death (apoptosis and necrosis) in human PMNs after brief exposure to different hypertonic and isotonic fluids.
Methods : Blood from 12 volunteers was incubated for 1 hour at 37[degrees]C in normal saline, 6.0% dextran-70, 7.5% hypertonic saline, and 7.5% hypertonic saline with 6% dextran-70. Isolated PMNs were double labeled with fluorescein-Annexin V and propidium iodide, and apoptosis and necrosis were measured using flow cytometry. Caspase activation was also detected with flow cytometry using pan-caspase inhibitor (carbobenzoxy-valyl-alanyl-aspartyl-fluoromethylketone) in non-isolated whole blood samples to determine apoptosis. Finally, cDNA macroarrays were used to evaluate the expression of 23 genes involved in the regulation of cell cycling and apoptosis.
Results : Exposure to hypertonic fluids (hypertonic saline and 7.5% hypertonic saline with 6% dextran-70) significantly ($p < 0.05$) increased necrosis in isolated PMNs. In whole blood samples, PMNs exposed to dextran demonstrated significant apoptosis as

evidenced by increased caspase activation. Dextran was the only fluid that affected leukocyte gene expression, inducing significant up-regulation of Rb gene transcription.
Conclusion : Hypertonic fluids and dextran decrease human polymorphonuclear cell survival through necrotic and apoptotic pathways, respectively.

349. The effect of N-acetylcysteine on nuclear factor-[kappa]B activation, interleukin-6, interleukin-8, and intercellular adhesion molecule-1 expression in patients with sepsis
Paterson, R.L., Galley, H. and Webster, N.
Crit. Care Med., 31(11), 2574-2578 (2003)

Objective: Expression of inflammatory mediators is controlled in part at the transcriptional level via nuclear factor-[kappa]B. Inhibition of nuclear factor-[kappa]B activation may be beneficial in critically ill patients. N-acetylcysteine is an antioxidant that inhibits nuclear factor-[kappa]B activation in vitro. In this pilot study we investigated the effect of N-acetylcysteine on nuclear factor-[kappa]B activation and circulating cytokine and adhesion molecules in patients with sepsis.
Design: Prospective, randomized, double blind, placebo-controlled pilot trial.
Setting: Eight-bed intensive care unit in a university teaching hospital.
Patients: Twenty consecutive patients within 12 hrs of fulfilling the consensus criteria for sepsis.
Interventions: A bolus of 150 mg/kg N-acetylcysteine in 100 mL of 0.9% saline over 15 mins, then 50 mg/kg in 100 mL of 0.9% saline over 4 hrs as a loading dose, and then a maintenance infusion of 50 mg/kg in 200 mL of 0.9% saline over each 24-hr period for a total of 72 hrs, or an equivalent volume of saline.
Measurements and Main Results: Nuclear factor-[kappa]B activation was measured in mononuclear leukocytes using electrophoretic mobility shift assay, at baseline and 24, 48, 72, and 96 hrs later. Activation decreased significantly in patients treated with N-acetylcysteine ($p = .016$) but not placebo and was significantly reduced at 72 hrs compared with both preinfusion values ($p = .028$) and patients receiving placebo ($p = .01$). Plasma interleukin-6, interleukin-8, and soluble intercellular adhesion molecule-1 concentrations were measured using enzyme immunoassay. Interleukin-6 concentrations were high initially and then decreased in all patients, regardless of whether they received N-acetylcysteine or placebo. Interleukin-8 decreased significantly only in those who received N-acetylcysteine ($p = .0081$). Soluble intercellular adhesion molecule-1 concentrations remained unchanged in all patients.
Conclusions: Administration of N-acetylcysteine results in decreased nuclear factor-[kappa]B activation in patients with sepsis, associated with decreases in interleukin-8 but not interleukin-6 or soluble intercellular adhesion molecule-1. These pilot data suggest that antioxidant therapy with N-acetylcysteine may be useful in blunting the inflammatory response to sepsis. Further studies are warranted.

350. Effects of rewarming on nuclear factor-[kappa]B and interleukin 8 expression in cold-preserved alveolar epithelial cells
Inoue, K et al
Transplantation, 76(2), 409-415 (2003)

Background. Nuclear factor-[kappa]B (NF-[kappa]B) and interleukin (IL)-8 play important roles in the pathophysiology of acute lung injury after lung transplantation. Because alveolar epithelium is one of the most important sites at which IL-8 production takes place after reperfusion of donor lungs, we examined the effects of cold/rewarming on NF-[kappa]B and IL-8 expression in alveolar epithelial cells.
Methods. A549 cells were preserved at 4[degrees]C for 5 hr and then rewarmed for up to 20 hr. NF-[kappa]B was analyzed by electrophoretic mobility shift assay. IL-8 mRNA expression was examined by reverse transcription-polymerase chain reaction. IL-8 concentration in the cell culture medium after rewarming was measured by enzyme-linked immunosorbent assay.
Results. NF-[kappa]B was increased in the nuclear extracts as early as 30 min after rewarming. There was a marked increase in the IL-8 mRNA expression at 1 and 3 hr after rewarming. IL-8 concentration in the cell culture medium was progressively increased during 20 hr following rewarming. The cell culture medium inhibited apoptosis of neutrophils significantly. The cold/rewarming-induced IL-8 production was reduced to approximately 50% by introducing an antisense oligonucleotide for the p65 subunit of NF-[kappa]B and by treatment with N-acetyl-leucinyl-leucinyl-norleucinal and pyrrolidine dithiocarbamate. The effect of dexamethasone treatment was dose dependent (reduced to approximately 30% at 10-5 M dexamethasone).

351. Influence of Steroids on Oxidant Generation in Activated Human Granulocytes and Mononuclear Leukocytes.
Cassidy, R.A.
Shock, 2081), 85-90 (2003)

Steroids, in particular, 17[beta]-estradiol (E2), have been reported to improve the response to trauma in animal models. In these models, the leukocyte plays a critical role in the inflammatory cascade. We examined the affects of E2, hydrocortisone (H), progesterone (P4), and E2 with P4 on oxidant production in human granulocytes (PMNs) and mononuclear leukocytes (MNCs). Each cell type was loaded with 2,7-dichlorodihydrofluorescein and then simultaneously activated with human cytokines (tumor necrosis factor [alpha], interleukin-1[beta], and interferon [gamma]) and hemoglobin and inhibited with and without equimolar concentrations of each steroid treatment or nitric oxide (NO) synthesis inhibitors. After incubations of 1 or 5 h, intracellular oxidants were quantified by flow

cytometry. Activation by cytokines combined with hemoglobin, resulted in a 450-575% increase in oxidant production that was synergistically greater than the sum of either component alone. Pharmacological levels of E2 decreased oxidants in MNCs at 1 h. In contrast at 5 h, H decreased oxidants more than E2. The addition of P4 to E2 concentrations almost eliminated oxidants from 1 h-activated MNCs. None of the steroids significantly reduced oxidants in PMNs, suggesting that the E2 effect on MNCs was not caused by its nonreceptor-mediated antioxidant properties. Because L-NMMA inhibited at least 55% of the total oxidants, part of E2 dampening effects would be attributed to NO. These results suggest that steroid-attenuated MNC-derived NO may reduce autocrine and paracrine effects on inflammation if appropriate doses of steroids are given soon after injury.

352. Expression Patterns of the Lipopolysaccharide Receptor CD14, and the FC[gamma] Receptors CD16 and CD64 on Polymorphonuclear Neutrophils: Data from Patients with Severe Bacterial Infections and Lipopolysaccharide-Exposed Cells
Wagner, C. et al
Shock, 19(1), 5-12 (2003)

In polymorphonuclear neutrophils (PMN) CD14, one of the receptors for lipopolysaccharides (LPS) is stored intracellularly as a preformed protein, with only few receptors expressed on the surface. We now report that in patients with severe bacterial infections, CD14 expression is profoundly upregulated, as is CD64 (Fc[gamma]RI), the high-affinity receptor for IgG, whereas CD16 (Fc[gamma]RIII) was partly lost from the surface. To further analyze regulation of these receptors, PMN of healthy donors were exposed to low doses of LPS. By brief exposure (10-120 min) to LPS, CD14 was transferred to the surface in a cytochalasin B-sensitive manner, as were CD16 and CD64. Prolonged culture (up to 48 h) resulted in a further upregulation of CD14, sustained expression of CD64, and profound decline of CD16, yielding a similar pattern of receptor expression as seen in the patients. Subsequent studies revealed that LPS induced de novo synthesis of CD14: the increase of surface expression could be inhibited by cycloheximide and by interfering with a known LPS-induced signaling event, the translocation of NF[kappa]B. Moreover, an up to 10-fold increase of specific mRNA was seen, as was incorporation into CD14 of 35S-methionine. The de novo synthesis prolonged expression of CD14, whereas the CD16 expression declined, generating a PMN phenotype characteristic for severe infection and indicative of escape from apoptosis of a PMN subpopulation.

353. The Early Increase in Intestinal Permeability and Systemic Endotoxin Exposure in Patients with Severe Acute Pancreatitis Is Not Associated with Systemic Bacterial Translocation: Molecular Investigation of Microbial DNA in the Blood
Ammori, B.J., Fitzgerald, P., Hawkey, P. and McMahon, M.J.
Pancreas, 26(1), 18-22 (2003)

Introduction: Sepsis is the main cause of late mortality in patients with severe acute pancreatitis and is largely attributed to secondary infection of pancreatic necrosis with gram-negative enteric organisms. This is commonly preceded by a significant increase in intestinal colonization with such microbes and with early increases in intestinal permeability, thus suggesting a mechanism of local bacterial translocation. Whilst cultures of blood specimens from these patients often remain sterile, it is conceivable that bacteria might translocate systemically in small volumes with detrimental effects but elude detection by standard microbial culture techniques.
Aims: To investigate the incidence and frequency with which bacterial DNA may exist in the systemic circulation of patients with acute pancreatitis and to relate that to disease severity, changes in intestinal permeability, and systemic endotoxin exposure.
Methodology: Blood samples were obtained at admission and on days 3 and 7 from 26 patients with acute pancreatitis (seven with severe cases) and from 10 healthy controls for DNA extraction and standard microbial cultures. Polymerase chain reaction techniques were used to amplify a gene region (16S ribosomal RNA) found in all bacteria. Levels of serum endotoxin and antibodies to endotoxin core (EndoCAb) were measured at admission, and intestinal permeability to the macromolecule polyethylene glycol 3350 was determined within 72 hours of the onset of symptoms.
Results: Blood cultures yielded Escherichia coli and enterococci for one patient with a severe attack and coagulase-negative staphylococci for another patient with a mild attack. No bacterial DNA was found in any of the samples. Endotoxemia was detected in 20 patients (five with severe cases), and levels of serum IgM EndoCAb were depleted in patients with severe attacks but remained relatively unchanged during mild attacks ($p = 0.033$). Intestinal permeability was significantly increased in patients with severe attacks of acute pancreatitis but remained unchanged during mild attacks ($p < 0.05$).
Conclusions: Whilst severe attacks of acute pancreatitis are associated with early derangement in gut barrier function and systemic endotoxin translocation, there is no molecular evidence for associated systemic bacterial "translocation."

354. Chemotactic activity of CXCL5 in cerebrospinal fluid of children with bacterial meningitis
Zwijnenberg, P.J., de Bie, H.M.A., Roord, J.J., van der Poll, T. And van Furth, M.
J. Neuroimmunol., 145(1-2), 148-153 (2003)

CXCL5 (epithelial-cell-derived neutrophil-activating protein (ENA-)78) is a CXC-chemokine that specifically acts on neutrophils. To obtain insight into the extent of local presence and action of CXCL5 during bacterial meningitis, we measured its concentrations in cerebrospinal fluid (CSF) of patients with culture-proven bacterial meningitis (*n*=14), aseptic meningitis (*n*=6), and controls (*n*=32) and compared these results with levels of other CXC-chemokines, CXCL8- (interleukin-8) and CXCL1-related oncogene (growth-related oncogene (GRO)-α). Patients with bacterial meningitis had profoundly elevated CSF concentrations of all three chemokines. CXCL5

was not detectable in patients with aseptic meningitis or control subjects. CSF from patients with bacterial meningitis exerted chemotactic activity towards neutrophils, which was partially inhibited by neutralizing antibodies against CXCL5 and CXCL8, but not CXCL1. CSF from controls exerted minor chemotactic activity, which could be strongly enhanced by the addition of recombinant CXCL5, CXCL8 or CXCL1. During bacterial meningitis, CXCL5 is elevated in CSF, where it is involved in the recruitment of neutrophils to the central nervous system.

355. Influence of helenanolide-type sesquiterpene lactones on gene transcription profiles in Jurkat T cells and human peripheral blood cells: anti-inflammatory and cytotoxic effects
Gertsch, J., Sticher, O., Schmidt, T. and Heilmann, J.
Biochem. Pharmacol., 66(11), 2141-2153 (2003)

Sesquiterpene lactones (SLs) are known to have potent anti-inflammatory and cytotoxic properties. So far, the anti-inflammatory effects have mainly been attributed to their inhibition of DNA-binding of the transcription factor NF-κB (p65), which is a pivotal regulator of the cellular immune response. Since NF-κB is involved in the transcriptional control of several pro-inflammatory and regulatory genes, we investigated the effects of one bifunctional NF-κB (p65) inhibiting and two monofunctional NF-κB (p65) inactive helenanolide-type SLs on PMA and LPS-induced mRNA expression in $CD4^+$ Jurkat T and human peripheral blood mononuclear cells (PBMCs) with reverse transcription real-time PCR (RT-rt-PCR). The monofunctional SLs 11α,13-dihydrohelenalin acetate (DHAc) and chamissonolide significantly reduced mitogen-induced cytokine and iNOS mRNA levels in PBMCs and Jurkat T cells at low micromolar concentrations. DHAc also showed significant effects on the gene expression of the house-keeping genes GAP-DH and β-actin, as well as on NF-ATc, p65, I-κBα, bcl-2, and cyclin D1. The bifunctional NF-κB inhibitor helenalin not only effectively inhibited pro-inflammatory gene expression, but also strongly down-regulated all investigated mRNA levels in a time-dependent manner. Flow cytometry and caspase-8 and -3 assays revealed that helenalin strongly and DHAc moderately induced apoptosis in Jurkat T cells, whereas chamissonolide caused cytoprotective effects. In PBMCs, DHAc and chamissonolide did not inhibit NF-κB (p65) DNA-binding at concentrations effective on the transcriptome. Thus, it can be concluded that the biological effects of SLs are not only due to NF-κB inhibition, but must be coupled to other mechanisms.

356. Regional hypothermia reduces mucosal NF-κB and PMN priming via gut lymph during canine mesenteric Ischemia/Reperfusion,
Hassoun, H.T. et al
J. Surg. Res., 115(1), 121-126 (2003)

Background. Mesenteric ischemia/reperfusion (I/R) activates pro-inflammatory mediators that exacerbate gut reperfusion injury and prime circulating neutrophils that cause remote organ injury. We have shown that regional intraischemic hypothermia protects the intestinal mucosa during I/R in rats. In this study, we examined the effects of regional hypothermia on I/R-induced transvascular protein clearance, NF-κB DNA binding activity, and polymorphonuclear neutrophil (PMN) priming via gut lymph in a canine mesenteric lymphatic fistula model.
Materials and methods. Conditioned dogs underwent 60 min of mesenteric ischemia, with or without regional intraischemic hypothermia, and 3 h reperfusion. A mesenteric lymphatic fistula model was used to measure transvascular protein clearance and harvest lymph. Biopsies of distal ileum were obtained at baseline and 0, 180 min of reperfusion for NF-κB DNA binding activity using electrophoretic mobility shift assay (EMSA). A kinetic spectrophotometric assay was used to determine fMLP stimulated PMN superoxide production after priming by gut lymph obtained at baseline and 180 min reperfusion.
Results. Transvascular protein clearance increased during reperfusion compared to baseline, and hypothermia had no significant effect on this I/R-induced protein clearance. NF-κB activity increased three-fold at the end of ischemia and hypothermia prevented this early activation. PMN superoxide production increased 19-fold during I/R (0.06 ± 0.04 *versus* 1.14 ± 0.50 nmol O_2, $P < 0.05$), but only 2.5-fold during I/R + hypothermia (0.28 ± 0.09 *versus* 0.70 ± 0.32 nmol O_2, $P = 0.2$).
Conclusions. Regional intraischemic hypothermia prevented early intestinal NF-κB activation, partially abrogated PMN priming via gut lymph, but had no significant effect on increased transvascular protein clearance during mesenteric I/R in dogs.

357. Carotenoid cleavage products modify respiratory burst and induce apoptosis of human neutrophils
Siems, W. et al
Biochim. Bophys. Acta, 1639(1), 27-33 (2003)

Carotenoid supplementation in the treatment of diseases associated with oxidative stress has been recently questioned because of the cell damage and the increased risk of lung cancer in male smokers. Because of the complex role of neutrophils in lung diseases, we investigated whether carotenoid derivatives could affect respiratory burst and apoptosis of human neutrophils purified from peripheral blood. Stimulation of superoxide production was induced by nanomolar and micromolar concentrations of carotenoid cleavage products with aliphatic chains of different length, but not by carotenoids lacking the carbonyl moiety. The stimulatory effect of carotenoid cleavage products was observed in cells activated by phorbol myristate acetate (PMA), while a slight inhibition of superoxide production was noticed with cells activated by the chemotactic tripeptide *N*-formyl-Met-Leu-Phe (f-MLP). At higher concentrations, carotenoid cleavage products inhibited superoxide production in the presence of both PMA and f-MLP. In the presence of 20 μM

carotenoid cleavage products, inhibition of superoxide production was accompanied by DNA fragmentation and increased level of intracellular caspase-3 activity.

358. Sulfur-related air pollutants induce the generation of platelet-activating factor, 5-lipoxygenase- and cyclooxygenase-products in canine alveolar macrophages via activation of phospholipases A2
Beck-Speier, I. Et al
Prostaglandins & Other Lipid Mediators, 71(3-4), 217-234 (2003)

Recent studies have shown that long-term in vivo exposure of dogs to neutral sulfur(IV)/sulfite aerosols induces mild inflammatory reactions, whereas the combination of neutral sulfite with acidic sulfur(VI)/sulfate aerosols evokes less pronounced effects. To understand underlying mechanisms, we studied in vitro the role of lipid mediators in the responses of alveolar macrophages (AMs) to sulfur-related compounds under neutral (pH 7) or moderate acidic (pH 6) conditions. Canine AMs incubated with sulfite at pH 7 released threefold higher amounts of platelet-activating factor than control (P<0.005). Generation of arachidonic acid, leukotriene B_4, 5-hydroxy-eicosatetraenoic acid, prostaglandin E_2, thromboxane B_2 and 12-hydroxyheptadecatrienoic acid increased twofold (P<0.0005). However, these metabolites remained unchanged following incubation of AMs with sulfite at pH 6 or with sulfate at pH 7 or pH 6. Mediator release by sulfite-treated AMs at pH 7 stimulated respiratory burst activity of neutrophils. Inhibition of MAPK pathway by PD 98059, of cytosolic ($cPLA_2$) and secretory phospholipases A_2 by $AACOCF_3$ and thioetheramide-PC, respectively, reduced sulfite-induced eicosanoid formation in AMs. Sulfite activated $cPLA_2$ activity twofold at pH 7. This mechanism of sulfite-stimulated responses in phospholipid metabolism predicts that chronic exposure to sulfur(IV)/sulfite is associated with a considerable health risk.

359. Characterization of Siglec-5 (CD170) expression and functional activity of anti–Siglec-5 antibodies on human phagocytes
Erickson-Miller, C.L. et al
Exp. Hematol., 31(5), 382-388 (2003)

Objective. The Siglec family of proteins consists of at least 10 members with immunoglobulin and lectin domains and with similar sialic acid–binding properties. Many Siglec family members are expressed on hematopoietic cells and are involved in cell/cell interactions. Some family members are suspected of regulating cellular processes through specific signaling pathways. Monoclonal antibodies were generated against specific epitopes of Siglec-5 (CD170) and were used to determine expression of Siglec-5 on normal blood and marrow cells and cell lines. The antibodies also were used to elucidate functional activity for Siglec-5 on blood neutrophils.
Methods. Flow cytometry and ELISA were used to determine the specificity of the monoclonal antibodies for Siglec-5 and to determine expression patterns. Chemiluminescence assays were employed to measure the oxidative burst activity of whole blood or purified neutrophils following treatment with the anti–Siglec-5 antibodies.
Results. Cell surface expression analysis demonstrated that the protein was expressed on gated human neutrophil and monocyte populations, both in the blood and bone marrow. Expression on neutrophils was enhanced by one-hour treatment with fMLP or TNF-α. Epitope-specific anti–Siglec-5 monoclonal antibodies did not directly activate human neutrophils; however, antibody binding augmented neutrophil oxidative burst activity as determined by fMLP-induced luminol-dependent chemiluminescence.
Conclusion. Data demonstrating expression of Siglec-5 on cells of the myelomonocytic lineage and alteration of its expression by inflammatory stimuli suggest a role for this protein in cell/cell interactions following microbial exposure.

360. Gemifloxacin: effects of sub-inhibitory concentrations on various factors affecting bacterial virulence
Dal Sasso, M., Culici, M., Bovio, C. and Braga, P.C.
Int. J. Antimicrobial Agents., 21(4), 325-333 (2003)

This study investigated the ability of sub-MICs of gemifloxacin to interfere with the bacterial virulence parameters of adhesiveness, haemagglutination, hydrophobicity and motility, as well as their interactions with host neutrophilic defences such as phagocytosis, killing and respiratory bursts. The adhesiveness of both *Escherichia coli* and *Staphylococcus aureus* was significantly reduced to a subinhibitory concentration of 1/32 MIC. Indirect fimbriation parameters, such as hydrophobicity and haemagglutination were significantly reduced at a concentration of 1/8 MIC, as was migration (swarming). Phagocytosis and the respiratory burst measured by means of chemiluminescence were not affected, but killing was significantly increased from 1/2 to 1/8 MIC. The interpolation of these pharmacodynamic findings with pharmacokinetic curves indicates that sub-MIC concentrations of gemifloxacin can prolong antimicrobial effects on virulence determinants up to 27 h after the antimicrobial concentration has fallen below the MIC value.

361. Platelets stimulated by IgG-coated surfaces bind and activate neutrophils through a selectin-dependent pathway
Wetterö, J., Tengvall, P. and Bengtsson, T.
Biomaterials, 24(9), 1559-1573 (2003)

Blood platelets bind rapidly to foreign surfaces and interact with adsorbed proteins and neutrophil granulocytes. We demonstrate by use of luminol-amplified chemiluminescence under stirred and non-stirred conditions that platelets at IgG-coated surfaces amplify the neutrophil extracellular release of reactive oxygen species (ROS). The neutrophil response involved tyrosine phosphorylation, but was only in part induced by neutrophil $F_{c\gamma}$-receptor stimulation. The platelet mediated effects were contact-dependent since the respiratory

burst was inhibited when the IgG-stimulated platelets were removed by filtration, but not when they were fixed in paraformaldehyde. Bodipyphallacidin-staining of filamentous actin (F-actin) revealed that an actin-dependent platelet adhesion supported the subsequent adhesion and spreading of neutrophils. The neutrophil ROS-response was lowered when the interaction between platelet P-selectin (CD62P) and neutrophil P-selectin glycoprotein ligand-l (PSGL-1 or CD162) was inhibited. The blocking of L-selectin (CD62L) or blocking of the interaction between platelet glycoprotein (Gp) IIb/IIIa and neutrophil complement receptor 3 (CR3) showed no effect. We conclude that platelet activation on immobilized IgG trigger a contact-dependent "frustrated" phagocytosis by neutrophils, associated with a release of toxic ROS.

362. Curcumin differentially modulates mRNA profiles in Jurkat T and human peripheral blood mononuclear cells
Gertsch, J., Güttinger, M., Heilmann, J. and Sticher, O.
Bioorganic & Medicinal Chem., 11(6), 1057-1063 (2003)

Curcumin, the yellow pigment of the rhizome of *Curcuma longa* is known to inhibit the transcription factors AP-1, Egr-1, NF-κB, c-myc and several important signaling kinases. We therefore investigated the differential effects of curcumin in concentation between 1.5 and 13.6 μM on gene expression in T Jurkat $CD4^+$ and human peripheral blood mononuclear cells (PBMCs). Relative quantification with reverse transcription real-time PCR (RT-rt-PCR) showed that low concentrations of curcumin significantly down-regulated mitogen-induced granulocyte macrophage colony stimulating factor (GM-CSF) mRNA (3- to 5-fold at 3 μM) in a dose- and time-dependent manner in both cell types. In comparison, the down-regulation of inducible nitric oxide (iNOS) mRNA levels was less pronounced, but interferon gamma (IFN-γ) mRNA was dose-dependently up-regulated with curcumin concentrations up to 8.2 μM. Cyclin D1 mRNA expression was specifically inhibited in Jurkat T cells and stimulated PBMCs. The transcription factors NF-κB and NF-ATc were not affected in PBMCs. Interleukin-2 (IL-2), and-6 (IL-6) mRNAs levels were not influenced markedly by curcumin in stimulated PBMCs, but significantly reduced in stimulated Jurkat T cells. In addition, cytotoxic effects and down-regulation of mRNAs, including p65 and the house-keeping genes could only be measured in Jurkat T cells. These findings confirm previous reports on the anti-neoplastic potential of curcumin and show that this compound differentially modulates the expression profile of Th1 cells and PBMCs.

363. Alternative splicing of myeloid IgA Fc receptor (FcαR, CD89) transcripts in inflammatory responses
Togo, S., Shimokawa, T., Fukuchi, Y. and Ra, C.
FEBS Lett., 535(1-3), 205-209 (2003)

More than 10 splice variants of the Fc receptor for IgA (FcαR, CD89) have been identified in human myeloid cells. In this study, we quantified FcαR splice transcripts ΔEC2 and Δ66EC2, which lack the entire and a part of the homologous immunoglobulin-like extracellular domain 2 (EC2), respectively. Tumor necrosis factor-α was found to specifically increase the ratio of ΔEC2 to the wild type CD89 in neutrophils and conversely decrease the ΔEC2 ratio in monocytes. We also observed a significant decrease in the neutrophil ΔEC2/CD89 ratio in pneumonia patients. These results suggest that ΔEC2 is differentially regulated and could be involved in immunoregulation of IgA-mediated host defense.

364. Inhibition of collagenase and metalloproteinases by aloins and aloe gel
Barrantes, E. and Guinea, M.
Life Sciences, 72(7), 843-850 (2003)

The effects of *Aloe barbadensis* gel and aloe gel constituents on the activity of microbial and human metalloproteinases have been investigated. *Clostridium histolyticum* collagenase (*ChC*) results dose-dependently inhibited by aloe gel and the activity-guided fractionation led to an active fraction enriched in phenolics and aloins. Aloins have been shown to be able to bind and to inhibit *ChC* reversibly and non-competitively. Aloe gel and aloins are also effective inhibitors of stimulated granulocyte matrix metalloproteinases (MMPs). The remarkable structural resemblances between aloins and the pharmacophore structure of inhibitory tetracyclines, suggest that the inhibitory effects of aloins are via an interaction between the carbonyl group at C_9 and an adjacent hydroxyl group of anthrone (C_1 or C_8) at the secondary binding site of enzyme, destabilizing the structure of granulocyte MMPs.

365. PECAM-1-dependent neutrophil transmigration is independent of monolayer PECAM-1 signaling or localization
O'Brien, C.D., Lim, P., Sun, J. and Abelda, S.M.
Blood, 101(7), 2816-2825 (2003)

Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), a tyrosine phosphoprotein highly expressed on endothelial cells and leukocytes, is an important component in the regulation of neutrophil transendothelial migration. Engagement of endothelial PECAM-1 activates tyrosine phosphorylation events and evokes prolonged calcium transients, while homophilic engagement of neutrophil PECAM-1 activates leukocyte β-integrins. Although PECAM-1 modulates polymorphoneutrophil transmigration via homophilic PECAM-1-PECAM-1 interaction, the mechanisms underlying endothelial PECAM-1 function are unknown. Proposed mechanisms include (1) formation of a haptotactic gradient that "guides" neutrophils to the cell-cell border, (2) service as a "passive ligand" for neutrophil PECAM-1, ultimately mediating activation of neutrophil β integrins, (3) regulation of endothelial calcium influx, and (4)

mediation of SH2 protein association, and/or (5) catenin and non-SH2 protein interaction. Utilizing PECAM-1-null "model" endothelial cells (REN cells), we developed a neutrophil transmigration system to study PECAM-1 mutations that specifically disrupt PECAM-1-dependent signaling and/or PECAM-1 cell localization. We report that interleukin-1β (IL-1β) elicits PECAM-1-dependent transmigration that requires homophilic PECAM-PECAM-1 engagement, but not heterophilic neutrophil PECAM-1 interactions, and is intercellular adhesion molecule-1 dependent. Conversely, whereas IL-8 and leukotriene-B_4-mediated transmigration is PECAM-1-independent, PECAM-1 and IL-8-dependent transmigration represent separable and additive components of cytokine-induced transmigration. Surprisingly, neither monolayer PECAM-1-regulated calcium signaling, cell border localization, nor the PECAM-1 cytoplasmic domain was required for monolayer PECAM-1 regulation of neutrophil transmigration. We conclude that monolayer (endothelial cell) PECAM-1 functions as a passive homophilic ligand for neutrophil PECAM-1, which after engagement leads to neutrophil signal transduction, integrin activation, and ultimately transmigration in a stimulus-specific manner.

366. Shortening of telomeres: Evidence for replicative senescence of T cells derived from patients with Wegener's granulomatosis
Vogt, S., Iking-Konert, C., Hug, F., Andrassy, K. and Hansch, G.M.
*Kidney Int., **63(6)**, 2144-2151 (2003)*

Background: Replicative senescence describes the fact that somatic cells undergo a finite and predictable number of cell divisions before entering an irreversible state of growth arrest. Progressive shortening of the telomeres, a consequence of cell division, is a reliable indicator of replicative senescence.
Method: We analyzed telomere length of DNA derived from T cells of patients suffering from Wegener's granulomatosis by Sourthern blotting. Moreover, expression of CD28, another marker for replicative senescence, was tested by cytofluorometry.
Results: In patients with disease for more than 5 years, short telomeres were detected in addition to telomeres of normal length, indicating replicative senescence of discrete T-cell clones. Reduced expression of CD28 was noted, particularly on CD8-positive T cells, derived from patients with disease for more than 5 years and short telomeres.
Conclusion: Our data provide evidence that a portion of T cells had undergone replicative senescence, which in turn indicates clonal expansion of T cells as consequence of activation.

367. Inducible nitric oxide synthase (NOS II) is constitutive in human neutrophils
Cedergren, J. et al
*APMIS, **111(10)**, 963-968 (2003)*

The objective was to study the expression of inducible nitric oxide synthase (NOS II) in and NO production by human blood neutrophils and in in vivo exudated neutrophils. Cellular expression of NOS II was evaluated by flow cytometry in whole blood, in isolated blood neutrophils, and in neutrophils obtained by exudation in vivo into skin chambers. Neutrophil NOS II was also demonstrated by Western blotting. Uptake of 3H-labelled L-arginine was studied in vitro and NOS activity measured in a whole cell assay by the conversion of 3H-arginine to 3H-citrulline. In contrast to unseparated blood cells, NOS II was demonstrable both in isolated blood neutrophils and exudated cells. The failure to detect NOS II by flow cytometry in whole blood cells thus proved to be due to the quenching effect of hemoglobin. Western blotting revealed a 130 kD band corresponding to NOS II in isolated blood neutrophils, but detection was dependent on diisopropylfluorophosphate for proteinase inhibition. L-arginine was taken up by neutrophils, but enzymatic activity could not be demonstrated. We conclude that human neutrophils constitutively express NOS II, but that its demonstration by FITC-labelling is inhibited by hemoglobin-mediated quenching in whole blood samples.

368. Cytoskeletal Reorganization Internalizes Multiple Transient Receptor Potential Channels and Blocks Calcium Entry into Human Neutrophils
Itagaki, K., Kannan, K.B., Singh, B.B. and Hauser, C.J.
J. Immunol., 172, 601 (2004)

Store-operated calcium entry (SOCE) is required for polymorphonuclear neutrophil (**PMN**) activation in response to G protein-coupled agonists. Some immunocytes express proteins homologous to the *Drosophila* transient receptor potential gene (*trp*) calcium channel. TRP proteins assemble into heterotetrameric ion channels and are known to support SOCE in overexpression systems, but the evidence that TRP proteins support SOCE and are functionally important in wild-type cells remains indirect. We therefore studied the expression and function of TRP proteins in primary human **PMN**. TRPC1, TRPC3, TRPC4, and TRPC6 were all expressed as mRNA as well as membrane proteins. Immunofluorescence microscopy demonstrated localization of TRPC1, TRPC3, and TRPC4 to the **PMN** cell membrane and their internalization after cytoskeletal reorganization by calyculin A (CalyA). Either TRPC internalization by CalyA or treatment with the inositol triphosphate receptor inhibitor 2-aminoethoxydiphenyl borane resulted in the loss of **PMN** SOCE. Cytochalasin D (CytoD) disrupts actin filaments, thus preventing cytoskeletal reorganization, and pretreatment with CytoD rescued **PMN** SOCE from inhibition by CalyA. Comparative studies of CytoD and 2-aminoethoxydiphenyl borane inhibition of **PMN** cationic entry after thapsigargin or platelet-activating factor suggested that SOCE occurs through both calcium-specific and nonspecific pathways. Taken together, these studies suggest that the multiple TRPC proteins expressed by human **PMN** participate in the formation of at least two store-operated calcium channels that have differing ionic permeabilities and regulatory characteristics.

369. C5a Mutants Are Potent Antagonists of the C5a Receptor (CD88) and of C5L2: POSITION 69 IS THE LOCUS THAT DETERMINES AGONISM OR ANTAGONISM

Otto, M. et al

J. Biol. Chem., 279, 142-151 (2004)

The anaphylatoxin C5a exerts a plethora of biologic activities critical in the pathogenesis of systemic inflammatory diseases. Recently, we reported on a C5a mutant, *jun*/*fos*-A8, as a potent antagonist for the human and mouse C5a receptor (CD88). Addressing the molecular mechanism accounting for CD88 receptor antagonism by site-directed mutagenesis, we found that a positively charged amino acid at position 69 is crucial. Replacements by either hydrophobic or negatively charged amino acids switched the CD88 antagonist *jun*/*fos*-A8 to a CD88 agonist. In addition to CD88, the seven-transmembrane receptor C5L2 has recently been found to provide high affinity binding sites for C5a and its desarginated form, C5adesArg^{74}. A *jun*/*fos*-A8 mutant in which the *jun*/ *fos* moieties and amino acids at positions 71–73 were deleted, A8$^{\Delta 71-73}$, blocked C5a and C5adesArg^{74} binding to CD88 and C5L2. In contrast, the cyclic C5a C-terminal analog peptide AcF-[OP-D-ChaWR] inhibited binding of the two anaphylatoxins to CD88 but not to C5L2, suggesting that the C5a core segment is important for high affinity binding to C5L2. Both receptors are coexpressed on human monocytes and the human mast cell line HMC-1; however, C5L2 expression on monocytes is weaker as compared with HMC-1 cells and highly variable. In contrast, no C5L2 expression was found on human neutrophils. A8$^{\Delta 71-73}$ is the first antagonist that blocks C5a and C5adesArg^{74} binding to both C5a receptors, CD88 and C5L2, making it a valuable tool for studying C5L2 functions and for blocking the biological activities of C5a and C5adesArg^{74} in mice and humans.

370. Antiinflammatory Roles of Peroxisome Proliferator–activated Receptor γ in Human Alveolar Macrophages

Asada, K., Sasaki, S., Suda, T., Chida, K. and Nakamura, H.

Am. J. Respir. Crit. Care Med., 169, 195 (2004)

Peroxisome proliferator–activated receptor γ (PPARγ) is a ligand-activated transcriptional factor belonging to the nuclear receptor superfamily. PPARγ, which is predominantly expressed in adipose tissue, plays a major regulatory role in glucose metabolism and adipogenesis. Interestingly, recent studies have demonstrated PPARγ expression in monocytes/macrophages and its antiinflammatory activities. However, it is unclear whether alveolar macrophages (AMs) express functional PPARγ. The present study was conducted to investigate the expression of PPARγ by AMs and to elucidate its functional role. Using reverse transcription–polymerase chain reaction and Western blotting, we demonstrated the strong expression of PPARs messenger RNA and protein in freshly isolated human AMs. Ligands of PPARγ, 15-deoxy-$\Delta^{12,14}$prostaglandin J_2, and troglitazone significantly decreased LPS-induced tumor necrosis factor-α production by AMs. These ligands markedly upregulated the expression of CD36, a scavenger receptor that mediates the phagocytosis of apoptotic neutrophils. Indeed, ligand-treated AMs ingested a significantly higher number of apoptotic neutrophils than untreated AMs. These data indicate that PPARγ expressed by AMs play an antiinflammatory role through inhibiting cytokine production and increasing their CD36 expression together with the enhanced phagocytosis of apoptotic neutrophils, which is an essential process for the resolution of inflammation. This suggests the potential therapeutic application of PPARγ ligands in inflammatory disorders of the lung.

371. The role of flow cytometric ANCA detection in screening for acute pauci-immune crescentic glomerulonephritis

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Nephrol. Dial. Transplant., 19, 365-370 (2004)

Background. Most cases of pauci-immune crescentic glomerulonephritis (PICGN) are associated with serum anti-neutrophil cytoplasmic antibodies (ANCA). This article studied the sensitivity and specificity of serum ANCA, determined by flow cytometry and indirect immunofluorescence (IIF), to identify patients with acute PICGN.

Methods. 577 adults presenting for first biopsy of their native kidneys with serum taken for ANCA (flow cytometry and IIF) determination were studied. A positive ANCA was defined using a flow cytometric ANCA assay as a screening test, followed by a slide-based indirect IIF technique. Pathological confirmation of acute PICGN was used to assess the sensitivity and specificity of this combined approach and its positive predictive value (PPV) and negative predictive value (NPV) in patients presenting for renal biopsy due to abnormal urinary sediment.

Results. Forty-nine patients were found to have acute PICGN on renal biopsy. Of these 47 were ANCA positive (sensitivity 95.9%). Overall 93 of the renal biopsy patients were ANCA positive, (specificity 91.3%). A further seven patients (two ANCA positive) had advanced sclerosing disease consistent with PICGN but without evidence of current disease activity. The PPV and NPV of ANCA, assessed by flow cytometry and slide IIF, in predicting that patients presenting with undifferentiated renal disease would have acute PICGN was 50.5 and 99.8%, respectively.

Conclusions. Flow cytometric screening of serum for ANCA in patients undergoing renal biopsy has a high NPV for determining those with acute PICGN. It may provide a rapid, simple screening test for this lesion in laboratories using diagnostic flow cytometry and may complement IIF/ELISA in evaluating ANCA positive patients.

372. Binding and Agglutination of Streptococcus pneumoniae by Human Surfactant Protein D (SP-D) Vary between Strains, but SP-D Fails To Enhance Killing by Neutrophils
Jounblat, R. et al
Infect. Immun., 72, 709-715 (2004)

Recombinant human surfactant protein D (SP-D) expressed in *Escherichia coli*, consisting of the head and neck regions of the native molecule, bound to all strains of *Streptococcus pneumoniae* that were tested, but the extent of binding varied between strains of differing capsular serotypes. The recombinant protein expressed in the yeast *Pichia pastoris* did not bind. Full-length native SP-D aggregated pneumococci in a calcium-dependent manner that was inhibited by maltose acting as a competitive sugar. The ability of SP-D to modulate the uptake and killing of pneumococci by human neutrophils was also addressed. Neither recombinant truncated SP-D nor native full-length SP-D enhanced the killing of pneumococci by human neutrophils. Aggregation of pneumococci varied not only between strains of the same multilocus sequence type and different serotypes but also between strains of the same serotype. However, use of recombinant strains in which the serotype had been changed showed that the degree of aggregation was influenced by the capsular type. Indeed, a 19F serotype strain which was not aggregated by SP-D did exhibit aggregation when the original isogenic strain was capsule switched to capsular serotype 3. However, although our results show that SP-D is capable of aggregating most pneumococci, no correlation between the degree of aggregation and the capsule or multilocus sequence type of the pneumococcus was clearly apparent. Therefore, although the capsule serotype is not the only determinant of aggregation by SP-D, the data presented here indicate that it does have a role to play.

373. Granzyme B and perforin: constitutive expression in human polymorphonuclear neutrophils
Wagner, C. et al
Blood, 103, 1099-1104 (2004)

Polymorphonuclear neutrophils (PMNs) produce an abundance of bactericidal and cytotoxic molecules consistent with their role as first-line defense against bacterial infection. PMNs, however, also cause efficient cellular cytotoxicity when targeted through Fc receptors to appropriate antibody-coated target cells. Although this so-called antibody-dependent cellular cytotoxicity (ADCC) was described many years ago, the mechanism of killing is still elusive. We now have found that PMNs contain perforin and granzyme B, the 2 molecules known as the cytotoxic entity of natural killer cells and of cytotoxic T lymphocytes as well. Lysates of PMNs were lytic for chicken erythrocytes in a time-, temperature-, and Ca^{2+}-dependent manner. Moreover, apoptosis of Jurkat cells was induced, consistent with the observation that the PMN lysates contain enzymatically active granzyme B. Taken together, our data provide evidence for the presence of perforin and granzyme B within the cytotoxic arsenal of PMNs.

374. Streptococcal M5 Protein Prevents Neutrophil Phagocytosis by Interfering with CD11b/CD18 Receptor-Mediated Association and Signaling
Weineisen, M., Sjöbring, U., Fällman, M. and Andersson, T.
J. Immunol., 172, 3798 (2004)

Group A streptococci (GAS) are common human pathogens that express major surface-associated virulence factors designated M proteins. In this study, we explored directly the cellular mechanisms behind their supposed ability to prevent phagocytosis. Isolated human neutrophils killed an M-negative GAS mutant (ΔM5), but not the wild-type parent strain (M5). After 3 h, 3–4 times as many ΔM5 as M5 bacteria were associated with the neutrophils, and more ΔM5 than M5 bacteria were ingested. However, there was no statistically significant difference between ΔM5 and M5 bacteria in regard to the percentage of the neutrophil-associated bacteria that were ingested, indicating that M5 protein prevents an adhesion receptor-dependent association with neutrophils and not the phagocytic machinery per se. Different Abs against CD11b/CD18 (CR3) blocked adhesion and killing of ΔM5 bacteria, whereas the blocking of two other complement receptors, CD11c/CD18 (CR4) and CD35 (CR1), did not. The CD11b/CD18-mediated killing of ΔM5 bacteria resulted in protein tyrosine phosphorylations and Cdc42 activation. Furthermore, inhibition of CD11b/CD18 receptor engagement or tyrosine kinase activity blocked the ΔM5-induced activation of Cdc42 as well as the killing of these bacteria. We conclude that M5 protein interferes with the CD11b/CD18-dependent association between GAS and neutrophils, and thereby blocks subsequent ingestion of the bacteria.

375. Granule Localization of Glutaminase in Human Neutrophils and the Consequence of Glutamine Utilization for Neutrophil Activity
Castell, L., Vance, C., Abbott, R., Marques, J. and eggleton, P.
J. Biol. Chem., 279, 13305-13310 (2004)

The provision of glutamine *in vivo* has been observed to reduce to normal levels the neutrophilia observed after exhaustive exercise and to decrease the neutrophil chemoattractant, interleukin-8. Thus, the role for glutamine in the regulation of inflammatory mediators of human neutrophil activation was investigated. The study sought to establish whether glutamine supplementation *in vitro* affects neutrophil function at rest and whether glutaminase, the major enzyme that metabolizes glutamine, is present in human polymorphonuclear neutrophils (PMN). During *in vitro* studies, the addition of 2 mM glutamine increased the respiratory burst of human

PMN stimulated with both phorbol myristate acetate (PMA) and formyl-methionyl-leucyl-phenylalanine. These observations were made using a highly sensitive, real time chemiluminescent probe, Pholasin®. Glutamine alone did not stimulate the release of reactive oxygen species. In a novel finding using glutaminase-specific antibodies in combination with flow cytometry and confocal microscopy, glutaminase was shown to be present on the surface of human PMN. Subcellular fractionation revealed that the enzyme was enriched in the secondary granules and could be released into cell culture medium upon stimulation with PMA. In conclusion, human PMN appeared to utilize glutamine and possess the appropriate glutaminase enzyme for metabolizing glutamine. This may depress some pro-inflammatory factors that occur during prolonged, exhaustive exercise.

376. Identification and Characterization of a Novel Human Myeloid Inhibitory C-type Lectin-like Receptor (MICL) That Is Predominantly Expressed on Granulocytes and Monocytes
Marshall, A.S.J. et al
J. Biol. Chem., 279, 14792-14802 (2004)

Inhibitory and activatory C-type lectin-like receptors play an important role in immunity through the regulation of leukocytes. Here, we report the identification and characterization of a novel myeloid inhibitory C-type lectin-like receptor (MICL) whose expression is primarily restricted to granulocytes and monocytes. This receptor, which contains a single C-type lectin-like domain and a cytoplasmic immunoreceptor tyrosine-based inhibitory motif, is related to LOX-1 (lectin-like receptor for oxidized low density lipoprotein-1) and the β-glucan receptor (Dectin-1) and is variably spliced and highly *N*-glycosylated. We demonstrate that it preferentially associates with the signaling phosphatases SHP-1 and SHP-2, but not with SHIP. Novel chimeric analyses with a construct combining MICL and the β-glucan receptor show that MICL can inhibit cellular activation through its cytoplasmic immunoreceptor tyrosine-based inhibitory motif. These data suggest that MICL is a negative regulator of granulocyte and monocyte function.

377. Triggering Receptor Expressed on Myeloid Cells-1 in Neutrophil Inflammatory Responses: Differential Regulation of Activation and Survival
Radsak, M.P., Salih, H.R., Rammensee, H-G. And Schild, H.
J. Immunol., 172, 4956-4963 (2004)

Polymorphonuclear neutrophils (PMN) are crucial in the innate host defense by their ability to rapidly accumulate in inflamed tissues and clear a site of infection from microbial pathogens by their potent effector mechanisms. The triggering receptor expressed on myeloid cells (TREM)-1 is a recently described activating receptor on PMN with an important role in inflammation. However, the effects of TREM-1 stimulation on a cellular level remain to be further defined. To characterize TREM-1-mediated activation of human PMN, we evaluated the effect of receptor ligation on PMN effector functions. Activation via TREM-1 induces immediate degranulation of neutrophilic granules resulting in the release of IL-8, respiratory burst, and phagocytosis. TREM-1 ligation synergizes with the activation by the Toll-like receptors (TLR) ligands LPS, Pam_3Cys, and R-848. In contrast, no synergy between TREM-1- and TLR-mediated stimulation was observed concerning PMN survival, whereas TLR-mediated stimuli protect PMN from apoptosis, concurrent TREM-1 activation neutralizes these anti-apoptotic effects. These results give a new perspective for the regulation of neutrophil inflammatory responses emphasizing the importance of TREM-1 in innate immunity.

378. Design and Use of Highly Specific Substrates of Neutrophil Elastase and Proteinase 3
Korkmaz, B. et al
Am. J. Respir. Cell Mol. Biol., 30, 801-807 (2004)

We have exploited differences in the structures of S2' subsites of proteinase 3 (Pr3) and human neutrophil elastase (HNE) to prepare new fluorogenic substrates specific for each of these proteases. The positively charged residue at position 143 in Pr3 prevents it from accommodating an arginyl residue at S2' and improves the binding of P2' aspartyl-containing substrates, as judged by the decreased K_m. As a result, the k_{cat}/K_m for Abz-VADCADQ-EDDnp is over 500 times greater for Pr3 than for HNE, and that for Abz-APEEIMRRQ-EDDnp is over 500 times greater for HNE than for Pr3. This allows each protease activity to be measured in the presence of a large excess of the other, as might occur *in vivo*. Placing a prolyl residue in position P2' greatly impaired substrate binding to both HNE and Pr3, which further emphasizes the importance of S' subsites in these proteases. HNE and Pr3 activities were measured with these substrates at the surface of fixed polymorphonuclear leukocytes (PMNs) before and after activation. This demonstrated that their active site remains accessible when they are exposed to the cell surface. Both membrane-bound proteases were strongly inhibited by low M_r serine protease inhibitors, but only partially by inhibitors of larger M_r such as α1-protease inhibitor, the main physiologic inhibitor in lung secretions. Most of membrane-bound HNE and Pr3 can be released from the membrane surface of fixed cells by a buffer containing detergent, suggesting that hydrophobic interactions are involved in membrane binding.

379. Volume-Sensitive Chloride Channels Do Not Mediate Activation-Induced Chloride Efflux in Human Neutrophils
Perez-Cornejo, P., Arreola, J., Law, F-Y., Schultz, J.B. and Krauf, P.A.
J. Immunol., 172, 6988-6993 (2004)

Many agents that activate neutrophils, enabling them to adhere to venular walls at sites of inflammation, cause a rapid Cl^- efflux. This Cl^- efflux and the increase in the number and affinity of β_2 integrin surface adhesion molecules (up-regulation) are all inhibited by ethacrynic acid and certain aminomethyl phenols. The effectiveness of the latter compounds correlates with their inhibition of swelling-activated Cl^- channels (I_{Clvol}), suggesting that I_{Clvol} mediates the activator-induced Cl^- efflux. To test this hypothesis, we used whole-cell patch clamp in hypotonic media to examine the effects of inhibitors of up-regulation on I_{Clvol} in neutrophils and promyelocytic leukemic HL-60 cells. Both the channel blocker 5-nitro-2-(3-phenylpropylamino)benzoic acid and [3-methyl-1-*p*-sulfophenyl-5-pyrazolone-(4)]-[1,3-dibutylbarbituric acid]-pentamethine oxonol (WW781), a nonpenetrating oxonol, inhibited I_{Clvol} at concentrations similar to those that inhibit β_2 integrin up-regulation. However, ethacrynic acid, at the same concentration that inhibits activator-induced Cl^- efflux and up-regulation, had no effect on I_{Clvol} and swelling-activated Cl^- efflux, providing evidence against the involvement of I_{Clvol} in the activator-induced Cl^- efflux.

380. Granulocyte Macrophage Colony-stimulating Factor Signaling and Proteasome Inhibition Delay Neutrophil Apoptosis by Increasing the Stability of Mcl-1
Derouet, M., Thomas, L., Cross, A., Moots, R.J. and Edwards, S.W.
J. Biol. Chem., 279, 26915-26921 (2004)

Human neutrophils normally have a very short half-life and die by apoptosis. Cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) can delay this apoptosis via increases in the cellular levels of Mcl-1, an anti-apoptotic protein of the Bcl-2 family with a rapid turnover rate. Here we have shown that inhibition of the proteasome (*a*) decreases the rate of Mcl-1 turnover within neutrophils and (*b*) significantly delays apoptosis. This led us to determine whether GM-CSF could enhance neutrophil survival by altering the rate of Mcl-1 turnover. Addition of GM-CSF to neutrophils enhanced Mcl-1 stability and delayed apoptosis by signaling pathways requiring PI3K/Akt and p44/42 Erk/Mek, because inhibitors of these pathways completely abrogated the GM-CSF-mediated effect on both Mcl-1 stability and apoptosis delay. Conversely, induction of Mcl-1 hyperphosphorylation by the phosphatase inhibitor, okadaic acid, significantly accelerated both Mcl-1 turnover and apoptosis. Neither the calpain inhibitor, carbobenzoxy-valinyl-phenylalaninal, nor the pan caspase inhibitor, benzyloxycarbonyl-VAD-fluoromethylketone, had any effect on Mcl-1 stability under these conditions. These observations indicate that profound changes in the rate of neutrophil apoptosis following cytokine signaling occur via dynamic changes in the rate of Mcl-1 turnover via the proteasome.

381. TNF-Induced β_2 Integrin Activation Involves Src Kinases and a Redox-Regulated Activation of p38 MAPK
Bouaouina, M., Blouin, E., Halbwaschs-Mecarelli, L., Lesavre, P. and Rieu, P.
J. Immunol., 173, 1313-1320 (2004)

We previously demonstrated that the TNF-α-induced inside-out signaling leading to β_2 integrin activation is redox regulated. To identify kinases involved in this pathway, the effects of kinase inhibitors on the expression of β_2 integrin activation neoepitope (clone 24) were investigated. We show that both p38 MAPK (inhibited by SB203580) and Src kinases (inhibited by PP2) are involved in β_2 integrin activation by TNF and oxidants in human neutrophils. Src kinases appeared constitutively active in resting neutrophils and not further activated by TNF or oxidants in nonadherent conditions. However, PP2 blocked both TNF-induced expression of the 24 epitope and cell adhesion promoted by the integrin activating anti-CD18 KIM185 mAb, showing that both the inside-out and the outside-in signaling involve Src kinases. p38 MAPK was activated by TNF and oxidants in nonadherent conditions i.e., with 10 mM EDTA. This activation in EDTA resulted in CD11b, CD35 and CD66 up-regulation and in an oxidative response, all blocked by SB203580 and PP2. p38 MAPK was not activated upon direct integrin activation by KIM185 mAb. Thus, p38 activation allows the study to distinguish the initial transduction pathway leading to β_2 integrin activation from the signaling resulting from integrin engagement. Finally, p38 MAPK activation by TNF was blocked by diphenylene iodonium, an inhibitor of flavoprotein oxidoreductase, and by the free radical scavenger *N*-acetylcystein. Taken together, these results demonstrate, for the first time, that constitutively activated Src tyrosine kinases and a redox-regulated activation of p38 MAPK are involved in TNF inside-out signaling leading to β_2 integrin activation.

382. Anaplasma phagocytophilum Utilizes Multiple Host Evasion Mechanisms To Thwart NADPH Oxidase-Mediated Killing during Neutrophil Infection
Carlyon, J.A., Latif, D.A., Pypaert, M., Lacy, P. and Fikrig, E.
Infect. Immunol., 72, 4772-4783 (2004)

Anaplasma phagocytophilum, the etiologic agent of human anaplasmosis, is a bacterial pathogen that specifically colonizes neutrophils. Neutrophils utilize the NADPH oxidase complex to generate superoxide (O_2^-) and initiate oxidative killing of microorganisms. *A. phagocytophilum*'s unique tropism for neutrophils, however, indicates that it subverts and/or avoids oxidative killing. We therefore examined the effects of *A. phagocytophilum* infection on neutrophil NADPH oxidase assembly and reactive oxygen species (ROS) production. Following neutrophil binding, *Anaplasma* invasion requires at least 240 min. During its prolonged association with the neutrophil plasma membrane, *A. phagocytophilum* stimulates NADPH oxidase assembly, as indicated by increased cytochrome b_{558} mobilization to the membrane, as well as colocalization of Rac and $p22^{phox}$. This initial stimulation taxes the host neutrophil's finite oxidase reserves, as demonstrated by time- and bacterial-dose-dependent decreases in secondary activation by *N*-formyl-methionyl-

leucyl-phenylalanine (FMLP) or phorbol myristate acetate (PMA). This stimulation is modest, however, and does not diminish oxidase stores to nearly the extent that *Escherichia coli*, serum-opsonized zymosan, FMLP, or PMA do. Despite the apparent activation of NADPH oxidase, no change in ROS-dependent chemiluminescence is observed upon the addition of *A. phagocytophilum* to neutrophils, indicating that the bacterium may scavenge exogenous O_2^-. Indeed, *A. phagocytophilum* rapidly detoxifies O_2^- in a cell-free system. Once internalized, the bacterium resides within a protective vacuole that excludes $p22^{phox}$ and $gp91^{phox}$. Thus, *A. phagocytophilum* employs at least two strategies to protect itself from neutrophil NADPH oxidase-mediated killing.

383. Granzymes A and B are not expressed in human neutrophils
Grossman, W.J. et al
Blood, 104, 906-908 (2004)

Recent articles by Wagner et al[1] and Hochegger et al[2] reported the expression of perforin and granzymes A and B in human polymorphonuclear cells (PMNs; neutrophils) using flow cytometry assays. Both groups used an intracellular staining technique, including the use of secondary antibodies to detect granzymes A and B. Since previous studies have suggested that granzyme expression is restricted to cytotoxic lymphocytes (ie, natural killer [NK] cells and cytotoxic T cells),[3] we also analyzed purified PMNs for granzyme expression using an intracellular flow cytometry assay with primary-conjugated granzyme monoclonal antibodies that were proven to be specific, using competitive recombinant granzymes and knockout mice (W.J.G., James W. Verbsky, Benjamin L. Tollefson, Claudia Kemper, John P. Atkinson, T.J.L., manuscript submitted, 2004).

384. Modulation of airway inflammation and bacterial clearance by epithelial cell ICAM-1
Humlicek, A.L., Pang, L. and Look, D.C.
Am. J. Physiol. Lung Cell. Mol. Physiol., 287, L598-L607 (2004)

Many cell types in the airway express the adhesive glycoprotein for leukocytes intercellular adhesion molecule-1 (ICAM-1) constitutively and/or in response to inflammatory stimuli. In this study, we identified functions of ICAM-1 on airway epithelial cells in defense against infection with *Haemophilus influenzae*. Initial experiments using a mouse model of airway infection in which the bacterial inoculum was mixed with agar beads that localize inflammation in airways demonstrated that ICAM-1 expression was required for efficient clearance of *H. influenzae*. Airway epithelial cell ICAM-1 expression required few or no leukocytes, suggesting that epithelial cells could be activated directly by interaction with bacteria. Specific inhibition of ICAM-1 function on epithelial cells by orotracheal injection of blocking antibodies resulted in decreased leukocyte recruitment and *H. influenzae* clearance in the airway. Inhibition of endothelial cell ICAM-1 resulted in a similar decrease in leukocyte recruitment but did not affect bacterial clearance, indicating that epithelial cell ICAM-1 had an additional contribution to airway defense independent of effects on leukocyte migration. To assess this possibility, we used an in vitro model of neutrophil phagocytosis of bacteria and observed significantly greater engulfment of bacteria by neutrophils adherent to epithelial cells expressing ICAM-1 compared with nonadherent neutrophils. Furthermore, bacterial phagocytosis and killing by neutrophils after interaction with epithelial cells were decreased when a blocking antibody inhibited ICAM-1 function. The results indicate that epithelial cell ICAM-1 participates in neutrophil recruitment into the airway, but its most important role in clearance of *H. influenzae* may be assistance with neutrophil-dependent bacterial killing.

385. cAMP protects neutrophils against TNF-α-induced apoptosis by activation of cAMP-dependent protein kinase, independently of exchange protein directly activated by cAMP (Epac)
Krakstad, C., Christensen, A.E. and Døskeland, S.O.
J. Leukoc. Biol., 76, 641-647 (2004)

It is unclear by which receptor cyclic adenosine monophosphate (cAMP) acts to promote neutrophil survival. We found that 8-(4-chlorophenylthio)-2'-O-methyl-cAMP, a specific activator of the recently discovered cAMP receptor, cAMP-regulated guanosine 5'-triphosphate exchange protein directly activated by cAMP, failed to protect human neutrophils from cell death. In contrast, specific activators of cAMP-dependent protein kinase type I (cA-PKI) could protect against death receptor [tumor necrosis factor receptor 1 (TNFR-1), Fas]-mediated apoptosis as well as cycloheximide-accelerated "spontaneous" apoptosis. A novel "caged" cA-PK-activating analog, 8-bromo (8-Br)-acetoxymethyl-cAMP, was more than 20-fold more potent than 8-Br-cAMP to protect neutrophils challenged with TNF-α against apoptosis. This analog acted more rapidly than forskolin (which increases the endogenous cAMP production) and allowed us to demonstrate that cA-PK must be activated during the first 10 min after TNF-α challenge to protect against apoptosis. The protective effect was mediated solely through cA-PK activation, as it was abolished by the cA-PKI-directed inhibitor *R*p-8-Br-cAMPS and the general cA-PK inhibitor H-89. Neutrophils not stimulated by cAMP-elevating agents showed increased apoptosis when exposed to the cA-PK inhibitors *R*p-8-Br-cAMPS and H-89, suggesting that even moderate activation of cA-PK is sufficient to enhance neutrophil longevity and thereby contribute to neutrophil accumulation in chronic inflammation.

386. Anti-GPVI–associated ITP: an acquired platelet disorder caused by autoantibody-mediated clearance of the GPVI/FcRγ-chain complex from the human platelet surface
Boylan, B. et al
Blood, 104, 1350-1355 (2004)

Platelet glycoprotein (GP) VI is a 62-kDa membrane glycoprotein that exists on both human and murine platelets in a noncovalent complex with the Fc receptor (FcR) γchain. The GPVI/FcRγ-chain complex serves as the major activating receptor for collagen, as evidenced by observations that platelets genetically deficient in GPVI or the FcRγ chain are highly refractory to collagen-induced platelet activation. Recently, several different rat anti–murine GPVI monoclonal antibodies, termed JAQs 1, 2, and 3, were produced that had the unique property of "immunodepleting" GPVI from the murine platelet surface and rendering it unresponsive to collagen or GPVI-specific agonists like convulxin or collagen-related peptide (CRP). Herein, we describe a patient with a mild bleeding disorder and a moderately reduced platelet count whose platelets fail to become activated in response to collagen or CRP and inefficiently adhere to and form thrombi on immobilized collagen under conditions of arterial shear. Although the amount of GPVI platelet mRNA and the nucleotide sequence of the GPVI gene were found to be normal, both GPVI and the FcRγ chain were nearly absent from the platelet surface and were markedly reduced in wholeplatelet detergent lysates. Patient plasma contained an autoantibody that bound specifically to GPVI-positive, normal platelets, and cleared soluble GPVI from the plasma, suggesting that the patient suffers from a rare form of idiopathic thrombocytopenic purpura caused by a GPVI-specific autoantibody that mediates clearance of the GPVI/FcRγ-chain complex from the platelet surface. Since antibody-induced GPVI shedding now has been demonstrated in both humans and mice, these studies may provide a rationale for developing therapeutic reagents that induce temporary depletion of GPVI for the treatment of clinical thrombosis.

387. Regulation of Human Polymorphonuclear Leukocytes Functions by the Neuropeptide Pituitary Adenylate Cyclase-Activating Polypeptide after Activation of MAPKs
Harfi, I., D'Hondt, S., Corazza, F. and Sariban, E.
J. Immunol., 173, 4154-4163 (2004)

Anti-inflammatory activities of pituitary adenylate cyclase-activating protein (PACAP) are mediated in part through specific effects on lymphocytes and macrophages. This study shows that in human polymorphonuclear neutrophils (PMNs), PACAP acts as a proinflammatory molecule. In PMNs, vaso-intestinal peptide/PACAP receptor 1 (VPAC-1) was the only receptor found to be expressed by RT-PCR. Using VPAC-1 Ab, we found that VPAC-1 mRNA was translated into proteins. In PMNs, PACAP increases cAMP, inositol triphosphate metabolites, and calcium. It activates two of the three members of the MAPK superfamily, the ERK and the stress-activated MAPK p38. U73122, an inhibitor of phospholipase C (PLC), inhibits PACAP-induced ERK activation, whereas p38 MAPK phosphorylation was unaffected. Using specific pharmalogical inhibitors of ERK (PD098059) and p38 MAPK (SB203580), we found that PACAP-mediated calcium increase was ERK and PLC dependent and p38 independent. PACAP primes fMLP-associated calcium increase; it also primes fMLP activation of the respiratory burst as well as elastase release, these last two processes being ERK and PLC dependent and p38 MAPK independent. PACAP also increases membrane expression of CD11b and release of lactoferrin and metallo proteinase-9 (MMP-9). These effects were PLC dependent (CD 11b, lactoferrin, MMP-9), ERK dependent (CD 11b, lactoferrin, MMP-9), and p38 dependent (CD11b, lactoferrin). We conclude that PACAP is a direct PMN activator as well as an effective PMN priming agent that requires PLC, ERK, and p38 MAPK activities.

388. Characterization of Antibodies to Capsular Polysaccharide Antigens of Haemophilus influenzae Type b and Streptococcus pneumoniae in Human Immune Globulin Intravenous Preparations
Mikolajczyk, M.G. et al
Clin. Diagn. Lab. Immunol., 11, 1158-1164 (2004)

The most common infections in primary immune deficiency disease (PIDD) patients involve encapsulated bacteria, mainly *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (pneumococcus). Thus, it is important to know the titers of Hib- and pneumococcus-specific antibodies that are present in immune globulin (Ig) intravenous (IGIV) preparations used to treat PIDD. In this study, seven IGIV preparations were tested by enzyme-linked immunosorbent assay and opsonophagocytic activity for antibody titers to the capsular polysaccharides of Hib and five pneumococcal serotypes. Differences in Hib- and pneumococcus-specific antibody titer were observed among various IGIV preparations, with some products having higher- or lower-than-average titers. Opsonic activity also varied among preparations. As expected, IgG2 was the most active subclass of both binding and opsonic activity except against pneumococcal serotype 6B where IgG3 was the most active. This study determines antibody titers against capsular polysaccharides of Hib and pneumococcus in seven IGIV products that have been shown to be effective in reducing infections in PIDD patients. As donor antibody levels and manufacturing methods continue to change, it may prove useful from a regulatory point of view to reassess IGIV products periodically, to ensure that products maintain antibody levels that are important for the health of IGIV recipients.

389. Rac2-Deficient Murine Macrophages Have Selective Defects in Superoxide Production and Phagocytosis of Opsonized Particles
Yamauchi, A. et al
J. Immunol., 173, 5971-5979 (2004)

The Rho family GTPase Rac is a crucial participant in numerous cellular functions and acts as a molecular switch for signal transduction. Mice deficient in hemopoietic-specific Rac2 exhibited agonist-specific defects in neutrophil functions including chemoattractant-stimulated filamentous actin polymerization and chemotaxis, and superoxide production elicited by phorbol ester,

fMLP, or IgG-coated particles, despite expression of the highly homologous Rac1 isoform. In this study, functional responses of Rac2-null murine macrophages were characterized to examine whether Rac2 also has nonredundant functions in this phagocytic lineage. In contrast to murine neutrophils, in which Rac1 and Rac2 are present in similar amounts, Rac1 was ~4-fold more abundant than Rac2 in both bone marrow-derived and peritoneal exudate macrophages, and macrophage Rac1 levels were unchanged by the absence of Rac2. Accumulation of exudate macrophages during peritoneal inflammation was reduced in *rac2*$^{-/-}$ mice. FcγR-mediated phagocytosis of IgG-coated SRBC was also significantly decreased in Rac2-null macrophages, as was NADPH oxidase activity in response to phorbol ester or FcγR stimulation. However, phagocytosis and oxidant production stimulated by serum-opsonized zymosan was normal in *rac2*$^{-/-}$ macrophages. Macrophage morphology was also similar in wild-type and Rac2-null cells, as was actin polymerization induced by FcγR-mediated phagocytosis or M-CSF. Hence, Rac2-null macrophages have selective defects paralleling many of the observed functional defects in Rac2-null neutrophils. These results provide genetic evidence that although Rac2 is a relatively minor isoform in murine macrophages, it plays a nonoverlapping role with Rac1 to regulate host defense functions in this phagocyte lineage.

390. The Use of Real-Time Reverse Transcription-PCR for Prostate-Specific Antigen mRNA to Discriminate between Blood Samples from Healthy Volunteers and from Patients with Metastatic Prostate Cancer
Patel, K. et al
Clin. Cancer Res., 10, 7511-7519 (2004)

Purpose: A clinical role for nonquantitative reverse transcription-PCR (RT-PCR) using prostate-specific antigen in blood samples from patients with prostate cancer remains undefined. Assay variation and detection of prostate-specific antigen mRNA illegitimate transcription may explain inconsistent results between studies. Defining levels of prostate-specific antigen mRNA expression in blood samples from healthy volunteers and patients with prostate cancer would allow cutoffs to be established to distinguish the two groups. *Experimental Design:* Quantitative real-time RT-PCR for prostate-specific antigen mRNA was established and levels of prostate-specific antigen mRNA measured in bloods samples from healthy volunteers (n = 21) and patients with localized (n = 27) and metastatic (n = 40) prostate cancer.
Results: Levels of prostate-specific antigen mRNA were significantly higher in blood samples from patients with metastatic prostate cancer than in blood samples from patients with localized prostate cancer ($P < 0.001$) or in blood samples from healthy volunteers ($P < 0.01$); levels between patients with localized prostate cancer and healthy volunteers were no different. Assay sensitivity to detect patients with metastatic prostate cancer was 68% with specificity of 95%. In patients with newly diagnosed metastatic prostate cancer, monitoring response to hormonal therapy was possible with this assay. No correlation between levels of prostate-specific antigen mRNA and serum prostate-specific antigen protein levels was found, suggesting that prostate-specific antigen mRNA and serum prostate-specific antigen protein levels reflect different features of prostate cancer, *i.e.*, circulating tumor cells and total tumor bulk, respectively. *Conclusions:* Quantitative RT-PCR discriminates patients with metastatic prostate cancer from healthy volunteers and patients with localized prostate cancer but cannot discriminate patients with localized prostate cancer from healthy volunteers. A role for quantitative RT-PCR has been identified in the assessment and monitoring of patients with metastatic prostate cancer.

391. Functional Activity of In Vivo Primed Granulocytes: A Comparative Study
Ribeiro, D. et al
Blood, 104, 3818 (2004)

Recombinant granulocyte colony-stimulating factor (G-CSF) has been widely used in the treatment of chemotherapy-induced neutropenia as well as in mobilization of peripheral blood stem cells in context with autologous bone marrow transplantation. Recombinant G-CSF expressed in E.coli (Filgrastim) and G-CSF expressed in CHO-cells (Lenograstim), are in clinical use. Here we study the effects of the different G-CSF on functional activity of granulocytes including chemotaxis, oxidative burst and antigen expression.

Granulocytes were obtained from patients with hematological malignancies before and after the administration of one of the three G-CSFs and isolated using a **polymorphprep** density gradient. Chemotactic properties were assessed using a Boyden chamber assay in combination with an under agarose assay, both using fMLP as chemotactic stimulus. Release of superoxide anions served as measure of the oxidative burst after stimulation with PMA using a chemiluminescence assay. The viability and surface antigen expression were assessed by FACS.
FACS analysis showed a decrease in CD10, CD11b and CD62L of the selectin family contrary to an increased expression of the VLA-5 alpha chain CD49, the LPS-receptor CD14 and the IgG receptor FcγRI (CD64). A stronger effect of lenograstim on CD11b and CD14 could be assessed contrary to filgrastim showing a stronger effect on CD62 and CD64. We observed a decrease of chemotactic activity and non-directed random migration in patients receiving filgrastim, as opposed to the results in patients receiving lenograstim. No obvious differences were found in production of superoxide anions. Whether the superior chemotactic activity and migration of Lenograstim- vs. Filgrastim-primed granulocytes translates into improved antibacterial activity and improved clinical endpoints warrants further study in the clinical setting.

392. Activation of the complement system generates antibacterial peptides
Andersson, E. et al

PNAS, 101, 16879-16884 (2004)

The complement system represents an evolutionary old and significant part of the innate immune system involved in protection against invading microorganisms. Here, we show that the anaphylatoxin C3a and its inactivated derivative C3a-desArg are antibacterial, demonstrating a previously unknown direct antimicrobial effect of complement activation. The C3a peptide, as well as functional epitopes in the sequence, efficiently killed the Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa*, and the Gram-positive *Enterococcus faecalis*. In mice, a C3a-derived peptide suppressed infection by Gram-positive *Streptococcus pyogenes* bacteria. Fluorescence and electron microscopy demonstrated that C3a binds to and induces breaks in bacterial membranes. C3a was also found to induce membrane leakage of liposomes. These findings provide an interesting link between the complement system and antimicrobial peptides, which are two important branches of innate immunity.

393. Protein FOG – a streptococcal inhibitor of neutrophil function
Johansson, H.M., Mörgelin, M. and Frick, I-M.
Microbiology, 150, 4211-4221 (2004)

Several strains of group G streptococci (GGS) form aggregates when grown *in vitro*. Aggregating strains interact with fibrinogen, and this study reports the isolation of a novel self-associating and fibrinogen-binding protein of GGS, denoted protein FOG. Sequencing of the *fog* gene revealed structural similarity with M proteins of both GGS and group A streptococci (GAS). Analogous to GAS, GGS were found to multiply in human blood. All strains of GGS express protein G, a protein known to interact with the constant region of immunoglobulin G and albumin. Surprisingly, a clinical isolate expressing protein G, but lacking protein FOG, was killed in human whole blood; however, the addition of intact soluble protein FOG restored the ability of the bacteria to survive and multiply in human blood. This is believed to be the first report of a soluble M-like protein salvaging an M-negative strain from being killed. The antibactericidal property of protein FOG is dependent on its fibrinogen-binding activity. Thus, in plasma, FOG precipitates fibrinogen, and when added to whole blood, protein FOG triggers the formation of visible aggregates comprising fibrinogen and neutrophils that are disabled in their killing of the bacteria. Moreover, the results emphasize the importance of an intact FOG molecule, as presented on the bacterial surface, for full protective effect.

394. Membrane retrieval in neutrophils during phagocytosis: inhibition by M protein-expressing S. pyogenes bacteria
Bauer, S. and Tapper, H.
J. Leukoc. Biol., 76, 1142-1150 (2004)

During phagocytosis and phagosome maturation, complex membrane traffic events must be coordinated. We have observed, using fluorescent fluid-phase and membrane markers, that in the human neutrophil, internalization of nonopsonized, Gram-positive bacteria, but not of latex beads, is accompanied by a rapid and localized formation of pinosomal structures. This pinocytic response is calcium-dependent but insensitive to actin cytoskeleton disruption and wortmannin treatment. Contrary to what we observe, endosomal structures usually are considered to participate in phagosome formation by providing necessary membrane to forming phagosomes. Instead, our results show a coupling between neutrophil secretory and membrane-retrieval processes during phagosome maturation, and we suggest that the observed, localized pinocytic response is linked to the secretion of azurophilic granules toward nascent phagosomes. Accordingly, M and M-like protein-expressing *Streptococcus pyogenes* bacteria, which are able to survive inside neutrophil phagosomes, inhibit both the secretion of azurophilic granules to phagosomes and pinosome formation.

395. Constitutive membrane expression of proteinase 3 (PR3) and neutrophil activation by anti-PR3 antibodies
Van Rossum, A.P. et al
J. Leukoc. Biol., 76, 1162-1170 (2004)

Antineutrophil cytoplasm autoantibodies with specificity for proteinase 3 (PR3) are thought to play a major role in the pathogenesis of Wegener's granulomatosis (WG), presumably by their potential to activate neutrophils. In patients with WG, high expression of PR3 on the surface of nonprimed neutrophils is associated with an increased incidence and rate of relapse. In this study, we analyzed the functional significance of constitutive PR3 expression for neutrophil activation as induced by anti-PR3 antibody. Therefore, primed and nonprimed neutrophils were stimulated with the monoclonal anti-PR3 antibody PR3G-3. Activation was measured as actin polymerization by the phalloidin assay as an early, detectable activation event and oxidative burst by the dihydrorhodamine assay, as a late, detectable activation event. In contrast to the oxidative burst, we found that anti-PR3 antibody-induced actin polymerization could be triggered in neutrophils without priming with tumor necrosis factor α (TNF-α). In addition, a correlation was found between the level of PR3 expression on the surface of these nonprimed neutrophils and the degree of actin polymerization. However, after priming with TNF-α, no correlation was found between membrane expression of PR3 and the level of actin polymerization or respiratory burst as induced by anti-PR3 antibody. These data suggest that the presence of PR3 on the surface of nonprimed neutrophils has consequences for their susceptibility to the initial activation step by anti-PR3 antibodies. These data may be relevant in view of the observed relation between membrane expression of PR3 on nonprimed neutrophils of patients with WG and their susceptibility for relapses.

396. Evidence for a Potent Antiinflammatory Effect of Rosiglitazone
Mohanty, P. et al
J. Clin. Endocrinol. Metab., 89, 2728-2735 (2004)

We have recently demonstrated a potent antiinflammatory effect of troglitazone, an agonist of peroxisome proliferator-activated receptor γ (PPARγ) and a partial agonist of PPARα in both the nondiabetic obese and diabetic obese subjects. We have now investigated the antiinflammatory actions of rosiglitazone, a selective PPARγ agonist. Eleven nondiabetic obese subjects and 11 obese diabetic subjects were each given 4 mg of rosiglitazone daily for a period of 6 wk. Fasting blood samples were obtained at 0, 1, 2, 4, 6, and 12 wk (6 wk after the cessation of rosiglitazone). Eight obese subjects and five obese diabetic subjects were also included in the study as control groups. Fasting blood samples were obtained from the control groups at 0, 1, 2, 4, and 6 wk only. Nuclear factor κB (NFκB)-binding activity in mononuclear cells, plasma monocyte chemoattractant protein-1 (MCP-1), TNF-α, soluble intercellular adhesion molecule-1, C-reactive protein (CRP), and serum amyloid A (SAA) were measured.

397. Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia
Aras, O. et al
Blood, 103, 4545-4553 (2004)

The precise role of intravascular tissue factor (TF) remains poorly defined, due to the limited availability of assays capable of measuring circulating TF procoagulant activity (PCA). As a model of inflammation-associated intravascular thrombin generation, we studied 18 volunteers receiving an infusion of endotoxin. A novel assay that measures microparticle (MP)-associated TF PCA from a number of cellular sources (but not platelets) demonstrated an 8-fold increase in activity at 3 to 4 hours after endotoxin administration ($P < .001$), with a return to baseline by 8 hours. TF antigen-positive MPs isolated from plasma were visualized by electron microscopy. Interindividual MP-associated TF response to lipopolysaccharide (LPS) was highly variable. In contrast, a previously described assay that measures*total* (cell and MP-borne) whole-blood TF PCA demonstrated a more modest increase, with a peak in activity (1.3-fold over baseline; $P < .000\ 01$) at 3 to 4 hours, and persistence for more than 24 hours. This surprisingly modest increase in whole-blood TF activity is likely explained by a profound although transient LPS-induced monocytopenia. MP-associated TF PCA was highly correlated with whole-blood TF PCA and total number of circulating MPs, and whole-blood TF PCA was highly correlated with TF mRNA levels.

398. Circulating Mononuclear Cells in the Obese Are in a Proinflammatory State
Ghanim, H. et al
Circulation, 110, 1564-1571 (2004)

Background— In view of the increase in plasma concentrations of proinflammatory mediators tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) in obesity, we investigated whether peripheral blood mononuclear cells (MNC) from obese subjects are in a proinflammatory state.
Methods and Results— MNC were prepared from fasting blood samples of obese (n=16; body mass index [BMI]=37.7±5.0 kg/m^2) and normal-weight control (n=16; BMI=23.8±1.9 kg/m^2) subjects. Nuclear factor κB (NF-κB) binding to DNA in nuclear extracts was elevated ($P<0.05$) and the inhibitor of NFκB-ß (IκB-ß) was significantly lower ($P<0.001$) in the obese group. Reverse transcription–polymerase chain reaction revealed elevated levels of migration inhibitor factor (MIF), IL-6, TNF-α, and matrix metalloproteinase-9 (MMP-9) mRNA expression in the obese subjects ($P<0.05$). Plasma concentrations of MIF, IL-6, TNF-α, MMP-9, and CRP were also significantly higher. Plasma glucose, insulin, and free fatty acids (FFAs) were measured, and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Plasma FFA concentration related significantly to BMI, IL-6, and TNF-α mRNA expression and plasma CRP levels but not to HOMA-IR. On the other hand, the inflammatory mediators were significantly related to BMI and HOMA-IR.
Conclusions— These data show (1) for the first time that MNC in obesity are in a proinflammatory state with an increase in intranuclear NF-κB binding, a decrease in IκB-ß, and an increase in the transcription of proinflammatory genes regulated by NF-κB; (2) that plasma FFAs are a modulator of inflammation; and (3) that insulin resistance is a function of inflammatory mediators.

399. Induction of De Novo Subcortical Actin Filament Assembly by Treponema denticola Major Outer Sheath Protein
Amin, M. et al
Infect. Immun., 72, 3650-3654 (2004)

Treponema denticola and its major outer sheath protein (Msp) induce actin reorganization in fibroblasts. We adapted a barbed-end labeling/imaging assay to monitor Msp-induced subcortical actin filament assembly in neutrophils and fibroblasts. Msp, at an actin-reorganizing concentration, inhibited migration of these dissimilar cell types, whose cytoskeletal functions in locomotion and phagocytosis are crucial for immunity and healing of peripheral infections.

400. Uropathogenic Escherichia coli Triggers Oxygen-Dependent Apoptosis in Human Neutrophils through the Cooperative Effect of Type 1 Fimbriae and Lipopolysaccharide
Blomgran, R., Zheng, L. and Stendahl, O.

Infect. Immun., 72, 4570-4578 (2004)

Type 1 fimbriae are the most commonly expressed virulence factor on uropathogenic *Escherichia coli*. In addition to promoting avid bacterial adherence to the uroepithelium and enabling colonization, type 1 fimbriae recruit neutrophils to the urinary tract as an early inflammatory response. Using clinical isolates of type 1 fimbriated *E. coli* and an isogenic type 1 fimbria-negative mutant (CN1016) lacking the FimH adhesin, we investigated if these strains could modulate apoptosis in human neutrophils. We found that *E. coli* expressing type 1 fimbriae interacted with neutrophils in a mannose- and lipopolysaccharide (LPS)-dependent manner, leading to apoptosis which was triggered by the intracellular generation of reactive oxygen species. This induced neutrophil apoptosis was abolished by blocking FimH-mediated attachment, by inhibiting NADPH oxidase activation, or by neutralizing LPS. In contrast, CN1016, which did not adhere to or activate the respiratory burst of neutrophils, delayed the spontaneous apoptosis in an LPS-dependent manner. This delayed apoptosis could be mimicked by adding purified LPS and was also observed by using fimbriated bacteria in the presence of D-mannose. These results suggest that LPS is required for *E. coli* to exert both pro- and antiapoptotic effects on neutrophils and that the difference in LPS presentation (i.e., with or without fimbriae) determines the outcome. The present study showed that there is a fine-tuned balance between type 1 fimbria-induced and LPS-mediated delay of apoptosis in human neutrophils, in which altered fimbrial expression on uropathogenic *E. coli* determines the neutrophil survival and the subsequent inflammation during urinary tract infections.

401. α1-antitrypsin and its C-terminal fragment attenuate effects of degranulated neutrophil-conditioned medium on lung cancer HCC cells, in vitro

Zelvyte, I., Stevens, T., Westin, U. and Janciauskine, S.
Cancer Cell Int., 4(7), (2004)

Background
Tumor microenvironment, which is largely affected by inflammatory cells, is a crucial participant in the neoplastic process through promotion of cell proliferation, survival and migration. We measured the effects of polymorphonuclear neutrophil (PMN) conditioned medium alone, and supplemented with serine proteinase inhibitor α-1 antitrypsin (AAT) or its C-terminal fragment (C-36 peptide), on cultured lung cancer cells.
Methods
Lung cancer HCC cells were grown in a regular medium or in a PMN-conditioned medium in the presence or absence of AAT (0.5 mg/ml) or its C-36 peptide (0.06 mg/ml) for 24 h. Cell proliferation, invasiveness and release of IL-8 and VEGF were analyzed by [^{3}H]-thymidine incorporation, Matrigel invasion and ELISA methods, respectively.
Results
Cells exposed to PMN-conditioned medium show decreased proliferation and IL-8 release by 3.9-fold, $p < 0.001$ and 1.3-fold, $p < 0.05$, respectively, and increased invasiveness by 2-fold ($p < 0.001$) compared to non-treated controls. In the presence of AAT, PMN-conditioned medium loses its effects on cell proliferation, invasiveness and IL-8 release, whereas VEGF is up-regulated by 3.7-fold ($p < 0.001$) compared to controls. Similarly, C-36 peptide abolishes the effects of PMN-conditioned medium on cell invasiveness, but does not alter its effects on cell proliferation, IL-8 and VEGF release. Direct HCC cell exposure to AAT enhances VEGF, but inhibits IL-8 release by 1.7-fold ($p < 0.001$) and 1.4-fold ($p < 0.01$) respectively, and reduces proliferation 2.5-fold ($p < 0.01$). In contrast, C-36 peptide alone did not affect these parameters, but inhibited cell invasiveness by 51.4% ($p < 0.001$), when compared with non-treated controls.
Conclusions
Our data provide evidence that neutrophil derived factors decrease lung cancer HCC cell proliferation and IL-8 release, but increase cell invasiveness. These effects were found to be modulated by exogenously present serine proteinase inhibitor, AAT, and its C-terminal fragment, which points to a complexity of the relationships between tumor cell biological activities and local microenvironment.

402. A truncated form of CKβ8-1 is a potent agonist for human formyl peptide-receptor-like 1 receptor

Elagoz, A. et al
Br. J. Pharmacol., 141, 37-46 (2004)

Human formyl peptide-receptor-like-1 (FPRL-1) is a promiscuous G protein-coupled receptor (GPCR), and belongs to a chemoattractant receptor family protein. This receptor has been reported to interact with various host-derived peptides and lipids involved in inflammatory responses. We described here, a novel role for FPRL-1 as a high-affinity β-chemokine receptor for an N-terminally truncated form of the CKβ8 (CCL23/MPIF-1) splice variant CKβ8-1 (22−137 aa).
RT-PCR analysis of mRNA derived from human tissues and cells revealed a predominant expression of FPRL-1 in inflammatory cells, particularly in neutrophils.
Intracellular calcium mobilisation assay, used as screening tool, in recombinant Chinese hamster ovary (CHO-K1) and human embryonic kidney (HEK293s) cells coexpressing FPRL-1 and $G\alpha_{16}$, demonstrated FPRL-1 is a functional high-affinity receptor for CKβ8-1 (46−137 aa, sCKβ8-1), with pEC_{50} values of 9.13 and 8.85, respectively.
The FPRL-1 activation in CHO-K1 cells is mediated by $G\alpha_i/G\alpha_o$ proteins, as assessed by pertussis toxin sensitivity and inhibition of forskolin-induced cyclic AMP accumulation.

Binding experiments were performed with a radio-iodinated synthetic peptide, [^{125}I]-WKYMVm, a known potent FPRL-1 agonist. CHO-K1 cell membranes expressing FPRL-1 bound [^{125}I]-WKYMVm with a K_d value of 9.34. Many known FPRL-1 agonists were tested and sCKβ8-1 was the most effective nonsynthetic ligand in displacing the radiolabelled agonist, with a pIC_{50} of 7.97.
The functional significance of sCKβ8-1 interaction with FPRL-1 was further demonstrated by the activation of polymorphonuclear leukocytes (PMNs) calcium mobilisation and chemotaxis. These interactions were shown to be *via* FPRL-1 by specific blockade of PMNs activation in the presence of an FPRL-1 antibody.

403. Icodextrin-induced peritonitis: Study of five cases and comparison with bacterial peritonitis
Toure, F. et al
Kidney Int., 65, 654-660 (2004)

Background

An epidemic of aseptic peritonitis related to the presence of peptidoglycan contaminant in some batches of icodextrin solution (Extraneal®, Baxter Healthcare Corporation) occurred in Europe in the first six months of 2002.
Methods

By case-control study we examined the clinical and biologic features of 5 patients with icodextrin-induced peritonitis (group AP) and compared them with 7 patients with bacterial peritonitis (group BP) recruited in our clinical center between January and June 2002.
Results

Diagnosis of icodextrin-induced peritonitis was confirmed in all cases by a positive reintroduction test with contaminated batches of icodextrin. No recurrence was observed on re-exposure to icodextrin free of peptidoglycan. Skin tests were positive with contaminated icodextrin in 2 of 5 patients, while they were negative with icodextrin solution free of peptidoglycan (<0.6 ng/mL). During peritonitis, serum level of C-reactive protein (CRP) was lower in group AP (42.4 ±34 mg/L) than in group BP (135 ±59 mg/L) ($P = 0.01$). Leukocyte number in peritoneal dialysis effluent was lower in group AP (284 ±101/mm^3), with a lower neutrophil/monocyte ratio (N/M = 0.67) than in group BP (1410 ±973/mm^3; N/M = 4) ($P < 0.05$). A low number of peritoneal fluid eosinophilia (11 ±8%) was detected in group AP.
Conclusion

Icodextrin-induced peritonitis was associated with a burst of intraperitoneal cytokines. The phenotype of peritoneal neutrophils was different between aseptic and bacterial peritonitis, indicating that inflammatory stimuli that activate neutrophils in both types of peritonitis are clearly distinct. Finally, peritoneal injury measured by weight gain, peritoneal permeability, and CA125 concentration seemed to be less severe during icodextrin-induced peritonitis than during bacterial peritonitis.

404. Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility
Hacking, D. et al
Genes & Immunity, 5, 274-282 (2004)

Interleukin-8 (IL-8) has been implicated in the pathogenesis of RSV-induced bronchiolitis. Previously, we have described an association between bronchiolitis disease severity and a specific IL-8 haplotype comprising six single-nucleotide polymorphisms (SNPs) (-251A/+396G/+781T/+1238delA/+1633T/+2767T, haplotype 2). Here we investigated the functional basis for this association by measuring haplotype-specific transcription *in vivo* in human primary cells. We found a significant increase in transcript level derived from the IL-8 haplotype 2 relative to the mirror haplotype 1 (-251T/+396T/+781C/+1238insA/+1633C/+2767A) in respiratory epithelial cells but not in lymphocytes. A promoter polymorphism, -251A, present on the high producer haplotype, had no significant affect on the allele-specific level of transcription when analyzed in reporter gene experiments in human respiratory epithelial A549 cells. We proceeded to systematically screen for allele-specific protein-DNA binding in this functional haplotype, which revealed significant differential binding at the +781T/C polymorphism. C/EBPβwas identified as being part of a transcription factor binding complex that preferentially bound in the presence of the +781 T allele. These results suggest that the mechanism for disease susceptibility to RSV-induced bronchiolitis may occur through a haplotype-specific increase in IL-8 transcription, which may be mediated by functional polymorphisms within that haplotype.

405. Relocalization of Endothelial Cell ß-Catenin After Coculture With Activated Neutrophils From Patients Undergoing Cardiac Surgery With Cardiopulmonary Bypass
Scholz, M., Nowak, P. and Blaheta, R.
Med. Mycol., 43(6), 143-149 (2004)

Cardiac surgery with cardiopulmonary bypass (CPB) is associated with neutrophil activation, inflammation, and consecutive edema. The impairment of endothelial junction molecules, and thus, hyperpermeability elicited by the interaction of activated neutrophils with endothelial cells may be important in this regard. Cocultures with human endothelial cells and neutrophils from 10 cardiac surgery

patients with CPB were used to evaluate the role of neutrophils in modifications of the endothelial zonula adherens molecules VE-cadherin and β-catenin. Laser scan microscopic analyses showed that neutrophils, which were isolated after the beginning of CPB, significantly impaired intracellular redistribution of endothelial β-catenin with regard to membrane association ($p < .0002$) and staining pattern ($p < .0001$). VE-cadherin localization was not found to be significantly modified. Western blots with total cell extracts showed that amounts of β-catenin did not vary significantly after co-culture with activated neutrophils. Activated neutrophils during cardiac surgery with CPB may induce endothelial dysfunction by impairing β-catenin localization and thus contribute to endothelial hyperpermeability.

406. Lymphatic Diversion Prevents Myocardial Edema Following Mesenteric Ischemia/Reperfusion
Cox, C., Fischer, U. and Allen, S.
Microcirculation, 11(1), 1-8 (2004)

Objective:Mesenteric ischemia/reperfusion (I/R) is associated with cardiac dysfunction. Mesenteric lymph primes polymorphonuclear leukocytes (PMNs) for increased superoxide release following I/R. We hypothesized that mesenteric I/R causes myocardial edema resulting in myocardial dysfunction, and that diverting mesenteric lymph would preserve myocardial function.Methods:Two canine groups were studied: lymphatic diversion (LD) and no lymphatic diversion (No LD). Preload recruitable stroke work,±dp/dtmax, isovolumic relaxation (tau), cardiac output, and myocardial water content (MWC) were determined. I/R consisted of 60 min of ischemia followed by 180 min of reperfusion. Myocardial myeloperoxidase (MPO) was measured as an index of PMN leukosequestration. In addition, mesenteric lymph harvested after I/R was infused into normalcaninesand all variables measured.Results:MWC increased from baseline in No LD. Tau and−dp/dtmaxwere significantly affected in No LD, but not in LD. After mesenteric I/R, mesenteric lymph primed PMNs for increased superoxide production. Lymph diversion resulted in significantly lower myocardial MPO. With reinfusion of I/R lymph, MWC and tau increased. MPO was also increased post I/R mesenteric lymph reinfusion.Conclusions:Our data indicate that myocardial dysfunction after mesenteric I/R is due to lymph-induced, PMN-mediated microvascular alterations and myocardial edema.

407. Genetic deficiency of CD16, the low-affinity receptor for immunoglobulin G, has no impact on the functional capacity of polymorphonuclear neutrophils
Wagner, C. and Hänsch, G.M.
Eur. J. Clin. Invest., 34(2), 149-155 (2004)

Background Of the three receptors for immunoglobulin G (IgG), the low-affinity receptor CD16 is constitutively expressed on polymorphonuclear neutrophils (PMNs), monocytes and NK-cells. CD16 participates in various effector functions, notably phagocytosis of opsonized particles or of immune complexes, and in antibody-dependent cellular cytotoxicity (ADCC). In the present study we report a case of total CD16 deficiency on PMNs and monocytes.
Design Polymorphonuclear neutrophils, monocytes and NK-cells were analyzed for surface-receptor expression by cytofluorometry and laser scan microscopy. Moreover, CD16-specific mRNA was assessed by RT-PCR. As functional parameters, phagocytosis of opsonized bacteria was tested, as was superoxide production.
Results Polymorphonuclear neutrophils and monocytes totally deficient in CD16 were detected by chance in an apparently healthy individual. Further analysis revealed that two more members of his family, his father and sister, were also deficient in CD16. All were healthy and there was no evidence of an increased frequency, or of exceptionally severe or persistent infections. Despite the lack of CD16, phagocytosis of antibody-coated bacteria was within the normal range, as was the superoxide production.
Conclusion Deficiency of CD16 does not compromise the host defence. Apparently, the other receptors for IgG, CD32 and CD64, can compensate for the lack of CD16.

408. *Aspergillus fumigatus* antigens activate innate immune cells via toll-like receptors 2 and 4
Braedel, S. et al
Br. J. Hematol., 12583), 392-399 (2004)

Invasive aspergillosis (IA) is a leading cause of mortality in haematological patients. Appropriate activation of the innate immune system is crucial for the successful clearance of IA. Therefore, we studied the *Aspergillus fumigatus*-mediated activation of human granulocytes and monocyte-derived immature dendritic cells (DCs), as well as murine bone marrow-derived DCs (BMDCs) from wild type, toll-like receptor (TLR)4-deficient, TLR2 knockout, and TLR2/TLR4 double deficient mice. *Aspergillus fumigatus* antigens induced the activation and maturation of immature DCs as characterized by CD83 expression, upregulation of major histocompatibility complex and co-stimulatory molecules. Moreover, fungal antigens enhanced the phagocytosis and production of interleukin (IL)-8 in granulocytes. The release of IL-12 by BMDCs in response to *A. fumigatus* antigens was dependent on the expression of TLR2, whereas the release of IL-6 was dependent on the expression of functional TLR4 molecules. The protein precipitate of *A. fumigatus* supernatant provided strong stimulation of DCs and granulocytes, indicating that a factor secreted by *A. fumigatus* might activate innate immune cells. In conclusion, *A. fumigatus* antigens induced the activation of DCs and granulocytes. Our results indicated that this activation was mediated via TLR2 and TLR4. Future studies are needed to assess the clinical impact of these findings in patients at high risk for IA.

409. Proinflammatory effects of copper deficiency on neutrophils and lung endothelial cells
Lominadze, D., Saari, J.T., Percival, S.S. and Schuschke, D.A.
Immunol. Cell Biol., 82(3), 231-238 (2004)

Dietary copper deficiency increases the accumulation of circulating neutrophils in the rat lung microcirculation. This process includes neutrophil adhesion to, migration along, and emigration though the vascular endothelium. The current study was designed to examine the role of copper in each of these steps. Neutrophils were isolated from rats fed either a copper-adequate (CuA, 6.1 μg Cu/g diet) or copper-deficient diet (CuD, 0.3 μg Cu/g diet) for 4 weeks. First, transient and firm adhesion of neutrophils to P-selectin in a flow chamber showed there were more adhered CuD neutrophils than CuA ones. This effect is probably caused by the increased expression of CD11b that was observed in the current study. Second, the evaluation of neutrophil migration under agarose showed that the CuD neutrophils moved farther than the CuA group in response to IL-8 but not *f*MLP; this suggests an increased sensitivity to a CD11/CD18-independent signalling pathway. Third, the contractile mechanism of endothelial cells was studied. Elevated F-actin formation in Cu-chelated lung microvascular endothelial cells suggests that neutrophil emigration may be promoted by enhanced cytoskeletal reorganization of the endothelium during copper deficiency. Combined, these results support the theory that dietary copper deficiency has proinflammatory effects on both neutrophils and the microvascular endothelium that promote neutrophil−endothelial interactions.

410. Polymorphonuclear neutrophils in posttraumatic osteomyelitis: cells recovered from the inflamed site lack chemotactic activity but generate superoxide
Wagner, C. et al
Shock, 22(2), 108-115 (2004)

The pathogenesis of posttraumatic osteomyelitis, one of the major complications after orthopedic surgery, is not yet understood. Formation of bacterial biofilms on the implant is presumed, conferring resistance to antibiotic therapy and probably also to the host defense mechanisms. In that context, the polymorphonuclear neutrophils (PMN) having infiltrated the infected site were recovered and characterized phenotypically and functionally. Loss of CD62L and upregulation of CD14 were seen, as was expression of CD83. Expression of the latter is dependent on de novo protein synthesis and thus is indicative of an extended life span and a transdifferentiation of the PMN at the infected site. The infiltrated PMN had lost their chemotactic activity, whereas the capacity to produce superoxides was preserved and in some patients even enhanced. In vitro experiments done in parallel showed that long-term culture with interferon-[gamma] resulted in similar alterations of PMN: loss of chemotactic activity, whereas other functions of PMN, such generation of superoxides and phagocytosis of opsonized bacteria, were preserved or even enhanced. The loss of the migratory capacity of PMN having already emigrated from the blood vessel to the infected site is not expected to affect the host defense negatively. Assuming, however, that bacteria are organized as a biofilm and that infiltration into this biofilm is required for phagocytosis of the bacteria, our data could to some extent explain why despite being activated, the PMN are not able to control the infection. By releasing their cytotoxic, proteolytic, and collagenolytic potential, PMN might instead contribute to tissue destruction and eventually to osteolysis.

411. Irsogladine, an anti-ulcer drug, suppresses superoxide production by inhibiting phosphodiesterase type 4 in human neutrophils
Kyoi, T., Noda, K., Oka, M. and Ukai, Y.
Life Sciences, 76(1), 71-83 (2004)

Neutrophil superoxide production is implicated in the pathogenesis of gastric mucosal damage induced by various ulcerative agents and *Helicobacter pylori* infection. We investigated here the effects of an anti-ulcer drug irsogladine [2, 4-diamino-6-(2, 5-dichlorophenyl)-*s*-triazine maleate] on cAMP formation in isolated human neutrophils. The cAMP level in human neutrophils was elevated by a phosphodiesterase (PDE) type 4 selective inhibitor rolipram, but not by any inhibitors of PDE1, PDE2 and PDE3. Irsogladine also increased cAMP formation in a concentration-dependent manner in neutrophils. A non-selective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) alone significantly increased cAMP level, whereas irsogladine was unable to further increase cAMP level in the presence of IBMX. Irsogladine inhibited concentration-dependently the superoxide (O_2^-) production induced by various stimuli including formyl-methionyl-leucyl-phenylalanine, opsonized zymosan, guanosine 5′-[gamma-thio] triphosphate, A23187 and phorbol 12-myristate 13-acetate. These effects of irsogladine were mimicked by rolipram, IBMX and dibutyryl cAMP. The inhibitory effects of irsogladine and rolipram on the O_2^- production were reversed by a protein kinase A inhibitor H-89. These results indicate that irsogladine inhibits the superoxide production in human neutrophils by the increase of cAMP content by PDE 4 inhibition, which in turn contributing to the anti-ulcer effects of irsogladine on gastric mucosal lesions associated with oxidative stress.

412. Differential inside-out activation of β2-integrins by leukotriene B4 and fMLP in human neutrophils
Patcha, V. et al
Exp. Cell Res., 300(2), 308-319 (2004)

We have investigated how LTB_4, an endogenous chemoattractant encountered early in the inflammatory process, and fMLP, a bacteria-derived chemotactic peptide emanating from the site of infection, mediate inside-out regulation of the β_2-integrin. The role of the two chemoattractants on β_2-integrin avidity was investigated by measuring their effect on β_2-integrin clustering and surface mobility,

whereas their effect on β_2-integrin affinity was measured by the expression of a high affinity epitope, a ligand-binding domain on β_2-integrins, and by integrin binding to s-ICAM. We find that the two chemoattractants modulate the β_2-integrin differently. LTB_4 induces an increase in integrin clustering and surface mobility, but only a modest increase in integrin affinity. fMLP evokes a large increase in β_2-integrin affinity as well as in clustering and mobility. Lipoxin, which acts as a stop signal for the functions mediated by pro-inflammatory agents, was used as a tool for further examining the inside-out mechanisms. While LTB_4-induced integrin clustering and mobility were inhibited by lipoxin, only a minor inhibition of fMLP-induced β_2-integrin avidity and no inhibition of integrin affinity were detected. The different modes of the inside-out regulation of β_2-integrins suggest that distinct mechanisms are involved in the β_2-integrin modulation induced by various chemoattractants.

413. Propofol scavenges reactive oxygen species and inhibits the protein nitration induced by activated polymorphonuclear neutrophils

Thiry, J-C. et al

Eur.J. Pharmacol., 499(1-2), 29-33 (2004)

Activated polymorphonuclear neutrophils may damage tissues through the release of biochemical mediators. Among them, peroxynitrite is responsible for hydroxylation reactions and nitration of proteins, or is metabolised into nitrate. We investigated the effect of propofol on the production of reactive oxygen species, the nitration of proteins and the formation of nitrate by activated human polymorphonuclear neutrophils. Propofol dose-dependently inhibited chemiluminescence, nitration of proteins and nitrate production in a concentration range from 10^{-3} to 10^{-6} mM. A significant correlation was observed between the logarithm of propofol concentration and the intensity of chemiluminescence (r^2=0.90), the nitration of proteins (r^2=0.67) and the production of nitrate (r^2=0.79). Those results are consistent with the scavenging effect of propofol on peroxynitrite and could confer a protective property to propofol in pathological situations involving polymorphonuclear neutrophils activation.

414. Effects of uridine, isomatitol and 4-thiouridine on in vitro cell adhesion and in vivo effects of 4-thiouridine in a lung inflammation model

Uppugunduri, S. and Gautam, C.

Int. Immunopharmacol., 4(9), 1241-1248 (2004)

Since leukocyte adhesion to endothelial cells is crucial for extravasation of leukocytes to sites of inflammation, inhibition of cell–cell adhesion has been suggested as a means to achieve selective modulation of the immune system. We have, using a static in vitro adhesion assay involving adhesion of granulocytes to tumor necrosis factor alpha (TNFα)-stimulated human umbilical vein endothelial cells (HUVEC), found three substances—uridine, isomaltitol and 4-thiouridine-that, independently and significantly, reduced leukocyte adhesion by approximately 30–65%. 4-Thiouridine was also tested in an in vivo model of Sephadex (SDX)-induced lung inflammation with Sprague–Dawley rats. Intratracheal instillation of Sephadex (5 mg/kg) alone resulted in a dramatic increase in lung edema and total leukocyte count after 24 h. A differential count of bronchoalveolar lavage (BAL) cells indicated an increased influx of macrophages, eosinophils and neutrophils. Co-administration of 4-thiouridine significantly reduced lung edema by 38%. There was also a significant reduction of the total leukocyte count by 58%. The differential leukocyte count indicated that eosinophil influx alone was reduced by 70%. After Sephadex challenge, we found elevated levels of TNFα—an important inflammatory mediator—in the bronchoalveolar lavage fluid (BALF). TNFα levels were significantly reduced by more than 80% by co-administration of 4-thoiuridine. These results suggest that uridine, isomaltitol and, especially, 4-thiouridine affect adhesion between leukocytes and activated endothelium, and warrant further in vitro and in vivo studies.

415. Functional effects of NAD(P)H oxidase p22phox C242T mutation in human leukocytes and association with thrombotic cerebral infarction

Shimo-Nakanishi, Y. et al

Atherosclerosis, 175(1), 109-115 (2004)

Background: Previous study showed that polymorphism of the NAD(P)H oxidase $p22^{phox}$ gene is associated with atherosclerosis, although others could not confirm such association. We investigated the association between $p22^{phox}$ C242T polymorphism and thrombotic cerebral infarction and the role of this polymorphism on superoxide-production activity in human neutrophils and promyelocytic HL-60 cells as a model system. *Methods:* PCR-RFLP analysis revealed that genotype and allele frequencies of C242T polymorphism in 120 patients with thrombotic cerebral infarction and 177 control subjects. The superoxide-production activity in neutrophils was determined by cytochrome c reduction assay. To clarify the role of $p22^{phox}$ C242T polymorphism on NAD(P)H oxidase activity, we used transgenic HL-60 cells transfected with expression plasmids carrying $p22^{phox}$ cDNAs with or without C242T polymorphism. *Results:* Genotype and allele frequencies of C242T polymorphism in patients and control subjects were not significantly different. The superoxide-production activity in neutrophils with T allele was higher than in neutrophils without T allele. Moreover, expression analysis showed that superoxide-production activity in $p22^{phox}$ C242T-expressing HL-60 cells were significantly higher than in $p22^{phox}$-expressing HL-60 cells. *Conclusions:* We conclude that C242T of $p22^{phox}$ gene is not involved in thrombotic cerebral infarction but more likely in increased NAD(P)H oxidase activity in phagocytes.

416. Lipid–drug conjugate nanoparticles of the hydrophilic drug diminazene—cytotoxicity testing and mouse serum adsorption
Olbrich, C., Gessner, A., Schröder, W., Kayser, O. and Müller, R.H.
J. Contolled Release, 96(3), 425-435 (2004)

Sleeping sickness is a widely distributed disease in great parts of Africa. It is caused by *Trypanosoma brucei gambiense* and *rhodiense*, transmitted by the Tse–Tse fly. After a hemolymphatic stage, the parasites enter the central nervous system where they cannot be reached by hydrophilic drugs. To potentially deliver the hydrophilic antitrypanosomal drug diminazene diaceturate to the brain of infected mice, the drug was formulated as lipid–drug conjugate (LDC) nanoparticles (NP) by combination with stearic- (SA) and oleic acid (OA). To estimate the in vivo compatibility, the particles were incubated with human granulocytes. Because as potential delivery mechanism the absorption of specific serum proteins (ApoE, Apo AI and Apo AIV) was found to be responsible for the delivery of nanoparticles to the brain, demonstrated using PBCA nanoparticles coated with polysorbate 80 (LDL uptake mechanism) the nanoparticles were incubated with mouse serum and the adsorption pattern was determined using the 2-D PAGE technique. As a result of this study, the cytotoxic potential was shown to decrease when diminazene is part of the particle matrix compared to pure fatty acid nanoparticles and the mouse serum protein adsorption pattern differs from the samples studied earlier in human serum. Especially, the fact concerning Apo-E that could be detected when the particles were incubated in human serum is absent after the mouse serum incubation, potentially, is a critical point for the delivery via the LDL-uptake mechanism but the data demonstrate that LDC nanoparticles, with 33% (wt/wt) drug loading capacity possess the potential to act as a delivery system for hydrophilic drugs like diminazene diaceturate and that further studies have to demonstrate the usability as a brain delivery system.

417. Hg2+ and small-sized polyethylene glycols have inverse effects on membrane permeability, while both impair neutrophil cell motility
Loitto, V-M. and Magnusson, K-E.
Biochem. Biophys. Res. Comm., 316(2), 370-378 (2004)

effects after exposure to mercury are well documented in human. Little is, however, known about how Hg^{2+} affect host defense in general and neutrophil functions in particular. We show here that exposure of human neutrophils to $HgCl_2$ dose-dependently impairs chemoattractant-stimulated motility. Long-term exposure (5–10 min) to Hg^{2+} yields a rapid influx of extracellular Ca^{2+} followed by leakage of cytosolic fluorophores, as assessed using fura-2 and ratio imaging microscopy. The inhibition on motility was partly reversible, since pre-treated neutrophils placed in an Hg^{2+}-free environment displayed higher migration rates. The Hg^{2+}-induced fluxes were prevented by addition of small-sized polyethylene glycols (PEG 200–400), which also dose-dependently inhibited neutrophil transmigration. Localized, minute micropipette additions of Hg^{2+} or PEG caused retraction of the leading edge and redirection of cell migration. Since Hg^{2+} increases and PEGs decrease membrane permeability in a partially competitive manner, we suggest that the known aquaporin-inhibitor Hg^{2+} alters membrane permeability by affecting the bidirectional flux through the leukocyte aquaporin-9 (AQP9) while small-sized PEGs yield decreased membrane permeability by becoming trapped in the promiscuous channel. The local additions of Hg^{2+} or PEG probably force other cell regions to take over from those with blocked AQPs. Hence, the cells turn direction of motility away from the micromanipulator needle.

418. Divergent effects of α1-antitrypsin on neutrophil activation, in vitro
Janciauskiene, S., Zelvyte, I., Jansson, L. and Stevens, T.
Biochem. Biophys. Res. Comm., 315(2), 288-296 (2004)

α1-Antitrypsin (AAT) is a major circulating serine proteinase inhibitor in humans. The anti-proteinase activity of AAT is inhibited by chemical modification. These include inter- or intramolecular polymerisation, oxidation, complex formation with target proteinases (e.g., neutrophil elastase), and/or cleavage by multi-specific proteinases. In vivo, several modified forms of AAT have been identified which stimulate biological activity in vitro unrelated to inhibition of serine proteinases. In this study we have examined the effects of native and polymerised AAT and C-36 peptide, a proteolytic cleavage product of AAT, on human neutrophil activation, in vitro. We show that the C-36 peptide displays striking concentration-dependent pro-inflammatory effects on human neutrophils, including induction of neutrophil chemotaxis, adhesion, degranulation, and superoxide generation. In contrast to C-36 peptide, native and polymerised AAT at similar and higher concentrations showed no effects on neutrophil activation. These results suggest that cleavage of AAT may not only abolish its proteinase inhibitor activity, but can also generate a powerful pro-inflammatory activator for human neutrophils.

419. Common trafficking pathway for variant antigens destined for the surface of the Plasmodium falciparum-infected erythrocyte
Haeggström, M. et al
Mol. Biochem. Parasitol., 133(1), 1-14 (2004)

Intraerythrocytic *Plasmodium falciparum* exports proteins to the cytosol and to the plasma membrane of the host cell. We here present data revealing the existence of a unique common pathway for the surface bound traffic of the clonally variant antigens, repeated-interspersed-antigen (RIFINS) and *P. falciparum* erythrocyte-membrane-protein-1 (PfEMP1). RIFIN- and PfEMP1-specific antibodies were found to stain single small vesicles (SSV) that bud off from the parasitophorus vacuolar membrane (PMV) at 6–10 h post-

invasion. Large multimeric vesicle (LMV) assemblies, composed of subunits each of a similar size to that of a SSV, appeared as the dominant vesicle type carrying the variant antigens in the cytosol as the parasites developed into early trophozoite stages (≥16 h post-invasion). Later, more than 24 h post-invasion, large spinle-like vesicles (LSLV) built up as the LMV approached and accumulated underneath the erythrocyte membrane. LMV were found to associate both with the Maurer's cleft antigen Pf332 and with lipids as seen by fluorescent BODIPY-Ceramide staining. Co-traffic of Pf332 with RIFINS and PfEMP1 occurred in sub-compartmentalized LMV, as the variant antigens co-localized at the outer rim while Pf332 occupied the core of the vesicle complex. Formation of LMV for the trafficking of RIFINS and PfEMP1 is a prominent feature of freshly isolated *P. falciparum* and of in vitro propagated K^+ as well as K^- parasites, seemingly independent of the knob-associated histidine-rich protein (KAHRP). In vitro cultured 3D7 clones lack LMV formation and traffic the variant antigens in vesicles of a similar size to that of the SSV.

420. The effect of TNF-α, PMA, and LPS on plasma and cell-associated IL-8 in human leukocytes
Lund, T. and Østerud, B.
Thromb. Res., 113(1), 75-83 (2004)

Introduction/aim: This study was performed to examine the proficiency of mononuclear cells (MNC) and polymorphonuclear cells (PMN) in a whole blood model to expressing interleukin-8 (IL-8) in response to various stimuli. *Methods*: Isolated cells that had been recombined with heparinized plasma were incubated with lipopolysaccharide (LPS), phorbol myristate acetate (PMA) and tumor necrosis factor (TNF)-α. *Results*: IL-8 release by MNC was most potently induced by LPS, reaching significant levels after 2-h incubation in the presence of 0.2 ng/ml LPS. In contrast, 5.0 ng/ml LPS was required for PMN to release significant amounts of the cytokine (P<0.001). When PMN and MNC were coincubated (MNC/PMN), LPS-induced IL-8 release was reduced compared to the release from MNC alone, regardless of the concentration of LPS used. IL-8 release by PMN was much more strongly induced by TNF-α, increasing by 1050% in the presence of 10 ng/ml TNF-α (P<0.005), whereas MNC or MNC/PMN subjected to this stimulus alone did not significantly enhance their IL-8 release. PMA had no effect on IL-8 release from either cell type. Since a high portion of IL-8 in blood is associated with cells, the IL-8 levels in isolated and lysed cell suspensions were also quantified. Thus, a considerably higher level of IL-8 was found in freshly isolated PMN (0.58±0.09 ng/ml) than in MNC (0.010±0.007 ng/ml). PMN remained the main source for cell-associated IL-8 after 2-h incubation in the absence of any added stimuli, harbouring a relatively high level of the cytokine (3.37±1.38 ng/ml), which was significantly enhanced in the presence of TNF-α (8.99±1.46 ng/ml, P<0.001). *Conclusion*: This study shows that LPS is an effective inducer of IL-8 in MNC, whereas TNF-α is a potent agonist for IL-8 release from PMN. The main portion of cell-associated IL-8 is present in PMN when the cells are stimulated in their normal environment of plasma.

421. Neutrophils are stimulated by syncytiotrophoblast microvillous membranes to generate superoxide radicals in women with preeclampsia
Aly, A.S. et al
Am. J. Obstet. Gynecol., 190(1), 252-258 (2004)

Objective
This study was undertaken to determine whether syncytiotrophoblast microvillous membranes (STBMs) stimulate maternal neutrophils to produce superoxide radicals in women with preeclampsia.
Study design
Serum levels of tissue polypeptide antigen (TPA), which is a marker for STBM, were measured in 25 nulliparous women (10 with mild preeclampsia, 6 with severe preeclampsia, and 9 controls). Superoxide production by maternal neutrophils from cases and controls and by donor neutrophils cocultured with the STBMs from cases and controls was measured spectrophotometrically by reduction of ferricytochrome C.
Results
Maternal TPA levels were significantly greater among cases than controls (P = .005). Superoxide production by maternal neutrophils and donor neutrophils cultured with STBM from cases of preeclampsia was greater than controls (P values .006 and .019, respectively), and dose-response relationships were observed. Superoxide production by maternal leukocytes was correlated with superoxide induction by STBMs in culture (P = .007).
Conclusion
STBMs in maternal blood induce neutrophils to generate superoxide radicals that may cause endothelial dysfunction in women with preeclampsia.

422. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-κB in mononuclear cells
Dhindsa, S. et al
Metabolism, 53(3), 330-334 (2004)

It has previously been shown that oral intake of 300 calories of glucose (75 g), lipid, or protein increases reactive oxygen species (ROS) generation by polymorphonuclear cells (PMNL) and mononuclear cells (MNCs). We investigated the effects of 75 g glucose on proinflammatory transcription factor, nuclear factor-κB (NFκB), in mononuclear cells. To further investigate whether the effects of

macronutrient-induced oxidative stress are due to consumption of calories or are nutrient specific, we investigated the effects of acute oral challenge of equicaloric amounts of alcohol (300 calories) on ROS generation and NF-κB activation in MNCs and PMNL and compared them with those of glucose and water (control). Sixteen normal healthy adult volunteers were given either vodka (10 subjects), glucose solution (10 subjects), or 300 mL water (7 subjects). Vodka and glucose drinks were equivalent to 300 calories. We measured ROS generation and intranuclear NF-κB activation by PMNL cells and MNCs at 1 hour, 2 hours, and 3 hours following ingestion. ROS generation by both MNC and PMNL increased significantly ($P < .05$ for MNC and $P < .01$ for PMNL) following intake of glucose solution, but did not change significantly following alcohol or water. NF-κB binding activity in MNC nuclear extracts also increased ($P < .001$) following ingestion of glucose solution, but did not change after the administration of alcohol or water. We conclude that (1) 75 g oral glucose increases NF-κB binding activity in MNCs. (2) While 75 g glucose (300 calories) induces an increase in ROS generation and intranuclear NF-κB, equicaloric amounts of alcohol did not produce these effects.

423. Shear-Dependent Capping of L-Selectin and P-Selectin Glycoprotein Ligand 1 by E-Selectin Signals Activation of High-Avidity β_2-Integrin on Neutrophils

Green, C.E., Pearson, D.N., Camphausen, R.T., Staunton, D.E. and Simon, S.I.
J. Immunol., 172, 7780-7790 (2004)

Two adhesive events critical to efficient recruitment of neutrophils at vascular sites of inflammation are up-regulation of endothelial selectins that bind sialyl Lewis^x ligands and activation of β_2-integrins that support neutrophil arrest by binding ICAM-1. We have previously reported that neutrophils rolling on E-selectin are sufficient for signaling cell arrest through β_2-integrin binding of ICAM-1 in a process dependent upon ligation of L-selectin and P-selectin glycoprotein ligand 1 (PSGL-1). Unresolved are the spatial and temporal events that occur as E-selectin binds to human neutrophils and dynamically signals the transition from neutrophil rolling to arrest. Here we show that binding of E-selectin to sialyl Lewis^x on L-selectin and PSGL-1 drives their colocalization into membrane caps at the trailing edge of neutrophils rolling on HUVECs and on an L-cell monolayer coexpressing E-selectin and ICAM-1. Likewise, binding of recombinant E-selectin to **PMN**s in suspension also elicited coclustering of L-selectin and PSGL-1 that was signaled via mitogen-activated protein kinase. Binding of recombinant E-selectin signaled activation of β_2-integrin to high-avidity clusters and elicited efficient neutrophil capture of β_2-integrin ligands in shear flow. Inhibition of p38 and p42/44 mitogen-activated protein kinase blocked the cocapping of L-selectin and PSGL-1 and the subsequent clustering of high-affinity β_2-integrin. Taken together, the data suggest that E-selectin is unique among selectins in its capacity for clustering sialylated ligands and transducing signals leading to neutrophil arrest in shear flow.

424. Chemotaxis and Calcium Responses of Phagocytes to Formyl Peptide Receptor Ligands Is Differentially Regulated by Cyclic ADP Ribose

Partida-Sanches, S. et al
J. Immunol., 172, 1896-1906 (2004)

Cyclic ADP ribose (cADPR) is a calcium-mobilizing metabolite that regulates intracellular calcium release and extracellular calcium influx. Although the role of cADPR in modulating calcium mobilization has been extensively examined, its potential role in regulating immunologic responses is less well understood. We previously reported that cADPR, produced by the ADP-ribosyl cyclase, CD38, controls calcium influx and chemotaxis of murine neutrophils responding to fMLF, a peptide agonist for two chemoattractant receptor subtypes, formyl peptide receptor and formyl peptide receptor-like 1. In this study, we examine whether cADPR is required for chemotaxis of human monocytes and neutrophils to a diverse array of chemoattractants. We found that a cADPR antagonist and a CD38 substrate analogue inhibited the chemotaxis of human phagocytic cells to a number of formyl peptide receptor-like 1-specific ligands but had no effect on the chemotactic response of these cells to ligands selective for formyl peptide receptor. In addition, we show that the cADPR antagonist blocks the chemotaxis of human monocytes to CXCR4, CCR1, and CCR5 ligands. In all cases, we found that cADPR modulates intracellular free calcium levels in cells activated by chemokines that induce extracellular calcium influx in the apparent absence of significant intracellular calcium release. Thus, cADPR regulates calcium signaling of a discrete subset of chemoattractant receptors expressed by human leukocytes. Since many of the chemoattractant receptors regulated by cADPR bind to ligands that are associated with clinical pathology, cADPR and CD38 represent novel drug targets with potential application in chronic inflammatory and neurodegenerative disease.

425. Staphylococcus aureus, but not Staphylococcus epidermidis, modulates the oxidative response and induces apoptosis in human neutrophils

Nilsdottir-Augustinsson, A. et al
*APMIS, **112(2)**, 109-118 (2004)*

S. epidermidis is the most common isolate in foreign body infections. The aim of this study was to understand why S. epidermidis causes silent biomaterial infections. In view of the divergent inflammatory responses S. epidermidis and S. aureus cause in patients, we analyzed how they differ when interacting with human neutrophils. Neutrophils interacting with S. epidermidis strains isolated either from granulation tissue covering infected hip prostheses or from normal skin flora were tested by measuring the oxidative response as

chemiluminescence and apoptosis as annexin V binding. Different S. aureus strains were tested in parallel. All S. epidermidis tested were unable to modulate the oxidative reaction in response to formyl-methionyl-leucyl-phenylalanine (fMLP) and did not provoke, but rather inhibited, apoptosis. In contrast, some S. aureus strains enhanced the oxidative reaction, and this priming capacity was linked to p38-mitogen-activated-protein-kinase (p38-MAPK) activation and induction of apoptosis. Our results may explain why S. epidermidis is a weak inducer of inflammation compared to S. aureus, and therefore responsible for the indolent and chronic course of S. epidermidis biomaterial infections.

426. Autoantibodies to neutrophil cytoplasmic antigens (ANCA) do not bind to polymorphonuclear neutrophils in blood
Abdel-Salam, B., et al
*Kidney Int., **66(3)**, 1009-1017 (2004)*

Background: Autoantibodies to neutrophil cytoplasmic antigens (ANCA), particularly to proteinase 3 (PR3), are found in the majority of patients with systemic Wegener's granulomatosis. The autoantibodies are widely used as diagnostic markers. Their role in the development and progression of the disease, however, is still under investigation. The primary target of ANCA, PR3, is located in the cytoplasm of polymorphonuclear neutrophils (PMN) or monocytes and is translocated to the cell surface upon stimulation. In patients with Wegener's granulomatosis PR3 is up-regulated most prominently during active disease. Despite the fact that both autoantibodies to PR3 and PMN expressing PR3 are present in patients with Wegener's granulomatosis, there is no evidence for binding of the autoantibodies to PMN. The present study was designed to analyze binding characteristics of autoantibodies to PR3 on PMN.
Methods and Results: PMN of patients with active Wegener's granulomatosis (N = 10) were tested for autoantibody binding. Despite high autoantibody titer and PR3 expression on the PMN, no surface-bound IgG was found on PMN ex vivo. When ANCA-containing plasma from patients was incubated with isolated PMN, stimulated to express PR3, again no specific binding of the autoantibody could be detected. Also keeping the samples on ice did not allow surface detection of IgG, ruling out degradation or internalization of the autoantibodies. Only when purified IgG fractions were used, binding to PMN was seen in 14 of 25 patients. Already 1% of plasma, however, was sufficient to greatly reduce the IgG binding. Reduced binding of the IgG fraction was also seen when a larger reaction volume was used.
Conclusion: Our data indicate that autoantibodies to PR3 have a rather low affinity for surface-associated PR3 on PMN. This, in turn, argues against the hypothesis that ANCA contributes to the pathogenesis of the disease by stimulating viable PMN in whole blood.

427. Real-Time quantitative PCR analysis of factor XI mRNA variants in human platelets
Podmore, A., Smith, M., Savidge, G. and Alhaq, A.
*J. Thrombosis and Hemostasis, **2(10)**, 1713-1719 (2004)*

Summary. Coagulation factor XI (FXI) plays an essential role in blood coagulation. A deficiency of FXI is an unusual hemorrhagic diathesis in that the bleeding tendency can be highly variable, ranging from severe deficiencies with no symptoms to mild and moderate deficiencies requiring multiple blood transfusions for hemorrhages. This variability in bleeding has been attributed to a number of factors including the presence of a novel form of FXI associated with platelets, which ameliorates the bleeding in some cases of FXI deficiency. However, the nature of this platelet FXI molecule is controversial. Hsu *et al*. (*J Biol Chem* 1998; **273**: 13787–93) suggest that it is a product of normal FXI – but lacking exon V whilst Martincic *et al*. (*Blood* 1999; **94**: 3397–404) were unable to detect this alternatively spliced variant using RT-PCR. In order to resolve this controversy, we have employed the highly sensitive technique of real-time quantitative RT-PCR using RNA isolated from FXI-deficient patients. Our results indicate that the platelets of both normal and FXI deficient individuals contain FXI mRNA that is identical to the mRNA found in liver. An exon V deleted splice variant was not detected. Thus the FXI message is not alternatively spliced in platelets and therefore would not be able to produce an unusual FXI protein.

428. Cardiac Surgery With Extracorporeal Circulation: Neutrophil Transendothelial Migration Is Mediated by β_1 Integrin (CD29) in the Presence of TNF-Alpha
Scholz, M. et al
*J.Invest. Surg., **17(5)**, 239-247 (2004)*

Cardiac surgery with extracorporeal circulation is associated with neutrophil activation, inflammation, and edema. Endothelial hyperpermeability elicited by the interaction of activated neutrophils and/or cytokines with endothelial cells may be critical in this regard. However, the immune and cellular mechanisms involved are not fully understood. Cocultures with human endothelial cells and neutrophils from cardiac surgery patients were used to evaluate the role of β1 integrin activity and the proinflammatory cytokine tumor necrosis factor (TNF)-α in neutrophil transendothelial migration and in impairment of the integrity of endothelial cell-to-cell contacts. Blocking of CD29 (heavy chain of β1 integrins) totally prevented neutrophil adhesion and transendothelial migration. Pretreatment of neutrophils with either a CD29-stimulating monoclonal antibody or the addition of TNF-α (0.1-10 U/ml) to the coculture failed to induce transendothelial migration. However, coculture of endothelial cells with CD29-stimulated neutrophils in the presence of 0.1-10 U/ml TNF-α strongly induced neutrophil transmigration. CD29/TNF-α-mediated transmigration was associated with intracellular redistribution of endothelial β-catenin. We further showed that CD29/TNF-α-mediated effects involved PI3K and tyrosine kinase-

dependent signaling via MAPK but were independent of nuclear transcription factor (NF)-κB activity. Inhibition of CD29/TNF-α might be a therapeutic option to limit endothelial dysfunction following cardiac surgery with extracorporeal circulation.

429. Fibronectin on activated T lymphocytes is bound to gangliosides and is present in detergent insoluble microdomains
Blum, S., Hug, F., Hänsch, G.M. and Wagner, C.
Immunol. Cell Biol., 83(2), 167-174 (2005)

Fibronectin (FN) is a multifunctional extracellular matrix glycoprotein, which participates in cell migration and signalling to adhering cells. Due to alternative splicing and post-translational modifications, different isoforms of FN are generated by a wide variety of cells. T lymphocytes synthesize a surface-associated isoform of FN, containing the two 'extradomains' EDA and EDB. In the present study, we identified gangliosides within the T-cell membrane as specific binding sites for the N-terminal 30 kDa fragment of FN. When T cells were activated with anti-CD3 coated beads, FN, together with the ganglioside GM1, converged at the contact zone. Moreover, endogenous FN was present in the detergent insoluble microdomain. The function of FN in T cells is still under investigation; however, its presence together with gangliosides at the activation site suggests participation in T-cell signalling.

430. Role of L-Ficolin/Mannose-Binding Lectin-Associated Serine Protease Complexes in the Opsonophagocytosis of Type III Group B Streptococci
Aoyagi, Y. et al
J. Immunol., 174, 418-425 (2005)

Serotype III group B streptococci (GBS) are a common cause of neonatal sepsis and meningitis. Although deficiency in maternal capsular polysaccharide (CPS)-specific IgG correlates with susceptibility of neonates to the GBS infection, serum deficient in CPS-specific IgG mediates significant opsonophagocytosis. This IgG-independent opsonophagocytosis requires activation of the complement pathway, a process requiring the presence of both Ca^{2+} and Mg^{2+}, and is significantly reduced by chelating Ca^{2+} with EGTA. In these studies, we defined a role of L-ficolin/mannose-binding lectin-associated serine protease (MASP) complexes in Ca^{2+}-dependent, Ab-independent opsonophagocytosis of serotype III GBS. Incubation of GBS with affinity-purified L-ficolin/MASP complexes and C1q-depleted serum deficient in CPS-specific Ab supported opsonophagocytic killing, and this killing was inhibited by fluid-phase *N*-acetylglucosamine, the ligand for L-ficolin. Binding of L-ficolin was proportional to the CPS content of individual strains, and opsonophagocytic killing and C4 activation were inhibited by fluid-phase CPS, suggesting that L-ficolin binds to CPS. Sialic acid is known to inhibit alternative complement pathway activation, and, as expected, the bactericidal index (percentage of bacteria killed) for individual strains was inversely proportional to the sialic acid content of the CPS, and L-ficolin-initiated opsonophagocytic killing was significantly increased by addition of CPS-specific IgG2, which increased activation of the alternative pathway. We conclude that binding of L-ficolin/MASP complexes to the CPS generates C3 convertase C4b2a, which deposits C3b on GBS. C3b deposited by this lectin pathway forms alternative pathway C3 convertase C3bBb whose activity is enhanced by CPS-specific IgG2, leading to increased opsonophagocytic killing by further deposition of C3b on the GBS.

431. Protease-activated Receptor-1 Activation of Endothelial Cells Induces Protein Kinase Cα-dependent Phosphorylation of Syntaxin 4 and Munc18c: ROLE IN SIGNALING P-SELECTIN EXPRESSION
Anjaparavanda, J.F., Naren, P., Gao, X., Ahmmed, G.U. and Malik, a.B.
J. Biol. Chem., 280, 3178-3184 (2005)

Endothelial cells exhibit regulated exocytosis in response to inflammatory mediators such as thrombin and histamine. The exocytosis of Weibel-Palade bodies (WPBs) containing von Willebrand factor, P-selectin, and interleukin-8 within minutes after stimulation is important for vascular homeostasis. SNARE proteins are key components of the exocytic machinery in neurons and some secretory cells, but their role in regulating exocytosis in endothelial cells is not well understood. We examined the function of SNARE proteins in mediating exocytosis of WPBs in endothelial cells. We identified the presence of syntaxin 4, syntaxin 3, and the high affinity syntaxin 4-regulatory protein Munc18c in human lung microvascular endothelial cells. Small interfering RNA-induced knockdown of syntaxin 4 (but not of syntaxin 3) inhibited exocytosis of WPBs as detected by the reduction in thrombin-induced cell surface P-selectin expression. Thrombin ligation of protease-activated receptor-1 activated the phosphorylation of syntaxin 4 and Munc18c, which, in turn, disrupted the interaction between syntaxin 4 and Munc18. Protein kinase Cα activation was required for the phosphorylation of syntaxin 4 and Munc18c as well as the cell surface expression of P-selectin. We also observed that syntaxin 4 knockdown inhibited the adhesion of neutrophils to thrombin-activated endothelial cells, demonstrating the functional role of syntaxin 4 in promoting endothelial adhesivity. Thus, protease-activated receptor-1-induced protein kinase Cα activation and phosphorylation of syntaxin 4 and Munc18c are required for the cell surface expression of P-selectin and the consequent binding of neutrophils to endothelial cells.

432. Distinct Signaling Pathways Are Involved in Leukosialin (CD43) Down-regulation, Membrane Blebbing, and Phospholipid Scrambling during Neutrophil Apoptosis

Nusbaum, P. et al

J. Biol. Chem., 280, 5843-5853 (2005)

Although leukosialin (CD43) membrane expression decreases during neutrophil apoptosis, the CD43 molecule, unexpectedly, is neither proteolyzed nor internalized. We thus wondered whether it could be shed on bleb-derived membrane vesicles. Membrane blebbing is a transient event, hardly appreciated during the asynchronous, spontaneous apoptosis of neutrophils. Cell pre-synchronization at 15 °C made it possible to observe numerous blebbing neutrophils for a short 1-h period at 37 °C. CD43 down-regulation co-occurred with the blebbing stage and phosphatidylserine externalization, shortly after mitochondria depolarization and before nuclear condensation. Blebs detaching from the cell body were observed by time lapse fluorescence microscopy, and the release of bleb-derived vesicles was followed by flow cytometry. Phosphatidylserine externalization required caspases and protein kinase C (PKC) but not the myosin light chain kinase (MLCK). By contrast, bleb formation and release was caspase- and PKC-independent but required an active MLCK, whereas CD43 down-regulation involved caspases but neither PKC nor MLCK. Furthermore, CD43 appeared mostly excluded from membrane blebs by electron microscopy. Thus, CD43 down-regulation does not result from the release of bleb-derived vesicles. Ultracentrifugation of apoptotic cell supernatants made it possible to recover <1 μm microparticles, which contained the entire CD43 molecule. These microparticles expressed neutrophil membrane markers such as CD11b, CD66b, and CD63, together with CD43. In conclusion, we show that the three early membrane events of apoptosis, namely blebbing, phosphatidylserine externalization, and CD43 down-regulation, result from different signaling pathways and can occur independently from one another. CD43 down-regulation results from the shedding of microparticles released during apoptosis but unrelated to the blebbing.

433. Heterogeneity of functional responses in differentiated myeloid cell lines reveals EPRO cells as a valid model of murine neutrophil functional activation

Gaines, P., Chi, J. and Berliner, N.

J. Leukoc. Biol., 77, 669-679 (2005)

Mature neutrophils display multiple functional responses upon activation that include chemotaxis, adhesion to and transmigration across endothelial cells, phagocytosis, and pathogen destruction via potent microbicidal enzymes and reactive oxygen species. We are using myeloid cell line models to investigate the signaling pathways that govern neutrophil functional activation. To facilitate these studies, we have performed a direct comparison of functional responses of human and murine myeloid cell line models upon neutrophil differentiation. Our results show that EPRO cells, promyelocytes that undergo complete neutrophil maturation, demonstrate a full spectrum of functional responses, including respiratory burst, chemotaxis toward two murine chemokines, and phagocytosis. We also extend previous studies of granulocyte-colony stimulating factor-induced 32Dcl3 cells, showing they domonstrate chemotaxis and phogocytosis but completely lack a respiratory burst as a result of the absent expression of a critical oxidase subunit, $gp91^{phox}$. Induced human leukemic NB4 and HL-60 cells display a respiratory burst and phagocytosis but have defective chemotaxis to multiple chemoattractants. We also tested each cell line for the ability to up-regulate cell-surface membrane-activated complex-1 (Mac-1) expression upon activation, a response mediating neutrophil adhesion and a surrogate marker for degranulation. We show that EPRO cells, but not 32Dcl3 or NB4, significantly increase Mac-1 surface expression upon functional activation. Together, these data show that EPRO and MPRO cells demonstrate complete, functional activation upon neutrophil differentiation, suggesting these promyelocytic models accurately reflect the functional capacity of mature murine neutrophils.

434. Expression of PrPC on cellular components of sheep blood

Halliday, S., Houston, F. and Hunter, N.

J. Gen. Virol., 86, 1571-1579 (2005)

PrP^C, a glycosylphosphatidylinositol-linked glycoprotein, plays a central role in the pathogenesis of transmissible spongiform encephalopathies (TSEs), undergoing a conformational alteration to the disease-associated isoform, commonly designated PrP^{Sc}. PrP^C is expressed in many tissues other than the nervous system, although its precise function(s) remains unclear. It has previously been demonstrated that TSEs can be transmitted by blood transfusion in sheep. The aim of this work was to identify which components of blood carried the infection. As an initial step, the distribution of PrP^C on cellular components of sheep blood was examined to identify potential targets for infection. Cell-surface expression of PrP^C was found only on peripheral blood mononuclear cells (PBMCs); however, platelets also contained significant amounts of intracellular PrP^C. The level of PrP^C expressed on the cell surface of PBMCs was influenced by PrP genotype, with the highest levels found in scrapie-susceptible VRQ/VRQ sheep and the lowest levels in scrapie-resistant ARR/ARR sheep. In susceptible sheep, PrP^C was expressed at varying levels on all major subsets of PBMCs, with the highest levels on the $CD21^+$ subset of B cells, and PrP expression was upregulated dramatically on $CD21^+$ B cells in some scrapie-infected sheep.

435. Neutrophil-Derived Heparin-Binding Protein (HBP/CAP37) Deposited on Endothelium Enhances Monocyte Arrest under Flow Conditions

Soehnlein, O. et al

Am. J. Respir. Cell Mol. Biol., 32, 553-559 (2005)

In acute inflammation, infiltration of neutrophils often precedes a second phase of monocyte invasion, and data in the literature suggest that neutrophils may directly stimulate mobilization of monocytes via neutrophil granule proteins. In this study, we present a role for neutrophil-derived heparin-binding protein (HBP) in monocyte arrest on endothelium. Adhesion of neutrophils to bovine aorta endothelial cells (ECs) or HUVEC-triggered secretion of HBP and binding of the protein to the EC surface. Blockade of neutrophil adhesion by treatment with a mAb to CD18 greatly reduced accumulation of HBP. In a flow chamber model, immobilized recombinant HBP induced arrest of human monocytes or monocytic Mono Mac 6 (MM6) cells to activated EC or plates coated with recombinant adhesion molecules (E-selectin, P-selectin, VCAM-1). However, immobilized recombinant HBP did not influence arrest of neutrophils or lymphocytes. Treatment of MM6 cells with recombinant HBP evoked a rapid and clear-cut increase in cytosolic free Ca^{2+} that was found to be critical for the HBP-induced monocyte arrest inasmuch as pretreatment with the intracellular calcium chelating agent BAPTA-AM abolished the evoked increase in adhesion. Thus, secretion of a neutrophil granule protein, accumulating on the EC surface and promoting arrest of monocytes, could contribute to the recruitment of monocytes at inflammatory loci.

436. Respiratory Syncytial Virus Infection of Human Lung Endothelial Cells Enhances Selectively Intercellular Adhesion Molecule-1 Expression

Arnhold, R. and König, W.

J. Immunol., 174, 7359-7367 (2005)

Respiratory syncytial virus (RSV) is worldwide the most frequent cause of bronchiolitis and pneumonia in infants requiring hospitalization. In the present study, we supply evidence that human lung microvascular endothelial cells, human pulmonary lung aorta endothelial cells, and HUVEC are target cells for productive RSV infection. All three RSV-infected endothelial cell types showed an enhanced cell surface expression of ICAM-1 (CD54), which increased in a time- and RSV-dose-dependent manner. By using noninfectious RSV particles we verified that replication of RSV is a prerequisite for the increase of ICAM-1 cell surface expression. The up-regulated ICAM-1 expression pattern correlated with an increased cellular ICAM-1 mRNA amount. In contrast to ICAM-1, a de novo expression of VCAM-1 (CD106) was only observed on RSV-infected HUVEC. Neither P-selectin (CD62P) nor E-selectin (CD62E) was up-regulated by RSV on human endothelial cells. Additional experiments performed with neutralizing Abs specific for IL-1α, IL-1β, IL-6, and TNF-α, respectively, excluded an autocrine mechanism responsible for the observed ICAM-1 up-regulation. The virus-induced ICAM-1 up-regulation was dependent on protein kinase C and A, PI3K, and p38 MAPK activity. Adhesion experiments using polymorphonuclear neutrophil granulocytes (PMN) verified an increased ICAM-1-dependent adhesion rate of PMN cocultured with RSV-infected endothelial cells. Furthermore, the increased adhesiveness resulted in an enhanced transmigration rate of PMN. Our in vitro data suggest that human lung endothelial cells are target cells for RSV infection and that ICAM-1 up-regulated on RSV-infected endothelial cells might contribute to the enhanced accumulation of PMN into the bronchoalveolar space.

437. Competition between Elastase and Related Proteases from Human Neutrophil for Binding to α1-Protease Inhibitor

Korkmaz, B. et al

Am. J. Respir. Cell Mol. Biol., 32, 553-559 (2005)

The protease–antiprotease imbalance that is characteristic of most inflammatory lung disorders depends on the spatial-temporal regulation of active inhibitor and protease concentrations in lung secretions. We have studied the competition between the three main serine proteases from human neutrophil primary granules in their binding to α1-Pi, the main serine proteases inhibitor in lung secretions. Elastase was the only target of α1-Pi when identical molar amounts of purified inhibitor and the three proteases were tested together. The other two proteases were only inhibited once elastase was saturated. Elastase remained the preferred target of inhibitors when bronchoalveolar lavage fluids from patients with lung pneumonia and acute respiratory distress syndrome were used as the source of inhibitors, in spite of the presence of additional inhibitors in lung secretions. Since neutrophil proteases are expressed at the neutrophil surface, we also measured residual activities of membrane-bound proteases after purified neutrophils were incubated with bronchoalveolar fluids. Again, elastase was the preferred target of the inhibitors. We conclude that protease 3 and cathepsin G are not controlled as efficiently as elastase in lung secretions, a feature that must be taken into account when developing inhibitor-based anti-inflammatory therapies.

438. TNF-α promotes a stop signal that inhibits neutrophil polarization and migration via a p38 MAPK pathway

Lokuta, M.A. and Huttenlocher, A.

J. Leukoc. Biol., 78, 210-219 (2005)

Neutrophils are a major component of the inflammatory response in patients with asthma and other inflammatory conditions. Proinflammatory cytokines, such as tumor necrosis factor α(TNF-α), are increased in the airway of patients with severe asthma and have been implicated in the recruitment of neutrophils into areas of inflammation. Here, we show that TNF-α induces a stop signal that

promotes firm neutrophil adhesion and inhibits neutrophil polarization and chemotaxis to chemoattractants including interleukin-8 and C5a. TNF-α treatment of neutrophils plated on a fibrinogen-coated surface promotes firm neutrophil adhesion and the formation of vinculin-containing focal complexes. TNF-α induces a more than tenfold increase in p38 mitogen-activated protein kinase (MAPK) but not extracellular signal-regulated kinase phosphorylation. Inhibition of p38 MAPK in neutrophils treated with TNF-α causes neutrophil polarization and motility. These findings suggest that TNF-α initiates a stop signal through a p38 MAPK pathway, which may promote the retention of neutrophils in inflammatory sites. Together, our data suggest that inhibition of p38 MAPK may be an attractive target to limit inflammatory responses that are mediated by TNF-α.

439. Antimicrobial and Chemoattractant Activity, Lipopolysaccharide Neutralization, Cytotoxicity, and Inhibition by Serum of Analogs of Human Cathelicidin LL-37

Ciornei, C.D., Sigurdardottir, T., Schmidtchen, A. and Bodelsson, M.
Antimicrob. Agents Chemother., 49, 2845-2850 (2005)

Antimicrobial peptides have been evaluated in vitro and in vivo as alternatives to conventional antibiotics. Apart from being antimicrobial, the native human cathelicidin-derived peptide LL-37 (amino acids [aa] 104 to 140 of the human cathelicidin antimicrobial peptide) also binds and neutralizes bacterial lipopolysaccharide (LPS) and might therefore have beneficial effects in the treatment of septic shock. However, clinical trials have been hampered by indications of toxic effects of LL-37 on mammalian cells and evidence that its antimicrobial effects are inhibited by serum. For the present study, LL-37 was compared to two less hydrophobic fragments obtained by N-terminal truncation, named 106 (aa 106 to 140) and 110 (aa 110 to 140), and to a previously described more hydrophobic variant, the 18-mer LLKKK, concerning antimicrobial properties, lipopolysaccharide neutralization, toxicity against human erythrocytes and cultured vascular smooth muscle cells, chemotactic activity, and inhibition by serum. LL-37, fragments 106 and 110, and the 18-mer LLKKK inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* in a radial diffusion assay, inhibited lipopolysaccharide-induced vascular nitric oxide production, and attracted neutrophil granulocytes similarly. While fragments 106 and 110 caused less hemolysis and DNA fragmentation in cultured cells than did LL-37, the 18-mer LLKKK induced severe hemolysis. The antibacterial effect of fragments 106 and 110 was not affected by serum, while the effect of LL-37 was reduced. We concluded that the removal of N-terminal hydrophobic amino acids from LL-37 decreases its cytotoxicity as well as its inhibition by serum without negatively affecting its antimicrobial or LPS-neutralizing action. Such LL-37-derived peptides may thus be beneficial for the treatment of patients with sepsis.

440. Calcium-sensing soluble adenylyl cyclase mediates TNF signal transduction in human neutrophils

Han, H. et al
J. Exp. Med., 202, 353-361 (2005)

Through chemical screening, we identified a pyrazolone that reversibly blocked the activation of phagocyte oxidase (phox) in human neutrophils in response to tumor necrosis factor (TNF) or formylated peptide. The pyrazolone spared activation of phox by phorbol ester or bacteria, bacterial killing, TNF-induced granule exocytosis and phox assembly, and endothelial transmigration. We traced the pyrazolone's mechanism of action to inhibition of TNF-induced intracellular Ca^{2+} elevations, and identified a nontransmembrane ("soluble") adenylyl cyclase (sAC) in neutrophils as a Ca^{2+}-sensing source of cAMP. A sAC inhibitor mimicked the pyrazolone's effect on phox. Both compounds blocked TNF-induced activation of Rap1A, a phox-associated guanosine triphosphatase that is regulated by cAMP. Thus, TNF turns on phox through a Ca^{2+}-triggered, sAC-dependent process that may involve activation of Rap1A. This pathway may offer opportunities to suppress oxidative damage during inflammation without blocking antimicrobial function.

441. Impaired Deformability of Copper-Deficient Neutrophils

Gordon, S.A., Lominadze, D., Saari, J.T., Lentsch, A.B. and Schuschke, D.A.
Exp. Biol. and Med., 230, 543-548 (2005)

We have previously shown that dietary copper deficiency augments neutrophil accumulation in the lung microvasculature. The current study was designed to determine whether a diet deficient in copper promotes neutrophil chemoattraction within the lung vasculature or if it alters the mechanical properties of the neutrophil, thus restricting passage through the microvessels. Sprague-Dawley rats were fed purified diets that were either copper adequate (6.3 μg Cu/g diet) or copper deficient (0.3 μg Cu/g diet) for 4 weeks. To assess neutrophil chemoattraction, bronchoalveolar lavage fluid was assayed for the neutrophil chemokine macrophage inflammatory protein-2 (MIP-2) by enzyme-linked immunosorbent assay. Neutrophil deformability was determined by measuring the pressure required to pass isolated neutrophils through a 5-μm polycarbonate filter. The MIP-2 concentration was not significantly different between the dietary groups (Cu adequate, 435.4 ± 11.9 pg/ml; Cu deficient, 425.6 ± 14.8 pg/ml). However, compared with controls, more pressure was needed to push Cu-deficient neutrophils through the filter (Cu adequate, 0.150 ± 0.032 mm Hg/sec; Cu deficient, 0.284 ± 0.037 mm Hg/sec). Staining of the filamentous actin (F-actin) with FITC-Phalloidin showed greater F-actin polymerization and shape change in the Cu-deficient group. These results suggest that dietary copper deficiency reduces the deformability of neutrophils by promoting F-actin polymerization. Because most neutrophils must deform during passage from arterioles to venules in the lungs, we propose that copper-deficient neutrophils accumulate in the lung because they are less deformable.

442. Inhibition of Neutrophil Elastase by α1-Protease Inhibitor at the Surface of Human Polymorphonuclear Neutrophils
Korkmaz, B., Attucci, S., Jourdan, M-L., Juliano, L. and Gauthier, F.
J. Immunol., 175, 3329-3338 (2005)

The uncontrolled proteolytic activity in lung secretions during lung inflammatory diseases might be due to the resistance of membrane-bound proteases to inhibition. We have used a new fluorogenic neutrophil elastase substrate to measure the activity of free and membrane-bound human neutrophil elastase (HNE) in the presence of α1-protease inhibitor (α1-Pi), the main physiological inhibitor of neutrophil serine proteases in lung secretions. Fixed and unfixed neutrophils bore the same amounts of active HNE at their surface. However, the HNE bound to the surface of unfixed neutrophils was fully inhibited by stoichiometric amounts of α1-Pi, unlike that of fixed neutrophils. The rate of inhibition of HNE bound to the surface of unfixed neutrophils was the same as that of free HNE. In the presence of α1-Pi, membrane-bound elastase is almost entirely removed from the unfixed neutrophil membrane to form soluble irreversible complexes. This was confirmed by flow cytometry using an anti-HNE mAb. HNE activity rapidly reappeared at the surface of HNE-depleted cells when they were triggered with the calcium ionophore A23187, and this activity was fully inhibited by stoichiometric amounts of α1-Pi. HNE was not released from the cell surface by oxidized, inactive α1-Pi, showing that active inhibitor is required to interact with active protease from the cell surface. We conclude that HNE activity at the surface of human neutrophils is fully controlled by α1-Pi when the cells are in suspension. Pericellular proteolysis could be limited to zones of contact between neutrophils and subjacent protease substrates where natural inhibitors cannot penetrate.

443. Neutrophil Adherence to Bladder Microvascular Endothelial Cells following Platelet-Activating Factor Acetylhydrolase Inhibition
Vinson, S.M., Rickard, A., Ryerse, J.S. and McHowat, J.
J. Pharmacol. Exp. Ther., 314, 1241-1247 (2005)

Interstitial cystitis (IC) is an inflammatory bladder condition of unknown etiology. Tryptase released from elevated numbers of activated mast cells is a proposed mediator of the inflammatory process in IC. We have previously shown that tryptase increases human bladder microvascular endothelial cell (HBMEC) calcium-independent phospholipase A_2 ($iPLA_2$) activity, resulting in the production of multiple biologically active phospholipid metabolites, including platelet-activating factor (PAF), that can mediate inflammation. Because the design of selective PLA_2 inhibitors may provide a useful therapeutic strategy to reduce the inflammatory process in IC, we tested several frequently used PLA_2 inhibitors on PAF production in tryptase-stimulated HBMEC. Among the inhibitors tested, methyl arachidonyl fluorophosphonate (MAFP) was found to be a potent inhibitor of PAF-acetylhydrolase activity. Pretreatment of HBMEC with MAFP significantly increased PAF production in both unstimulated and tryptase-stimulated cells. In addition, MAFP pretreatment of tryptase-stimulated HBMEC increased both surface expression of P-selectin and polymorphonuclear leukocyte adherence to the HBMEC monolayer. These effects suggest that MAFP has a proinflammatory effect, irrespective of its ability to inhibit PLA_2.

444. Transdifferentiation of polymorphonuclear neutrophils to dendritic-like cells at the site of inflammation in rheumatoid arthritis: evidence for activation by T cells
Iking-Konert, C. Et al
Ann. Rheum. Dis., 64, 1436-1442 (2005)

Objectives: To investigate infiltrated cells in the synovial fluid (SF) of inflamed joints of patients with rheumatoid arthritis (RA), with special reference to polymorphonuclear neutrophils (PMN) and their interaction with T cells.
Methods: Expression on PMN of activation associated receptors CD14, CD64, CD83, and major histocompatibility complex (MHC) class II was examined in the SF of 15 patients with RA, as were the infiltrated T cells. SF cytokines were determined by enzyme linked immunosorbent assay (ELISA). To mimic the in vivo situation, co-culture experiments were carried out using PMN and T cells of healthy donors.
Results: The SF contained activated T lymphocytes and abundant PMN. SF PMN expression of CD14 and CD64 was enhanced compared with peripheral blood. Of special interest was the observation that only the SF PMN expressed MHC class II antigens and CD83. Exposure to SF, which contained considerable amounts of cytokines (for example, interferon γ(IFNγ), tumour necrosis factor α, and interleukin 2), induced a similar receptor pattern on blood derived PMN of healthy donors. Furthermore, PMN acquired MHC class II and CD83 within 24 to 48 hours, when co-cultured with autologous T cells or T cell lines. This effect was also achieved by T cell supernatants, was dependent on protein synthesis, and could be inhibited by antibodies against IFNγ.
Conclusions: SF PMN from patients with RA undergo major alterations, including transdifferentiation to cells with dendritic-like characteristics, probably induced by T cell derived cytokines. Because MHC class II positive PMN are known to activate T cells, the mutual activation of PMN and T cells might contribute to the perpetuation of the local inflammatory process, and eventually to the destructive process in RA.

445. D-Alanylation of Teichoic Acids Promotes Group A Streptococcus Antimicrobial Peptide Resistance, Neutrophil Survival, and Epithelial Cell Invasion
Kristian, S.A. et al
J. Bacteriol., 187, 6719-6725 (2005)

Group A streptococcus (GAS) is a leading cause of severe, invasive human infections, including necrotizing fasciitis and toxic shock syndrome. An important element of the mammalian innate defense system against invasive bacterial infections such as GAS is the production of antimicrobial peptides (AMPs) such as cathelicidins. In this study, we identify a specific GAS phenotype that confers resistance to host AMPs. Allelic replacement of the *dltA* gene encoding D-alanine-D-alanyl carrier protein ligase in an invasive serotype M1 GAS isolate led to loss of teichoic acid D-alanylation and an increase in net negative charge on the bacterial surface. Compared to the wild-type (WT) parent strain, the GAS Δ*dltA* mutant exhibited increased susceptibility to AMP and lysozyme killing and to acidic pH. While phagocytic uptake of WT and Δ*dltA* mutants by human neutrophils was equivalent, neutrophil-mediated killing of the Δ*dltA* strain was greatly accelerated. Furthermore, we observed the Δ*dltA* mutant to be diminished in its ability to adhere to and invade cultured human pharyngeal epithelial cells, a likely proximal step in the pathogenesis of invasive infection. Thus, teichoic acid D-alanylation may contribute in multiple ways to the propensity of invasive GAS to bypass mucosal defenses and produce systemic infection.

446. Domaln 5 of High Molecular Weight Kininogen Is Antibacterial
Andersson Nordahl, E., Rydengård, V., Mörgelin, M. And Schmidtchen, A.
J. Biol. Chem., 280, 34832-34839 (2005)

Antimicrobial peptides are important effectors of the innate immune system. These peptides belong to a multifunctional group of molecules that apart from their antibacterial activities also interact with mammalian cells and glycosaminoglycans and control chemotaxis, apoptosis, and angiogenesis. Here we demonstrate a novel antimicrobial activity of the heparin-binding and cell-binding domain 5 of high molecular weight kininogen. Antimicrobial epitopes of domain 5 were characterized by analysis of overlapping peptides. A peptide, HKH20 (His^{479}-His^{498}), efficiently killed the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the Gram-positive *Enterococcus faecalis*. Fluorescence microscopy and electron microscopy demonstrated that HKH20 binds to and induces breaks in bacterial membranes. Furthermore, no discernible hemolysis or membrane-permeabilizing effects on eukaryotic cells were noted. Proteolytic degradation of high molecular weight kininogen by neutrophil-derived proteases as well as the metalloproteinase elastase from *P. aeruginosa* yielded fragments comprising HKH20 epitopes, indicating that kininogen-derived antibacterial peptides are released during proteolysis.

447. Effects of Anaplasma phagocytophilum on Host Cell Ferritin mRNA and Protein Levels
Carlyon, J.A., Ryan, D., Archer, K. and Fikrig, E.
Infect. Immun., 73, 7629-7636 (2005)

Ferritin is a major intracellular iron storage protein and also functions as a cytoprotectant by sequestering iron to minimize the formation of reactive oxygen species. *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, is an obligate intracellular bacterium that colonizes neutrophils. We have previously reported that human promyelocytic HL-60 cells infected with *A. phagocytophilum* demonstrate increased transcription of ferritin heavy chain and also that the bacterium stimulates neutrophil NADPH oxidase assembly and degranulation during the initial hours of infection (J. A. Carlyon, W. T. Chan, J. Galan, D. Roos, and E. Fikrig, J. Immunol. **169:**7009-7018, 2002, and J. A. Carlyon, D. Abdel-Latif, M. Pypaert, P. Lacy, and E. Fikrig, Infect. Immun. 72:4772-4783, 2004). In this study, we assessed ferritin mRNA and protein levels during *A. phagocytophilum* infection in vitro using HL-60 cells and neutrophils and in vivo using neutrophils from infected mice. The addition of *A. phagocytophilum*, as well as *Escherichia coli* and serum-opsonized zymosan, to neutrophils results in a pronounced increase in ferritin light-chain transcription and a concomitant rise in ferritin protein levels. Neutrophils from *A. phagocytophilum*-infected mice demonstrate elevated ferritin heavy-chain mRNA expression, a phenomenon consistent with infections by intracellular pathogens. Notably, ferritin protein levels of infected HL-60 cells were markedly diminished in a dose- and time-dependent manner. These studies provide insight into the effects *A. phagocytophilum* has on the ferritin levels of its host cell.

448. Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils
Genstier, A-L. et al
J. Clin. Invest., 115, 3117-3127 (2005)

Panton-Valentine leukocidin (PVL) is a pore-forming toxin secreted by *Staphylococcus aureus* that has recently been associated with necrotizing pneumonia. In the present study, we report that in vitro, PVL induces polymorphonuclear cell death by necrosis or by apoptosis, depending on the PVL concentration. PVL-induced apoptosis was associated with a rapid disruption of mitochondrial homeostasis and activation of caspase-9 and caspase-3, suggesting that PVL-induced apoptosis is preferentially mediated by the mitochondrial pathway. Polymorphonuclear cell exposure to PVL leads to mitochondrial localization of the toxin, whereas Bax, 1 of the 2 essential proapoptotic members of the Bcl-2 family, was still localized in the cytosol. Addition of PVL to isolated mitochondria induced the release of the apoptogenic proteins cytochrome *c* and Smac/DIABLO. Therefore, we suggest that PVL, which belongs to the pore-forming toxin family, could act at the mitochondrion level by creating pores in the mitochondrial outer membrane. Furthermore, LukS-PV, 1 of the 2 components of PVL, was detected in lung sections of patients with necrotizing pneumonia together with DNA

fragmentation, suggesting that PVL induces apoptosis in vivo and thereby is directly involved in the pathophysiology of necrotizing pneumonia.

449. Functional Activity of Granulocytes Primed In Vivo with Glycosylated Granulocyte Colony-Stimulating Factor (G-CSF) Is Superior To Priming with Non-Glycosylated G-CSF.
Ribeiro, D. et al
Blood, 106, 3865 (2005)

Recombinant granulocyte colony-stimulating factor (G-CSF) is widely used in the treatment of chemotherapy-induced neutropenia as well as in mobilization of peripheral blood stem cells in context with autologous bone marrow transplantation. Beside recombinant non-glycosylated G-CSF expressed in E.coli (Filgrastim) and glycosylated G-CSF expressed in CHO-cells (Lenograstim), pegylated filgrastim (Pegfilgrastim) has been introduced for single-administration into clinical use. Previous studies suggest a different functional activity of glycosylated vs. non-glycosylated G-CSF. Here we study the effects of these different G-CSF on chemotaxis, oxidative burst and antigen expression of granulocytes including and correlated the results with G-CSF serum levels.
Granulocytes were obtained from 27 patients with hematological malignancies before and after the administration of one of the three G-CSFs (10 Lenograstim, 9 Filgrastim, 8 Pegfilgrastim) and isolated using a **polymorphprep** density gradient. Chemotactic properties were assessed using a Boyden chamber assay in combination with an under agarose assay, both using fMLP as chemotactic stimulus. Release of superoxide anions served as measure of the oxidative burst after stimulation with PMA using a chemiluminescence assay. The viability and surface antigen expression were assessed by FACS. In addition G-CSF serum levels were determined by ELISA. Patients receiving Filgrastim showed a significantly impaired chemotaxis contrary to patients receiving Lenograstim ($p<0.05$), in the Pegfilgrastim group patients showed a strong, yet not significant reduction when compared to Lenograstim. No significant effects could be shown in production of superoxide anions in a chemiluminescence assay.
In all three groups, FACS analysis showed a decrease in expression of CD10, CD11b, CD18 and CD62L and a significant increase of the LPS-receptor CD14 as well as an increased expression of the IgG receptor FcγRI (CD64) following G-CSF treatment. The increase of CD64 in the Pegfilgrastim group was significantly stronger compared to the other groups. Moreover, we observed a significantly stronger increase of CD14 in patients receiving Lenograstim when compared to the Filgrastim group. We did not observe significant differences in G-CSF serum levels between the three groups, the highest mean serum concentrations were found in the Pegfilgrastim group.
Our data argue for an improved functionality of granulocytes primed with glycosylated G-CSF.

450. Potential role for mast cell tryptase in recruitment of inflammatory cells to endothelium
Myer, M.C., Creer, M.H. and McHowat, J.
Am. J. Physiol. Cell Physiol., 289, C1485-C1491 (2005)

Recent research suggests that activation of protease-activated receptors (PARs) on the surface of endothelial and epithelial cells may play a role in general mechanisms of inflammation. We hypothesized that mast cell tryptase activation of endothelial cell PAR-2 is coupled to increased calcium-independent PLA_2 ($iPLA_2$) activity and increased platelet-activating factor (PAF) production that may play a role in inflammatory cell recruitment at sites of vascular injury. Stimulation of human coronary artery endothelial cells (HCAEC) with 20 ng/ml tryptase increased $iPLA_2$ activity, arachidonic acid release, and PAF production. These tryptase-stimulated responses were inhibited by pretreatment with the $iPLA_2$-selective inhibitor bromoenol lactone (BEL; 5 μM, 10 min). Similar patterns of increased $iPLA_2$ activity and PAF production were also seen when HCAEC were treated with SLIGKV, which represents the tethered ligand sequence for the human PAR-2 once the receptor is cleaved by tryptase. Tryptase stimulation also increased cell surface expression of P-selectin, decreased electrical resistance, and increased neutrophil adherence to the endothelial cell monolayer. The tryptase-stimulated increases in both cell surface P-selectin expression and neutrophil adhesion were also inhibited with BEL pretreatment. We conclude that tryptase stimulation of HCAEC contributes importantly to early inflammatory events after vascular injury by activation of $iPLA_2$, leading to arachidonic acid release, PAF production, cell surface P-selectin expression, and increased neutrophil adherence.

451. Lysophosphatidic acid triggers calcium entry through a non-store-operated pathway in human neutrophils
Itagaki, K., Kannan, K.B. and Hauser, C.J.
J. Leukoc. Biol., 77, 181-189 (2005)

Lysophosphatidic acid (LPA) is a bioactive lipid, which is structurally similar to sphingosine 1-phosphate (S1P) and which can mobilize Ca^{2+} in multiple cell types. We recently showed that S1P induces Ca^{2+} entry directly through store-operated Ca^{2+} entry (SOCE) channels in human polymorphonuclear neutrophils (**PMN**) [1]. We therefore examined the mechanisms by which LPA induces intracellular Ca^{2+} mobilization in **PMN**. External application of low micromolar LPA caused dose-dependent Ca^{2+} influx without releasing Ca^{2+} stores, whereas G-protein-coupled (GPC) LPA receptors respond to nanomolar LPA. Additive Ca^{2+} influx by LPA compared with 100 nM ionomycin-induced Ca^{2+} influx suggests that LPA-induced Ca^{2+} influx does not pass through SOCE channels. Ca^{2+} influx was resistant to inhibition of $G_{i/o}$ by pertussis toxin, of phospholipase C by U73122, and of $G_{12/13}$/*Rho* by Y27632, all demonstrating GPC receptor independence. This Ca^{2+} influx was inhibited by Gd^{3+}, La^{3+}, Zn^{2+}, or MRS1845 but not by Ni^{2+} or the sphingosine kinase inhibitor dimethylsphingosine. In addition, we found that LPA has no effect on neutrophil chemotaxis; however, it has stimulatory effects on

neutrophil respiratory burst in a dose-response manner. These findings suggest that LPA-induced Ca^{2+} influx in **PMN** occurs through a mechanism other than SOCE channels, independent of Ca^{2+} store-depletion and S1P synthesis, and that the characteristics of LPA-induced Ca^{2+} influx are similar to those of S1P-induced influx in terms of sensitivity to inorganic inhibitors. Unlike S1P, LPA has stimulatory effects on neutrophil respiratory burst.

452. Functional Implication of the Hydrolysis of Platelet Endothelial Cell Adhesion Molecule 1 (CD31) by Gingipains of Porphyromonas gingivalis for the Pathology of Periodontal Disease
Yun, P.L.W., Decarlo, A.A., Chapple, C.C. and Hunter, N.
Infect. Immun., 73, 1386-1398 (2005)

Periodontitis is a response of highly vascularized tissues to the adjacent microflora of dental plaque. Progressive disease has been related to consortia of anaerobic bacteria, with the gram-negative organism *Porphyromonas gingivalis* particularly implicated. The gingipains, comprising a group of cysteine proteinases and associated hemagglutinin domains, are major virulence determinants of this organism. As vascular expression of leukocyte adhesion molecules is a critical determinant of tissue response to microbial challenge, the objective of this study was to determine the capacity of gingipains to modulate the expression and function of these receptors. Given the potential multifunctional role of platelet endothelial cell adhesion molecule 1 (PECAM-1) in the vasculature, the effect of gingipains on PECAM-1 expression by endothelial cells was examined. Activated gingipains preferentially down-regulated PECAM-1 expression on endothelial cells compared with vascular cell adhesion molecule 1 and endothelial-leukocyte adhesion molecule 1, but the reduction in PECAM-1 expression was completely inhibited in the presence of the cysteine proteinase inhibitor TLCK (*N*α-*p*-tosyl-L-lysine chloromethyl ketone). Endothelial monolayers treated with activated gingipains demonstrated progressive intercellular gap formation that correlated with reduced intercellular junctional PECAM-1 expression as determined by Western blotting and immunofluorescence microscopy. This was accompanied by enhanced transfer of both albumin and neutrophils across the monolayer. The results suggest that degradation of PECAM-1 by gingipains contributes to increased vascular permeability and neutrophil flux at disease sites.

453. Free Fatty Acid-Induced Insulin Resistance in the Obese Is Not Prevented by Rosiglitazone Treatment
Dhindsa, S. et al
J. Clin. Endocrinol. Metab., 90, 5058-5063 (2005)

Objective: Elevation of free fatty acids (FFAs) by the infusion of triglyceride-heparin emulsion infusion (TG-Hep) causes insulin resistance (IR). We examined the effect of insulin sensitizer (rosiglitazone) on FFA-induced IR.
Design: Nine obese subjects underwent a 6-h infusion of TG-Hep before and after 6 wk of rosiglitazone (8 mg/d) treatment. Hyperinsulinemic euglycemic clamps were performed during 0–2 and 4–6 h of TG-Hep.
Results: After rosiglitazone for 6 wk, fasting FFA concentration fell, but not significantly (489 ± 63 at 0 wk; 397 ± 58 μmol/liter at 6 wk; $P = 0.16$), whereas C-reactive protein (4.26 ± 0.95 at 0 wk; 2.03 ± 0.45 μg/ml at 6 wk) and serum amyloid A (17.36 ± 4.63 at 0 wk; 8.77 ± 1.63 μg/ml at 6 wk) decreased significantly. At 0 wk, TG-Hep infusion caused a decrease in glucose infusion rate (GIR) from 4.49 ± 0.95 mg/kg·min to 3.02 ± 0.59 mg/kg·min ($P = 0.018$). Rosiglitazone treatment resulted in an increase in baseline GIR to 6.29 ± 0.81 mg/kg·min ($P = 0.03$ *vs*. 0 wk), which decreased to 4.52 ± 0.53 mg/kg·min ($P = 0.001$) after 6 h of TG-Hep infusion. The decrease in GIR induced by TG-Hep infusion was similar before and after rosiglitazone therapy [1.47 ± 0.50 *vs*. 1.77 0.3 mg/kg·min (28.9 ± 6.5 *vs*. 26.4 ± 3.7%); $P = 0.51$]. The rise in FFAs and triglycerides after TG-Hep infusion was significantly lower at 6 wk ($P = 0.006$ for FFAs; $P = 0.024$ for triglycerides).
Conclusions: We conclude that rosiglitazone: 1) causes a significant increase in GIR; 2) induces a decrease in inflammatory mediators, C-reactive protein, and serum amyloid A; 3) decreases the rise in FFAs and triglycerides after TG-Hep infusion; and 4) does not prevent FFA-induced IR.

454. E. coli virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/lysis and lung injury in a rat pneumonia model
Russo, T.A. et al
Am. J. Lung Cell Mol. Physiol., 289, L207-L216 (2005)

Enteric gram-negative bacilli, such as *Escherichia coli* are the most common cause of nosocomial pneumonia. In this study a wild-type extraintestinal pathogenic strain of *E. coli* (ExPEC)(CP9) and isogenic derivatives deficient in hemolysin (Hly) and cytotoxic necrotizing factor (CNF) were assessed in vitro and in a rat model of gram-negative pneumonia to test the hypothesis that these virulence factors induce neutrophil apoptosis and/or necrosis/lysis. As ascertained by in vitro caspase-3/7 and LDH activities and neutrophil morphology, Hly mediated neutrophil apoptosis at lower *E. coli* titers ($1 \times 10^{5-6}$ cfu) and necrosis/lysis at higher titers ($\geq 1 \times 10^{7}$ cfu). Data suggest that CNF promotes apoptosis but not necrosis or lysis. We also demonstrate that annexin V/7-amino-actinomycin D staining was an unreliable assessment of apoptosis using live *E. coli*. The use of caspase-3/7 and LDH activities and neutrophil morphology supported the notion that necrosis, not apoptosis, was the primary mechanism by which neutrophils were affected in our in vivo gram-negative pneumonia model using live *E. coli*. In addition, in vivo studies demonstrated that Hly mediates lung injury. Neutrophil necrosis was not observed when animals were challenged with purified lipopolysaccharide, demonstrating the importance of using live bacteria. These findings establish that Hly contributes to ExPEC virulence by mediating neutrophil toxicity, with

necrosis/lysis being the dominant effect of Hly on neutrophils in vivo and by lung injury. Whether Hly-mediated lung injury is due to neutrophil necrosis, a direct effect of Hly, or both is unclear.

455. Methionine Sulfoxide and Proteolytic Cleavage Contribute to the Inactivation of Cathepsin G by Hypochlorous Acid: AN OXIDATIVE MECHANISM FOR REGULATION OF SERINE PROTEINASES BY MYELOPEROXIDASE
Shao, B., Belaaouaj, A., Verlinde, C.L.M.J., Fu, X. and Heinecke, J.W.
J. Biol. Chem., 280, 29311-29321 (2005)

Using myeloperoxidase and hydrogen peroxide, activated neutrophils produce high local concentrations of hypochlorous acid (HOCl). They also secrete cathepsin G, a serine protease implicated in cytokine release, receptor activation, and degradation of tissue proteins. Isolated cathepsin G was inactivated by HOCl but not by hydrogen peroxide *in vitro*. We found that activated neutrophils lost cathepsin G activity by a pathway requiring myeloperoxidase, suggesting that oxidants generated by myeloperoxidase might regulate cathepsin G activity *in vivo*. Tandem mass spectrometric analysis of oxidized cathepsin G revealed that loss of a peptide containing Asp^{108}, which lies in the active site, associated quantitatively with loss of enzymatic activity. Catalytic domain peptides containing Asp^{108} were lost from the oxidized protein in concert with the conversion of Met^{110} to the sulfoxide. Release of this peptide was blocked by pretreating cathepsin G with phenylmethylsulfonyl fluoride, strongly implying that oxidation introduced proteolytic cleavage sites into cathepsin G. Model system studies demonstrated that methionine oxidation can direct the regiospecific proteolysis of peptides by cathepsin G. Thus, oxidation of Met^{110} may contribute to cathepsin G inactivation by at least two distinct mechanisms. One involves direct oxidation of the thioether residue adjacent to the aspartic acid in the catalytic domain. The other involves the generation of new sites that are susceptible to proteolysis by cathepsin G. These observations raise the possibility that oxidants derived from neutrophils restrain pericellular proteolysis by inactivating cathepsin G. They also suggest that methionine oxidation could render cathepsin G susceptible to autolytic cleavage. Myeloperoxidase may thus play a previously unsuspected role in regulating tissue injury by serine proteases during inflammation.

456. Prolastin, a pharmaceutical preparation of purified human α1-antitrypsin, blocks endotoxin-mediated cytokine release
Nita, I., Hollander, C., Westin, U. and Janciauskiene, S.M.
Resp. Res., 6(12) (2005)

Background

α1-antitrypsin (AAT) serves primarily as an inhibitor of the elastin degrading proteases, neutrophil elastase and proteinase 3. There is ample clinical evidence that inherited severe AAT deficiency predisposes to chronic obstructive pulmonary disease. Augmentation therapy for AAT deficiency has been available for many years, but to date no sufficient data exist to demonstrate its efficacy. There is increasing evidence that AAT is able to exert effects other than protease inhibition. We investigated whether Prolastin, a preparation of purified pooled human AAT used for augmentation therapy, exhibits anti-bacterial effects.

Methods

Human monocytes and neutrophils were isolated from buffy coats or whole peripheral blood by the Ficoll-Hypaque procedure. Cells were stimulated with lipopolysaccharide (LPS) or zymosan, either alone or in combination with Prolastin, native AAT or polymerised AAT for 18 h, and analysed to determine the release of TNFα, IL-1β and IL-8. At 2-week intervals, seven subjects were submitted to a nasal challenge with sterile saline, LPS (25 μg) and LPS-Prolastin combination. The concentration of IL-8 was analysed in nasal lavages performed before, and 2, 6 and 24 h after the challenge.

Results

In vitro, Prolastin showed a concentration-dependent (0.5 to 16 mg/ml) inhibition of endotoxin-stimulated TNFα and IL-1β release from monocytes and IL-8 release from neutrophils. At 8 and 16 mg/ml the inhibitory effects of Prolastin appeared to be maximal for neutrophil IL-8 release (5.3-fold, $p < 0.001$ compared to zymosan treated cells) and monocyte TNFα and IL-1β release (10.7- and 7.3-fold, $p < 0.001$, respectively, compared to LPS treated cells). Furthermore, Prolastin (2.5 mg per nostril) significantly inhibited nasal IL-8 release in response to pure LPS challenge.

Conclusion

Our data demonstrate for the first time that Prolastin inhibits bacterial endotoxin-induced pro-inflammatory responses *in vitro* and *in vivo*, and provide scientific bases to explore new Prolastin-based therapies for individuals with inherited AAT deficiency, but also for other clinical conditions.

457. Candida albicans-infected oral epithelial cells augment the anti-fungal activity of human neutrophils in vitro
Dongari-Bagtzoglou, A., Cuntha Villar, C. and Kashleva, H.
Med. Mycol., 43(6), 545-549 (2005)

Oropharyngeal candidiasis (OPC) is the most common opportunistic infection in immunosuppressed patients. In OPC, *Candida albicans* persists intraepithelially triggering inflammatory events, without generally causing invasive infection. Since neutrophils play an important role in preventing invasive infection and since they establish contact with the microorganisms only within the epithelial cell layer, we examined the ability of *Candida*-infected oral epithelial cells to augment neutrophil-mediated hyphal damage *in vitro*. We found that challenge of neutrophils with hyphal organisms in the presence of *C. albicans*-infected oral epithelial cell supernatants

resulted in a significantly greater suppression of hyphal cell metabolic activity compared to basal neutrophil anti-fungal function. Anti-hyphal activity in response to these supernatants was partly inhibited by neutralizing anti-IL-1α antibody and IL-1 receptor antagonist. Control supernatants from uninfected oral epithelial cells, as well as *C. albicans* conditioned-medium had a much less pronounced effect on neutrophil anti-fungal activity, which was not inhibited by these cytokine antagonists. We conclude that oral epithelial cells can act as activators of neutrophil anti-hyphal function, an effect that can be partly attributed to the generation of immunomodulatory cytokines during the interaction of oral mucosal cells with the pathogen.

458. A Quantitative Nitroblue Tetrazolium Assay for Determining Intracellular Superoxide Anion Production in Phagocytic Cells
Choi, H.S., Kim, J.W. and Cha, Y.N.
J. Immunoassay and Immunochem., 27(1), 31-44 (2005)

Conventionally, a semi□quantitative microscopic nitroblue tetrazolium (NBT) assay is used to determine the production of superoxide anion (O_2^-) in various phagocytic cells. This microscopic assay is conducted by counting the cells containing blue NBT formazan deposits, which are formed by reduction of the membrane permeable, water□soluble, yellow□colored, nitroblue tetrazolium (Y□NBT) by O_2^-. However, this assay is semi□quantitative and is prone to observer bias. In the present study, we modified the NBT assay by dissolving the blue formazan particles using 2 M potassium hydroxide and dimethylsulfoxide and then measured its absorbance using a microplate reader at 620 nm. The absorbance of dissolved NBT increased in proportion to cell number (r=0.9907), incubation time, and stimulus concentration. To test the usefulness of this modified assay, we compared the abilities of a number of types of phagocytic cells to produce O_2^-. The cells examined included murine macrophage cell lines (RAW 264.7 and J774), freshly prepared murine peritoneal macrophages and neutrophils, a human myeloid cell line (PLB□985), and freshly prepared human peripheral blood neutrophils. In addition, we demonstrate that nitric oxide produced by RAW 264.7 cells does not interfere with the modified colorimetric NBT assay. Taken together, our results indicate that the modified colorimetric NBT assay is simple, sensitive, and quantitative, and that it can be used to determine the amounts of intracellular O_2^- produced by phagocytic cells. Thus, this assay is sensitive enough to measure, quantitatively, even the small amounts of O_2^- produced in monocytes and macrophages that are not detectable by the conventional microscopic NBT assay.

459. Adherence of *Staphylococcus epidermidis* to extracellular matrix proteins and effects of fibrinogen-bound bacteria on oxidase activity and apoptosis in neutrophils
Nilsdottir-Augustinsson, Å, Claesson, C., Lindgren, P-E., Lundquist-Gustavsson, H. and Ôhman, L.
APMIS, 113(5), 361-373 (2005)

Staphylococcus epidermidis often causes foreign-body infections such as those associated with hip prostheses, but the underlying pathogenic mechanisms are not fully understood. We performed spectrophotometry to study the ability of *S. epidermidis* to bind to immobilised fibrinogen, fibronectin, vitronectin, and collagen. The strains were isolated from infected hip prostheses or from normal flora and the well-known protein-binding strain *Staphylococcus aureus* Cowan was used as positive control. We also analysed the interaction between neutrophils and a fibrinogen-bound prosthesis-derived strain of *S. epidermidis*by measuring chemiluminescence to determine the neutrophil oxidative response and binding of annexin V to indicate neutrophil apoptosis. We found that binding of *S. epidermidis* to extracellular matrix proteins varied under different growth conditions, and that prosthesis isolates adhered better to vitronectin than did strains from normal flora. The oxidative response caused by fibrinogen-bound *S. epidermidis* was not above the background level, which was in marked contrast to the distinct response induced by fibrinogen-associated *S. aureus* Cowan. Furthermore, fibrinogen-adhering *S. epidermidis* retarded neutrophil apoptosis. We conclude that surface-bound *S. epidermidis* induces only a weak inflammatory response, which in combination with the ability of the adherent bacteria to retard neutrophil apoptosis may contribute to low-grade inflammation and loosening of prostheses.

460. Peptidoglycan of staphyloccocus aureus induces enhanced levels of matrix metalloproteinase-9 in human blood originating from neutrophils
Wang, Y.Y. et al
Shock, 24(3), 214-218 (2005)

Enhanced plasma levels of matrix metalloproteinase 9 (MMP-9) detected in patients with severe sepsis are thought to contribute to the development of organ dysfunction in endotoxemia. We have recently reported that peptidoglycan, the major wall component of gram-positive bacteria, increases MMP-9 levels in lung and liver and organ injury in the rat. Thus far, it is unclear whether MMP-9 is part of the septic response to peptidoglycan in human blood. The aim of the present study was to examine the regulation of MMP-9 by peptidoglycan in human leukocytes. The addition of peptidoglycan to whole human blood caused enhanced levels of MMP-9 after 1 h of incubation (306 vs. 75 ng/mL, P <= 0.05) and onward, as measured by enzyme-linked immunoabsorbant assay. In neutrophil cultures, MMP-9 values increased significantly after 30 min of incubation with peptidoglycan (242 vs. 121 ng/mL, P <= 0.05), whereas muramyl dipeptide had no effect. In contrast, adherent monocytes released insignificant amounts of MMP-9. To examine whether the released MMP-9 resulted from de novo synthesis, intracellular and secreted MMP-9 was measured during stimulation of neutrophils. The total MMP-9 values (the sum of intracellular and secreted MMP-9) before and after stimulation were mainly unaltered. The enhanced MMP-9 levels induced by peptidoglycan was attenuated by inhibitors of p38 mitogen-activated protein kinases (MAPK),

(SB202190, 25 [mu]M) and ERK1/2 (PD98059, 25 [mu]M) and inhibitors of Src Tyrosine kinase (PP2, 5 [mu]M) and PI3-K (LY294002, 25 [mu]M).

461. Interleukin 8 mRNA gene expression in peripheral and intra-abdominal neutrophils during human secondary peritonitis
Holzer, K. et al
Shock, 23(6), 501-506 (2005)

The aim of the study was to investigate interleukin 8 (IL-8) mRNA gene expression in circulating and emigrated intra-abdominal neutrophils during human secondary peritonitis intra- and postoperatively (until 96 h). Patients with secondary peritonitis were allocated to two groups, e.g., patients with no complications (n = 10) and patients with complications (organ failure, septic shock, etc., n = 9). Patients with elective abdominal surgery (n = 11) and a group with healthy volunteers (n = 7) were studied as controls. Neutrophil RNA was isolated and semiquantitative reverse transcription-PCR was performed. The PCR products were compared with corresponding GAPDH bands (=100%). The highest amount of IL-8 mRNA could be assessed in blood neutrophils of healthy volunteers (87.4% +/- 7.4%). Complicated peritonitis was associated with the lowest concentration of IL-8 mRNA in blood neutrophils intraoperatively (24% +/- 7%, $P < 0.05$), which showed no recovery throughout the observation period (34% +/- 8%, 96 h postoperatively). IL-8 mRNA concentration in blood neutrophils of patients with uncomplicated peritonitis and patients with elective abdominal surgery was higher intraoperatively (55.2% +/- 9% (uncomplicated peritonitis); 68% +/- 15% (elective abdominal surgery, $P < 0.05$ versus complicated peritonitis). Thereafter, IL-8 mRNA decreased slightly in both groups, but was distinctly higher than in patients with complicated peritonitis. Emigration to the abdominal cavity resulted in an approximately 2-fold, in some cases 3-fold, increase in the concentration of IL-8 mRNA in emigrated intra-abdominal neutrophils when compared with circulating cells. This increase could be observed in all groups. The long-lasting down-regulation of constitutive gene expression of IL-8 mRNA in blood neutrophils during complicated peritonitis is worrying because IL-8 is an important activator and chemoattractant for neutrophils themselves. It is encouraging that migration to another compartment, e.g., infected abdominal cavity, resulted in an increase in neutrophil IL-8 mRNA during complicated and uncomplicated peritonitis.

462. Mitochondrial gene knockout HL60ρ0 cells show preferential differentiation into monocytes/macrophages
Herst, P.M., Levine, D.M. and Berrigde, M.V.
Leukemia Res., 29(10), 1163-1170 (2005)

This study compares the differentiation potential of mitochondrial gene knockout (ρ^0) and parental HL60 cells in response to 1.25% dimethylsulfoxide (DMSO) and 10 nM phorbol myristate acetate (PMA). Compared to HL60 cells, undifferentiated HL60ρ^0 cells showed partial monocyte/macrophage differentiation, with increased CD11c and CD14 expression, decreased CD71 expression, and weak non-specific esterase staining. Differentiation along the monocyte/macrophage pathway (PMA) was more pronounced in HL60ρ^0 than parental HL60 cells with increased CD11c and CD14 expression and stronger non-specific esterase staining. DMSO-exposure resulted in a poorly differentiated nuclear morphology, small respiratory burst and marginal up-regulation of CD15 expression in HL60ρ^0 cells.

463. Assessing the interaction between Helicobacter pylori and human neutrophils by freeze-fracture replica labeling
Petersson, C. and Magnusson, K-E.
Micron, 36(6), 558-562 (2005)

We recently introduced a freeze-fracture replica labeling method adapted to studies of bacterial envelopes. This report describes a further development of this detergent-digested freeze-fracture replica labeling technique, thus more exactly the conception of this explicit methodology for visualization of bacteria–host cell interactions.
Our experimental model employs human neutrophils and the gastric pathogenic bacterium *Helicobacter pylori*. The phagocytic process performed by the neutrophils represents a crucial element of the host defense system against invading microorganisms, and by so doing, it allows direct observation of the interplay between bacteria and host cells at an ultrastructural level.
The here launched methodology can be used as a tool to investigate the events taking place between pathogenic microbes and phagocytes, as well as for pinpoint targeting of other cell–cell communications in the field of cell biology.

464. Degradation of microvascular brain endothelial cell β-catenin after co-culture with activated neutrophils from patients undergoing cardiac surgery with prolonged cardiopulmonary bypass
Schuller, A.M. et al
Biochem. Biophys. Res. Comm., 329(2), 616-623 (2005)

The adhesion of highly activated neutrophils to cerebral microvascular endothelial cells (MVECs) may contribute to disruption and hyperpermeability of the blood–brain barrier (BBB) after cardiac surgery with prolonged cardiopulmonary bypass (CPB). A correlation between CPB duration and neutrophil-mediated BBB damage has not been investigated on the cellular level yet. Therefore, we studied the effects of neutrophils from cardiac surgery patients with CPB time <80 min (group I; $n = 8$) and >80 min (group II; $n = 8$) on the integrity of cultured porcine MVEC. Ex vivo, neutrophils of group II but not of group I significantly degraded the *zonula adherens*

molecule β-catenin whereas VE-cadherin and occludin were not modified. The transendothelial electric resistance as a measure for the integrity of the endothelial monolayers was reduced over time in both groups. In conclusion, prolonged CPB time entails neutrophil-mediated decrease in MVEC β-catenin expression, and thus may be an important trigger for BBB disruption.

465. The effects of allergen-specific immunotherapy on polymorphonuclear leukocyte functions in patients with seasonal allergic rhinitis

Gürer, Ü.S. et al

Int. Immunopharmacol., 5(4), 661-666 (2005)

Immunotherapy plays an important role in the therapy of allergic rhinitis and bronchial asthma. However, there is not much information about the effects of allergen-specific immunotherapy (SIT) on the polymorphonuclear leukocyte functions.
The aim of this study was to investigate the effects of specific immunotherapy on phagocytic and intracellular killing activities of polymorphonuclear leukocytes (PMN) derived from patients with seasonal allergic rhinitis. Twenty-four patients with seasonal allergic rhinitis documented to be sensitive to grass pollen were included in this study. Patients were divided into 3 groups. Group 1 (n=7) received conventional immunotherapy whereas patients in Group 2 (n=7) were treated with short-term immunotherapy and the third group (n=10) were given placebo during the study process. Both phagocytic and intracellular killing activities were significantly increased (p=0.002, p<0.0001, respectively) by conventional immunotherapy when compared to the first determination. In the short-term immunotherapy group, phagocytic activity was increased very significantly (p=0.0001), whereas intracellular killing activity was not affected (p=0.252). There were no changes in these parameters in the placebo group. These results suggest that allergen-specific immunotherapy has an enhancing effect on PMNs functions in the patients with seasonal allergic rhinitis. It should be clarified by further studies whether this enhancement might be considered as another beneficial effect of the immunotherapy.

466. Inhibition of human neutrophil reactive oxygen species production and p67phox translocation by cigarette smoke extract

Dunn, J.S., Freed, B.M., Gustafson, D.L. and Stringer, K.A.

Atheroscerosis, 179(2), 261-267 (2005)

The association between cigarette smoking and atherogenesis is well established. Inflammatory cells may participate in atherogenesis via activation of the NADPH oxidase and the subsequent production of reactive oxygen species (ROS), which exacerbates endothelial injury. However, little is known about the ability of cigarette smoke (CS) to modulate NADPH oxidase protein function. In this study, we investigated the ability of a CS extract derived from a high tar cigarette to alter human neutrophil ROS production and the translocation of two NADPH oxidase proteins, p47*phox* and p67*phox*.
Phorbol ester-induced intracellular and extracellular production of ROS was reduced following CS treatment as measured by enhanced luminol or isoluminol chemiluminescence, respectively, (luminol AUC was reduced by 59%, $p \leq 0.0001$; isoluminol by 49%, $p \leq 0.001$). The phorbol ester-induced phosphorylation and translocation of p47*phox* from the cytosol to the membrane was not changed by CS treatment but the translocation of p67*phox* was reduced. Cigarette smoke treatment alone did not provoke neutrophil ROS production.
These findings demonstrate that CS treatment reduced agonist-induced human neutrophil ROS production independent of p47*phox* phosphorylation and translocation from the cytosol to the membrane. However, this inhibition could be attributed to a reduction in translocation of another cytosolic NADPH oxidase protein, p67*phox.* Although neutrophil-generated ROS have been implicated in the pathogenesis of atherosclerosis, this does not appear to be the mechanism by which CS induces vascular injury.

467. Local and systemic expression of inducible nitric oxide synthase in comparison with that of cyclooxygenase-2 in rat carrageenin-induced pleurisy

Fujisawa, H. et al

Nitric Oxide, 12(2), 80-88 (2005)

Expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) is up-regulated in response to inflammatory stimuli. To evaluate the extent to which local pleural inflammation involves additional site in the pleural cavity and elsewhere, we investigated the time course of the levels of iNOS and its product in the inflammatory and other sites, and compared those with a level of COX-2 in rat carrageenin-induced pleurisy. The exudate and plasma NO_x levels rose, reaching peaks at 9 and 14 h, respectively. Both COX-2 and iNOS became detectable in exudate leukocytes, their levels reaching peaks at 3 and 9 h after irritation, respectively. COX-2 was detectable mainly in neutrophils, but iNOS was detectable in both neutrophils and mononuclear leukocytes. Furthermore, iNOS became detectable in neutrophils and mononuclear leukocytes in enlarged parathymic lymph nodes from 3 h in addition to those in peripheral blood and Kupffer cells from 3 to 14 h, respectively. The gene product is also detectable in thymic large dendritic cells of pleurisy-induced rats as well as normal control rats. COX-2 became detectable in stellar dendritic cells of the enlarged draining lymph nodes from 14 h. Thus, these gene products were induced in the immediate proximity of regional lymph nodes, and even at a considerable distance of liver by the local inflammatory stimulus. Although their expression pattern was quite different from each other, these gene products were detectable in phagocytic cells.

468. Detection of the 47-kilodalton membrane immunogen gene of Treponema pallidum in various tissue sources of patients with syphilis

Kouznetsov, A.V., Weisenseel, P., Trommler, P., Multhaup, S. and Prinz, J.C.

Diagn. Microbiol. Infect. Dis., 51(2), 143-145 (2005)

Polymerase chain reaction (PCR) was used to detect the 47-kDa immunogen gene of *Treponema pallidum* in peripheral blood mononuclear cells (PBMCs), skin lesions, and serum, but less consistently in purified granulocytes or ejaculates of patients with manifest and latent syphilis. Therefore, skin lesions and PBMCs may serve as the most reliable sources for a PCR-based diagnosis of syphilis.

469. Epidermal growth factor enhances TNF-α-induced priming of human neutrophils

Lewkowicz, P., Tchorzewski, H., Dytnerska, K., Banasik, M. and Lewkowicz, N.

Immunol. Lett., 96(2), 203-210 (2005)

The intensity of neutrophil inflammatory response could be rapidly amplified by priming with pro-inflammatory mediators such as TNF-α, GM-CSF or LPS at low concentrations prior to stimuli. We proposed that epidermal growth factor (EGF) increases TNF-α-induced priming of human neutrophils. This study showed that EGF enhanced TNF-α-induced activation of neutrophils functions. The addition of EGF to neutrophils cultured with TNF-α resulted in increased respiratory burst and phagocytic activity of polymorphonuclear leukocytes (PMN) and up-regulation of adhesion molecule CD11b. Moreover, EGF enhanced IL-8 production by TNF-α-primed PMN. EGF alone was able to prime CD11b expression and IL-8 production by PMN. EGF receptor selective tyrosine kinase inhibitor, tyrphostin AG-1517, blocked the effect of priming with EGF, whereas the status of non-primed and TNF-α-primed neutrophils remained unaffected. EGFR expression on neutrophils was confirmed by flow cytometry and CELISA methods. These data provide the original evidence that EGF significantly enhances TNF-α-induced priming of human neutrophils acting through EGFR tyrosine kinase pathway. The observed effect may be a result of co-operative action of EGF, TNF-α and reactive oxygen intermediates (ROI).

470. A phase I study of T900607 given once every 3 weeks in patients with advanced refractory cancers; National Cancer Institute of Canada Clinical Trials Group (NCIC–CTG) IND 130

Gelmon, K.A. et al

Invest. New Drugs, 23(5), 445-453 (2005)

Background: T900607 is a novel tubulin active agent which disrupts microtubule polymerization by a unique mechanism of action. This phase I trial was conducted to determine the maximum tolerated dose, recommended phase II dose, pharmacokinetic properties and toxicities of this agent. *Patients and methods*: Patients with advanced and/or metastatic solid malignancies were enrolled, for an open dose escalation of T900607 administered intravenously over 30 minutes every 21-days. *Results*: Thirty patients were enrolled on 7 dose levels ranging from 15 to 270 mg/m^2. No DLTs were seen until 270 mg/m^2, the sixth dose level, with 1 patient experiencing Grade 3 thrombocytopenia, 1 grade 4 troponin increase and 1 grade 5 myocardial infarction in an expanded cohort of 6 patients. The dose was decreased to 180 mg/m^2 with increased cardiac monitoring and at this dose 3/4 patients experienced cardiac toxicity. Further animal cardiotoxicity studies failed to reveal any cardiac effects and the study was reopened at 130 mg/m^2; of 6 enrolled patients, 1 had grade 3 drug related lethargy considered to be a DLT and this dose was considered the RP2D. No objective responses were seen but stable disease was reported in 7/20. Pharmacokinetic analysis showed that AUC and C_{max} increased with dose with considerable intrapatient variability, a short half life of < 1 hour, and no apparent dose dependency clearance. *Conclusions*: The recommended phase II dose for T900607 is 130 mg/m^2 given as an intravenous infusion over 60 minutes on a 21-day cycle. Cardiac toxicity was seen with this schedule.

471. Feasibility of Tissue Plasminogen Activator Formulated for Pulmonary Delivery

Dunn, J.S. et al

Pharmaceut. Res., 22(10), 1700-1707 (2005)

Purpose This study was conducted to assess the feasibility of a pulmonary formulation of tissue plasminogen activator (tPA) for nebulization into the airway by measuring protein stability, biologic activity, particle size, and estimating human lung distribution. Methods Formulations were derived by varying the surfactant and protein concentrations. Protein stability and recovery of each nebulized tPA formulation were assessed by ultraviolet spectroscopy. Formulations that met protein stability feasibility criteria were assessed for biologic and fibrinolytic activities. Biologic activity was determined by their ability to inhibit superoxide anion production by human neutrophils. Fibrinolytic activity was assessed by the cleavage of plasminogen to plasmin. Aerodynamic properties were assessed using a cascade impactor, and an estimation of human airway deposition was made via a human lung replica. Results Twenty-seven tPA formulations were initially assessed, 15 of which met protein stability criteria. Subsequently, three of these formulations maintained biologic and fibrinolytic activities. These formulations exhibited particle sizes of 2.4–3.1 μm, and had

respirable doses ≥65%. A formulation of 1mg mL^{-1} tPA and 0.1% Tween 80 exhibited a 45% deposition in the lower airways of a human lung replica.
Conclusions A suitable pulmonary tPA formulation was identified that, following nebulization, maintained protein stability as well as biologic and fibrinolytic activities, and resulted in an optimal respirable dose and human airway deposition. This formulation may be applicable in the treatment of lung diseases, such as acute respiratory distress syndrome by permitting targeted pulmonary delivery of a therapeutic protein to the lungs.

472. The link between the insecticide heptachlor epoxide, estradiol, and breast cancer
Cassidy, R.A., Natarajan, S. and Vaughan, G.M.
Breast Cancer Res. and Treatment., 90(1), 55-64 (2005)

Given the suspected effects of estrogens on breast cancer, xenoestrogenic insecticides may be a risk factor. Studies of the weak xenoestrogen, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), have failed to demonstrate a causal relationship, though another estrogenic organochlorine insecticide, dieldrin, belonging to the cyclodiene family, has recently been linked to breast cancer. Other cyclodienes such as heptachlor epoxide (HE) and oxychlordane (OC) present in breast tissue have not been evaluated as rigorously, presumably due to their lower concentration and lower recovery using solvent extraction procedures. We used sparging extraction coupled with gas chromatography to determine the levels of HE, OC, and DDE in adipose tissue within breast biopsies in a series of 34 women evaluated for breast abnormality. Of the three insecticides tested, only HE (p=0.007) was positively associated with prevalence of breast cancer in the biopsies. In rapid, non-genomic studies using isolated human leukocytes, flow cytometric methods were used to measure HE-induced oxidants and DNA damage. These studies indicated that HE, at concentrations similar to those in breast biopsies, induced an inverted-U increase in intracellular oxidants and DNA strand breaks [both blocked by specific nitric oxide- (NO-) synthesis blockade with$_L$-NMMA] in human polymorphonuclear leukocytes (PMNs). HE-treated PMNs also induced damage to surrounding lymphocytes in mixed-leukocyte incubations (also inhibited by NO blockade). The HE-induced changes in NO were inhibited by 17β-estradiol-(17β-E_2) receptor antagonists and were mimicked by similar concentrations of 17β-E_2. The addition of tumor necrosis factor-α (TNF-α) increased intracellular oxidants and DNA damage and shifted the responses to lower HE concentrations. This study, along with others, suggests that HE-induced NO production may contribute to initiation, promotion, and progression of cancer.

473. Thromsbospondin-1 binds to the heavy chain of elastase activated coagulation factor V (FVaHNE) and enhances thrombin generation on the surface of a promyelocytic cell line
Isordia-Salas, I., Manns, J.M., Sainz, I., Parekh, H. and DeLa Cadena, R.A.
Thromb. Res., 116, 533-543 (2005)

Introduction
Thrombospondin 1 (TSP1) has the ability to bind to HL-60 cells and to reversibly inhibit human neutrophil elastase (HNE). Human factor V (FV) can be cleaved by HNE thereby providing FV with cofactor activity (FVa_{HNE}). Experiments were performed to evaluate the ability of HNE expressed on the surface of HL-60 cells to generate FVa_{HNE} to support thrombin generation, and to determine the effect of TSP1 on this reaction.
Results
Western blot analysis showed TSP1 forming a complex with FVa_{HNE} within a region corresponding to the heavy chain of FV. Enzymatic reactions were performed to determine the role of TSP1–HNE–FVa_{HNE} on the surface of HL-60 cells, namely the assembly of the prothrombinase complex. Thrombin generation was measured by the chromogenic substrate S2238. Exposure of factor V to HL-60 cells prior to the addition of prothrombin and activated factor X provided FV with cofactor activity. HL-60 cells were found capable of synthesizing factor V with cofactor activity, but HL-60 cells failed to synthesize and/or to provide factor X with enzymatic activity. The ability of HL-60 cells to synthesize FV and TSP1 was demonstrated. The addition of exogenous TSP1 enhanced both the rate and amount of thrombin generated on the HL-60 cell surface.
Conclusion
Despite the ability of TSP1 to reversibly inhibit HNE in a purified system, TSP1 expression favors the reactions leading to thrombin generation on the HL-60 cell surface. These observations are relevant to clinical conditions where there is a prothrombotic state such as malignant tumors.

474. Non-specific effects of 4-chloro-m-cresol may cause calcium flux and respiratory burst in human neutrophils
Hauser, C.J., Kannan, K.B., Deitch, E.A. and Itagaki, K.
*Biochem. Biophys. Res. Comm., **336(4)**, 1087-1095 (2005)*

We examined the effects of 4-chloro-*m*-cresol (4-CmC, a potent and specific activator of ryanodine receptors) on Ca^{2+}-release/influx and respiratory burst in freshly isolated human PMN as well as HL60 cells. 4-CmC induces Ca^{2+} store-depletion in a dose-dependent manner at concentrations between 400 μM and 3 mM, however no dose-dependent effect on Ca^{2+}-influx was found. 4-CmC depleted Ca^{2+} stores that were shared with the GPC agonists such as fMLP and PAF, and therefore 4-CmC presumably depletes Ca^{2+} from ER.

Since the authentic ligand for RyR is cyclic ADP-ribose (cADPR), we assessed the functional relevance of RyR in PMN by studying the presence and function of membrane-bound ADP-ribosyl cyclase (CD38) in PMN. First, expression of CD38 was confirmed by RT-PCR using cDNA from HL60 cells. Second, PMN from trauma patients showed significantly enhanced CD38 expression than those from healthy volunteers. In addition, although no chemotaxis effect was detected by 4-CmC, it stimulated respiratory burst in PMN in a dose-dependent manner. Our findings suggest that RyRs exist in human PMN and that RyR pathway may play an active role in inflammatory PMN calcium signaling. 8-Br-cADPR and cyclic 3-deaza-ADP did not have inhibitory effects either on 4-CmC-induced Ca^{2+} store-depletion or on respiratory burst, on the other hand, PLC inhibitor, U73122, completely attenuated both 4-CmC-induced Ca^{2+} store-depletion and respiratory burst. Although it has been used as a specific activator of RyR, 4-CmC has non-specific effects which cause Ca^{2+} store-depletion and respiratory burst at least in human PMN.

475. A novel exopolysaccharide from a clinical isolate of Prevotella nigrescens: purification, chemical characterization and possible role in modifying human leukocyte phagocytosis
Yamane, K. et al
Oral Microbiol. Immunol., ***20(1)****, 1-9 (2005)*

Prevotella nigrescens, a gram-negative black-pigmented anaerobic rod, has frequently been isolated from periodontitis and periapical periodontitis lesions. We have isolated an exopolysaccharide-producing P. nigrescens, strain 22, from a chronic periodontitis lesion. The purpose of this study was to determine the chemical composition and function of the exopolysaccharide associated with this clinical isolate. The chemical composition and structure of the purified exopolysaccharide from strain 22 were determined by high performance liquid chromatography and methylation analysis. To define the biological function of this exopolysaccharide, a chemically induced exopolysaccharide nonproducing mutant, strain 328, which was derived from strain 22, was established. The biological effects of exopolysaccharide were determined by comparing the ability of strain 22, strain 328 or heat-killed strain 22 to form abscesses in mice and to interfere with the phagocytic activity of peripheral blood polymorphonuclear leukocytes. Chemical analysis showed that isolated exopolysaccharide consisted of mannose (521.6 [mu]g/mg), glucose (25.6 [mu]g/mg), fructose (65.8 [mu]g/mg), galactose (12.5 [mu]g/mg), arabinose (6.2 [mu]g/mg), xylose (3.2 [mu]g/mg), rhamnose (6.1 [mu]g/mg), and ribose (0.6 [mu]g/mg). Methylation analysis of exopolysaccharide indicated that the linkages of mannose were primarily (1->2, 1->6) (1->2) (1->6), and (1->3). Strain 22 and, to a lesser extent, its heat-killed counterpart induced greater abscess formation in mice than strain 328, even though the enzymatic profile of strain 22 was similar to that of strain 328. The ability of strain 328 to induce abscess formation was restored by adding the purified exopolysaccharide isolated from strain 22 to the cell suspension of strain 328. Exopolysaccharide alone failed to induce abscess formation in mice. Further, strain 328 but not the untreated or heat-killed strain 22, was phagocytosed by polymorphonuclear leukocytes both in the presence and in the absence of opsonic factors. The results suggest that these polysaccharides isolated from strain 22, which primarily consisted of mannose, may play a key role in the development of the chronic inflammatory lesion from which this strain was isolated.

476. Mutational analysis of the group A streptococcal operon encoding streptolysin S and its virulence role in invasive infection
Datta, V. et al
Mol. Microbiol., ***56(3)****, 681-695 (2005)*

The pathogen group A Streptococcus (GAS) produces a wide spectrum of infections including necrotizing fasciitis (NF). Streptolysin S (SLS) produces the hallmark [beta]-haemolytic phenotype produced by GAS. The nine-gene GAS locus (sagA-sagI) resembling a bacteriocin biosynthetic operon is necessary and sufficient for SLS production. Using precise, in-frame allelic exchange mutagenesis and single-gene complementation, we show sagA, sagB, sagC, sagD, sagE, sagF and sagG are each individually required for SLS production, and that sagE may further serve an immunity function. Limited site-directed mutagenesis of specific amino acids in the SagA prepropeptide supports the designation of SLS as a bacteriocin-like toxin. No significant pleotrophic effects of sagA deletion were observed on M protein, capsule or cysteine protease production. In a murine model of NF, the SLS-negative M1T1 GAS mutant was markedly diminished in its ability to produce necrotic skin ulcers and spread to the systemic circulation. The SLS toxin impaired phagocytic clearance and promoted epithelial cell cytotoxicity, the latter phenotype being enhanced by the effects of M protein and streptolysin O. We conclude that all genetic components of the sag operon are required for expression of functional SLS, an important virulence factor in the pathogenesis of invasive M1T1 GAS infection.

477. Detection and clinical evaluation of antineutrophil antibodies in neonates who had a blood transfusion or exchange transfusion
Ören, H., Duman, N., Anal, Ö, Özkan, H. and Irken, G.
Pediatrics Int., ***47(5)****, 519-522 (2005)*

Abstract*Background*: The aim of this study was to detect and investigate the clinical effects of antineutrophil antibodies in neonates who had received a blood transfusion or exchange transfusion.
Methods: Venous blood samples were drawn from 34 neonates at pretransfusion (sample 0), immediately after transfusion (sample 1), 2–3 weeks (sample 2) and 8–12 weeks (sample 3) after transfusion. Ten healthy neonates were in the control group. Antineutrophil antibodies were detected using flow cytometric assay.

Results: Antineutrophil antibody was detected in the sera of 20 (58.8%) neonates in the study group. Of these 20 neonates, nine had antineutrophil antibodies in serum samples 0, 1 and 2, which were probably due to the passive transfer of maternal antibodies. Nine neonates had antineutrophil antibodies in serum samples 1 and 2, which were probably due to neutrophil antibodies being present in the donor's blood. In two neonates, antineutrophil antibodies were not detected in samples 0 and 1, but appeared in sample 2, which were probably actively produced by the neonates. All of the antineutrophil antibodies disappeared in the serum samples, except in one neonate. Only one preterm newborn developed neutropenia, which resolved spontanously in a week. The presence of antineutrophil antibody in transfused neonates was significantly higher than in non-transfused neonates.
Conclusions: The presence of neutrophil specific antibodies in transfused neonates is not rare and antineutrophil antibodies may be found more often in transfused neonates compared to non-transfused neonates. The clinical significance of those antibodies needs to be assessed since they are transient and their clinical effects are not evident.

478. CYSTIC FIBROSIS SPUTUM STIMULATES CD18-INDEPENDENT NEUTROPHIL MIGRATION ACROSS ENDOTHELIAL CELLS
Mackarel, A.J. et al
Exp. Lung Res., ***31(4)****, 377-390 (2005)*

Excessive neutrophil recruitment to the lung underlies inflammatory-mediated lung damage in cystic fibrosis (CF). Neutrophils can migrate to the lung using either a CD18-dependent or CD18-independent mechanism. To determine if one of these migratory pathways is preferentially utilized by neutrophils migrating to the CF airways, this study examined the CD18 dependency of neutrophil transendothelial migration stimulated by the soluble fraction of CF sputum (SOL). Results demonstrate the preferential use of the CD18-independent migratory mechanism by both control and CF neutrophils and suggest that selective blocking of the CD18-independent migration pathway may offer a means of decreasing neutrophil influx to the CF airways.

479. Chemical inhibitors of TNF signal transduction in human neutrophils point to distinct steps in cell activation
Han, H. et al
J. Leukoc. Biol., 79, 147-154 (2006)

Chemical screening identified three small compounds that selectively inhibited activation of the respiratory burst (RB) of human neutrophils in response to tumor necrosis factor (TNF) and formylated peptide but not phorbol ester and spared the ability of neutrophils to kill bacteria. These compounds partially inhibited TNF-triggered cytoskeletal rearrangements without blocking adhesion or transmigation of polymorphonuclear neutrophils through TNF-activated monolayers of endothelial cells. The compounds were nontoxic to neutrophils and endothelial cells. They had no direct inhibitory effect on the tyrosine kinases Src, Syk, or Pyk2. However, their differential effects on cell spreading, bacteria-induced RB, TNF-induced degranulation, TNF-induced protein tyrosine phosphorylation, and TNF-induced Syk activation suggested that each may act on different elements of neutrophil signaling pathways.

480. Sodium Salicylate Promotes Neutrophil Apoptosis by Stimulating Caspase-Dependent Turnover of Mcl-1
Derouet, M. et al
J. Immunol., 176, 957-965 (2006)

Mcl-1 is an antiapoptotic member of the Bcl-2 family of proteins that plays a central role in cell survival of neutrophils and other cells. The protein is unusual among family members in that it has a very short half-life of 2–3 h. In this report, we show that sodium salicylate (at 10 mM) greatly enhances the rate at which neutrophils undergo apoptosis and, in parallel, greatly accelerates the turnover rate of Mcl-1, decreasing its half-life to only 90 min. Whereas constitutive and GM-CSF-modified Mcl-1 turnover is regulated by the proteasome, the accelerated sodium salicylate-induced Mcl-1 turnover is mediated largely via caspases. Sodium salicylate resulted in rapid activation of caspase-3, -8, -9, and -10, and salicylate-accelerated Mcl-1 turnover was partly blocked by caspase inhibitors. Sodium salicylate also induced dramatic changes in the activities of members of the MAPK family implicated in Mcl-1 turnover and apoptosis. For example, sodium salicylate blocked GM-CSF-stimulated Erk and Akt activation, but resulted in rapid and sustained activation of p38-MAPK, an event mimicked by okadaic acid that also accelerates Mcl-1 turnover and neutrophil apoptosis. These data thus shed important new insights into the dynamic and highly regulated control of neutrophil apoptosis that is effected by modification in the rate of Mcl-1 turnover.

481. Novel Compounds That Interact with Both Leukotriene B4 Receptors and Vanilloid TRPV1 Receptors
McHugh, D. et al
J. Pharmacol. Exp. Ther., 316, 955-965 (2006)

The aim of this study was to investigate the interaction of a series of novel compounds with leukotriene B_4 receptors (BLT) and vanilloid receptor (TRPV1). First, we characterized leukotriene B_4 (LTB_4) ethanolamide. In guinea pig isolated lung parenchyma, LTB_4 ethanolamide antagonized the contractile action of LTB_4 with an apparent K_B value of 7.28 nM. Using a Boyden chamber assay, we demonstrated that this compound stimulated human neutrophil migration in a similar manner to LTB_4 but with lower efficacy. In rat TRPV1 (rTRPV1)-expressing Chinese hamster ovary (CHO) cells and dorsal root ganglion (DRG) neurons, LTB_4 and LTB_4 ethanolamide acted as low-efficacy agonists, increasing intracellular calcium concentration ($[Ca^{2+}]_i$) in a capsazepine-sensitive manner. These results prompted us to hypothesize that a molecule may possess pharmacophores such that it is capable of dual antagonism of BLT and TRPV1 receptors. Two novel compounds, *N*-{2-fluoro-4-[3-(11 hydroxyheptadec-8-enyl)-thioureiomethyl]-phenyl}-methanesulfonamide (O-3367) and *N*-{4-[3-(11 hydroxyheptadec-8-enyl)-thioureio-methyl]-phenyl}-methanesulfonamide (O-3383), were synthesized. In human neutrophils, both compounds acted as antagonists, significantly attenuating the BLT receptor-mediated ability of LTB_4 to induce migration, with pIC_{50} values of 7.22 ± 0.17 and 5.95 ± 0.16, respectively. In rTRPV1-expressing CHO cells, they caused a significant rightward shift in the log concentration-response curve for the TRPV1 receptor agonist capsaicin (3-methoxy-4-hydroxy)benzyl-8-methyl-6-nonenamide). In DRG neurons O-3367 significantly attenuated the capsaicin-induced increases in $[Ca^{2+}]_i$ with a pIC_{50} value of 5.94 ± 0.004. O-3367 and O-3383 represent novel structural templates for generating compounds possessing dual antagonism at BLT and TRPV1 receptors. In view of the crucial role of both TRPV1 and BLT receptors in the pathophysiology of inflammatory conditions, such compounds may betoken a novel class of highly effective therapeutics.

482. LIGHT enhances the bactericidal activity of human monocytes and neutrophils via HVEM
Heo, S-K. et al
J. Leukoc. Biol., 79, 330-338 (2006)

Human monocytes and neutrophils play major roles in clearing bacteria from human blood and tissues. We found that the herpes virus entry mediator (HVEM) was highly expressed in monocytes and neutrophils, and its interaction with "homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM/tumor necrosis factor (TNF)-related 2" (LIGHT) enhanced bactericidal activity against *Listeria monocytogenes* and *Staphylococcus aureus*. The LIGHT-HVEM interaction increased levels of phagocytosis, interleukin (IL)-8, TNF-α, nitric oxide (NO), and reactive oxygen species (ROS) in monocytes and neutrophils. Anti-HVEM monoclonal antibody was able to block LIGHT-induced bactericidal activity, cytokine production (IL-8 and TNF-α), and ROS generation. Moreover, inhibition of ROS and NO production blocked LIGHT-induced bactericidal activity. Our results indicate that the LIGHT/HVEM interaction in monocytes and neutrophils contributes to antibacterial activity.

483. Modulation of Human Neutrophil Functions In Vitro by Treponema denticola Major Outer Sheath Protein
Puthengady Thomas, B., Sun, C.X., Bajenova, E., Ellen, R.P. and Glogauer, M.
Infect. Immun., 74, 1954-1957 (2006)

In this study of human polymorphonuclear leukocytes (PMNs), pretreatment with *Treponema denticola* major outer sheath protein (Msp) inhibited formyl-methionyl-leucyl-phenylalanine (fMLP)-induced chemotaxis, phagocytosis of immunoglobulin G-coated microspheres, fMLP-stimulated calcium transients, and actin assembly. Msp neither altered oxidative responses to phorbol myristate or fMLP nor induced apoptosis. Msp selectively impairs chemotaxis and phagocytosis by impacting the PMN cytoskeleton.

484. Functional Polymorphisms in the Vascular Endothelial Growth Factor Gene Are Associated with Development of End-Stage Renal Disease in Males
Doi, K. et al
J. Am. Soc. Nephrol., 17, 823-830 (2006)

This study elucidates the genetic role of vascular endothelial growth factor (VEGF) as a predisposing factor for progression of chronic kidney disease. Single-nucleotide polymorphisms were genotyped and haplotype structures were determined in the 3' untranslated region (UTR) of VEGF gene, and the distribution of each haplotype in male patients with ESRD (n = 101) and healthy male control subjects (n = 189) was examined. The 936C/T and 1451C/T polymorphisms in the 3' UTR were in nearly absolute linkage disequilibrium, and haplotype analysis demonstrated that they were the primary responsible single-nucleotide polymorphisms. The distribution of the 936CC-1451CC genotype was significantly more frequent among patients with ESRD than among the age-matched healthy control subjects. In addition to case-control association study, the 936CC-1451CC genotype was also associated with significantly higher plasma VEGF levels in healthy individuals, but a significant association was found only in males, not in females. We also examined the effect of the 936C-1451C haplotype on mRNA stability. Consistent with the results of plasma VEGF levels, mRNA carrying 936C-1451C haplotype showed higher stability. The 936CC-1451CC genotype in the 3' UTR showed not only susceptibility for ESRD but also higher plasma VEGF levels and mRNA stability, indicating the contribution of VEGF to chronic kidney disease progression, especially in males.

485. Biomechanics of P-Selectin PSGL-1 Bonds: Shear Threshold and Integrin-Independent Cell Adhesion
Xiao, Z., Goldsmith, H.L., MvIntosh, F.A., Shankaran, H. and Neelamegham, S.
Biophys. J., 90, 2221-2234 (2006)

Platelet-leukocyte adhesion may contribute to thrombosis and inflammation. We examined the heterotypic interaction between unactivated neutrophils and either thrombin receptor activating peptide (TRAP)-stimulated platelets or P-selectin-bearing beads (Ps-beads) in suspension. Cone-plate viscometers were used to apply controlled shear rates from 14 to 3000/s. Platelet-neutrophil and bead-neutrophil adhesion analysis was performed using both flow cytometry and high-speed videomicroscopy. We observed that although blocking antibodies against either P-selectin or P-selectin glycoprotein ligand-1 (PSGL-1) alone inhibited platelet-neutrophil adhesion by ~60% at 140/s, these reagents completely blocked adhesion at 3000/s. Anti-Mac-1 alone did not alter platelet-neutrophil adhesion rates at any shear rate, though in synergy with selectin antagonists it abrogated cell binding. Unstimulated neutrophils avidly bound Ps-beads and activated platelets in an integrin-independent manner, suggesting that purely selectin-dependent cell adhesion is possible. In support of this, antagonists against P-selectin or PSGL-1 caused dissociation of previously formed platelet-neutrophil and Ps-bead neutrophil aggregates under shear in a variety of experimental systems, including in assays performed with whole blood. In studies where medium viscosity and shear rate were varied, a shear threshold for P-selectin PSGL-1 binding was also noted at shear rates <100/s when Ps-beads collided with isolated neutrophils. Results are discussed in light of biophysical computations that characterize the collision between unequal-size particles in linear shear flow. Overall, our studies reveal an integrin-independent regime for cell adhesion and weak shear threshold for P-selectin PSGL-1 interactions that may be physiologically relevant.

486. Cellular inflammatory response to persistent localized *Staphylococcus aureus* infection: phenotypical and functional characterization of polymorphonuclear neutrophils (PMN)
Wagner, C. et al
Clin. Exp. Immunol., 143(1), 70-77 (2006)

Persistent, localized *Staphylococcus aureus* infections, refractory to antibiotic treatment, can result in massive tissue destruction and surgical intervention is often the only therapeutic option. In that context, we investigated patients with *S. aureus*-induced infection at various sites, apparent as either olecranon bursitis, empyema of the knee joint or soft tissue abscess formation. As expected, a prominent leucocyte infiltrate was found, consisting predominantly of polymorphonuclear neutrophils (PMN) (up to 75%) and to a lesser extent of T lymphocytes and natural killer (NK) cells. In line with their bactericidal capacity, PMN expressed the high-affinity receptor for IgG, CD64 and the lipopolysaccharide (LPS) receptor CD14; moreover, the oxygen radical production in response to the bacterial peptide f-MLP was enhanced, while chemotactic activity was greatly reduced. The more intriguing finding, however, was that a portion of PMN had acquired major histocompatibility complex (MHC) class II antigens and CD83, indicative of a transdifferentiation of PMN to cells with dendritic-like characteristics. Of note is that a similar transdifferentiation can be induced in PMN *in vitro*, e.g. by gamma interferon or by tumour necrosis factor alpha. Co-cultivation of transdifferentiated PMN with autologous T lymphocytes resulted in prominent T cell proliferation, provided that *S. aureus* enterotoxin A was added. Taken together, persistent *S. aureus* infection induces PMN to acquire characteristics of dendritic cells, which in turn might promote the local immune response.

487. Autoantibodies in Argentine Women with Recurrent Pregnancy Loss
Bustos, D. et al
Am. J. Reprod. Immunol., 55(3), 201-207 (2006)

To determine the presence or absence of subclinical autoimmunity in Caucasian Argentine healthy women with first trimester recurrent pregnancy loss (RPL), the sera of 118 healthy women with a history of three or more consecutive abortions and 125 fertile control women without abortions and two children were analyzed for the presence of autoantibodies: immunoglobulin (Ig)G and IgM anticardiolipin, antinuclear (ANA), antismooth muscle (ASMA), antimitocondrial (AMA), antiliver-kidney-microsomal fraction (LKM), antigastric parietal cells (GPC), antineutrophil cytoplasmatic (ANCA) and antibodies antigliadin type IgA and IgG and IgA antitransglutaminase related with celiac disease (CD).
ANA, ASMA, AMA, anti-LKM, antibodies to GPC and ANCA were determined by indirect immunofluorescence (IFI) and anticardiolipin, antigliadina and antitransglutaminase antibodies were measured by enzyme-linked immunosorbent assays (ELISA).
There was no significant difference between controls and patients with ANA, ASMA, AMA, LKM, ANCA and GPC. The prevalence of anticardiolipin antibodies in RPL was significantly higher than controls ($P < 0,01$) and the prevalence of positive antibodies for antigliadina type IgA and IgG and IgA antitransglutaminase in RPL was significantly higher than controls ($P < 0.04$).
We show that Caucasian Argentine women with RPL showed significantly higher incidence of anticardiolipin antibodies than normal controls and finally we recommended the screening of IgA and IgG antigliadina and IgA antitransglutaminase antibodies in pregnancy, because of the high prevalence of subclinical CD in RPL and the chance of reversibility through consumption of a gluten free diet.

488. *Streptococcus pyogenes* bacteria modulate membrane traffic in human neutrophils and selectively inhibit azurophilic granule fusion with phagosomes
Staali, L., Bauer, S., Mörgelin, M., Björk, L. and Tapper, H.
Cell. Microbiol., 8(4), 690-703 (2006)

We recently reported that the human pathogen *Streptococcus pyogenes* of the M1 serotype survives and replicates intracellularly after being phagocytosed by human neutrophils. These data raised the possibility that the generation of reactive oxygen metabolites by

neutrophils, and the release of microbicidal molecules from their azurophilic and specific granules into phagosomes, can be modulated by *S. pyogenes* bacteria expressing surface-associated M and/or M-like proteins. We now demonstrate, using flow cytometry, immunofluorescence microscopy and transmission electron microscopy, that live wild-type *S. pyogenes*, after internalization by human neutrophils, inhibits the fusion of azurophilic granules with phagosomes. In contrast, azurophilic granule-content is efficiently delivered to phagosomes containing bacteria not expressing M and/or M-like proteins. Also, when heat-killed wild-type bacteria are used as the phagocytic prey, fusion of azurophilic granules with phagosomes is observed. The inhibition caused by live wild-type *S. pyogenes* is specific for azurophilic granule–phagosome fusion, because the mobilization of specific granules and the production of reactive oxygen species are induced to a similar extent by all strains tested. In conclusion, our results demonstrate that viable *S. pyogenes* bacteria expressing M and M-like proteins selectively prevent the fusion of azurophilic granules with phagosomes.

489. The effects of some antibiotics on polymorphonuclear leukocyte functions of elderly patients in vitro before and after zinc supplementation
Gürer, Ü.S. et al
Int.Immunopharmacol., 6(5), 808-816 (2006)

The effects of ciprofloxacin, cefodizime, rifampicine, doxycycline and cefodizime + rifampicine combination on polymorphonuclear leukocyte (PMN) functions (phagocytosis and intracellular killing activity) were investigated *in vitro* in elderly patients and compared with those of healthy young volunteers before and after zinc supplementation. PMNs of 13 elderly hypertensive patients and 10 healthy young volunteers were isolated by Ficoll-Hypaque gradient centrifugation method from venous blood with EDTA. The subjects were given 22 mg/daily/oral zinc supplementation for 1 month. Serum zinc levels before and after supplementation were measured by flame atomic absorbtion spectrophotometer and the effects of each drug on PMN functions at therapeutic concentrations were investigated. Ciprofloxacin significantly increased the PMN's phagocytic activity of elderly patients ($p = 0.002$) before zinc supplementation and significantly increased both PMN functions of elderly patients ($p = 0.002$) after zinc supplementation. The same antibiotic significantly increased both PMN functions of healthy young volunteers ($p = 0.005$ and $p < 0.05$, respectively) before and after zinc supplementation when compared with the control (drug-free). Cefodizime significantly increased the PMN's phagocytic activity of elderly patients ($p = 0.003$, $p = 0.002$) before and after zinc supplementation when compared with the control (drug-free). It also significantly increased both PMN functions of healthy young volunteers ($p = 0.005$ and $p < 0.05$, respectively) before and after zinc supplementation when compared with the control (drug-free). Doxycycline significantly increased PMN's intracellular killing activity of healthy young volunteers before zinc supplementation ($p < 0.05$) when compared with the control (drug-free) values. Rifampicine significantly decreased PMN's phagocytic activity of elderly patients ($p < 0.05$) after zinc supplementation. Cefodizime + rifampicine combination significantly increased PMN's phagocytic activity at therapeutic concentrations of healthy young volunteers ($p = 0.005$) before zinc supplementation and PMN's phagocytic activity of elderly patients ($p < 0.05$) after zinc supplementation when compared with the control (drug-free). Consequently, in the present study from the antibiotics ciprofloxacin, cefodizime and cefodizime + rifampicine combination, which are accepted as biological response modifiers have demonstrated stimulatory effects by significantly increasing polymorphonuclear leucocyte functions (phagocytosis and/or intracellular killing activity) of elderly patients and healthy young volunteers in vitro before and after zinc supplementation. Additionally zinc supplementation has more immunostimulatory effects on PMN functions of healthy young volunteers than elderly patients.

490. Effects of CNI-1493 on human granulocyte functions
Abdalla, H., Forslund, T., Schön, T., Stendahl, O. and Sundqvist, T.
Immunobiology, 211(3), 191-197 (2006)

During acute bacterial infections such as sepsis and meningitis, activation of inflammatory mediators such as nitric oxide (NO) plays a crucial role in both pathogenesis and host defense. We have previously reported that CNI-1493, a macrophage deactivator, reduced mortality in infant rats infected with *Haemophilus influenzae* type b (Hib) with associated decrease in the number of granulocytes in the infected tissue. The aim of the present study was to investigate how CNI-1493 affects granulocytes and macrophages in vitro. Murine macrophages (RAW 264.7) pre-incubated with CNI-1493 prior to activation with lipopolysaccharide (LPS)/interferon gamma (IFNγ) had decreased NO production measured as NO_2^-/NO_3^- levels and reduction in inducible NO-synthase (iNOS) expression. Reactive oxygen species (ROS) production was increased in formylmethionyl-leucyl-phenylalanine (FMLP)-stimulated granulocytes following CNI-1493 treatment, whereas F-actin content, motility and chemotaxis were decreased under the same conditions. The effects of CNI-1493 on both NO production in LPS/IFNγ-activated macrophages and ROS production, F-actin content, motility and chemotaxis in granulocytes, may contribute to the reduced inflammatory response and increased survival in Hib-infected animals treated with CNI-1493.

491. A multiparametric flow cytometry method for detection of modifications of antigen expression in polymorphonuclear cells infected by human cytomegalovirus
Bressollette-Bodin, C., Andre-Garnier, E., Robillard, N.,Billaudel, S. and Imbert-Marcille, B.M.
J. Virol. Methods, 132(1-2), 32-39 (2006)

Human cytomegalovirus (CMV) has been shown to alter adhesion molecule expression on permissive cells such as endothelial cells. The aim of the present study was to investigate expression of receptors for these molecules on CMV infected polymorphonuclear leukocytes (PMNLs). CMV-induced variations on cellular integrin expression were examined using an in vitro system to obtain infected PMNLs. A triparametric flow cytometry approach was developed, which allows combined detection, in a single experiment, of both viral intranuclear antigen in the selected PMNLs and cellular CD11/CD18 expression. Comparison of infected PMNLs with uninfected cells showed a decrease of up to 50% in the expression of CD11b, CD11c, and CD18. This study thus demonstrates that the presence of CMV in PMNLs, which characterizes active infection, modifies the expression of integrins and may thus affect cell-to-cell interactions and immune functions.

492. Human peripheral blood mononuclear cells express GABAA receptor subunits
Alam, S., Laughton, D.L., Walding, A. and Wolstenholme, A.J.
Mol. Immunol., 43(9), 1432-1442 (2006)

The polymerase chain reaction was used to screen human peripheral blood mononuclear cells (PBMC) and Jurkat cells for the presence of $GABA_A$ receptor subunit mRNAs. Positive signals were detected for the α1, α3, β2, β3, δ and ε subunit mRNAs in both cell populations, with the Jurkat cells giving a positive signal for some additional species. Real-time PCR was used to confirm that PBMC, lymphocytes and monocytes contained significant levels of the α1 subunit mRNA and that PBMC and lymphocytes contained low levels of β2 mRNA. The α1 subunit was detected in PBMC and fractionated T-cell populations, as well as Jurkat and HL-60 cell lines, by Western blotting and immunofluorescence using a specific antibody. The application of 1 mM GABA reduced the specific increase in intracellular PBMC Ca^{2+} levels produced by addition of 1 nM fMLP: this effect was mimicked by muscimol, but not glycine, and was blocked by bicuculline. The inhibitory effect of GABA was limited to a subset of PBMC. We conclude that cells within the human PBMC population, including lymphocytes, express functional $GABA_A$ receptors and these receptors may modulate immune responses.

493. Effects of a collagenolytic cell wall component from Fusobacterium necrophorum subsp. necrophorum on rabbit tissue-culture cells
Okamoto, K., Kanoe, M., Yagauchi, Y., Inoue, T. and Watanabe, T.
Vet. J., 171(2), 380-382 (2006)

The effects on rabbit tissue-cultured cells of collagenolytic cell wall component (CCWC) from *Fusobacterium necrophorum* subsp. *necrophorum* were investigated. Scanning electron microscopy demonstrated that CCWC damaged the cell surfaces of the rabbit granulocytes and hepatocytes but the effects of the cells differed from each other. Granulocytes appeared smooth and morphologically irregular whereas hepatocytes looked rough and had tiny holes in the cell membranes. Differences in cell viability were observed in MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt) assay. The findings suggest that cytotoxic activity in vivo may well contribute to the establishment of an initial injury in visceral tissues, and the action of CCWC could increase the chances of survival for an invading *F. necrophorum* subsp. *necrophorum* at the first stages of infection.

494. Role of the actin cytoskeleton during respiratory burst in chemoattractant-stimulated neutrophils
Bengtsson, T., Orselius, K. and Wetterö, J.
Cell Biol. Int., 30(2), 154-163 (2006)

The aim of this study was to clarify the role of the actin cytoskeleton during chemotactic peptide fMet-Leu-Phe (fMLF)-stimulated respiratory burst in human neutrophil granulocytes. Reactive oxygen species (ROS) was measured as luminol-amplified chemiluminescence (CL) and F-actin content as bodipy phallacidin fluorescence in neutrophils treated with latrunculin B or jasplakinolide, an inhibitor and activator of actin polymerization, respectively. Latrunculin B markedly decreased, whereas jasplakinolide increased, the F-actin content in neutrophils, unstimulated or stimulated with fMLF. Latrunculin B enhanced the fMLF-triggered ROS-production more than tenfold. Jasplakinolide initially inhibited the fMLF-induced CL-response, however, caused a potent second sustained phase (>400% of control). Both actin drugs triggered a substantial CL-response when added 5–25 min after fMLF. This was also valid for chemotactic doses of fMLF, where latrunculin B and jasplakinolide amplified the ROS-production 5–10 times. By using specific signal transduction inhibitors, we found that the NADPH oxidase activation triggered by destabilization of the actin cytoskeleton occurs downstream of phospholipase C and protein kinase C but is mediated by Rho GTPases and tyrosine phosphorylation. In conclusion, rearrangements of the actin cytoskeleton are a prerequisite in connecting ligand/receptor activation, generation of second messengers and assembly of the NADPH oxidase in neutrophil granulocytes.

495. 5-Oxo-Eicosatetraenoic Acid-Induced Chemotaxis: Identification of a Responsible Receptor hGPCR48 and Negative Regulation by G Protein G12/13
Koike, D. Et al
J. Biochem., 139(3), 543-549 (2006)

While screening genes encoding G protein–coupled receptors (GPCRs) in the human genome, we and other groups have identified a GPCR named hGPCR48 as a high affinity receptor for 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE), which is arachidonic acid

metabolite and an endogenous chemoattractant for granulocytes. Using Chinese hamster ovary (CHO) cells stably expressing hGPCR48, we show here that activation of the receptor causes the chemotaxis of the cells toward 5-oxo-ETE. We also show that the chemotaxis of human granulocytes toward 5-oxo-ETE is inhibited by pretreatment with anti-hGPCR48 antibodies, indicating that hGPCR48 is an endogenous receptor responsible for chemotaxis of granulocytes toward 5-oxo-ETE. In addition, we show that the chemotaxis of CHO cells expressing hGPCR48 is suppressed by pretreatment with pertussis toxin, and enhanced by overexpression of the carboxy terminal peptides of $G\alpha_{12/13}$ subunits or a regulator of the G protein signaling domain of p115RhoGEF, both of which are known to suppress $G_{12/13}$-dependent signaling pathways. These results indicate that hGPCR48 couples with $G_{i/o}$ and $G_{12/13}$ proteins, which then initiate or attenuate the chemotaxis of the cells toward 5-oxo-ETE, respectively.

496. Production of soluble triggering receptor expressed on myeloid cells by lipopolysaccharide-stimulated human neutrophils involves de novo protein synthesis
Mahdy, A.M., Lowes, D.A., Galley, H.F., Bruce, J.E. and Webster, N.R.
*Clin. Vaccine Immunol.,**13**, 492-495 (2006)*

The triggering receptor expressed on myeloid cells (TREM-1) is a recently identified receptor expressed on neutrophils and monocytes. Activation of the receptor induces neutrophils to release the enzyme myeloperoxidase and inflammatory cytokines such as interleukin-8. TREM-1 has an alternatively spliced variant that lacks the transmembrane region, resulting in the receptor being secreted in a soluble form (sTREM-1). Soluble TREM-1 has been detected in plasma during experimental and clinical sepsis and has been advocated as a diagnostic marker of infection for pneumonia and as a prognostic marker for patients with septic shock. We studied TREM-1 surface expression, using flow cytometry, and simultaneously measured sTREM-1 concentrations in culture supernatants of lipopolysaccharide (LPS)-stimulated neutrophils. TREM-1 surface expression was constitutive and was not upregulated upon LPS stimulation. However, sTREM-1 release from neutrophils was significantly upregulated by LPS stimulation ($P < 0.0001$), an effect that was abrogated by cycloheximide. Soluble TREM-1 is therefore secreted by human neutrophils in response to LPS challenge in a process involving de novo protein synthesis that is not accompanied by an upregulation of the TREM-1 receptor on the surfaces of the cells.

497. Granulocyte function is stimulated by a novel hexapeptide WKYMVm, in chemotherapy-treated cancer patients
Kim, H. et al
Exp. Hematol., 34, 407-413 (2006)

Bacterial infections are major life-threatening complications in patients receiving cytotoxic drugs. These infections generally occur during periods of neutropenia. It has been suggested that the incidence of neutropenia correlates with the incidence of infections. A synthetic hexapeptide, WKYMVm, which stimulates phosphoinositide hydrolysis in leukocytes, has been shown to activate microbicidal activities of human polymorphonuclear neutrophils. In this study, we evaluate whether WKYMVm stimulates bactericidal activity in neutrophils obtained from patients who received chemotherapy for solid tumors when they were neutropenic. Eight patients and 11 healthy controls were recruited for the study. Patient neutrophils, on day 0 and at 2 weeks after chemotherapy, were collected. Expression of the WKYMVm peptide receptor, on leukocytes, was analyzed by fluorescein-activated cell sorting. Neutrophil bactericidal assays were performed using both reactive oxygen species generation and intracellular killing. Expression of the WKYMVm peptide receptor on leukocytes showed no difference in the treated patients compared to healthy controls. WKYMVm increased bactericidal activities, in a dose-dependent fashion, of control neutrophils compared to treated patient neutrophils obtained on day 0. WKYMVm markedly stimulated bactericidal activity of treated patient neutrophils obtained at 2 weeks after chemotherapy compared to treated patient neutrophils obtained on day 0. WKYMVm augmented neutrophil bactericidal activity was noted at low concentration but was suppressed at higher concentrations of 5-fluorouracil. WKYMVm augmented neutrophil bactericidal activity was not suppressed by cisplatin. WKYMVm has the potential for increasing neutrophil bactericidal activity in chemotherapy-treated cancer patients.

498. G protein-mediated signal transduction is affected in primary biliary cirrhosis
Lesma, E.m et al
*Hepatol. Res., **35**, 45-52 (2006)*

Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by severe intraepatic cholestasis. Pruritus often occurs during the course of the illness. We designed a study aimed at assessing whether pruritus is associated with dysfunction of signal transduction. Seventeen female patients affected by PBC were enrolled into the study and divided in two groups according to severity of liver disease. Leukocytes were isolated from peripheral blood and Gi, Go and Gs protein expressions were evaluated by western blotting, while G protein function was assessed by measuring cyclic adenosine phosphate formation. The expression of all types of G proteins was increased in leukocytes of PBC patients. The basal adenylate activity was significally higher than control in patients with less severe liver disease, while it was lower than normal in those with severe liver disease. Incubation of patient leukocytes with guanosine triphosphate-γ-S and Gs protein activators failed to enhance cAMP production, while *N*-formyl-met-leu-phe was more effective in reducing cAMP production. The expression of all G proteins was non-selectively increased in PBC leukocytes, while adenylate cyclase activity was significantly modified. However, the observed changes in G proteins expression and in adenylate cyclase activity are not related to the presence of pruritus.

499. Simultaneous five cell-lineage flow cytometric analysis system for detection of leucocyte antibodies
Matsuyama, N. et al
Transfusion Med., *16(2), 111-118 (2006)*

Although flow cytometric (FCM) analysis is one of the most widely used approaches to screen the presence of leucocyte antibodies, it has several drawbacks. First, neutrophils and, especially, monocytes exhibit high background reactivity. Second, to determine antibody specificity, it is often necessary to examine not only neutrophils and monocytes but also other lineage cells including T cells, B cells and platelets. Therefore, we attempted to establish an FCM analysis system in which four lineages of leucocytes and platelets are simultaneously tested with low background. FCM analysis was performed using ethylene diamine tetraacetic acid-anticoagulated whole blood as cell sample without any cell preparation. Discrimination of five cell lineages was carried out based on the differences in forward vs. side scatter distribution and in the expression of CD4, CD20 and CD14. When anti-HNA (human neutrophil antigen) 1b antiserum was applied to HNA 1b-positive blood samples, only neutrophils were unambiguously positive. When anti-Naka (anti-CD36) antiserum was applied, only platelets and monocytes were positive. The background reactivity of neutrophils and monocytes was low enough. When anti-human leucocyte antigen (HLA) class II antiserum was tested, only B-lymphocytes and monocytes were positive. When anti-HLA class I antiserum was tested, all the five-lineage cells were positive.

500. Cytokine Responses to CpG DNA in Human Leukocytes
Ågren, J., Thiemermann, C., Foster, S.J., Wang, J.E. and Aasen, A.O.
Scand. J. Immunol., ***64(1)****, 61-68 (2006)*

Previous studies have implicated a role of bacterial DNA, containing unmethylated cytosine-phosphate-guanosine (CpG) motifs, in the initiation of systemic inflammation. This is based on the ability of CpG-DNA to act in synergy with lipopolysaccharide (LPS) to trigger tumor necrosis factor alpha (TNFα) production in murine monocytes and to enhance LPS toxicity in rodents. In this study we investigated the capacity of CpG-DNA to trigger and modulate cytokine responses in human leukocytes.
A human blood assay, as well as isolated cultures of monocytes and neutrophils, was exposed to the synthetic oligodeoxynucleotides (ODNs) CpG ODN (2006) and GpC ODN (2006-GC), alone or in combination with peptidoglycan or LPS. Plasma or supernatants were isolated and analyzed for TNFα, interleukin-1 beta (IL-1β), IL-6 and IL-8 by ELISA.
In the blood, 2006 (but not 2006-GC) induced the release of TNFα ($P < 0.05$) and possibly IL-1β and IL-6. IL-8 was induced in a CpG-independent manner. When co-administered with peptidoglycan, both ODNs enhanced the release of cytokines, but not consistently CpG dependent. When co-administered with LPS, only IL-8 values were enhanced, whereas IL-6 was suppressed at early time points. In monocyte and neutrophil cultures, CpG dependent induction of cytokine release was not observed. However, both ODNs inhibited LPS-induced IL-6.

501. Effects of Peripheral Cannabinoid Receptor Ligands on Motility and Polarization in Neutrophil-like HL60 Cells and Human Neutrophils
Kurihara, R. et al
J. Biol. Chem., 281(18), 12908-12918 (2006)

The possible role of the peripheral cannabinoid receptor (CB2) in neutrophil migration was investigated by using human promyelocytic HL60 cells differentiated into neutrophil-like cells and human neutrophils isolated from whole blood. Cell surface expression of CB2 on HL60 cells, on neutrophil-like HL60 cells, and on human neutrophils was confirmed by flow cytometry. Upon stimulation with either of the CB2 ligands JWH015 and 2-arachidonoylglycerol (2-AG), neutrophil-like HL60 cells rapidly extended and retracted one or more pseudopods containing F-actin in different directions instead of developing front/rear polarity typically exhibited by migrating leukocytes. Activity of the Rho-GTPase RhoA decreased in response to CB2 stimulation, whereas Rac1, Rac2, and Cdc42 activity increased. Moreover, treatment of cells with RhoA-dependent protein kinase (p160-ROCK) inhibitor Y27632 yielded cytoskeletal organization similar to that of CB2-stimulated cells. In human neutrophils, neither JWH015 nor 2-AG induced motility or morphologic alterations. However, pretreatment of neutrophils with these ligands disrupted *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced front/rear polarization and migration and also substantially suppressed fMLP-induced RhoA activity. These results suggest that CB2 might play a role in regulating excessive inflammatory response by controlling RhoA activation, thereby suppressing neutrophil migration.

502. Induction of polymorphonuclear leukocyte response by human cytomegalovirus
Jerström Skarman, P., Rahbar, A., Xie, X. and Söderberg-Naucler, C.
Microbes and Infection, 8, 1592-1601 (2006)

Neutrophils are important in the defense against bacterial infections, by ingesting and killing invading microorganisms. Because of the higher incidence of bacterial infections in patients with active human cytomegalovirus (HCMV) infections, we hypothesized that HCMV-infected neutrophils were inefficient in eliminating the bacteria. Therefore, we mock infected or infected neutrophils with HCMV by contact with HCMV-infected human pulmonary artery endothelial cells. We found that HCMV infection without *N*-formyl-

methionyl-leucyl-phenylalanine (fMLP) stimulation increased the surface expression of CD11b to the same extent as fMLP stimulation of mock infected cells. Also, HCMV-infected neutrophils became more efficient in phagocytosing serum opsonized yeast particles than mock infected cells. Furthermore, we observed an increase in intracellular free calcium and chemiluminescence in HCMV-infected cells, in response to fMLP compared to fMLP-treated mock cells. We also found that apoptosis was significantly inhibited in HCMV-infected neutrophils. In conclusion, our results suggest that neutrophils become more effective in performing their effector functions when infected with HCMV. Thus, the higher incidence of bacterial infections in HCMV patients might not be due directly to a dysfunction in the neutrophils. Instead, the fact that apoptosis is inhibited may cause over-reactive neutrophils to remain in the tissues, where they will start leaking their contents, damaging the tissues and contributing to inflammatory processes.

503. Species- and cell type-specific interactions between CD47 and human SIRPα
Subramanian, S., Parthasarathy, R., Sen, S., Boder, E.T. and Discher, D.E.
Blood, 107(6), 2548-2556 (2006)

CD47 on red blood cells (RBCs) reportedly signals "self" by binding SIRPα on phagocytes, at least in mice. Such interactions across and within species, from mouse to human, are not yet clear and neither is the relation to cell adhesion. Using human SIRPα1 as a probe, antibody-inhibitable binding to CD47 was found only with human and pig RBCs (not mouse, rat, or cow). In addition, CD47-mediated adhesion of human and pig RBCs to SIRPα1 surfaces resists sustained forces in centrifugation (as confirmed by atomic force microscopy) but only at SIRPα-coating densities far above those measurable on human neutrophils, monocytes, and THP-1 macrophages. While interactions strengthen with deglycosylation of SIRPα1, low copy numbers explain the absence of RBC adhesion to phagocytes under physiologic conditions and imply that the interaction being studied is not responsible for red cell clearance in humans. Evidence of clustering nonetheless suggests mechanisms of avidity enhancement. Finally, using the same CD47 antibodies and soluble SIRPα1, bone marrow-derived mesenchymal stem cells were assayed and found to display CD47 but not bind SIRPα1 significantly. The results thus demonstrate that SIRPα-CD47 interactions, which reportedly define self, exhibit cell type specificity and limited cross-species reactivity.

504. Dynamic shifts in LFA-1 affinity regulate neutrophil rolling, arrest, and transmigration on inflamed endothelium
Green, C.E. et al
Blood, 107(5), 2101-2111 (2006)

Polymorphonuclear leukocyte (**PMN**) recruitment to vascular endothelium during acute inflammation involves cooperation between selectins, G-proteins, and β_2-integrins. LFA-1 (CD11a/CD18) affinity correlates with specific adhesion functions because a shift from low to intermediate affinity supports rolling on ICAM-1, whereas high affinity is associated with shear-resistant leukocyte arrest. We imaged **PMN** adhesion on cytokine-inflamed endothelium in a parallel-plate flow chamber to define the dynamics of β_2-integrin function during recruitment and transmigration. After arrest on inflamed endothelium, high-affinity LFA-1 aligned along the uropod-pseudopod major axis, which was essential for efficient neutrophil polarization and subsequent transmigration. An allosteric small molecule inhibitor targeted to the I-domain stabilized LFA-1 in an intermediate-affinity conformation, which supported neutrophil rolling but inhibited cell polarization and abrogated transmigration. We conclude that a shift in LFA-1 from intermediate to high affinity during the transition from rolling to arrest provides the contact-mediated signaling and guidance necessary for **PMN** transmigration on inflamed endothelium.

505. A specific p47phox -serine phosphorylated by convergent MAPKs mediates neutrophil NADPH oxidase priming at inflammatory sites
Dang, P. et al
J. Clin. Invest., 116, 2033-2043 (2006)

Neutrophil NADPH oxidase plays a key role in host defense and in inflammation by releasing large amounts of superoxide and other ROSs. Proinflammatory cytokines such as GM-CSF and TNF-α prime ROS production by neutrophils through unknown mechanisms. Here we used peptide sequencing by tandem mass spectrometry to show that GM-CSF and TNF-α induce phosphorylation of Ser345 on $p47^{phox}$, a cytosolic component of NADPH oxidase, in human neutrophils. As Ser345 is located in the MAPK consensus sequence, we tested the effects of MAPK inhibitors. Inhibitors of the ERK1/2 pathway abrogated GM-CSF–induced phosphorylation of Ser345, while p38 MAPK inhibitor abrogated TNF-α–induced phosphorylation of Ser345. Transfection of HL-60 cells with a mutated $p47^{phox}$ (S345A) inhibited GM-CSF– and TNF-α–induced priming of ROS production. This event was also inhibited in neutrophils by a cell-permeable peptide containing a TAT-$p47^{phox}$-Ser345 sequence. Furthermore, ROS generation, $p47^{phox}$-Ser345 phosphorylation, and ERK1/2 and p38 MAPK phosphorylation were increased in synovial neutrophils from rheumatoid arthritis (RA) patients, and TAT-Ser345 peptide inhibited ROS production by these primed neutrophils. This study therefore identifies convergent MAPK pathways on Ser345 that are involved in GM-CSF– and TNF-α–induced priming of neutrophils and are activated in RA. Inhibition of the point of convergence of these pathways might serve as a novel antiinflammatory strategy.

506. Do Native and Polymeric α1-Antitrypsin Activate Human Neutrophils In Vitro?
Persson, C., Subramaniyam, D., Stevens, T. and Janciauskiene, S.

Chest, 129, 1683-1692 (2006)

Background: α_1-Antitrypsin (AAT)-Z deficiency is a risk factor for the development of COPD. Compared to wild-type M, AAT-Z has an increased tendency to polymerize, rendering it inactive as a serine proteinase inhibitor. It has been demonstrated that wild-type M- and Z-deficiency AAT polymers are chemotactic for human neutrophils. However, our own studies dispute a proinflammatory role for polymerized AAT-M and AAT-Z, suggesting rather that they are predominantly antiinflammatory, exhibiting inhibitory effects on lipopolysaccharide-stimulated human monocyte activation. The discrepancies between these observations prompted us to re-examine the effects of AAT.
Methods and results: The effects of native and polymerized AAT-M and AAT-Z with varying levels of endotoxin contamination (0.08 to 2.55 endotoxin units [EU]/mg protein) on human neutrophil chemotaxis and interleukin (IL)-8 release, *in vitro*, were evaluated. Neither native nor polymerized (M- or Z-deficient) AAT contaminated with low levels of endotoxin ($\leq$ 0.08 EU/mg protein) stimulated neutrophil chemotaxis, whereas N-formyl methionyl leucyl phenylalanine (fMLP), a positive control, increased chemotaxis fourfold. A small but nonsignificant increase in neutrophil chemotaxis, however, was observed with AAT preparations containing higher levels of endotoxin ($\geq$ 0.88 EU/mg protein), and significant chemotaxis occurred when AAT was spiked with either endotoxin or zymosan. In support, native and polymeric AAT-M with low endotoxin contamination completely inhibited neutrophil IL-8 release triggered by the zymosan, while AATs with high endotoxin contamination strongly induced IL-8 release and did not inhibit zymosan-stimulated IL-8 release.
Conclusions: The proinflammatory effects of native and polymeric AAT may be critically dependent on the presence of other cell activators, bacterial or otherwise, while pure preparations of AAT appear to exert predominantly antiinflammatory activity.

507. Imaging of Myeloperoxidase in Mice by Using Novel Amplifiable Paramagnetic Substrates
Chen, J.W., Sans, M.Q., Bogdanov, A. Jr. and Weissleder, R.
Radiology, 240, 473-481 (2006)

Purpose: To evaluate whether contrast agents for molecular magnetic resonance (MR) imaging can demonstrate the in vivo activity of myeloperoxidase, an enzyme that is secreted by stimulated polymorphonuclear leukocytes, monocytes, and macrophages during inflammation.
Materials and Methods: Animal experiments were approved by the animal care committee. Protocols for the procurement and use of human blood were approved by the institutional review board. Informed consent was obtained from each donor, and HIPAA guidelines were followed for humans. Two paramagnetic myeloperoxidase substrates—that is, gadolinium-5-hydroxytryptamide–tetraazacyclododecane tetraacetic acid (Gd-5-HT-DOTA) and Gd-*bis*-5-HT–diethylenetriaminepentaacetic acid (Gd-*bis*-5-HT-DTPA)—were synthesized. Indium 111-labeled *bis*-5-HT-DTPA was used to determine biodistribution and target localization. A total of 22 mice were used in three models. In the first model, human myeloperoxidase was embedded in a basement membrane matrix gel and was injected intramuscularly. In the second model, lipopolysaccharide (LPS) from *Escherichia coli* was embedded in a basement membrane matrix gel and was injected intramuscularly to induce endogenous myeloperoxidase secretion. In the third model, LPS was injected intramuscularly to induce myositis. Statistical significance was calculated for contrast-to-noise ratio (CNR) curves by using the Kolmogorov-Smirnov test.
Results: After the administration of Gd-*bis*-5-HT-DTPA, strong MR signal enhancement (up to 2.5-fold increase in CNR, $P < .001$) was observed in vivo for implants that contained human myeloperoxidase. In the LPS-induced myositis model, a smaller visible difference was seen (1.3-fold increase in CNR, $P < .001$), which was consistent with the fact that endogenous mouse myeloperoxidase is only about 10%–20% as active as human myeloperoxidase. Prolonged contrast material enhancement was observed in the myeloperoxidase-containing areas that were injected with Gd-5-HT-DOTA or Gd-*bis*-5-HT-DTPA but was not observed in areas that were injected with Gd-DTPA or Gd-dopamine-DOTA ($P < .05$). Single photon emission computed tomography combined with computed tomography was used to confirm the increased retention of contrast agents at sites that contained human myeloperoxidase, and the results of biodistribution studies demonstrated a more than fourfold increase radiotracer accumulation at these sites.
Conclusion: Human and mouse myeloperoxidase activity in myeloperoxidase implants and inflamed tissues can be visualized and reported in vivo by using myeloperoxidase-sensitive "smart" molecular imaging probes.

508. Taurine chloramine inhibits PMA-stimulated superoxide production in human neutrophils perhaps by inhibiting phosphorylation and translocation of $p47^{phox}$
Choi, H.S., Cha, Y-N. and Kim, C.
Int. Immunopharmacol.,6, 1431-1440 (2006)

Neutrophils produce microbicidal oxidants to destroy the invading pathogens using nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a membrane-associated enzyme complex that generates superoxide anion (O_2^-). Upon stimulation, the cytosolic components of NADPH oxidase, $p47^{phox}$ and $p67^{phox}$ and the small GTPase Rac move to phagosomal and plasma membranes where they become associated with the membrane components of NADPH oxidase, $gp91^{phox}$ and $p22^{phox}$ and express enzyme activity. We previously showed that taurine chloramine (Tau-Cl) inhibits O_2^- production in mouse peritoneal neutrophils (Kim, 1996). In the present study, we investigated the mechanisms underlying Tau-Cl-derived inhibition on O_2^- production using a human myeloid leukemia cell

line, PLB-985 cell, which has been differentiated into neutrophil-like cell. Tau-Cl inhibited the phorbol myristate acetate (PMA)-elicited O_2^- production as previously observed in murine peritoneal neutrophils. Translocation of $p47^{phox}$, $p67^{phox}$ and Rac was increased in response to PMA, and Tau-Cl inhibited the PMA-stimulated translocation of $p47^{phox}$ and $p67^{phox}$ to plasma membrane without affecting the translocation of Rac. In addition, Tau-Cl inhibited the PMA-derived phosphorylation of $p47^{phox}$, a requirement for the translocation of cytosolic NADPH oxidase component to the plasma membrane. These results suggest that Tau-Cl inhibits PMA-elicited O_2^- production in PLB-985 granulocytes by inhibiting phosphorylation of $p47^{phox}$ and translocation of $p47^{phox}$ and $p67^{phox}$, eventually blocking the assembly of NADPH oxidase complex.

509. Myeloperoxidase genetic polymorphisms modulate human neutrophil enzyme activity: Genetic determinants for atherosclerosis?
Chevrier, I., Tregouet, D-A., Massonnet-Castel, S., Beaune, P. and Loriot, M-A.
Atherosclerosis, 188, 150-154 (2006)

Objective
Myeloperoxidase (MPO), an abundant leukocyte hemoprotein has been linked to atherosclerosis and cardiovascular disease. We previously found new genetic polymorphisms in *MPO* gene. The purpose of this study was to evaluate the influences of these polymorphisms on human neutrophil MPO activity by means of haplotype analysis.
Methods and results
Neutrophils from 102 blood donors were isolated and MPO activity was measured, while subjects were genotyped for polymorphisms located in *MPO* gene 5′non-coding region (−1940A > G, −638C > A, −463G > A and −129G > A) and in coding region (V53F, M251T, A332V, I642L and I717V). Single-point analysis showed that the −638C > A and the V53F polymorphisms were significantly associated with MPO activity, and haplotype analysis confirmed that two haplotypes, one carrying the −638A allele and the other carrying the 53F allele, resulted an increase in MPO activity.
Conclusion
Since MPO is suspected to be a bio-marker in cardiovascular disease, −638C > A and V53F polymorphisms associated with increased enzymatic activity could be genetic determinants for cardiovascular disease risk.

510. Electrochemical characterization and application of azurin-modified gold electrodes for detection of superoxide
Shleev, S., Wetterö, J., Magnusson, K-E. and Ruzgas, T.
*Biosensors and Bioelectronics, **22**, 213-219 (2006)*

A novel biosensor for superoxide radical ($O_2^{\bullet -}$) detection based on *Pseudomonas aeruginosa* azurin immobilized on gold electrode was designed. The rate constant of azurin reduction by $O_2^{\bullet -}$ was found to be 10^5 M^{-1} s^{-1} in solution and five times lower, i.e., 0.2×10^5 M^{-1} s^{-1}, for azurin coupled to gold by 3,3′-dithiobis(sulfosuccinimidylpropionate) (DTSSP). The electron transfer rate between the protein and the electrode ranged from 2 to 6 s^{-1}. The sensitivity of this biosensor to $O_2^{\bullet -}$ was 6.8×10^2 A m^{-2} M^{-1}. The response to the interference substances, such as uric acid, H_2O_2, and dimethylsulfoxide was negligible below 10 μM. The electrode was applied in three $O_2^{\bullet -}$ generating systems: (i) xanthine oxidase (XOD), (ii) potassium superoxide (KO_2), and (iii) stimulated neutrophil granulocytes. The latter was compared with luminol-amplified chemiluminescence. The biosensor responded to $O_2^{\bullet -}$ in all three environments, and the signals were antagonized by superoxide dismutase.

511. In Silico Identification and Biological Evaluation of Antimicrobial Peptides Based on Human Cathelicidin LL-37
Sigurdardottir, T et al
*Antimicrob. Agent Chemother., **50**, 2983-2989 (2006)*

Bacterial lipopolysaccharides (LPS) are important triggers of the widespread inflammatory response, which contributes to the development of multiple organ failure during sepsis. The helical 37-amino-acid-long human antimicrobial peptide LL-37 not only possesses a broad-spectrum antimicrobial activity but also binds and neutralizes LPS. However, the use of LL-37 in sepsis treatment is hampered by the fact that it is also cytotoxic. To find a less toxic analog of LL-37, we used in silico analysis to identify amphipathic helical regions of LL-37. A 21-amino-acid fragment (GKE) was synthesized, the biological actions of which were compared to those of two equally long peptides derived from the N and C termini of LL-37 as well as native LL-37. GKE displayed antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Candida parapsilosis* that was similar to or even stronger than LL-37. GKE, as well as the equally long control peptides, attracted granulocytes in a fashion similar to that of LL-37, while only GKE was as potent as LL-37 in inhibiting LPS-induced vascular nitric oxide production. GKE caused less hemolysis and apoptosis in human cultured smooth muscle cells than LL-37. In summary, we have identified an active domain of LL-37, GKE, which displays antimicrobial activity in vitro and LPS-binding activity similar to those of LL-37 but is less toxic. GKE therefore holds promise as a template for the development of peptide antibiotics for the treatment of sepsis.

512. Interaction of TLR2 and TLR4 Ligands with the N-terminal Domain of Gp96 Amplifies Innate and Adaptive Immune Responses
Warger, T. et al

J. Biol. Chem., ***281(32)****, 22545-22553 (2006)*

Activation of dendritic cells by ligands for Toll-like receptors (TLR) is a crucial event in the initiation of innate and adaptive immune responses. Several classes of TLR ligands have been identified that interact with distinct members of the TLR-family. TLR4 ligands include lipopolysaccharide derived from different Gram-negative bacteria and viral proteins. Recent reports have demonstrated the TLR-mediated activation of dendritic cells by heat shock proteins (HSPs). However, doubts were raised as to what extent this effect was due to lipopolysaccharide contaminations of the HSP preparations. We re-examined this phenomenon using Gp96 or its N-terminal domain, nominally endotoxin-free (<0.5 enzyme units/mg). As described previously, innate immune cells are activated by Gp96 at high concentrations (≥50 μg/ml) but not at lower concentrations. However, preincubation of low amounts of Gp96 with TLR2 and TLR4 ligands at concentrations unable to activate dendritic cells by themselves results in the production of high levels of proinflammatory cytokines, up-regulation of activation markers, and amplification of T cell activation. Our results provide significant new insights into the mechanism of HSP-mediated dendritic cell activation and present a new function of HSPs in the amplification of dendritic cell activation by bacterial products and induction of adaptive immune responses.

513. The phosphoinositide-binding protein p40phox activates the NADPH oxidase during FcγIIA receptor–induced phagocytosis
Suh, C-I. Et al
J. Exp. Med., ***203(8)****, 1915-1925 (2006)*

Superoxide produced by the phagocyte reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is essential for host defense. Enzyme activation requires translocation of $p67^{phox}$, $p47^{phox}$, and Rac-GTP to flavocytochrome b_{558} in phagocyte membranes. To examine the regulation of phagocytosis-induced superoxide production, flavocytochrome b_{558}, $p47^{phox}$, $p67^{phox}$, and the FcγIIA receptor were expressed from stable transgenes in COS7 cells. The resulting COS^{phox}FcγR cells produce high levels of superoxide when stimulated with phorbol ester and efficiently ingest immunoglobulin (Ig)G-coated erythrocytes, but phagocytosis did not activate the NADPH oxidase. COS7 cells lack $p40^{phox}$, whose role in the NADPH oxidase is poorly understood. $p40^{phox}$ contains SH3 and phagocyte oxidase and Bem1p (PB1) domains that can mediate binding to $p47^{phox}$ and $p67^{phox}$, respectively, along with a PX domain that binds to phosphatidylinositol-3-phosphate (PI(3)P), which is generated in phagosomal membranes. Expression of $p40^{phox}$ was sufficient to activate superoxide production in COS^{phox}FcγR phagosomes. FcγIIA-stimulated NADPH oxidase activity was abrogated by point mutations in $p40^{phox}$ that disrupt PI(3)P binding, or by simultaneous mutations in the SH3 and PB1 domains. Consistent with an essential role for PI(3)P in regulating the oxidase complex, phagosome NADPH oxidase activation in primary macrophages ingesting IgG-coated beads was inhibited by phosphatidylinositol 3 kinase inhibitors to a much greater extent than phagocytosis itself. Hence, this study identifies a role for $p40^{phox}$ and PI(3)P in coupling FcγR-mediated phagocytosis to activation of the NADPH oxidase.

514. Role of DNA methylation for expression of novel stem cell marker CDCP1 in hematopoietic cells
Kimura, H. et al
Leukemia, ***20****, 1551-1556 (2006)*

CDCP1, a novel stem cell marker, is expressed in hematopoietic cell line K562 but not in Jurkat. When *CDCP1* promoter was transfected exogenously, Jurkat showed comparable promoter activity with K562, suggesting that the factor to enhance transcription was present but interfered to function in Jurkat. The reporter assay and si-RNA-mediated knockdown experiment revealed that zfp67, a zinc-finger protein, enhanced *CDCP1* transcription. Amount of zfp67 in Jurkat was comparable with K562, but chromatin immunoprecipitation showed that zfp67 bound to *CDCP1* promoter in K562 but not in Jurkat. There are CpG sequences around the promoter of *CDCP1*, which were heavily methylated in Jurkat but not in K562. Addition of demethylating reagent to Jurkat induced *CDCP1* expression, and increased the zfp67 binding to *CDCP1* promoter. Among normal hematopoietic cells such as $CD34^{+}CD38^{-}$ cells, lymphocytes and granulocytes, inverse correlation between proportion of methylated CpG sequences and *CDCP1* expression level was found. Demethylation of CpG sequences in lymphocytes, in which CpG sequences were heavily methylated, induced *CDCP1* expression and its expression level further increased through zfp67 overexpression. The methylation of DNA appeared to regulate the cell-type-specific expression of *CDCP1* through the control of interaction between chromatin DNA and transcription factors.

515. Identification of Antigenic Components of Staphylococcus epidermidis Expressed during Human Infection
Pourmand, M.R., Clarke, S.R., Schuman, R.F., Mond, J.J. and Foster, S.J.
Infect. Immun., ***74(8)****, 4644-4654 (2006)*

A spectrum of in vivo-expressed *Staphylococcus epidermidis* antigens was identified by probing a bacteriophage lambda library of *S. epidermidis* genomic DNA with human serum from infected and uninfected individuals. This analysis resulted in identification of 53 antigen-encoding loci. Six antigenic polypeptides were expressed from these loci and purified. These polypeptides were the propeptide, mature amidase, and repeat sequence domains of the major autolysin AtlE, GehD (lipase), and two members of a conserved family of surface proteins (ScaA [AaE] and ScaB). AtlE, ScaA, and ScaB all exhibit human ligand binding capacity. Screening a bank of human serum samples revealed that there were significant increases in the amounts of reactive immunoglobulin G in infected individuals compared to the amounts in healthy individuals for the repeat sequence and mature amidase domains of AtlE, ScaB, and GehD.

Vaccination of mice with recombinant antigens stimulated an immune response which in vitro opsonized *S. epidermidis*. In this study we identified prospective candidate antigens for prophylaxis or immunotherapy to control disease.

516. Species- and cell type-specific interactions between CD47 and human SIRPα
Subramanian, S., Parthasarathy, R., Sen, S., Boder, E.T. and Discher, D.E.
*Blood, **107(6)**, 2548-2556 (2006)*

CD47 on red blood cells (RBCs) reportedly signals "self" by binding SIRPα on phagocytes, at least in mice. Such interactions across and within species, from mouse to human, are not yet clear and neither is the relation to cell adhesion. Using human SIRPα1 as a probe, antibody-inhibitable binding to CD47 was found only with human and pig RBCs (not mouse, rat, or cow). In addition, CD47-mediated adhesion of human and pig RBCs to SIRPα1 surfaces resists sustained forces in centrifugation (as confirmed by atomic force microscopy) but only at SIRPα-coating densities far above those measurable on human neutrophils, monocytes, and THP-1 macrophages. While interactions strengthen with deglycosylation of SIRPα1, low copy numbers explain the absence of RBC adhesion to phagocytes under physiologic conditions and imply that the interaction being studied is not responsible for red cell clearance in humans. Evidence of clustering nonetheless suggests mechanisms of avidity enhancement. Finally, using the same CD47 antibodies and soluble SIRPα1, bone marrow-derived mesenchymal stem cells were assayed and found to display CD47 but not bind SIRPα1 significantly. The results thus demonstrate that SIRPα-CD47 interactions, which reportedly define self, exhibit cell type specificity and limited cross-species reactivity.

517. Genetic heterogeneity of granulocytes for the JAK2 V617F mutation in essential thrombocythaemia: implications for mutation detection in peripheral blood
Stevenson, W.S. et al
*Pathology, **38(4)**, 336-342 (2006)*

Aims: The molecular pathogenesis of essential thrombocythaemia (ET) is heterogeneous. We aimed to determine the relative sensitivity of four separate molecular assays used to detect the presence of the *JAK2* V617F mutation in peripheral blood from patients with essential thrombocythaemia and related myeloproliferative disorders.
Methods: Purified granulocytes from 60 patients were analysed for the presence of the *JAK2* V617F mutation by direct sequencing, denaturing high□performance liquid chromatography (DHPLC), allele□specific polymerase chain reaction (PCR) and allele□specific enrichment. Clinical data were collected for all patients and correlated with assay results.
Results: Direct sequencing and DHPLC were relatively insensitive assays for mutation detection, together identifying only 53% of the *JAK2* V617F positive cases of ET. Allele□specific PCR and allele□specific enrichment were significantly more sensitive assays and were able to identify additional ET patients that were positive for the *JAK2* V617F mutation in only a minority of circulating granulocytes. Enrichment for the mutation was demonstrated in blood platelets from two of these patients.
Conclusions: The observed biological difference in circulating granulocyte involvement by the *JAK2* V617F clone necessitates a sensitive molecular assay for the diagnostic investigation of thrombocytosis.

518. Correlation analysis of two-dimensional gel electrophoretic protein patterns and biological variables
Van Belle, W. et al
BMC Bioinformatics, 7, 1-16 (2006)

Background
Two-dimensional gel electrophoresis (2DE) is a powerful technique to examine post-translational modifications of complexly modulated proteins. Currently, spot detection is a necessary step to assess relations between spots and biological variables. This often proves time consuming and difficult when working with non-perfect gels. We developed an analysis technique to measure correlation between 2DE images and biological variables on a pixel by pixel basis. After image alignment and normalization, the biological parameters and pixel values are replaced by their specific rank. These rank adjusted images and parameters are then put into a standard linear Pearson correlation and further tested for significance and variance.
Results
We validated this technique on a set of simulated 2DE images, which revealed also correct working under the presence of normalization factors. This was followed by an analysis of p53 2DE immunoblots from cancer cells, known to have unique signaling networks. Since p53 is altered through these signaling networks, we expected to find correlations between the cancer type (acute lymphoblastic leukemia and acute myeloid leukemia) and the p53 profiles. A second correlation analysis revealed a more complex relation between the differentiation stage in acute myeloid leukemia and p53 protein isoforms.
Conclusion
The presented analysis method measures relations between 2DE images and external variables without requiring spot detection, thereby enabling the exploration of biosignatures of complex signaling networks in biological systems.

519. Molecular profiling of signalling proteins for effects induced by the anti-cancer compound GSAO with 400 antibodies
Cadd, V.A., Hogg, P.J., Harris, A.L. and Feller, S.M.
*BMC Cancer, **6**, 1-10 (2006)*

Background
GSAO (4-[N-[S-glutathionylacetyl]amino] phenylarsenoxide) is a hydrophilic derivative of the protein tyrosine phosphatase inhibitor phenylarsine oxide (PAO). It inhibits angiogenesis and tumour growth in mouse models and may be evaluated in a phase I clinical trial in the near future. Initial experiments have implicated GSAO in perturbing mitochondrial function. Other molecular effects of GSAO in human cells, for example on the phosphorylation of proteins, are still largely unknown.
Methods
Peripheral white blood cells (PWBC) from healthy volunteers were isolated and used to profile effects of GSAO vs. a control compound, GSCA. Changes in site-specific phosphorylations, other protein modifications and expression levels of many signalling proteins were analysed using more than 400 different antibodies in Western blots.
Results
PWBC were initially cultured in low serum conditions, with the aim to reduce basal protein phosphorylation and to increase detection sensitivity. Under these conditions pleiotropic intracellular signalling protein changes were induced by GSAO. Subsequently, PWBC were cultured in 100% donor serum to reflect more closely *in vivo* conditions. This eliminated detectable GSAO effects on most, but not all signalling proteins analysed. Activation of the MAP kinase Erk2 was still observed and the paxillin homologue Hic-5 still displayed a major shift in protein mobility upon GSAO-treatment. A GSAO induced change in Hic-5 mobility was also found in endothelial cells, which are thought to be the primary target of GSAO *in vivo*.
Conclusion
Serum conditions greatly influence the molecular activity profile of GSAO *in vitro*. Low serum culture, which is typically used in experiments analysing protein phosphorylation, is not suitable to study GSAO activity in cells. The signalling proteins affected by GSAO under high serum conditions are candidate surrogate markers for GSAO bioactivity *in vivo* and can be analysed in future clinical trials. GSAO effects on Hic-5 in endothelial cells may point to a new intracellular GSAO target.

520. Induction of Neutrophil Chemotaxis by the Quorum-Sensing Molecule N-(3-Oxododecanoyl)-L-Homoserine Lactone
Zimmermann, S. et al
*Infect. Immun., **74(10)**, 5687-5692 (2006)*

Acyl homoserine lactones are synthesized by *Pseudomonas aeruginosa* as signaling molecules which control production of virulence factors and biofilm formation in a paracrine manner. We found that *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL), but not its 3-deoxo isomer or acyl-homoserine lactones with shorter fatty acids, induced the directed migration (chemotaxis) of human polymorphonuclear neutrophils (PMN) in vitro. By use of selective inhibitors a signaling pathway, comprising phosphotyrosine kinases, phospholipase C, protein kinase C, and mitogen-activated protein kinase C, could be delineated. In contrast to the well-studied chemokines complement C5a and interleukin 8, the chemotaxis did not depend on pertussis toxin-sensitive G proteins, indicating that 3OC12-HSL uses another signaling pathway. Strong evidence for the presence of a receptor for 3OC12-HSL on PMN was derived from uptake studies; by use of radiolabeled 3OC12-HSL, specific and saturable binding to PMN was seen. Taken together, our data provide evidence that PMN recognize and migrate toward a source of 3OC12-HSL (that is, to the site of a developing biofilm). We propose that this early attraction of PMN could contribute to prevention of biofilm formation.

521. Gingipains of Porphyromonas gingivalis Modulate Leukocyte Adhesion Molecule Expression Induced in Human Endothelial Cells by Ligation of CD99
Yun, P.L.W., DeCarlo, A.A. and Hunter, N.
*Infect. Immun., **74(3)**, 1661-1672 (2006)*

Porphyromonas gingivalis has been implicated as a key etiologic agent in the pathogenesis of destructive chronic periodontitis. Among virulence factors of this organism are cysteine proteinases, or gingipains, that have the capacity to modulate host inflammatory defenses. Intercellular adhesion molecule expression by vascular endothelium represents a crucial process for leukocyte transendothelial migration into inflamed tissue. Ligation of CD99 on endothelial cells was shown to induce expression of endothelial leukocyte adhesion molecule 1, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and major histocompatibility complex class II molecules and to increase adhesion of leukocytes. CD99 ligation was also found to induce nuclear translocation of NF-κB. These results indicate that endothelial cell activation by CD99 ligation may lead to the up-regulation of adhesion molecule expression via NF-κB activation. However, pretreatment of endothelial cells with gingipains caused a dose-dependent reduction of adhesion molecule expression and leukocyte adhesion induced by ligation of CD99 on endothelial cells. The data provide evidence that the gingipains can reduce the functional expression of CD99 on endothelial cells, leading indirectly to the disruption of adhesion molecule expression and of leukocyte recruitment to inflammatory foci.

522. Nonimmune immunoglobulin binding and multiple adhesion characterize Plasmodium falciparum-infected erythrocytes of placental origin
Rasti, N. et al
*PNAS, **103(37)**, 13795-13800 (2006)*

The harmful effects of pregnancy-associated malaria (PAM) are engendered by the heavy sequestration of *Plasmodium falciparum*-parasitized RBCs in the placenta. It is well documented that this process is mediated by interactions of parasite-encoded variant surface antigens and placental receptors. A *P. falciparum* erythrocyte membrane protein 1 variant, VAR2CSA, and the placental receptor chondroitin sulfate A (CSA) are currently the focus of PAM research. A role for immunoglobulins (IgG and IgM) from normal human serum and hyaluronic acid as additional receptors in placental sequestration have also been suggested. We show here (*i*) that CSA and nonimmune IgG/IgM binding are linked phenotypes of *in vitro*-adapted parasites, (*ii*) that a VAR2CSA variant shown to bind CSA also harbors IgG- and IgM-binding domains (DBL2-X, DBL5-ε, and DBL6-ε), and (*iii*) that IgG and IgM binding and adhesion to multiple receptors (IgG/IgM/HA/CSA) rather than the exclusive binding to CSA is a characteristic of fresh Ugandan placental isolates. These findings are of importance for the understanding of the pathogenesis of placental malaria and have implications for the ongoing efforts to develop a global PAM vaccine.

523. The cannabinoid agonist WIN 55,212-2 inhibits TNF-α-induced neutrophil transmigration across ECV304 cells
Nilsson, O., Fowler, C.J. and Jacobsson, S.O.P.
*Eur. J. Pharmacol., **547**, 165-173 (2006)*

Cannabinoids are known to possess both anti-inflammatory and neuroprotective effects. In the present study, we have investigated the ability of cannabinoids to inhibit the transmigration of neutrophils in response to chemotaxic stimuli. The cannabinoid receptor agonist WIN 55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate) significantly decreased the number of migrating neutrophils across a monolayer of tumour necrosis factor alpha (TNF-α) activated ECV304 cells at concentrations ≥ 1 μM. In contrast, the agonists HU210 and CP 55,940 (0.01–1 μM) and the endocannabinoid anandamide (0.1–10 μM) were without significant effect on the response to TNF-α. The ability of WIN 55,212-2 to reduce the neutrophil transmigration was still seen in the presence of the cannabinoid CB_1 receptor antagonist/inverse agonist AM251 (0.1–1 μM) and the cannabinoid CB_2 receptor antagonist/inverse agonist AM630 (0.1–1 μM). TNF-α treatment of ECV304 cells caused release of interleukin-8 (IL-8), but WIN 55,212-2 did not affect either the ability of neutrophils to migrate across chemotaxis plates in response to an IL-8 stimulus, or to change the percentage of CXC 1 and CXC 2 receptors expressed by the neutrophils. WIN 55,212-2 at a concentration of 1 μM, but not at lower concentrations, produced a significant inhibition of IL-8 release from ECV304 cells in response to TNF-α-stimulation. Thus WIN 55,212-2 reduces the transmigration of neutrophils across a monolayer of TNF-α-activated ECV304 cells by an indirect action upon the release of IL-8 and/or other chemokine release from the ECV304 cells, and that this effect is brought about mainly by a cannabinoid CB receptor-independent mechanism.

524. SK channels mediate NADPH oxidase-independent reactive oxygen species production and apoptosis in granulocytes
Fay, A.J., Qian, X., Jan, Y.N. and Jan, L.Y.
*PNAS, **103(46)**, 17548-17553 (2006)*

Neutrophils are immune cells that bind to, engulf, and destroy bacterial and fungal pathogens in infected tissue, and their clearance by apoptosis is essential for the resolution of inflammation. Killing involves both oxidative and nonoxidative processes, the oxidative pathway requiring electrogenic production of superoxide by the membrane-bound NADPH oxidase complex. A variety of stimuli, from bacterial chemotactic peptides to complement- or IgG-opsonized microbes, can induce the production of reactive oxygen species (ROS) by neutrophils, presumably by means of NADPH oxidase. We report here that 1-ethyl-2-benzimidazolinone (1-EBIO), an activator of Ca^{2+}-activated potassium channels of small conductance (SK) and intermediate conductance (IK), causes production of superoxide and hydrogen peroxide by neutrophils and granulocyte-differentiated PLB-985 cells. This response can be partially inhibited by the SK blocker apamin, which inhibits a Ca^{2+}-activated K^+ current in these cells. Analysis of RNA transcripts indicates that channels encoded by the SK3 gene carry this current. The effects of 1-EBIO and apamin are independent of the NADPH oxidase pathway, as demonstrated by using a PLB-985 cell line lacking the gp91*phox* subunit. Rather, 1-EBIO and apamin modulate mitochondrial ROS production. Consistent with the enhanced ROS production and K^+ efflux mediated by 1-EBIO, we found that this SK opener increased apoptosis of PLB-985 cells. Together, these findings suggest a previously uncharacterized mechanism for the regulation of neutrophil ROS production and programmed cell death.

525. Peroxisome Proliferator-Activated Receptor α (PPARα) down-regulation in cystic fibrosis lymphocytes
Reynders, V. et al
Respiratory Res., 7, 104 (2006)

Background
PPARs exhibit anti-inflammatory capacities and are potential modulators of the inflammatory response. We hypothesized that their expression and/or function may be altered in cystic fibrosis (CF), a disorder characterized by an excessive host inflammatory response.
Methods
PPARα, β and γ mRNA levels were measured in peripheral blood cells of CF patients and healthy subjects via RT-PCR. PPARα protein expression and subcellular localization was determined via western blot and immunofluorescence, respectively. The activity of PPARα was analyzed by gel shift assay.
Results

In lymphocytes, the expression of PPARα mRNA, but not of PPARβ, was reduced (-37%; $p < 0.002$) in CF patients compared with healthy persons and was therefore further analyzed. A similar reduction of PPARα was observed at protein level (-26%; $p < 0.05$). The transcription factor was mainly expressed in the cytosol of lymphocytes, with low expression in the nucleus. Moreover, DNA binding activity of the transcription factor was 36% less in lymphocytes of patients ($p < 0.01$). For PPARα and PPARβ mRNA expression in monocytes and neutrophils, no significant differences were observed between CF patients and healthy persons. In all cells, PPARγ mRNA levels were below the detection limit.
Conclusion
Lymphocytes are important regulators of the inflammatory response by releasing cytokines and antibodies. The diminished lymphocytic expression and activity of PPARα may therefore contribute to the inflammatory processes that are observed in CF.

526. Lipopolysaccharide-Activated CD4+CD25+ T Regulatory Cells Inhibit Neutrophil Function and Promote Their Apoptosis and Death
Lewkowicz, P., Lewkowicz, N., Sasiak, A. and Tchorzewski, H.
*J. Immunol., **177**, 7155-7163 (2006)*

$CD4^+CD25^+$ T regulatory (Treg) cells play a central role in the suppression of immune response and prevention of autoimmune reactions. Pathogen recognition receptors expressed by immune cells, such as TLRs, may provide a critical link between the innate and adaptive immune systems. There is also evidence that TLR ligands can directly modulate the suppressive capacity of Treg cells. Here, we showed that $CD4^+CD25^+$ Treg cells affect neutrophil function and survival and that the TLR4 ligand is involved in the regulation of the cell interactions. We found that LPS-activated Treg cells inhibit reactive oxygen intermediates and cytokine production by neutrophils. Moreover, Treg cells reverse LPS-induced survival of neutrophils and promote their apoptosis and death. We also found that TCR-activated Treg cells induce the same effects on polymorphonuclear neutrophils as those achieved by TLR4 stimulation. Importantly, the suppressive potential of $CD4^+CD25^+$ Treg cells induced by LPS seems to be partially IL-10 and TGF-β dependent, whereas anti-CD3/CD28 stimulation is rather contact dependent. Together, these observations suggest that Treg cells have the ability to directly regulate neutrophil function and life span when both types of the cells are exposed to LPS.

527. The Role of Neutrophils and Oxygen Free Radicals in Post-Operative Adhesions
Ten Raa, S. et al
*J. Surg. Res., **136(1)**, 45-52 (2006)*

Background
Postoperative intra-abdominal adhesion formation remains a major surgical problem. Surgery induces an inflammatory reaction, which is responsible for adhesion formation. Neutrophils and their oxygen-free radicals are key mediators in the early post-operative inflammatory response. The present study evaluates the effect of either blocking the influx of neutrophils or its products by scavenging oxygen-free radicals on adhesion formation.
Materials and methods
Reproducible rat models were used to induce post-surgical intra-abdominal adhesions. In the first experiment anti-neutrophil serum (ANS) was used to prevent neutrophils from entering the peritoneal cavity after surgery. In a second experiment superoxide dismutase (SOD), catalase, and mannitol were tested, to scavenge the superoxide, hydrogen peroxide, and hydroxyl radicals, respectively.
Results
In positive control groups 69 to 76% of the area of interest contained adhesions. In all experimental groups, except for mannitol, a significant reduction in post-surgical adhesion formation could be achieved. ANS reduced adhesion formation by 38% ($P < 0.001$) and SOD/catalase by 42% ($P < 0.01$). Mannitol could not reduce adhesion formation.
Conclusions
Intra-abdominal influx of neutrophils after surgical peritoneal trauma plays an important role in post-operative adhesion formation. Preventing the intra-abdominal influx of neutrophils in the early post-operative inflammatory reaction can reduce adhesion formation, but an even more selective approach, by scavenging its products, proved as efficient.

528. TLR2/TLR4-Independent Neutrophil Activation and Recruitment upon Endocytosis of Nucleosomes Reveals a New Pathway of Innate Immunity in Systemic Lupus Erythematosus
Rönnefarth, V.M. et al
*J. Immunol., **177**, 7740-7749 (2006)*

The nucleosome is a major autoantigen in systemic lupus erythematosus (SLE); it can be detected as a circulating complex in the serum, and nucleosomes have been suggested to play a key role in disease development. In the present study, we show for the first time that physiological concentrations of purified nucleosomes trigger innate immunity. The nucleosomes are endocytosed and induce the direct activation of human neutrophils (polymorphonuclear leukocytes (PMN)) as revealed by CD11b/CD66b up-regulation, IL-8 secretion, and increased phagocytic activity. IL-8 is a neutrophil chemoattractant detected in high concentrations in the sera of patients, and IL-8 secretion might thus result in enhanced inflammation, as observed in lupus patients, via an amplification loop. Nucleosomes act as free complexes requiring no immune complex formation and independently of the presence of unmethylated CpG DNA motifs. Both normal

and lupus neutrophils are sensitive to nucleosome-induced activation, and activation is not due to endotoxin or high-mobility group box 1 contamination. In mice, i.p. injection of purified nucleosomes induces neutrophil activation and recruitment in a TLR2/TLR4-independent manner. Importantly, neutrophils have been suggested to link innate and adaptive immunity. Thus, nucleosomes trigger a previously unknown pathway of innate immunity, which may partially explain why peripheral tolerance is broken in SLE patients.

529. Inhalation of activated protein C inhibits endotoxin-induced pulmonary inflammation in mice independent of neutrophil recruitment
Slofstra, S.H. et al
*Immunopharmacol. and Inflammation, **149**, 740-746 (2006)*

Background and purpose:

Intravenous administration of recombinant human activated protein C (rhAPC) is known to reduce lipopolysaccharide (LPS)-induced pulmonary inflammation by attenuating neutrophil chemotaxis towards the alveolar compartment. Ideally, one would administer rhAPC in pulmonary inflammation at the site of infection to minimize the risk of systemic bleeding complications. In this study, we therefore assessed the effect of inhaled rhAPC in a murine model of acute lung injury.
Experimental approach:

Mice were exposed to LPS (0.5 mg kg^{-1}: intranasally) to induce acute lung injury. 30 minutes before and 3 hours after LPS exposure mice were subjected to vehicle or rhAPC inhalation (25 or 100 μg per mouse in each nebulization). In order to establish whether rhAPC inhalation affects neutrophil recruitment, neutrophil migration was determined *in vitro* using a trans-well migration assay.
Key results:

rhAPC inhalation dose-dependently decreased LPS-induced coagulation and inflammation markers in bronchoalveolar lavage fluid (BALF), reduced protein leakage into the alveolar space and improved lung function. In contrast, rhAPC did not prevent LPS-induced neutrophil recruitment into the alveolar space.
Neutrophil migration *in vitro* towards FCS or interleukin (IL)-8 was significantly inhibited by pretreatment with rhAPC (0.01-10 μg ml^{-1}], whereas rhAPC (10 μg ml^{-1}) added to the chemoattractant (modelling for topical rhAPC administration) did not affect neutrophil migration towards FCS or IL-8.
Conclusions and Implications:

rhAPC inhalation significantly diminished LPS-induced pulmonary inflammation. The benefit of inhaled rhAPC appeared not to involve attenuation of neutrophil recruitment, in contrast to its effects after intravenous administration.

530. Herpes virus entry mediator synergizes with Toll-like receptor mediated neutrophil inflammatory responses
Hasselmeyer, P. et al
*Immunology, **119(3)**, 404-411 (2006)*

In microbial infections polymorphnuclear neutrophils (PMN) constitute a major part of the innate host defence, based upon their ability to rapidly accumulate in inflamed tissues and clear the site of infection from microbial pathogens by their potent effector mechanisms. The recently described transmembrane receptor herpes virus entry mediator (HVEM) is a member of the tumour necrosis factor receptor super family and is expressed on many haematopoietic cells, including T cells, B cells, natural killer cells, monocytes and PMN. Interaction of HVEM with the natural ligand LIGHT on T cells has a costimulatory effect, and increases the bactericidal activity of PMN. To further characterize the function of HVEM on PMN, we evaluated the effect of receptor ligation on human PMN effector functions using an agonistic monoclonal antibody. Here we demonstrate that activation of HVEM causes activation of neutrophil effector functions, including respiratory burst, degranulation and release of interleukin-8 in synergy with ligands for Toll-like receptors or GM-CSF. In addition, stimulation via HVEM enhanced neutrophil phagocytic activity of complement opsonized, but not of non-opsonized, particles. In conclusion, these results indicate a new, as yet unknown, participation of HVEM in the innate immune response and points to a new link between innate and adaptive immunity.

531. Myeloid lineage-selective growth of revertant cells in Fanconi anaemia
Hamanoue, S. et al
*Br. J. Hematol., **132(5)**, 630-636 (2006)*

Fanconi anaemia (FA) is a genetically heterogeneous chromosome instability syndrome characterised by bone marrow failure and congenital anomalies. Although an increasing number of reports suggest that reversion mosaicism noted in peripheral blood lymphocytes (PBLs) is associated with mild haematopoietic failure in FA, myeloid cells are rarely directly examined. We here report a patient with prolonged mild pancytopenia in whom proliferation of revertant cells was detected in mature myeloid cells but not in PBLs. While this patient had inherited heterozygous mutations, 2546delC and 3720-3724del, in the major FA gene FANCA, Epstein-Barr

virus-immortalised lymphoblastoid cells from the patient had 2546C > T instead of 2546delC, resulting in expression of a functional missense protein. As the identical reversion was detected in polymorphonuclear granulocytes and mononuclear phagocytes, sustained haematopoiesis in the patient can be attributed to a selective growth advantage of revertant myeloid cells. It is noteworthy that such a myeloid lineage-selective mosaicism is overlooked in routine examination of PBLs. Recognition of this status will expand the role of reversion mosaicism in the pathophysiology of FA.

532. Increased neutrophil membrane expression and plasma level of proteinase 3 in systemic vasculitis are not a consequence of the -564 A/G promotor polymorphism
Abdgawad, M., Hellmark, T,m Gunnarsson, L., Westman, K.W.A. and Segelmark, M.
Clin. Exp. Immunol., ***145(1)****, 63-70 (2006)*

Several findings link proteinase 3 (PR3) to small vessel vasculitis. Besides being a major target of anti-neutrophil cytoplasm antibodies (ANCA), previous findings have shown increased circulating levels of PR3 in vasculitis patients, increased levels of neutrophil membrane-PR3 (mPR3) expression and a skewed distribution of the - 564 A/G polymorphism in the promotor region of the PR3 gene. In this study we elucidate how these three findings relate to each other. The plasma concentration of PR3 was measured by enzyme-linked immunosorbent assay (ELISA), mPR3 expression by fluorescence activated cell sorter (FACS) and the gene polymorphism by real-time polymerase chain reaction (PCR). We compared results from 63 patients with ANCA-associated systemic vasculitis (AASV) with 107 healthy blood donors. In accordance with previous reports, AASV patients had increased plasma concentrations of PR3 compared to healthy controls (mean 224 [mu]g/l versus 155 [mu]g/l, P < 0[middle dot]0001). They also showed an increased number of mPR3-positive neutrophils (60% versus 42%, P < 0[middle dot]001). However, contrary to a previous report, we found no skewed distribution of the polymorphism in PR3 gene. There was a weak correlation between mPR3 mean fluorescence intensity (MFI) and plasma PR3 among healthy controls and myeloperoxidase-ANCA (MPO-ANCA)-positive patients (r = 0[middle dot]24, P = 0[middle dot]015 and r = 0[middle dot]52, P = 0[middle dot]011, respectively). In conclusion, increased plasma PR3 and high expression of mPR3 are associated with small vessel vasculitis, but neither of them is a consequence of the - 564 A/G polymorphism of the PR3 gene promotor.

533. Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue
Hosokawa, I. et al
Clin. Exp. Immunol., ***146(2)****, 218-225 (2006)*

Anti-microbial peptides produced from mucosal epithelium appear to play pivotal roles in the host innate immune defence system in the oral cavity. In particular, human beta-defensins (hBDs) and the cathelicidin-type anti-microbial peptide, LL-37, were reported to kill periodontal disease-associated bacteria. In contrast to well-studied hBDs, little is known about the expression profiles of LL-37 in gingival tissue. In this study, the anti-microbial peptides expressed in gingival tissue were analysed using immunohistochemistry and enxyme-linked immunosorbent assay (ELISA). Immunohistochemistry revealed that neutrophils expressed only LL-37, but not hBD-2 or hBD-3, and that such expression was prominent in the inflammatory lesions when compared to healthy gingivae which showed very few or no LL-37 expressing neutrophils. Gingival epithelial cells (GEC), however, expressed all three examined anti-microbial peptides, irrespective of the presence or absence of inflammation. Moreover, as determined by ELISA, the concentration of LL-37 in the gingival tissue homogenates determined was correlated positively with the depth of the gingival crevice. Stimulation with periodontal bacteria in vitro induced both hBD-2 and LL-37 expressions by GEC, whereas peripheral blood neutrophils produced only LL-37 production, but not hBD-2, in response to the bacterial stimulation. These findings suggest that LL-37 displays distinct expression patterns from those of hBDs in gingival tissue.

534. Peripheral leukocyte response to oncological radiotherapy: Expression of heat shock proteins
Guisasola, M.C. et al
Int. J. Rad. Biol., ***82(3)****, 171-179 (2006)*

*Purpose:*To study Heat Shock Proteins (HSP) expression in patients subjected to radiotherapy and their potential use as biomarkers for radiation tolerance. An evaluation is also made of whether irradiated volume is critical to the outcome of normal tissue injury using polymorphonuclear neutrophils as biosensors, and whether HSP antibodies (Ab) may be involved in post-radiotherapy disease.

*Material and methods:*Twelve patients receiving the same total dose of radiotherapy, but in three different volumes, and four healthy volunteers used as controls were analysed. *hsp*27 and 70i mRNA were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Southern-blot, HSP by flow cytometry, and HSP-Ab by Enzyme-linked Immnoadsorbent Assay (ELISA). The clinical protocol included radiation related toxicity based on clinical and analytical scales.

*Results:*Radiotherapy caused *hsp* downregulation, maximum in patients with the largest irradiated volumes, and a decrease in intracellular HSP content. Patients with greatest intraleukocyte HSP levels before treatment suffered more severe radiation morbidity. Patients with endocrine neoplasms presented the highest HSP-Ab titers.

*Conclusions:*Radiotherapy downregulates *hsp*27 and 70i, which would enhance radiosensitivity. HSP content prior to treatment is suggested as a prognostic biomarker for radiation tolerance, with circulating leukocytes as biosensors. HSP-Ab may be biomarkers of tumor disease, but do not seem to be involved in the morbidity of acute post-radiotherapy disease, which is closely related to the volumes irradiated.

535. Spreading of Neutrophils: From Activation to Migration
Sengputa, K., Aranda-Espinoza, H., Smith, L., Janmey, P. and Hammer, D.
*Biophys. J., **91**, 4638-4648 (2006)*

Neutrophils rely on rapid changes in morphology to ward off invaders. Time-resolved dynamics of spreading human neutrophils after activation by the chemoattractant fMLF (formyl methionyl leucyl phenylalanine) was observed by RICM (reflection interference contrast microscopy). An image-processing algorithm was developed to identify the changes in the overall cell shape and the zones of close contact with the substrate. We show that in the case of neutrophils, cell spreading immediately after exposure of fMLF is anisotropic and directional. The dependence of spreading area, A, of the cell as a function of time, t, shows several distinct regimes, each of which can be fitted as power laws ($A \sim t^b$). The different spreading regimes correspond to distinct values of the exponent b and are related to the adhesion state of the cell. Treatment with cytochalasin-B eliminated the anisotropy in the spreading.

536. Insulin inhibits phagocytosis in normal human neutrophils via PKCα/β-dependent priming of F-actin assembly
Saiepour, D., Sehlin, J. and Oldenburg, P.A.
*Inflammation Res., **55(3)**, 85-91 (2006)*

Objective: This study investigated the effects of insulin on the phagocytosis of C3bi – and IgG-opsonized yeast particles in normal human neutrophils.
Methods: Neutrophils were incubated in different insulin concentrations for 30 minutes and stimulated by C3bi – or IgG-opsonized yeast particles. Phagocytosis was quantified by both light microscopy and FACscan flow cytometry. Laser confocal microscopy was used for quantification of F-actin levels.
Results: Elevated insulin concentrations decreased neutrophil phagocytosis of both types of targets. This defect was shown to be in part due to a delayed phagocytosis in the presence of insulin. Following a 30 minute incubation, insulin was found to increase the accumulation of cortical F-actin, without affecting the total cellular F-actin content. The specific PKCα/β inhibitor, Go6976, abolished the insulin-mediated increase in cortical F-actin content and both Go6976 and the PKCα/β/δ/ε-specific inhibitor GF109203X reversed the inhibitory effects of insulin on phagocytosis.
Conclusion: Hyperinsulinemia *in vitro* can inhibit phagocytosis of opsonized targets in normal human neutrophils. This effect of insulin is dependent on activation of PKCα and/or PKCβ, and these insulin signals may interfere with the dynamic assembly/disassembly and/or distribution of F-actin, which is required for the phagocytosis process.

537. Negative association between serum dioxin level and oxidative DNA damage markers in municipal waste incinerator workers
Yoshida, J. et al
*Int. Arch. Occupational and Environmental Health, **79(2)** , 115-122 (2006)*

Objectives: To investigate the effect of dioxin on the formation of oxidative DNA damage and urinary mutagenicity, we measured the concentrations of serum dioxins and lymphocytic 8-hydroxydeoxyguanosine (8-OH-dG) in 57 male waste incinerator workers, urinary 8-OH-dG and urinary mutagenicity in 29 male waste incinerator workers. Methods: Information about the subjects was obtained from a questionnaire. Concentrations of polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and coplanar-polychlorinated-biphenyl (Co-PCB) in serum samples from the workers were measured with a high-resolution gas chromatograph /high-resolution mass spectrometer. Lymphocytic and urinary 8-OH-dG levels were measured with a high-performance liquid chromatography-electrochemical detector system. The urinary mutagenicity was measured with umu assay. Results: The lymphocytic 8-OH-dG level showed a negative association with the serum dioxin level (total value of TEQ-PCDD, PCDF, and Co-PCB). Urinary 8-OH-dG did not show correlation with serum dioxin level, but showed positive correlation with the smoking index. Conclusions: With respect to the subjects‘ serum dioxin level, dioxin did not increase the urinary 8-OH-dG level by oxidative DNA damage, but upregulation of the primary defenses with oxidative damage and/or DNA repair system activity might have occurred.

538. Isolation and characterization of the potential receptor for wheat germ agglutinin from human neutrophils
Solorzano, C. et al
*Glycoconj. J., **23**, 591-598 (2006)*

Neutrophils participate in host protection and central to this process is the regulation of oxidative mechanisms. We purified by affinity chromatography the receptor for the GlcNAc-specific WGA from CD14+ CD16+ cell lysates (WGAr). The receptor is a 141 kDa glycoprotein constituted by two subunits of 78 and 63 kDa. It is mainly composed of Ser, Asx, and Gly, and, in a minor proportion, His, Cys, and Pro. Its glycan portion contains GlcNAc, Gal, and Man; NeuAc and GalNAc were identified in a minor proportion. The amino acid sequence of the WGA receptor was predicted from tryptic peptides by MALDI-TOF, both subunits showed homology with

cytokeratin type II (26 and 29% for the 78 and 63 kDa subunits, respectively); the 78 kDa subunit showed also homology with the human transferrin receptor (24%). Antibodies against WGAr induce higher oxidative burst than WGA, determined by NBT reduction; however, this effect was inhibited ($p < 0.05$) with GlcNAc suggesting that WGAr participates as mediator in signal transduction in neutrophils.

539. Human leukocytes express ephrinB2 which activates microvascular endothelial cells
Zamora, D., Babra, B., Pan, Y., Planck, S.R. and Rosenbaum, J.T.
*Cell. Immunol., **242**, 99-109 (2006)*

EphrinB2–EphB4 interaction modulates the migration/adhesion of various cell types, including endothelial cells (EC) and peripheral blood leukocytes (PBLs). We hypothesize that the Ephrin/Eph signaling mechanism plays a role in mediating EC/leukocyte interactions during inflammation. PBLs were isolated from human blood, stimulated with inflammatory mediators, and total RNA or protein assayed for EphrinB2 expression. PBLs demonstrated differential expression profiles of EphrinB2 mRNA or protein, depending on cell subtype and stimulus. Human iris tissue and iris EC (HIEC) were examined for the expression of EphB4 mRNA and protein. Some blood vessels were $EphB4^+$, while stimulation of purified HIEC did not alter their expression of EphB4. HIEC treated with sEphrinB2/Fc from 0 to 60 min did exhibit changes in their phospho-Erk1/2 levels. These observations indicate that stimulated lymphocytes express EphrinB2, which has the potential to activate EC. This suggests a novel mechanism by which EC and lymphocytes communicate to regulate cell activation/migration during inflammation.

540. Comparative Pharmacokinetics of Azithromycin in Serum and White Blood Cells of Healthy Subjects Receiving a Single-Dose Extended-Release Regimen versus a 3-Day Immediate-Release Regimen
Liu, P. et al
*Antimicrob. Agents Chemother., **51(1)**, 103-109 (2007)*

The pharmacokinetic profiles of azithromycin given as a single-dose regimen (2.0-g extended-release microspheres) were characterized in serum and white blood cells (WBC) and compared with those of a 3-day regimen (a 500-mg immediate-release tablet once daily; total dose, 1.5 g) in an open-label, randomized, parallel-group study of 24 healthy adult subjects. Serial blood samples were collected up to 5 days after the start of dosing for both regimens. Safety assessments were conducted throughout the study. A single 2.0-g dose of azithromycin microspheres achieved significantly higher exposures in serum and WBC during the first 24 h after the start of dosing than a 3-day regimen: an approximately threefold higher area under the curve from time zero to 24 h postdose (AUC_{0-24}) and an approximately twofold higher mean peak concentration on day 1. The single-dose regimen provided total azithromycin exposures in serum and WBC similar to those of the 3-day regimen, as evidenced by the similar AUC_{0-120} and trough azithromycin concentrations in serum and WBC (mononuclear leukocytes [MNL] and polymorphonuclear leukocytes [PMNL]). For both regimens, the average total azithromycin exposures in MNL and PMNL were approximately 300- and 600-fold higher than those in serum. Azithromycin concentrations in MNL and PMNL remained above 10 μg/ml for at least 5 days after the start of dosing for both regimens. This "front-loading" of the dose on day 1 is safely achieved by the extended-release microsphere formulation, which maximizes the drug exposure at the time when the bacterial burden is likely to be highest.

541. Granulocyte Macrophage Colony–Stimulating Factor Expression in Human Herpetic Stromal Keratitis: Implications for the Role of Neutrophils in HSK
Duan, R., Remeijer, L., van Dun, J.M., Osterhaus, A.D.M.E. and Verjans, G.M.G.M.
*Invest. Ophthalmol. Vis. Sci., **48**, 277-284 (2007)*

PURPOSE. Granulocyte macrophage colony–stimulating factor (GM-CSF) is thought to play a key role in chronic inflammatory diseases by governing the survival and function of infiltrating neutrophils. The objective of this study was to determine the putative role of GM-CSF in the pathogenesis of human herpetic stromal keratitis (HSK).
METHODS. Primary human corneal fibroblast (HCF) cultures and a telomerase-immortalized human corneal epithelial (HCE) cell line representative of native HCE were stimulated with the known HSK-inducing cytokines interferon (IFN)-γ, interleukin (IL)-1ß, and tumor necrosis factor (TNF)-α. Alternatively, the T-cell cytokine IL-17 was added solely or simultaneously. Human neutrophils were incubated with conditioned medium (CM) of the HCF and HCE stimulated with the aforementioned cytokines, or recombinant GM-CSF, and their viability or activation status was determined by flow cytometry. GM-CSF and IL-8 secretion levels in the CM were determined by ELISA. The antibody-dependent cellular cytotoxicity (ADCC) of neutrophils toward herpes simplex virus (HSV)-infected HCFs was determined by flow cytometry. The expression of GM-CSF was determined in HSK and control corneal buttons by real-time RT-PCR and immunohistology.
RESULTS. Compared with IFN-γ, CM of either cell type stimulated with IL-1ß, or in the case of HCE cells, stimulated with TNF-α or IL-17, delayed neutrophil apoptosis significantly. Only in HCFs did IL-17 exhibit a synergistic effect with TNF-α. The antiapoptotic activity was attributable in part to the GM-CSF secreted by the activated HCFs and HCE cells. GM-CSF stimulation of neutrophils

induced their activation and the secretion of IL-8. GM-CSF did not increase significantly the ADCC reaction of neutrophils toward HSV-infected HCFs. Finally, GM-CSF was expressed in corneas of the patients with HSK but not in control subjects. CONCLUSIONS. The data suggest that GM-CSF, expressed by cornea-resident cells such as HCFs and HCE cells, may play a role in the immunopathogenesis of HSK by prolonging the survival and modulating the effector function of corneal infiltrating neutrophils.

542. Fas ligand exerts its pro-inflammatory effects via neutrophil recruitment but not activation
Dupont, P.J. and Warrens, A.N.
*Immunology, **120(1)**, 133-139 (2007)*

Fas ligand (FasL) expression induces apoptosis of activated T cells and has been suggested as a strategy to inhibit graft rejection. Unfortunately, the use of FasL to confer 'immune privilege' in this setting has been hampered by the finding that it may also provoke a destructive granulocytic response. While the Fas/FasL-mediated apoptotic pathways are well defined, the pro-inflammatory effects of FasL are poorly understood. Our aim in this study was to define in vitro the biological effects of FasL on neutrophil recruitment and activation. DAP-3 cells expressing human FasL on the cell membrane (mFasL) potently induced apoptosis in human neutrophils and in activated T lymphocytes. Recombinant human soluble FasL (sFasL), by contrast, was a very weak inducer of apoptosis, even at high concentrations. This latter observation suggests that cleavage of mFasL by naturally occurring matrix metalloproteinases may serve to down-regulate FasL activity in vivo. However, in the presence of a cross-linking antibody, the efficiency of apoptosis-induction by sFasL was greatly increased, suggesting that the lesser pro-apoptotic potency of sFasL reflects an inability to induce trimerization of the Fas receptor. With regard to pro-inflammatory effects, we found that sFasL is a potent neutrophil chemoattractant and, given that it induces little apoptosis, the dominance of sFasL over mFasL may mean that graft-infiltrating neutrophils will survive to mediate inflammation. Neither sFasL nor mFasL produced neutrophil activation as assessed by chemiluminescence assay. This suggests that neutrophils recruited to an inflammatory site by FasL will be activated by mechanisms other than Fas-FasL signalling.

543. Group B Streptococcal Capsular Sialic Acids Interact with Siglecs (Immunoglobulin-Like Lectins) on Human Leukocytes
Carlin, A.F., Lewis, A.L., Varki, A. and Nizet, VZ.
*J. Bacteriol., **189(4)**, 1231-1237 (2007)*

Group B *Streptococcus* (GBS) is classified into nine serotypes that vary in capsular polysaccharide (CPS) architecture but share in common the presence of a terminal sialic acid (Sia) residue. This position and linkage of GBS Sia closely resembles that of cell surface glycans found abundantly on human cells. CD33-related Siglecs (CD33rSiglecs) are a family of Sia-binding lectins expressed on host leukocytes that engage host Sia-capped glycans and send signals that dampen inflammatory gene activation. We hypothesized that GBS evolved to display CPS Sia as a form of molecular mimicry limiting the activation of an effective innate immune response. In this study, we applied a panel of immunologic and cell-based assays to demonstrate that GBS of several serotypes interacts in a Sia- and serotype-specific manner with certain human CD33rSiglecs, including hSiglec-9 and hSiglec-5 expressed on neutrophils and monocytes. Modification of GBS CPS Sia by O acetylation has recently been recognized, and we further show that the degree of O acetylation can markedly affect the interaction between GBS and hSiglec-5, -7, and -9. Thus, production of Sia-capped bacterial polysaccharide capsules that mimic human cell surface glycans in order to engage CD33rSiglecs may be an example of a previously unrecognized bacterial mechanism of leukocyte manipulation.

544. Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils
Bauer, S. et al
*J. Leukoc. Biol., **81**, 458-464 (2007)*

Proteinase 3 (PR3) is found in granules of all neutrophils but also on the plasma membrane of a subset of neutrophils (mPR3). CD177, another neutrophil protein, also displays a bimodal surface expression. In this study, we have investigated the coexpression of these two molecules, as well as the effect of cell activation on their surface expression. We can show that CD177 is expressed on the same subset of neutrophils as mPR3. Experiments show that the expression of mPR3 and CD177 on the plasma membrane is increased or decreased in parallel during cell stimulation or spontaneous apoptosis. Furthermore, we observed a rapid internalization and recirculation of mPR3 and plasma membrane CD177, where all mPR3 is replaced within 30 min. Our findings suggest that the PR3 found on the plasma membrane has its origin in the same intracellular storage as CD177, i.e., secondary granules and secretory vesicles and not primary granules. PR3- and CD177-expressing neutrophils constitute a subpopulation of neutrophils with an unknown role in the innate immune system, which may play an important role in diseases such as Wegener's granulomatosis and polycythemia vera.

545. Haemophilus influenzae Induces Neutrophil Necrosis: A Role in Chronic Obstructive Pulmonary Disease?
Naylor, E.J. et al
*Am. J. Respir. Cell Mol. Biol., **37**, 135-143 (2007)*

Noncapsulate *Haemophilus influenzae* is commonly found in the airways of patients with chronic obstructive pulmonary disease (COPD), both during stable disease and during exacerbations. Neutrophils are also found in large numbers in sputum from patients with COPD, which also contains released neutrophil products such as elastase. Why *H. influenzae* colonizes the lungs of patients with COPD

in the presence of such large numbers of infiltrating neutrophils is not known. We set out to determine if abnormal interactions between *H. influenzae* and neutrophils could impact on COPD pathology. Noncapsulate *H. influenzae* clinical isolates were incubated *in vitro* with neutrophils from healthy volunteers, and respiratory burst activity, cytokine and chemokine production, phagocytosis and killing of bacteria, and neutrophil apoptosis and necrosis were measured. Neutrophil morphology was determined in sputum samples. *H. influenzae* were phagocytosed by neutrophils, thereby activating a respiratory burst and the secretion of the neutrophil chemoattractant IL-8. However, rather than kill the bacteria, the neutrophils themselves were killed (largely via necrosis) and released their granule contents into the extracellular environment. Neutrophil-derived IL-8, generated after the interaction of *H. influenzae* with neutrophils, may result in the further infiltration of neutrophils into the lungs, thereby amplifying the inflammatory response. However, the infiltrating neutrophils fail to kill the bacteria and instead release tissue-damaging products into the lung as they undergo necrosis. These results may help to explain the clinical picture in COPD.

546. TREM-1 ligand expression on platelets enhances neutrophil activation
Haselmayer, P., Grosse-Hovest, L., von Landenberg, P. and Radsak, M.P.
Blood, ***110(3)****, 1029-1035 (2007)*

The triggering receptor expressed on myeloid cells 1 (TREM-1) plays an important role in the innate immune response related to severe infections and sepsis. Modulation of TREM-1–associated activation improves the outcome in rodent models for pneumonia and sepsis. However, the identity and occurrence of the natural TREM-1 ligands are so far unknown, impairing the further understanding of the biology of this receptor. Here, we report the presence of a ligand for TREM-1 on human platelets. Using a recombinant TREM-1 fusion protein, we demonstrate specific binding of TREM-1 to platelets. TREM-1–specific signals are required for the platelet-induced augmentation of polymorphonuclear leukocyte (PMN) effector functions (provoked by LPS). However, TREM-1 interaction with its ligand is not required for platelet/PMN complex formation, which is dependent on integrins and selectins. Taken together, the results indicate that the TREM-1 ligand is expressed by platelets, and the TREM-1/ligand interaction contributes to the amplification of LPS-induced PMN activation. Our results shed new light on our understanding of TREM-1 and its role in the innate inflammatory response in infections and might contribute to the development of future concepts to treat sepsis.

547. High-Throughput mRNA Profiling Characterizes the Expression of Inflammatory Molecules in Sepsis Caused by Burkholderia pseudomallei
Joost Wiersinga, W. et al
Infect. Immun., ***75(6)****, 3074-3079 (2007)*

Sepsis is characterized by an uncontrolled inflammatory response to invading microorganisms. We describe the inflammatory mRNA profiles in whole-blood leukocytes, monocytes, and granulocytes using a multigene system for 35 inflammatory markers that included pro- and anti-inflammatory cytokines, chemokines, and signal transduction molecules in a case-control study with 34 patients with sepsis caused by the gram-negative bacterium *Burkholderia pseudomallei* (the pathogen causing melioidosis) and 32 healthy volunteers. Relative to healthy controls, patients with sepsis showed increased transcription of a whole array of inflammatory genes in peripheral blood leukocytes, granulocytes, and monocytes. Specific monocyte and granulocyte mRNA profiles were identified. Strong correlations were found between inflammatory mRNA expression levels in monocytes and clinical outcome. These data underline the notion that circulating leukocytes are an important source for inflammatory mediators in patients with gram-negative sepsis. Gene profiling such as was done here provides an excellent tool to obtain insight into the extent of inflammation activation in patients with severe infection.

548. Endogenous Interleukin-18 Improves the Early Antimicrobial Host Response in Severe Melioidosis
Joost-Wiersinga, W. et al
Infect. Immun., ***75(8)****, 3739-3746 (2007)*

Melioidosis is caused by the soil saprophyte *Burkholderia pseudomallei* and is endemic in Southeast Asia. The pathogenesis of melioidosis is still largely unknown, although gamma interferon (IFN-γ) seems to play an obligatory role in host defense. Previously, we have shown that IFN-γ production in melioidosis is controlled in part by interleukin-18 (IL-18). The aim of the present study was to determine the role of IL-18 in the immune response to *B. pseudomallei*. For this the following investigations were performed. (i) Plasma IL-18 and blood monocyte IL-18 mRNA levels were elevated in 34 patients with culture-proven melioidosis compared to the levels in 32 local healthy controls; in addition, IL-18 binding protein levels were markedly elevated in patients, strongly correlating with mortality. (ii) IL-18 gene-deficient (IL-18 knockout [KO]) mice showed accelerated mortality after intranasal infection with a lethal dose of *B. pseudomallei*, which was accompanied by enhanced bacterial growth in their lungs, livers, spleens, kidneys, and blood at 24 and 48 h postinfection, compared to wild-type mice. In addition, IL-18 KO mice displayed evidence of enhanced hepatocellular injury and renal insufficiency. Together, these data indicate that the enhanced production of IL-18 in melioidosis is an essential part of a protective immune response to this severe infection.

549. Cigarette smoke extract-induced suppression of caspase-3-like activity impairs human neutrophil phagocytosis
Stringer, K.A., Tobias, M., O'Neill, H.C. and Franklin, C.C.
Am. J. Physiol. Lung Cell. Mol. Physiol., ***L1572-L1579*** *(2007)*

Neutrophils are the primary inflammatory cell in smokers' lungs, but little is known about the ability of cigarette smoke to modulate neutrophil function. Neutrophils undergo caspase-3-dependent spontaneous, as well as phagocytosis-induced, apoptosis. This study investigated the ability of cigarette smoke extract (CSE) to alter neutrophil caspase-3 activity, apoptosis, and phagocytosis. CSE treatment resulted in a dramatic suppression of neutrophil caspase-3-like activity, which correlated with reduced cleavage of glutamate-L-cysteine ligase catalytic subunit, a known target of active caspase-3. CSE did not affect procaspase-3 processing to its active fragment, suggesting a direct effect of CSE on active caspase-3. Consistent with this, CSE inhibited active recombinant caspase-3 activity, which was abolished by dithiothreitol, suggesting a redox-sensitive mechanism. CSE-induced suppression of caspase-3 activity did not alter spontaneous apoptosis but did impair phagocytic activity. Since CSE treatment resulted in profound suppression of caspase-3 activity but did not alter apoptosis, the possibility of a threshold level of caspase-3 activity was investigated. CSE reduced caspase-3 activity in a concentration-dependent manner. Despite near complete suppression of caspase-3 activity, spontaneous apoptosis was not altered. Conversely, treatment with the pan-caspase inhibitor, Z-Val-Ala-Asp-fluoromethylketone, reduced spontaneous apoptosis. These data demonstrate that CSE does not suppress caspase-3 activity below a threshold level to prevent spontaneous apoptosis, but the level of inhibition is sufficient to impair neutrophil phagocytic activity. These divergent functions of caspase-3 may contribute to the persistence of neutrophils in the lungs of smokers, as well as be a factor in their higher incidence of community-acquired pneumonia.

550. Novel Role for Mitochondria: Protein Kinase C-Dependent Oxidative Signaling Organelles in Activation-Induced T-Cell Death
Kaminski, M., Kiessling, M., Süss, D., Krammer, P.H. and Gülow, K.
Mol. Cell. Biol., ***27(10)****, 3625-3639 (2007)*

Reactive oxygen species (ROS) play a key role in regulation of activation-induced T-cell death (AICD) by induction of CD95L expression. However, the molecular source and the signaling steps necessary for ROS production are largely unknown. Here, we show that the proximal T-cell receptor-signaling machinery, including ZAP70 (zeta chain-associated protein kinase 70), LAT (linker of activated T cells), SLP76 (SH2 domain-containing leukocyte protein of 76 kDa), PLC1 (phospholipase C1), and PKC (protein kinase C), are crucial for ROS production. PKC is translocated to the mitochondria. By using cells depleted of mitochondrial DNA, we identified the mitochondria as the source of activation-induced ROS. Inhibition of mitochondrial electron transport complex I assembly by small interfering RNA (siRNA)-mediated knockdown of the chaperone NDUFAF1 resulted in a block of ROS production. Complex I-derived ROS are converted into a hydrogen peroxide signal by the mitochondrial superoxide dismutase. This signal is essential for CD95L expression, as inhibition of complex I assembly by NDUFAF1-specific siRNA prevents AICD. Similar results were obtained when metformin, an antidiabetic drug and mild complex I inhibitor, was used. Thus, we demonstrate for the first time that PKC-dependent ROS generation by mitochondrial complex I is essential for AICD.

551. Coronin Function Is Required for Chemotaxis and Phagocytosis in Human Neutrophils
Yan, M., Di Ciano-Oliveira, C., Grinstein, S. and Trimble, W.S.
J. Immunol., ***178****, 5769-5778 (2007)*

Coronins are a family of conserved actin-associated proteins that have been implicated in a variety of cellular processes dependent on actin rearrangements. In this study, we show that in primary human neutrophils, coronins-1–4 and -7 are expressed. Coronin-1 accumulates at the leading edge of migrating neutrophils and at the nascent phagosome. Inhibition of coronin function by transduction of a dominant-negative form of the protein leads to inhibition of chemotaxis and a reduction in neutrophil spreading and adhesion. This inhibition appears to correlate with changes in the distribution of F-actin structures within the cell. In addition, phagocytosis is inhibited, but neither secretion nor activation of the NADPH oxidase appears to be affected. Together, these results show that coronins are required for actin-dependent changes in cell morphology that lead to migration and phagocytosis.

552. Potent inhibition of store-operated Ca2+ influx and superoxide production in HL60 cells and polymorphonuclear neutrophils by the pyrazole derivative BTP2
Steinckwich, N. et al
J. Leukoc. Biol., ***81****, 1054-1064 (2007)*

Store-operated calcium entry (SOCE) is a key regulator in the activation of leukocytes. 3,5-Bistrifluoromethyl pyrazole (BTP) derivatives have been identified recently as inhibitors of T lymphocyte activation. The inhibitory effect of one of these compounds, N-(4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]phenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (BTP2), appears to be a result of inhibition of SOC influx. Polymorphonuclear neutrophils provide effective protection against bacterial infection, but they are also involved in tissue damage during chronic inflammation. As for T lymphocytes, their activation relies on SOCE. We therefore investigated the effect of BTP2 on calcium homeostasis and functional responses of human neutrophils. BTP2 significantly inhibited the calcium influx after stimulation with thapsigargin or fMLF. This inhibition was seen after 5 min of incubation with 10 μM BTP2 and after 24 h with lower concentrations. With 24 h incubation, the effect appeared irreversible, as the removal of BTP2 3 h before the

experiment did not reduce this inhibition in granulocyte-differentiated HL60 cells. In human neutrophils, BTP2 reduced superoxide anion production by 82% after 24 h of incubation. On the contrary, phagocytosis, intraphagosomal radical production, and bacterial killing by neutrophils were not reduced significantly, even after 24 h treatment with 10 μM BTP2. This work suggests that BTP2 could become an important tool to characterize calcium signaling in neutrophils. Furthermore, BTP2 or related compounds could constitute a new approach to the down-regulation of neutrophils in chronic inflammatory disease without compromising antibacterial host defense.

553. Quantitative Determination of JAK2 V617F by TaqMan: An Absolute Measure of Averaged Copies per Cell That May Be Associated with the Different Types of Myeloproliferative Disorders
Hammond, E. et al
J. Mol. Diagn., ***9(2)****, 242-248 (2007)*

We report a novel TaqMan assay for *JAK2* V617F that measures averaged copies per cell in absolute terms, as opposed to a ratio of mutant to wild-type alleles. Measurements were obtained by comparing the *JAK2* V617F signal generated by the test samples to that generated by a set of external plasmid standards containing the sequence of interest. Specificity of the assay was demonstrated above 36 cycles of amplification, and endpoint titration experiments indicated sensitivity down to 0.05% clinical dilutions. The test measured linearly over a wide logarithmic range and exhibited good reproducibility. Combination of this assay with another TaqMan method for determining cell number allowed identification of 14 cases of myeloproliferative disease with greater than two copies per cell. Mutational frequency was 68% among polycythemia vera (n = 44), 59% (n = 37) among essential thrombocythemia and 46% (n = 13) among idiopathic myelofibrosis. Levels of the mutation were significantly higher in polycythemia vera compared with essential thrombocythemia (P = 0.0005) and correlated with the following jointly significant variables at diagnosis: *PRV-1*, hemoglobin, white cell count, neutrophil count, and red cell count, using multiple regression analyses (P = 0.015). This method should be useful for assessing the relationship of gene dose to phenotype and possibly for monitoring therapy.

554. α_1-ANTITRYPSIN EXERTS IN VITRO ANTI-INFLAMMATORY ACTIVITY IN HUMAN MONOCYTES BY ELEVATING cAMP
Janciauskiene, S., Nita, I.M. and Stevens, T.
J. Biol. Chem., ***282(12)****, 8573-8582 (2007)*

Regulation of serine protease activity is considered to be the sole mechanism for the function of α_1-antitrypsin (AAT). However, recent reports of the anti-inflammatory effects of AAT are hard to reconcile with this classical mechanism. We discovered that two key activities of AAT *in vitro*, namely inhibition of endotoxin-stimulated tumor necrosis factor-α and enhancement of interleukin-10 in human monocytes, are mediated by an elevation of cAMP and activation of cAMP-dependent protein kinase A. As expected with this type of mechanism, the AAT-mediated rise in cAMP and the impact on endotoxin-stimulated tumor necrosis factor-α and interleukin-10 was enhanced when the catabolism of cAMP was blocked by the phosphodiesterase inhibitor rolipram. These effects were still observed with modified forms of AAT lacking protease inhibitor activity.

555. Relevance of anti-reactive oxygen species activity to anti-inflammatory activity of components of Eviprostat®, a phytotherapeutic agent for benign prostatic hyperplasia
Oka, M. et al
Phytomed., ***14****, 465-472 (2007)*

Inflammation is a common finding in benign prostatic hyperplasia (BPH). The phytotherapeutic agent eviprostat is a popular treatment for BPH in Japan and Germany. This agent consists of five components; four are extracted from *Chimaphila umbellata*, *Populus tremula*, *Pulsatilla pratensis* and *Equisetum arvense* (coded as EVI-1, EVI-2, EVI-3 and EVI-4, respectively) and the fifth is germ oil from *Triticum aestivum* (coded as EVI-5). In this study, the effects of each component on the reactive oxygen species (ROS), superoxide anion (O_2^-) and hydroxyl radical ($OH^{\bullet}$) generated in cell-free systems and human neutrophils, and on carrageenin-induced paw edema in rats were investigated. EVI-1, EVI-2 and EVI-4 suppressed the O_2^- levels in the xanthine/xanthine oxidase system, and EVI-1, EVI-2, EVI-3 and EVI-4 abolished the $OH^{\bullet}$ produced in a Fenton-type reaction system, so that EVI-1, EVI-2 and EVI-4 possessed inhibitory action with respect to both O_2^- and $OH^{\bullet}$. EVI-1, EVI-2 and EVI-4 also reduced ROS levels in phorbol myristate acetate-stimulated neutrophils. The paw swelling was inhibited by a mixture of EVI-1, EVI-2, EVI-3, EVI-4 and EVI-5 (a mixture which is equivalent to eviprostat) or by a mixture of EVI-1, EVI-2 and EVI-4, even though each component alone did not significantly inhibit the swelling. These findings suggest that the suppression of ROS by EVI-1, EVI-2 and EVI-4 may partly contribute to the anti-inflammatory action of eviprostat, and this action may be implicated in its therapeutic effect on BPH.

556. Heparin-Binding EGF-like Growth Factor Decreases Neutrophil–Endothelial Cell Interactions
Rocourt , D.V., Mehta, V.B., Wu, D. and Besner, G.E.
J. Surg. Res., ***141****, 262-266 (2007)*

Background

Hyperadhesiveness of neutrophils (PMN) to vascular endothelial cells (EC) followed by neutrophil transendothelial migration play important roles in the initiation of ischemia/reperfusion (I/R)-mediated injury. We investigated whether the ability of heparin-binding EGF-like growth factor (HB-EGF) to decrease intestinal injury after intestinal I/R is mediated, in part, by its ability to affect PMN-EC interactions and EC junctional integrity.
Materials and methods
Human umbilical vein EC monolayers were treated with HB-EGF (100 ng/mL) or phosphate-buffered saline followed by anoxia/reoxygenation (A/R). Simultaneously, labeled human PMN were treated with HB-EGF or phosphate-buffered saline and then co-incubated with EC for determination of PMN-EC adherence and PMN transendothelial migration. EC junctional integrity was also determined.
Results
PMN-EC adhesion increased after exposure of EC to A/R compared to EC exposed to normoxia (87% *versus* 64% binding, $P < 0.05$, Wilcoxon rank sum test). A/R-induced PMN-EC hyperadherence was significantly decreased by treatment of PMN with HB-EGF compared to nontreated cells (51% *versus* 87% binding, $P < 0.05$). HB-EGF significantly decreased PMN transendothelial migration and also augmented EC tight junctional integrity after A/R.
Conclusions
HB-EGF significantly reduces A/R-induced PMN-EC adhesion and PMN transendothelial migration and augments junctional integrity *in vitro*. Thus, HB-EGF acts not only as a potent cytoprotective agent for the intestine, but as an anti-inflammatory agent as well.

557. Induction of Adaptive Anti-HER2/neu Immune Responses in a Phase 1B/2 Trial of 2B1 Bispecific Murine Monoclonal Antibody in Metastatic Breast Cancer (E3194): A Trial Coordinated by the Eastern Cooperative Oncology Group
Hoosein, B. et al
J. Immunotherapy, ***30(4)****, 455-467 (2007)*

2B1 is a bispecific murine monoclonal antibody that binds to the extracellular domains of HER2/neu and Fc[gamma]RIII. 2B1 efficiently promotes the lysis of tumor cells overexpressing HER2/neu by natural killer cells and mononuclear phagocytes that express the Fc[gamma]RIII A isoform. Here, we report the results of E3194, a phase 1B/2 trial conducted by the Eastern Cooperative Oncology Group that employed 2B1 therapy in 20 women with metastatic breast cancer. The median age was 51 years. All but 1 patient had received prior chemotherapy. After the first dose, 3 of the initial 8 patients experienced dose-limiting toxicities that required dose-reduction. The nature of these dose-limiting toxicities resulted in a reduced dose from 2.5 mg/m2/d to 1 mg/m2/d in the remaining 12 patients. Objective antitumor responses were not seen. However, 2B1 therapy induced adaptive immune responses to both intracellular and extracellular domains of HER2/neu. Even though 2B1 antibody therapy did not show activity in metastatic breast cancer at the current administered doses, the ability of this antibody to induce detectable immune responses against an important tumor antigen has implications for understanding the mechanisms by which antibodies that mediate antibody-directed cellular cytotoxicity may exert their clinical antitumor effects.

558. Differences in functional activity and antigen expression of granulocytes primed in vivo with filgrastim, lenograstim, or pegfilgrastim
Ribeiro, D. et al
Transfusion, ***47(6)****, 969-980 (2007)*

BACKGROUND: Granulocyte-colony-stimulating factor (G-CSF) is known to affect functional activity and antigen expression of neutrophil granulocytes. Beside nonglycosylated filgrastim and glycosylated lenograstim, pegylated filgrastim (pegfilgrastim) has recently been introduced for single administration into clinical use.
STUDY DESIGN AND METHODS: Here, granulocytes from 27 patients with nonmyeloid malignancies were compared functionally (migration, reactive oxygen species production, and G-CSF serum levels) and phenotypically (cell surface antigen expression) before and after G-CSF administration.
RESULTS: After exposure to G-CSF, chemotaxis was reduced significantly in the filgrastim group. Immunophenotypically, in vivo G-CSF-primed granulocytes were more mature in the lenograstim than in the filgrastim and to lesser extent in the pegfilgrastim groups as shown by the expression profile for CD11b, CD14, and CD16. Of note, G-CSF serum levels were similar among the groups.
CONCLUSION: Our data suggest that granulocytes exposed to glycosylated G-CSF in vivo seem to resemble more closely their steady-state phenotype than after treatment with nonglycosylated and to lesser extent pegylated G-CSF.

559. Endostatin plus interferon-[alpha]2b therapy for metastatic melanoma: a novel combination of antiangiogenic and immunomodulatory agents
Moschos, S.J. et al
Melanoma Res., ***17(3)****, 193-200 (2007)*

In patients with stage IIB-III disease, adjuvant high-dose interferon-[alpha]2b has shown clinical benefit, although metastatic melanoma is currently without any known survival-prolonging therapy. Angiogenesis has been considered important in melanoma progression, and endostatin is an angiogenesis inhibitor with antitumor activity that has shown promising results in murine model systems, prompting

investigation of a formulation of rh-Endostatin (EntreMed, Rockville, Maryland, USA) alone and with interferon in metastatic melanoma. Patients were randomly assigned to receive interferon [alpha]2b (Schering-Plough) 10 million units/m2 subcutaneously three times a week plus rh-Endostatin 45 mg/m2 subcutaneously every 12 h (arm A) vs. rh-Endostatin alone (arm B). Twenty-one patients (age range 31-77 years, median age 54, 12 men and nine women, 17 cutaneous, and four ocular melanomas) were enrolled. No antitumor responses were observed, and no significant differences were noted in time to progression or overall survival. Two patients had stable disease enduring more than 30 weeks on treatment. Serum endostatin levels increased significantly 4 weeks after treatment in both groups. Basic fibroblast growth factor levels in urine were significantly lower following treatment in patients on arm B (P=0.043). The percentage of circulating endothelial cells was increased in five evaluable patients 4 weeks after treatment. Low titer (<=1 : 25) IgG antibodies against the rh-Endo formulation were detected in two patients (one per arm) in cycle 4. In conclusion, interferon did not improve response rate of rh-Endo although prolonged disease stability was observed in two patients. Better laboratory correlates of antiangiogenic response are needed, and the predictive value of circulating endothelial cells warrants further evaluation.

560. Cathepsin-cleaved Bid promotes apoptosis in human neutrophils via oxidative stress-induced lysosomal membrane permeabilization
Blomgran, R., Zheng, L and Stendahl, O.
J. Leukoc. Biol., ***81(5)****, 1213-1223 (2007)*

Lysosomal membrane permeabilization (LMP) is emerging as an important regulator of cell apoptosis. Human neutrophils are highly granulated phagocytes, which respond to pathogens by exhibiting increased production of reactive oxygen species (ROS) and lysosomal degranulation. In a previous study, we observed that intracellular, nonphagosomal generation of ROS triggered by adherent bacteria induced ROS-dependent neutrophil apoptosis, whereas intraphagosomal production of ROS during phagocytosis had no effect. In the present study, we measured lysosomal membrane stability and leakage in human neutrophils and found that adherent, noningested, Type 1-fimbriated *Escherichia coli* bacteria induced LMP rapidly in neutrophils. Pretreatment with the NADPH oxidase inhibitor diphenylene iodonium markedly blocked the early LMP and apoptosis in neutrophils stimulated with Type 1-fimbriated bacteria but had no effect on the late LMP seen in spontaneously apoptotic neutrophils. The induced lysosomal destabilization triggered cleavage of the proapoptotic Bcl-2 protein Bid, followed by a decrease in the antiapoptotic protein Mcl-1. Involvement of LMP in initiation of apoptosis is supported by the following observations: Bid cleavage and the concomitant drop in mitochondrial membrane potential required activation of cysteine-cathepsins but not caspases, and the differential effects of inhibitors of cysteine-cathepsins and cathepsin D on apoptosis coincided with their ability to inhibit Bid cleavage in activated neutrophils. Together, these results indicate that in microbe-induced apoptosis in neutrophils, ROS-dependent LMP represents an early event in initiation of the intrinsic apoptotic pathway, which is followed by Bid cleavage, mitochondrial damage, and caspase activation.

561. Non-T Cell Activation Linker (NTAL) Negatively Regulates TREM-1/DAP12-Induced Inflammatory Cytokine Production in Myeloid Cells
Tessarz, A.S. et al
J. Immunol., ***178****, 1991-1999 (2007)*

The engagement of triggering receptor expressed on myeloid cells 1 (TREM-1) on macrophages and neutrophils leads to TNF-α and IL-8 production and enhances inflammatory responses to microbial products. For signal transduction, TREM-1 couples to the ITAM-containing adapter DNAX activation protein of 12 kDa (DAP12). In general, ITAM-mediated signals lead to cell activation, although DAP12 was recently implicated in inhibitory signaling in mouse macrophages and dendritic cells. To date, signals downstream of the TREM-1 and DAP12 complex in myeloid cells are poorly defined. By analyzing receptor-induced tyrosine phosphorylation patterns, we discovered that the ligation of TREM-1 leads to tyrosine phosphorylation of the non-T cell activation linker (NTAL; also called linker of activation in B cells or LAB) in a myelomonocytic cell line and primary human granulocytes. Using RNA interference to decrease the expression levels of NTAL, we demonstrate that in NTAL knockdown cell lines the phosphorylation of ERK1/2 is enhanced. In addition, low levels of NTAL are correlated with decreased and delayed mobilization of Ca^{2+} after TREM-1 triggering. Most importantly, we demonstrate that NTAL acts as a negative regulator of TNF-α and IL-8 production after stimulation via TREM-1. Our results show that activation signals delivered via DAP12 can be counterbalanced by the adaptor NTAL, identifying NTAL as gatekeeper of TREM-1/DAP12-induced signaling in myeloid cells.

562. Free Cholesterol Alters Lipid Raft Structure and Function Regulating Neutrophil Ca2+ Entry and Respiratory Burst: Correlations with Calcium Channel Raft Trafficking
Kannan, K.B., Barlos, D. and Hauser, C.J.
J. Immunol., ***178****, 5253-5261 (2007)*

Recent studies associate cholesterol excess and atherosclerosis with inflammation. The link between these processes is not understood, but cholesterol is an important component of lipid rafts. Rafts are thought to concentrate membrane signaling molecules and thus regulate cell signaling through G protein-coupled pathways. We used methyl β-cyclodextrin to deplete cholesterol from polymorphonuclear neutrophil (**PMN**) rafts and thus study the effects of raft disruption on G protein-coupled Ca^{2+} mobilization. Methyl β-cyclodextrin had no effect on Ca^{2+} store depletion by the G protein-coupled agonists platelet-activating factor or fMLP, but abolished

agonist-stimulated Ca^{2+} entry. Free cholesterol at very low concentrations regulated Ca^{2+} entry into **PMN** via nonspecific Ca^{2+} channels in a biphasic fashion. The specificity of cholesterol regulation for Ca^{2+} entry was confirmed using thapsigargin studies. Responses to cholesterol appear physiologic because they regulate respiratory burst in a proportional biphasic fashion. Investigating further, we found that free cholesterol accumulated in **PMN** lipid raft fractions, promoting formation and polarization of membrane rafts. Finally, the transient receptor potential calcium channel protein TRPC1 redistributed to raft fractions in response to cholesterol. The uniformly biphasic relationships between cholesterol availability, Ca^{2+} signaling and respiratory burst suggest that Ca^{2+} influx and **PMN** activation are regulated by the quantitative relationships between cholesterol and other environmental lipid raft components. The association between symptomatic cholesterol excess and inflammation may therefore in part reflect free cholesterol- dependent changes in lipid raft structure that regulate immune cell Ca^{2+} entry. Ca^{2+} entry-dependent responses in other cell types may also reflect cholesterol bioavailability and lipid incorporation into rafts.

563. Interplay between Shear Stress and Adhesion on Neutrophil Locomotion
Smith, L.A., Aranda-Espiinoza, H., Haun, J.B. and Hammer, D.A.
Biophys. J., ***92****, 632-640 (2007)*

Leukocyte locomotion over the lumen of inflamed endothelial cells is a critical step, following firm adhesion, in the inflammatory response. Once firmly adherent, the cell will spread and will either undergo diapedesis through individual vascular endothelial cells or will migrate to tight junctions before extravasating to the site of injury or infection. Little is known about the mechanisms of neutrophil spreading or locomotion, or how motility is affected by the physical environment. We performed a systematic study to investigate the effect of the type of adhesive ligand and shear stress on neutrophil motility by employing a parallel-plate flow chamber with reconstituted protein surfaces of E-selectin, E-selectin/PECAM-1, and E-selectin/ICAM-1. We find that the level and type of adhesive ligand and the shear rate are intertwined in affecting several metrics of migration, such as the migration velocity, random motility, index of migration, and the percentage of cells moving in the direction of flow. On surfaces with high levels of PECAM-1, there is a near doubling in random motility at a shear rate of 180 s^{-1} compared to the motility in the absence of flow. On surfaces with ICAM-1, neutrophil random motility exhibits a weaker response to shear rate, decreasing slightly when shear rate is increased from static conditions to 180 s^{-1}, and is only slightly higher at 1000 s^{-1} than in the absence of flow. The random motility increases with increasing surface concentrations of E-selectin and PECAM-1 under static and flow conditions. Our findings illustrate that the endothelium may regulate neutrophil migration in postcapillary venules through the presentation of various adhesion ligands at sites of inflammation.

564. Lack of hepatocellular CD10 along bile canaliculi is physiologic in early childhood and persistent in Alagille syndrome
Byrne, J.A., Meara, N.J., Rayner, A.C., Thompson, R.J. and Knisely, A.S.
Lab. Invest., ***87****, 1138-1148 (2007)*

Many tissues, including hepatobiliary cells, express neutral endopeptidase (CD10), encoded by *MME*. Serum neutral endopeptidase activity (NEA) has been recommended as a marker of cholestasis in adults but not in children with Alagille syndrome (AGS). We investigated ontogenic and disease-related differences in the expression of CD10. CD10 was found on canalicular surfaces of hepatocytes throughout the lobule in 16 adults and in 31 children aged ≥24 months, with and without cholestasis, but not in 39 children aged <24 months, with and without cholestasis. Ten AGS children aged 2 months to 6 years lacked any canalicular CD10 expression. Cholangiocyte apices and/or intrasinusoidal granulocytes marked for CD10 in all subjects. Liver membrane fractions from a child with cholestasis aged <24 months and from 2 AGS patients aged >24 months contained reduced levels of CD10. In contrast, AGS children and all controls expressed CD10 similarly on granulocytes. *MME* mRNA was found in the liver of children aged <24 months and of adults, all with cholestasis, and of AGS patients. Granulocyte *MME* mRNA levels were similar among all study subjects; however, liver *MME* mRNA levels were 6- to 140-fold less than in normal adults in all cholestatic subjects, including AGS children. Methylation of the *MME* promoter was not detected in the liver of AGS children. In conclusion, hepatocytes in early childhood physiologically lack immunohistochemically detectable CD10. Reduced *MME* mRNA in AGS is not due to *MME* promoter methylation. Liver CD10 in childhood appears to undergo reduced synthesis or rapid degradation, which persists in AGS. Absence of CD10 expression thus may limit NEA as a marker of cholestasis in young patients and in AGS.

565. Lysoplasmenylcholine increases neutrophil adherence to human coronary artery endothelial cells
White, M.C., Rastogi, P. and McHowat, J.
Am. J. Physiol. Cell Physiol., ***293****, C1467-C1471 (2007)*

We demonstrated previously that thrombin stimulation of human coronary artery endothelial cells (HCAEC) results in release of choline lysophospholipids [lysophosphatidylcholine (lysoPtdCho) and lysoplasmenylcholine (lysoPlsCho)]. These amphiphilic metabolites have been implicated in arrhythmogenesis following the onset of myocardial ischemia, but studies examining their direct effects on the vasculature remain limited. We and others have shown that thrombin and lysoPtdCho can increase cell surface adhesion molecules and adherence of circulating inflammatory cells to the endothelium. This study supports our hypothesis that these changes may be mediated, at least in part, by lysoPlsCho, thus implicating this metabolite as an inflammatory mediator in the coronary vasculature and a modulator of the progression of atherosclerosis. Apical stimulation of HCAEC with thrombin resulted in the production and release of choline lysophospholipids from the apical surface of the HCAEC monolayer. Basolateral stimulation had no effect on choline lysophospholipid

production or release from either the apical or basolateral surface of the HCAEC monolayer. Incubation of HCAEC with lysoPlsCho or lysoPtdCho resulted in similar increases in HCAEC surface expression of P-selectin and E-selectin. Furthermore, lysoPlsCho increased cell surface expression of P-selectin, E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 with a time course similar to that of thrombin stimulation. Increased presence of cell surface adhesion molecules may contribute to the significant increase in adherence of neutrophils to either thrombin- or lysoPlsCho-stimulated HCAEC. These results demonstrate that the presence of thrombin at sites of vascular injury in the coronary circulation, resulting in increased choline lysophospholipid release from the HCAEC apical surface, has the potential to propagate vascular inflammation by upregulation of adhesion molecules and recruitment of circulating inflammatory cells to the endothelium.

566. Identification of genomic targets downstream of p38 mitogen-activated protein kinase pathway mediating tumor necrosis factor-α signaling
Zer, C., Sachs, G. and Shin, J.M.
*Physiol. Genomics, **31**, 343-351 (2007)*

Inhibition of p38 MAPK suppresses the expression of proinflammatory cytokines such as TNF-α and IL-1ß in macrophages and fibroblast-like synoviocytes (FLS). However, there have been no genomewide studies on the gene targets of p38 MAPK signaling in synoviocytes. Microarray technology was applied to generate a comprehensive analysis of all genes regulated by the p38 MAPK signaling pathway in FLS. Gene expression levels were measured with Agilent oligonucleotide microarrays. Four independent sets of mRNA modulated by TNF-α and vehicle were used to measure the change of gene expression due to TNF-α, and three experiments were done to ascertain the effect of SB-203580, a p38 MAPK inhibitor, on TNF-α-induced genes. Microarray data were validated by RT-quantitative polymerase chain reaction. One hundred forty-one significantly expressed genes were more than twofold upregulated by TNF-α. Thirty percent of these genes were downregulated by the p38 inhibitor SB-203580, whereas 67% of these genes were not significantly changed. The SB-203580-inhibited genes include proinflammatory cytokines such as interleukins and chemokines, proteases including matrix metallopeptidases, metabolism-related genes such as cyclooxygenases and phosphodiesterase, genes involved in signal transduction, and genes encoding for transcription factors, receptors, and transporters. Approximately one-third of the TNF-α-induced genes in FLS are regulated by the p38 MAPK signal pathway, showing that p38 MAPK is a possible target for suppressing proinflammatory gene expressions in rheumatoid arthritis.

567. Standardised assessment of membrane proteinase 3 expression. Analysis in ANCA-associated vasculitis and controls
Van rasmussen, A.P. et al
*Ann. Rheum. Dis., **66**, 1350-1355 (2007)*

Objectives: Increased numbers of neutrophils expressing proteinase 3 on their membrane (mPR3) have been reported in anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) and are suggested to be involved in AAV immunopathogenesis. In most studies, neutrophils were analysed for mPR3 expression without priming with TNFα, suggesting that mPR3 expression on neutrophils is dependent on other priming events, such as isolation procedures. These priming events can be variable. Therefore, we analysed mPR3 expression on neutrophils before and after priming with TNFα to assess whether standardised assessment of mPR3 expression requires priming. Using neutrophils before and after priming with TNFα, we assessed percentages of $mPR3^+$ neutrophils in patients with AAV and in disease and healthy controls.
Methods: Neutrophils from patients with PR3-AAV and MPO-AAV, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), and from healthy controls were analysed before and after priming with TNFα for mPR3 expression.
Results: 42% of all individuals analysed showed minimal expression for mPR3 on all neutrophils before priming with TNFα, whereas after priming a clear $mPR3^+$ subset was observed next to $mPR3^-$ neutrophils, corresponding to bimodal mPR3 expression. In patients with PR3-AAV or MPO-AAV, the percentage of $mPR3^+$ neutrophils after priming with TNFα was significantly increased ($p<0.01$ and $p<0.05$, respectively) compared with healthy controls. Percentages of $mPR3^+$ PMN were also increased in patients with SLE ($p<0.01$) but not in RA.
Conclusion: Standardised assessment of proteinase 3 on the membrane of neutrophils requires priming with TNFα. Percentages of $mPR3^+$ PMN are increased in AAV and SLE, but not in RA.

568. MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival
Kim, Y. et al
*J. Exp. Med., **204(9)**, 2063-2074 (2007)*

The innate immune system relies on evolutionally conserved Toll-like receptors (TLRs) to recognize diverse microbial molecular structures. Most TLRs depend on a family of adaptor proteins termed MyD88s to transduce their signals. Critical roles of MyD88-1–4 in host defense were demonstrated by defective immune responses in knockout mice. In contrast, the sites of expression and functions of vertebrate MyD88-5 have remained elusive. We show that MyD88-5 is distinct from other MyD88s in that MyD88-5 is preferentially expressed in neurons, colocalizes in part with mitochondria and JNK3, and regulates neuronal death. We prepared MyD88-5/GFP transgenic mice via a bacterial artificial chromosome to preserve its endogenous expression pattern. MyD88-5/GFP was detected chiefly in the brain, where it associated with punctate structures within neurons and copurified in part with mitochondria. In vitro, MyD88-5

coimmunoprecipitated with JNK3 and recruited JNK3 from cytosol to mitochondria. Hippocampal neurons from MyD88-5–deficient mice were protected from death after deprivation of oxygen and glucose. In contrast, MyD88-5–null macrophages behaved like wild-type cells in their response to microbial products. Thus, MyD88-5 appears unique among MyD88s in functioning to mediate stress-induced neuronal toxicity.

569. Proteolysis of the endothelial cell protein C receptor by neutrophil proteinase 3
Villegas-Mendez, A. et al
J. Thrombosis and Hemostasis, ***5(5)****, 980-988 (2007)*

Summary. *Background:* The endothelial cell protein C receptor (EPCR) presents protein C to the thrombin:thrombomodulin complex on the endothelium of large vessels, and enhances the generation of activated protein C (APC) and activation of protease-activated receptor-1. A previous report has demonstrated binding of soluble (s) EPCR to activated neutrophils via surface proteinase 3 (PR3). *Methods:* We now report further characterization of this interaction. Activated neutrophils and purified PR3 both decrease endothelial cell (EC) surface EPCR, suggestive of its proteolysis. *Results:* When added to purified recombinant sEPCR, PR3 produced multiple cleavages, with early products including 20 kDa N-terminal and C-terminal (after Lys^{176}) fragments. The binding of active site blocked PR3 to sEPCR was studied by surface plasmon resonance. Estimates of the K_D of 18.5–102 nM were obtained with heterogeneous binding, suggestive of more than a single interaction site. *Conclusions:* This work demonstrates PR3 binding to and proteolysis of EPCR and suggests a mechanism by which anticoagulant and cell protective pathways can be down-regulated during inflammation.

570. Cathelicidin LL-37 induces the generation of reactive oxygen species and release of human α-defensins from neutrophils
Zheng, Y. et al
Br. J. Dermatol., ***157(6)****, 1124-1131 (2007)*

Background Psoriasis is characterized by epidermal infiltration of neutrophils that destroy invading microorganisms via a potent antimicrobial arsenal of oxidants and antimicrobial agents. In contrast to atopic dermatitis, psoriasis exhibits low levels of skin infections due to the presence of antimicrobial agents, including cathelicidin LL-37. LL-37 kills a broad spectrum of microbes, and activates neutrophil chemotaxis.
Objective To determine whether or not LL-37 could regulate additional neutrophil functions such as production of cytokines/chemokines, reactive oxygen species and release of neutrophil antimicrobial peptides.
Methods Human peripheral blood neutrophils were used in this study. The production of interleukin (IL)-8 and release of α-defensins were analysed by enzyme-linked immunosorbent assay, and real-time polymerase chain reaction (PCR) was used to quantify α-defensin gene expression. Phosphorylation of mitogen-activated protein kinase (MAPK) was determined by Western blotting. The generation of reactive oxygen species was examined using flow cytometry, and intracellular Ca^{2+} mobilization was measured using a calcium assay kit.
Results LL-37 enhanced the production of IL-8 under the control of MAPK p38 and extracellular signal regulated kinase (ERK), as evidenced by the inhibitory effects of p38 and ERK1/2 inhibitors on LL-37-mediated IL-8 production. Furthermore, LL-37 induced phosphorylation of p38 and ERK. We also revealed that LL-37 stimulated the generation of reactive oxygen species dose- and time-dependently, most probably via NADPH oxidase activation and intracellular Ca^{2+} mobilization. Finally, LL-37 induced both mRNA expression and protein release of α-defensins, known as human neutrophil peptide 1–3.
Conclusion Taken together, we suggest that in addition to its microbicidal properties, LL-37 may contribute to innate immunity by enhancing neutrophil host defence functions at inflammation and/or infection sites.

571. Functional polymorphisms of the FPR1 gene and aggressive periodontitis in Japanese
Gunji, T. et al
Biochem. Biophys. Res. Comm., ***364****, 7-13 (2007)*

Aggressive periodontitis (AgP), a severe and early onset type of periodontitis, is thought to be subject to significant genetic background effects. Formyl peptide receptor 1 (FPR1) is a gene strongly implicated in AgP. To determine whether variations in this gene are associated with AgP, we performed an association study with 49 AgP patients and 373 controls using 30 variations identified by sequencing the 21.1-kb gene region. Five polymorphisms (−12915C > T, −10056T > C, −8430A > G, 301G > C, and 546C > A) showed significant association with AgP. Polymorphonuclear neutrophils from subjects carrying the −12915T allele expressed significantly lower levels of *FPR1* transcripts than those homozygous for the −12915C allele. Furthermore, the −12915T allele decreased activity of transcriptional regulation in a luciferase assay. Haplotype association analysis with three SNPs (−12915C > T, 301G > C, and 546C > A) revealed that one haplotype (−12915T–301G–546C) was significantly represented in AgP patients ($p = 0.000020$). Thus, altered FPR1 function might confer increased risk to AgP.

572. Effects of low dose endotoxemia on endothelial progenitor cells in humans
Mayr, F.B. et al
Atherosclerosis, ***195****, e202-e206 (2007)*

Background
Endothelial progenitor cells (EPCs) are a specific subtype of hematopoietic stem cells that migrate from the bone marrow to the peripheral circulation where they contribute to the repair of injured endothelium and to the formation of new blood vessels. Levels of circulating EPCs have been investigated in different inflammatory disease states. However, data on circulating EPC levels and systemic inflammation remain scarce and contradictory.
Objective
We investigated a putative relationship of low grade experimental endotoxemia to changes in circulating EPC levels.
Methods
Randomized, double-blind, placebo-controlled parallel group trial in 36 healthy male volunteers. Thirty-two volunteers received 2 ng/kg LPS intravenously, the remaining four an equal volume of physiologic saline solution as placebo.
Results
Endothelial progenitor cells showed a significant decrease over the observation period among the 32 subjects challenged with LPS ($P < 0.0001$) and reached their nadir at 6 h, with a median decrease of 62% (interquartile range: 48–81%) compared with baseline levels. Circulating EPCs returned to values comparable to baseline 24 h after LPS challenge.
Conclusion
Infusion of 2 ng/kg LPS led to a significant decrease in peripheral EPCs. These results suggest that the early phase of acute inflammation is associated with a decrease in peripheral EPCs.

573. Gene expression signature in peripheral blood cells from medical students exposed to chronic psychological stress
Kawai, T. Et al
Biol. Physiol., ***76****, 147-155 (2007)*

To assess response to chronic psychological stress, gene expression profiles in peripheral blood from 18 medical students confronting license examination were analyzed using an original microarray. Total RNA was collected from each subject 9 months before the examination and mixed to be used as a universal control. At that time, most students had normal scores on the state-trait anxiety inventory (STAI). However, STAI scores were significantly elevated at 2 months and at 2 days before the examination. Pattern of the gene expression profile was more uniform 2 days before than 2 months before the examination. We identified 24 genes that significantly and uniformly changed from the universal control 2 days before the examination. Of the 24 genes, real-time PCR validated changes in mRNA levels of 10 (*PLCB2*, *CSF3R*, *ARHGEF1*, *DPYD*, *CTNNB1*, *PPP3CA*, *POLM*, *IRF3*, *TP53*, and *CCNI*). The identified genes may be useful to assess chronic psychological stress response.

574. α-Tocopherol increases caspase-3 up-regulation and apoptosis by β-carotene cleavage products in human neutrophils
Salerno, C., Capuozzo, E., Crifo, C. and Siems, W.
Biochem. Biophys. Acta, ***1772****, 1052-1056 (2007)*

It has been found that β-carotene cleavage products (CarCP), besides having mutagenic and toxic effects on mitochondria due to their prooxidative properties, also initiate spontaneous apoptosis of human neutrophils. Therefore, it was expected that antioxidants such as α-tocopherol would inhibit the stimulation of apoptosis and caspase-3 activity by CarCP. However, we found that α-tocopherol increases caspase-3 up-regulation and stimulation of apoptosis of human neutrophils by CarCP. Ascorbic acid does not alter this caspase-3 up-regulating and proapoptotic effect exerted by α-tocopherol. Both α-tocopherol and ascorbic acid, in the absence of CarCP, decrease intracellular caspase-3 activity and spontaneous apoptosis of neutrophils. Uric acid alone or in combination with CarCP does not exert apparent effects on caspase-3 activity and apoptosis. Up-regulating effect of α-tocopherol is not observed in the presence of retinol that markedly stimulates apoptosis by itself, whereas increase of caspase-3 activity is induced by concomitant addition of α-tocopherol and β-ionone, a cyclohexenyl degradation product of β-carotene with shorter aliphatic chain.

575. Protease activation of calcium-independent phospholipase A_2 leads to neutrophil recruitment to coronary artery endothelial cells
White, M.C. and McHowat, J.
Thromb. Res., ***120****, 597-605 (2007)*

Introduction
Thrombin or tryptase cleavage of protease-activated receptors (PAR) on human coronary artery endothelial cells (HCAEC) results in activation of a membrane-associated, calcium-independent phospholipase A_2 ($iPLA_2$) that selectively hydrolyzes plasmalogen phospholipids. Atherosclerotic plaque rupture results in a coronary ischemic event in which HCAEC in the ischemic area would be exposed to increased thrombin concentrations in addition to tryptase released by activated mast cells present in the plaque.
Materials and methods

HCAEC were stimulated with thrombin or tryptase in the absence or presence of bromoenol lactone (BEL), a selective $iPLA_2$ inhibitor, and $iPLA_2$ activation, accumulation of biologically active membrane phospholipid-derived metabolites, upregulation of cell surface P-selectin expression and neutrophil adherence were measured.

Results

HCAEC exposed to thrombin or tryptase stimulation demonstrated an increase in $iPLA_2$ activity and arachidonic acid release. Additionally, stimulated HCAEC demonstrated increased platelet-activating factor (PAF) production and cell surface P-selectin expression, resulting in increased adhesion of neutrophils to HCAEC monolayers. Pretreatment with bromoenol lactone to inhibit $iPLA_2$, blocked membrane phospholipid-derived metabolite production, increased cell surface P-selectin expression and neutrophil adherence.

Conclusions

The similar biochemical and cellular responses in HCAEC exposed to thrombin or tryptase stimulation suggest that the cleavage of two separate PAR serve to extend the range of proteases to which the cells respond rather than resulting in separate intracellular events. This suggests that in conditions such as thrombosis and atherosclerosis that multiple mechanisms can activate the inflammatory response.