CELL ADHESION MECHANISMS IN LEUKOCYTE TRAFFIC

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Differentiation of the Microvasculature

C 001 MATRIX ORGANIZATION: A MODULATOR OF MICROVASCULAR ENDOTHELIAL DIFFERENTIATION by Joseph A. Madri[1], Martin Marx[2] and June Rae Merwin,[1] [1]Yale University School of Medicine, New Haven, CT, USA 06510, [2]University of Erlangen, Germany.

Blood vessels are comprised of a variety of cell types including vascular endothelial and smooth muscle cells. In large vessels these two cell types appear to exhibit stable phenotypes. In contrast, in the microvasculature, endothelial cells and pericytes appear to exhibit "plastic" phenotypes in vivo and in vitro. In vivo, during inflammatory responses, microvascular endothelial cells have been shown by others to express PDGF receptors. In vitro, when cultured under certain conditions, microvascular endothelial cells have been shown to express α smooth muscle actin mRNA and protein as well as PDGF receptor α and β chains while retaining their Factor VIII related antigen expression. In addition both of these moieties (α-SM actin and PDGFRα & β chains) appear to be modulated in vitro by the composition and organization of the extracellular matrix. Microvascular endothelial cells plated and grown on type I collagen express α smooth muscle actin and PDGF receptor α and β chains. These cells exhibit a substantial proliferative response to PDGF BB, a moderate proliferative response to PDGF AB and no proliferative response to PDFG AA consistent with the notion that these cells express the ββ and αβ forms of the PDGF receptor. In addition, these cells do not readily undergo tube formation and do not exhibit organization of cell adhesion molecules (PECAM-1) or formation of tight junctional complexes. In contrast, in three dimensional culture, as these cells form complex, branching capillary-like networks in response to a variety of soluble factors. These cells lose their expression of α smooth muscle actin mRNA and protein, as well as their expression of PDGF receptors and responsiveness to PDGF isoforms and exhibit organization of PECAM-1 in areas of cell-cell contact and form tight junctional complexes as evidenced ultrastructurally and by the assembly of ZO-1. These findings are consistent with a "plastic" microvascular endothelial cell phenotype, which can be reversibly modulated between pericytic and endothelial phenotypes, depending, in part, upon the composition and organization of the surrounding extracellular matrix.

C 002 MOLECULAR GENETIC ANALYSIS OF P-SELECTIN FUNCTION, Denisa D. Wagner, New England Medical Center and Tufts University, Boston.

P-selectin, an adhesion receptor for leukocytes, is synthesized specifically by megakaryocytes and endothelial cells. It is not continually present on the plasma membrane but rather it is stored in the α-granules of platelets or in Weibel-Palade bodies of endothelial cells. Activation of these cells with agents leading to granule secretion causes rapid translocation of P-selectin to the plasma membrane. The surface expression of P-selectin is short lived as it is internalized by endocytosis. E-selectin, another receptor of the selectin family, is expressed on the endothelial cells after activation with cytokines by <u>de novo</u> synthesis. In contrast, it can be detected on the cell surface for several hours. We have found that this difference is not due to different rates of endocytosis of these two proteins but rather in the less synchronized expression of E-selectin as compared to P-selectin on the cell surface. While E-selectin after endocytosis appears to end up in lysosomes, P-selectin returns through the Golgi apparatus to nascent Weibel-Palade bodies. The exact biological function of P-selectin is not yet known. Deficiency in this relatively recently recognized protein has not been reported in animal nor man. We therefore decided to develop a mouse model of P-selectin deficiency to study the function of the protein by analyzing the defects resulting from its absence. Embryonic stems cells were transfected with a replacement vector containing modified 5'end of the mouse P-selectin gene. After double selection and screening of clones for an homologous recombination event, three clones were identified that contained the dysfunctional P-selectin gene allele. These embryonic stem cells were then injected into blastocyst to generate chimeric animals. Germ line transmission of the defective gene allowed us to obtain first heterozygous and finally homozygous offsprings. The existence of homozygous animals was determined by southern blot analysis of tail DNA and by the lack of platelet P-selectin by immunofluorescent staining with a polyclonal antibody to P-selectin. The homozygous animals are fertile and appear grossly normal. It therefore appears that P-selectin does not play a role in embryogenesis and angiogenesis. We will determine the effect of P-selectin gene alteration on circulating leukocytes and platelets. For example, we will examine whether the absence of P-selectin affects leukocytes rolling on the mesentery and/or their recruitment to sites of inflammation.

Regulation of CAM Expression: Transcriptional and Post-Transcriptional Controls

C 003 DYNAMIC REGULATION OF NCAM SPLICING AND EXPRESSION, Richard Akeson, Department of Basic Science Research,Childrens Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio, 45229

The neural cell adhesion molecule NCAM is a member of the immunoglobulin superfamily which was first detected on neural tissue. However polypeptide products of the single NCAM gene are widely expressed in a number of tissues including varying subsets of leukocytes in different species. NCAM primary transcripts are alternatively spliced at three positions to ultimately yield a predicted 192 distinct polypeptide products. This alternative splicing gives NCAM isoforms with varying or absent cytoplasmic domains, with varying numbers of small exons in the membrane proximal region containing the fibronectin-like repeats, and with a fourth immunoglobulin-like domain which either contains or lacks a 10 amino acid exon termed VASE. NCAM alternative splicing combinations in developing heart appear to be largely stochastic. However in neural tissue coordinate regulation of splicing is observed. NCAM isoforms with small and large cytoplasmic domains have very low levels of alternative small membrane proximal exons while the majority of NCAM mRNAs encoding the isoform which lacks a cytoplasmic domain contains these small exons. Splicing of the VASE exon is developmentally regulated among all isoforms in neural tissue. Only 1-2% of NCAM mRNAs from embryonic day 15 rat brain contain VASE while about 40% of mRNAs from adult brain express VASE. The functional significance of NCAM alternative splicing is a topic of intense current analysis. The distinct cytoplasmic domains of NCAM isoforms do not strongly modulate the ability of transfected NCAM molecules to mediate adhesion. NCAM isoforms with both small and large cytoplasmic domains as well as that lacking a cytoplasmic domain are capable of mediating adhesion. The possibility that these cytoplasmic domains could mediate differential signal transduction events remains open. Alternative small exons in the membrane proximal region may influence molecular conformation and potentially the ability of NCAM to span varying intercellular spaces. The fourth immunoglobulin-like domain is not known to be directly involved in either homophilic or heterophilic NCAM mediated adhesion. However the presence of the VASE exon in this domain alters the biological activities of transfected NCAM isoforms. Fibroblasts expressing NCAM isoforms lacking VASE self aggregate more rapidly than fibroblasts expressing NCAM+VASE. When tested as a growth substrate, cells expressing NCAM lacking VASE were more permissive for axon outgrowth than cells expressing NCAM+VASE. Similarly transfected cells expressing NCAM lacking VASE were more active in neurite outgrowth than cell expressing NCAM+VASE. These experiments indicate that the presence of the VASE exon has a significant modulatory effect on NCAM mediated cellular activities. Therefore complete analysis of potential NCAM function in diverse tissues including those of the immune system requires definition of the exact NCAM isoforms expressed.

C 004 E-SELECTIN (ELAM-1) GENE EXPRESSION IS REGULATED BY MODULATING NFκB ACTIVITY. J. Whelan, R. Hooft van Huijsduijnen, P. Ghersa, W. Kaszubska, J.F. DeLamarter, Glaxo Institute for Molecular Biology, Plan-les-Ouates, Geneva, Switzerland

The cytokine-induced cell-specific activity of the E-selectin gene is tightly regulated. We have identified three proximal promoter elements and their DNA binding factors which are essential for induced expression of the E-selectin gene. Mutation of any one of these elements markedly inhibits cytokine-induced promoter activity. One of these elements represents the binding site for NFκB, whose binding to this site is induced by IL-1 treatment of endothelial cells. However, the ability of NFκB to activate the E-selectin promoter is modulated by interaction with the other essential factors bound to the promoter. We refer to the additional factors as NF-ELAM1 and 2. Multimers of either the NF-ELAM1 or 2 binding sites alone fails to augment reporter gene expression when placed upstream of a heterologous enhancerless promoter. In contrast, insertion of multimers of an NFκB binding site upstream of the same promoter renders expression of the reporter gene IL-1-responsive. Insertion of combined NF-ELAM1/NFκB binding sites significantly increases this IL-1-responsiveness. These results indicate that NF-ELAM1 and 2 alone do not have enhancer activity. However, NF-ELAM1 acts as an enhancer in cooperation with NFκB.

We have isolated several cDNA clones which encode proteins which interact specifically with the NF-ELAM1 recognition sequence. These cDNA's encode proteins which are non-cAMP responsive members of the ATF family of transcription factors. Co-transfection expression experiments with various ATF factors and NFκB have demonstrated an ability of the ATF factors to modulate NFκB-induced E-selectin promoter activity. *In vivo*, cAMP induction fails to augment either uninduced or IL-1-induced E-selectin expression. These observations indicate that cytokine-induced E-selectin gene expression is modulated by interactions between NFκB and non-cAMP responsive members of the ATF family of transcription factors.

Signal Transduction and Affinity/Avidity State Changes of CAMs

C 005 ROLE AND REGULATION OF L-SELECTIN IN LEUKOCYTE ATTACHMENT TO ENDOTHELIUM, Thomas F. Tedder, Geoffrey S. Kansas, and Boris Schleiffenbaum, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

L-selectin is expressed on granulocytes, lymphocytes and monocytes, cell types with different patterns of recirculation and migration. These differences in migration are not attributable to differences in the structure of L-selectin as only a single protein core is expressed by all cell types and 13 distinct epitopes identified by monoclonal antibodies (mAb) are present on each cell type. Furthermore, L-selectin directly mediates both lymphocyte attachment to high endothelial venules (HEV) and attachment of all leukocytes classes to cytokine-activated endothelial cells. Transfection of L-selectin cDNA into cells which do not normally bind to endothelium confers adhesion. Furthermore, L-selectin transfected cells roll in rat mesenteric venules *in vivo* demonstrating that expression of L-selectin alone is sufficient to mediate leukocyte rolling[1]. Thus, leukocyte adhesion to endothelium through L-selectin may be differentially regulated in several ways. First, L-selectin is expressed by distinct leukocyte subsets during different stages of their differentiation and can be rapidly shed from the cell surface following cellular activation. Shed L-selectin (sL-selectin) from both lymphocytes and neutrophils is present at high levels in human plasma, with mean sL-selectin levels of 1.6 ± 0.8 μg/ml (n=63) in normal individuals. In addition, semipurified sL-selectin from plasma inhibits L-selectin-specific attachment of lymphocytes to cytokine-activated endothelium in a dose dependent manner. Attachment is completely inhibited at sL-selectin concentrations of 8 to 15 μg/ml, while physiological concentrations of sL-selectin cause a small but consistent inhibition of lymphocyte attachment. sL-selectin in plasma also inhibits anti-L-selectin mAb (2 to 5 μg/ml) binding to the surface of leukocytes. The presence of serum sL-selectin with functional activity indicates a potential role for sL-selectin in the regulation of leukocyte attachment to endothelium. Second, the affinity of L-selectin for ligand is transiently increased following leukocyte activation. Lineage specific activation signals induce a rapid (min) increase in receptor affinity for ligand followed by a decrease in ligand binding and shedding of the receptor.

This process can be recapitulated by the binding of a unique mAb to the EGF domain of L-selectin, suggesting the involvement of the EGF domain in this process. Interestingly, this same epitope present within the EGF-like domain of sL-selectin is lost in sL-selectin, suggesting a conformational change in the receptor after shedding. The affinity change in the receptor as well as receptor function is likely to be controlled by cellular signals generated from within the cell. This is suggested by the finding that the cytoplasmic domain of L-selectin is required for lymphocyte adhesion to HEV and is also required for leukocyte rolling[2]. Third, expression of the ligand by endothelial cells is induced following activation with inflammatory cytokines. Leukocyte attachment assays demonstrate that the L-selectin ligand is induced in both umbilical vein endothelium as well as kidney glomerular endothelium, suggesting a broad tissue distribution. Induced expression of the ligand also persists for long periods of time (>72 h). Furthermore, the L-selectin ligand is utilized by lymphocytes, neutrophils and monocytes to mediate the initial phase of leukocyte attachment to endothelium. Thus, the coordinated regulation of L-selectin and it's ligand may in part explain how a single receptor appears to mediate different functions on different cell types.

[1] Ley, K., Tedder, T.F., Kansas, G.S. Keystone Symposium on Cell Adhesion Mechanisms in Leukocyte Traffic;1992 (abstract).
[2] Kansas, G.S., Ley, K., Tedder, T.F. Keystone Symposium on Cell Adhesion Mechanisms in Leukocyte Traffic; 1992 (abstract).
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CAM Interactions with the Cytoskeleton/Junctional Integrity of Endothelium

C 006 CADHERINS AND ASSOCIATED PROTEINS IN EPITHELIAL CELL JUNCTIONS, Pierre D. McCrea and Barry M. Gumbiner, The Sloan-Kettering Institute, New York.

E-cadherin is a Ca^{2+}-dependent adhesion molecule that controls the state of the epithelial junctional complex. Its association with a bundle of actin filaments at the *zonula adhaerens* junction seems to be required for its function. A set of three putative cytoskeletal linker proteins, called alpha, beta, and gamma-catenins, interact tightly with the cytoplasmic tail of cadherins and are necessary for cadherin function. We have purified beta-catenin from *Xenopus* epithelial cells and cloned and sequenced the cDNA encoding it. *Xenopus* beta-catenin is highly homologous to *armadillo* (70% amino acid identity), a protein encoded by a *Drosophila* segment polarity gene, and to plakoglobin (63% amino acid identity), a mammalian protein isolated from desmosomal junctions. Beta-catenin and plakoglobin are actually distinct proteins expressed from different genes in the same epithelial cells. Plakoglobin also interacts with the cytoplasmic tail of E-cadherin, and therefore is a component of the cadherin-catenin protein complex. The phenotypes of *armadillo* mutations in early *Drosophila* development raises the possibility that beta-catenin is involved in transducing developmental signals in vertebrates. Our findings from preliminary studies of beta-catenin *function in Xenopus* embryos are consistent with such a role. Thus the E-cadherin-catenin complex may facilitate both intercellular communication and the physical associations between epithelial cells in vertebrates.

Carbohydrate Recognition: Selectins and Their Ligands (A) (Joint)

C 007 LEUKOCYTE INTERACTIONS WITH P-SELECTIN, Rodger P. McEver, W.K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, and Oklahoma Medical Research Foundation, Oklahoma City, OK.

P-selectin is an adhesion receptor for myeloid cells and lymphocyte subsets that is expressed by megakaryocytes/platelets and endothelial cells. A sorting signal in the cytoplasmic tail directs P-selectin to secretory granules in both platelets and endothelial cells; it is then rapidly redistributed to the cell surface in response to agonists such as thrombin. An alternatively spliced soluble form of P-selectin is secreted constitutively because its C-terminus no longer faces the cytoplasm where it can serve as a sorting signal. Membrane P-selectin and recombinant soluble P-selectin bind with *apparent high affinity* to a saturable number of protease-sensitive sites on myeloid cells. Cell recognition requires a conformational change in the lectin domain resulting from occupancy of two Ca^{2+}-binding sites. Peptides from discontinuous regions of the lectin domains of selectins block neutrophil adhesion to both P- and E-selectin. Ca^{2+} binds to peptides from two regions and enhances their ability to support myeloid cell adhesion. Although P-selectin, like the other selectins, binds to multivalent forms of the tetrasaccharides sialyl Lewis x and sialyl Lewis a, the carbohydrate structures of high affinity ligands on cell surfaces have not been defined. P-selectin binds preferentially to a single glycoprotein in extracts of myeloid cells that may correspond to the high affinity binding sites on intact neutrophils. The glycoprotein ligand appears to be a disulfide-linked homodimer with subunits of Mr 120,000. It is heavily sialylated, expresses the sialyl Lewis x epitope, and appears to contain only 1 or 2 one N-linked glycans but many clustered O-linked oligosaccharides. Further studies of this and other high affinity ligands for selectins may help clarify how selectins mediate rapid cell adhesion under flow conditions in the circulation.

Carbohydrate Recognition: Selectins and Their Ligands (B) (Joint)

C 008 **SELECTIN LIGANDS AND ANTAGONISTS - BEYOND SIALYL LEWIS X,** Brian K. Brandley, Mina Nashed, Falguni Dasgupta, Saeed Abbas, Darwin Asa, Carrol Foxall, and John Musser. Glycomed, Inc., Alameda, CA 94501.

The discovery of the selectins and the importance in leukocyte-endothelium adhesion has recently refocused attention on lectin mediated cell adhesion. Several potential carbohydrate ligands have been identified for the selectins. These can be broadly divided into two groups: 1) Sialyl Lewis X (sLe^x) and related oligosaccharides, and 2) sulfated carbohydrates. All three functional groups on sLe^x oligosaccharides, although with differing avidities. We have identified the functional groups on sLe^x analogs that replace sialic acid and fucose residues with simpler and more stable substituents. Although the process is ongoing, we have been successful at replacing the sialic acid residue with acetic or lactic acid groups. The second group of ligands all contain sulfate on a carbohydrate support, and bind to the selectins with characteristics that differ from sLe^x binding. Such compounds are recognized by L-Selectin and P-Selectin, but in general, not E-Selectin. The most recently discovered member of this class of ligands is an HNK-1 reactive epitope, the SulfoGlucuronlyNeoLacto (SGNL) structure. While the biological implication of this second group of potential ligands is unclear, our data may indicate the L-Selectin and P-Selectin can bind via a mechanism independent of, or in conjunction with, sLe^x like structures.

C 009 LEUKOCYTE ADHESION DEFICIENCY (LAD II) - DEFICIENCY IN SIALYL LEWIS X, A LIGAND FOR SELECTIN - DUE TO GENERAL FUCOSE DEFICIENCY, Amos Etzioni[1,2], John M. Harlan[3], M. Laurie Phillips[4], Shimon Pollack[1,2], Ruth Gershoni-Baruch[1,2] and James C. Paulson[4], Rambam Medical Center and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [3]University of Washington, Seattle, and [4]Cytel Corporation, San Diego, CA.

"It is in her moments of abnormality that nature reveals her secrets." (Goethe) The occurrence of recurrent bacterial infections, neutrophil dysfunction and normal expression of CD18 integrins in two unrelated children suggested an as yet undescribed adhesion deficiency. The fact that both children exhibited the rare Bombay blood group and were Lewis negative, each involving carbohydrates with different fucose linkages, a possible defect in the fucose containing ligand of E- and P-selectin, sialyl-Lewis-X (SLe^x), was considered. Indeed, using a monoclonal SLe^x antibody, no expression of SLe^x on the patients' neutrophils was detected. Adhesion to IL-1β or histamine activated endothelial cells was markedly decreased (<5% of control). The observation that the neutrophils did not bind to recombinant E- and purified P-selectins confirmed the SLe^x deficiency as the basis for the adhesion deficiency. Low binding of fucose specific lectins to EBV virus transformed patient β-lymphocytes was observed, while the binding of mannose specific lectins was normal, providing further evidence for a general fucose deficiency as the primary defect. The existence of the patients and their deficiency emphasizes the essential role of the endothelial cell selectin and the SLe^x ligands on the neutrophils in an early step of neutrophil recruitment to sites of infection. We would like to designate this syndrome as LAD II and the previously described CD18 deficiency as LAD I.

C 010 SELECTINS AS THERAPEUTIC TARGETS FOR INFLAMMATORY DISEASE, James C. Paulson[1], M. Laurie Phillips[1], Amos Etzioni[2], Hiroshi Asako[3], D. Neil Granger[3], John M. Harlan[4], Michael S. Mulligan[5], Peter A. Ward[5] and Robert K. Winn[4], [1]Cytel Corporation, San Diego, CA, [2]Rambam Medical Center and The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [3]Louisiana State University Medical Center, Shreveport, LA, [4]University of Washington, Seattle, WA, [5]University of Michigan, Ann Arbor, MI.

Recruitment of neutrophils to sites of inflammation in tissue injury involves the combined action of multiple adhesion molecules, cytokines, and chemoattractants. Three members of the selectin family, L-selectin, E-selectin and P-selectin, participate in the initial rolling of neutrophils on the activated endothelium of the blood vessel wall. The endothelial cell selectins, E-selectin and P-selectin, mediate adhesion by recognition of a carbohydrate ligand, sialyl Lewis X (SLe^x), expressed on the carbohydrate groups of glycoprotein receptors on the resting neutrophil. Based on 1) the discovery of a new human leukocyte adhesion deficiency (LAD II) involving the absence of SLe^x expression on patient neutrophils and 2) inhibition of neutrophil recruitment in response to E- and P-selectin antagonists, the interaction of the endothelial cell selectins with their carbohydrate ligand on neutrophils appears to be a prerequisite step for efficient neutrophil recruitment. These findings suggest that E- and P-selectin are suitable targets for development of therapeutic agents to prevent inflammatory disease mediated by neutrophils and other leukocytes recognized by these adhesion molecules.

Dynamics of Transendothelial Migration

C 011 TRANSENDOTHELIAL MIGRATION OF HUMAN T LYMPHOCYTES: A RECEPTOR MEDIATED PROCESS OF SPECIFIC T CELL SUBSETS, Nancy Oppenheimer-Marks and Peter E. Lipsky, The University of Texas Southwestern Medical Center, Dallas, TX 75235.

Using an *in vitro* assay of the transendothelial migration of T cells, the adhesion molecules mediating this process have been examined. The adhesion of T cells to EC that had been activated by IL-1 was markedly, but not completely inhibited by mAb to VCAM-1 as well as to its counter-receptor, VLA-4 (CD49d/CD29), whereas T cell binding to IL-1 activated EC was independent of the activities of ICAM-1 and its counter-receptor, LFA-1 (CD11a/CD18). In contrast, ICAM-1/LFA-1, but not VCAM-1/VLA-4, mediated much, but not all of the binding of T cells to unstimulated EC. Activation of T cells with the protein kinase C activator, phorbol dibutyrate, and the calcium ionophore, ionomycin, was found to alter the receptor counter-receptor pairs utilized for binding to EC. The binding of activated T cells to EC was less dependent on the activity of LFA-1 than that of resting T cells, and ICAM-1 played a minor role. Regardless of the activation status of the T cells or the EC, VCAM-1 was never found to function during transendothelial migration, even when it mediated the binding of T cells to EC. In contrast, ICAM-1 was found to play an important role in transendothelial migration under all of the conditions examined, including situations when T cell-EC binding was not dependent on ICAM-1. These studies confirm that the receptor pairs, ICAM-1/LFA-1 and VCAM-1/VLA-4 play specific roles in the various aspects of interactions between T cells and EC.

The nature of the T cells capable of transendothelial migration was the focus of additional studies. Another *in vitro* model was developed that permitted cells that bound to EC and those that migrated through EC to be harvested and analyzed. Of freshly prepared human peripheral blood T cells, 13±2% bound to EC and 10±1% migrated through EC after a 4 hour incubation at 37°C. The migratory capacity of T cells appeared to be an inherent property of these cells as migrated cells exhibited a markedly increased capacity to migrate on a second analysis. Analyses of the surface phenotype of the CD4(+) T cells in the migrated population revealed that these cells were mainly $CD29^{bright}$ and CD45RO(+), as well as L-selectin(-), but did not express activation molecules. In contrast, cells that neither bound nor migrated were enriched in $CD29^{dim}$/CD45RO(-) cells that expressed L-selectin. Pretreatment of the EC with the cytokines, IL-1 and IFN-γ, did not influence the phenotype of the CD4(+) T cells that were recovered in the migrated population. These results indicate that a subset of resting memory T cells exhibits an enhanced intrinsic capacity for transendothelial migration.

C 012 TRANSENDOTHELIAL MIGRATION OF NEUTROPHILS, C. Wayne Smith[1], B.J. Hughes[1], Scott Simon[2], Martyn Robinson[3], [1]Baylor College of Medicine, Houston, TX 77030, [2]University of New Mexico, Albuquerque, NM 87131, [3]Celltech Ltd., Slough, England.

The emigration of neutrophils at sites of inflammation initially involves migration through the endothelial lining of small venules. Several models of transendothelial migration have been developed *in vitro* that allow the investigation of specific stimuli and adhesion molecules critical to the process. Current evidence indicates that the endothelium plays an active role by expressing adhesion molecules that catch unstimulated neutrophils as they flow past the luminal surface of the endothelial cell, and they express chemotactic factors that activate adhesive and motile mechanisms in the attached neutrophils. Two members of the Selectin family have been shown to function under conditions of flow. L-selectin on the neutrophil and P-selectin on the endothelial cell each appear to be sufficient to initiate neutrophil rolling under conditions of flow. We have recently demonstrated that E-selectin when expressed on the surface of monolayers of L cells or on endothelial cells is also sufficient to support rolling of previously unstimulated neutrophils. This adhesion is largely inhibited by monoclonal antibodies (MAbs) against E-selectin and by MAbs against L-selectin, supporting previous work indicating a functional interaction between L-selectin and E-selectin. Chemotactic stimulation under conditions that shed L-selectin from the neutrophil surface markedly reduces that ability of E-selectin to catch flowing neutrophils. On E-selectin expressing L cells, neutrophil rolling proceeds with only transient periods of stopping. When stationary, the neutrophils remain spherical. In contrast, on IL-1-stimulated endothelial cells, rolling usually stops and the neutrophils change shape and undergo transendothelial migrating, usually within 2 min after stopping. This migration is not blocked by anti-E- or L-selectin MAbs, but is almost completely inhibited by anti-CD18 MAbs.

Optimal transendothelial migration of neutrophils requires both CD11a/CD18 and CD11b/CD18, as shown by the fact that MAbs specific for each integrin are partially inhibitory but when combined are completely inhibitory. We have investigated the contribution of CD11b/CD18 (Mac-1) in more detail, specifically the role of newly upregulated Mac-1. The response of human neutrophils to changing concentrations of chemotactic stimulus was evaluated by determining the amount of newly arrived surface Mac-1, and Mac-1-dependent locomotion. The results support the hypothesis that small increments in the chemotactic stimulus bring Mac-1 to the cell surface, and this newly mobilized Mac-1 is available for adherence-dependent locomotion with subsequent increases in the concentrations of the stimulus. In addition, these adhesive events are transient, with Mac-1 apparently increasing and then decreasing avidity for its ligand on the endothelial cell. The contribution that the return to low adhesion plays in adherence-dependent cell locomotion was evaluated using an anti-CD18 MAb (KIM127) that sustains the high avidity state of Mac-1. This MAb inhibited neutrophil detachment by >90%, and reduced chemokinetic migration on protein-coated glass by >85%. It also produced a highly significant reduction in transendothelial migration. Thus, migration of neutrophils through confluent monolayers of endothelial cells is a CD18-dependent process that requires a highly coordinated sequence of $\beta2$ integrin activation, upregulation and detachment. The Selectins appear to play no direct role in this process after they catch flowing neutrophils, placing them in the micro-environment were $\beta2$ activation can proceed.

C 013 THE DISTINCTIVE FUNCTIONS OF SELECTINS, INTEGRINS, AND IG FAMILY MOLECULES IN REGULATION OF LEUKOCYTE INTERACTION WITH ENDOTHELIUM, Timothy A. Springer, The Center for Blood Research, 200 Longwood Avenue, Boston, MA 02115.

Adhesion molecules together with cytokines and chemoattractants regulate leukocyte interaction with endothelium and subsequent events such as migration through the endothelium and within tissues, and cell-cell interactions that are important in inflammatory responses including those of granulocytes and monocytes with host cells and foreign pathogens, and antigen-specific responses of lymphocytes. Adhesion receptors are also used by cells to sense information about the environment that can stimulate signalling pathways. Three families of adhesion receptors have distinct functions in cell interactions. Ig family molecule density on the surface regulates cell interactions; some molecules are inducible by cytokines such as ICAM-1 and VCAM-1 and others are constitutively expressed such as ICAM-2. Integrin adhesive activity can be regulated independently of surface expression by intracellular signals acting on integrin cytoplasmic domains that appear to affect the conformation of the extracellular ligand binding domain. Selectins mediate attachment of leukocytes ot the vessel wall under flow conditions at which integrin and Ig family members are ineffective. The initial steps in leukocyte accumulation have been reconstituted in on vitro system. Neutrophils in a parallel plate flow chamber roll on artificial phospholipid bilayers containing the selectins CD62 or ELAM-1. The results are the same on bilayers containing CD62 and ICAM-1, expect when a chemoattractant is infused, activation of integrins causes the rolling neutrophils to arrest and then spread. The accompanying adhesion developed through the integrin-ICAM-1 interaction is more than 100-fold stronger than through selectins.

C 014 JUXTACRINE INTERACTIONS OF ENDOTHELIAL CELLS WITH LEUKOCYTES, Guy A. Zimmerman[1], Diane E. Lorant[1], Kamala D. Patel[1], Ralph E. Whatley[1], Rodger P. McEver[2], Thomas M. McIntye[1], Stephen M. Prescott[1]. [1]CVRTI and University of Utah School of Medicine, Salt Lake City, Utah, 84112, and [2]Oklahoma Medical Research Foundation and University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104.

The Interaction of circulating white blood cells with the endothelium of post-capillary venules and other vessels involves regulated expression of molecules on both the endothelial cell (EC) and the leukocyte. This is true of each of the major classes of leukocytes including granulocytes, monocytes, and lymphocytes. Time-dependent expression of different patterns of adhesion molecules by EC provides a mechanism for differential targeting of specific classes of leukocytes in acute and subacute inflammation. Our studies of the interaction of myeloid leukocytes with cultured human endothelium indicate that, in addition to adhesion, the functional state of the leukocyte is altered at the EC surface by molecules of diverse structure, mechanism of expression, and mechanism of action. For example, PMNs, a subset of granulocytes, bind rapidly and reversibly to EC induced to coexpress P-selectin and Platelet-activating Factor (PAF). In addition, the PMNs have an increase in $[Ca^{+2}]i$, functionally upregulate α_M/β_2 - integrin (CD11b/CD18), polarize, and become "primed" for enhanced degranulation when subsequently stimulated with chemotactic factors. Using purified P-selectin alone or incorporated in model membranes, and P-selectin expressed by transfected cells, we find that it induces none of the responses directly. However, tethering of the PMN to the EC surface is required for events such as priming to occur efficiently, as shown by experiments with blocking monoclonal antibodies. This indicates that tethering by P-selectin facilitates interaction of the PMN with a second molecule that is expressed by the EC and that mediates activation of the PMN. Again using purified molecular systems and stimulated EC, we find that PAF (which is coupled to Ca^{+2} regulatory elements and protein kinases in the PMN by a seven membrane-spanning, G-protein-linked receptor) mediates each of the functional responses and that P-selectin supports each when it is induced by PAF. Thus P-selectin and PAF are components of a costimulatory system but have distinct roles: P-selectin captures or tethers the PMN and PAF is the signaling molecule that mediates juxtacrine activation. This juxtacrine system provides a mechanism for regulated activation of the PMN at the endothelial surface without requiring a fluid phase chemotactic factor, and has the biologic advantage of spatially localizing the response(s). Cytokine-activated EC have a similar system of tethering and signaling molecules that regulate both adhesion and activation of PMNs; however, different molecules are used and, in addition, signaling molecules are released and can act in a paracrine, rather than juxtacrine, fashion.

Hemopoiesis, Immunologic Memory and Specialized Adhesion/Traffic Mechanisms

C 015 GP39-CD40, AN ESSENTIAL LIGAND RECEPTOR PAIR IN THE DEVELOPMENT OF HUMORAL IMMUNITY. **Randolph J. Noelle[1], Meenakshi, Roy,[1] Fiona Durie[1], Lisa S. Marshall[1], Alfons Van den Eertwegh[2], E. Claassen[2], Jeffrey A. Ledbetter[3], Alejand Aruffo[3], [1] Department of Microbiology, Dartmouth Medical School, Lebanon, N.H., [2] TNO Medical Biological, Rijswijk, The Netherlands, [3] Bristol Myers Squibb Pharmaceutical Research Institute, Seattle, Wa.**

Although the initial interactions of T_h and B cells is class II-restricted and antigen-specific, the molecular trigger for initiating B cell activation appears to be delivered by the interactions of two non-polymorphic molecules, gp39 and its receptor, CD40. CD40 is a mitogenic receptor on the surface of all mature B cells. During cognate interactions with helper T cells (T_h), T_h become activated and express the ligand for CD40, gp39. gp39 is transiently expressed by normal $CD4^+$ T cells and by T_h clones. The expression is cyclosporine-sensitive and the expression on normal T cells is an order of magnitude lower then that observed on T_h clones. Once gp39 is expressed, the binding of gp39 to CD40 triggers thymus-dependent (TD) humoral immunity. Transduction of the activation signal to the B cell appears to be via enhanced protein tyrosine phosphorylation. T_h-B cell interaction does not appear to induce Ca^{++} mobilization, PKC translocation or changes in cAMP levels. However, specific tyrosine phosphorylated substrates can be observed upon CD40 function ablates primary and secondary humoral immune responses *in vivo*, implicating gp39 as an essential molecule for signalling. Studies with anti-gp39 show that blocking of gp39 TD responses. In addition, the polyclonal Ig responses observed in graft-versus host disease are reversed by anti-gp39 therapy. *In situ* immunohistochemical analysis of gp39 expression in immune mice reveals that gp39 is expressed by 3 days following antigen exposure and found expressed by $CD4^+$ cells mainly in the outer periarteriolar sheaths and around the terminal arterioles. Cells expressing gp39 can be found proximal to the responding antigen-specific B cells and producing IL2, IL4 and/or IFNγ. Studies with recombinant forms of soluble gp39 indicate that co-stimulatory signals may be required for optimal responses via CD40. As with murine T cells, a ligand for CD40 is also expressed on human $CD4^+$ T cells. Further studies on the involvement gp39-CD40 interactions in the regulation of humoral immunity will be presented.

C 016 ADHESION MOLECULES UTILIZED BY LYMPHO-HEMOPOIETIC PROGENITOR CELLS TO INTERACT WITH THE BONE MARROW MICROENVIRONMENT, Paul W. Kincade, Qi He, Katsuhiko Ishihara, Kensuke Miyake, Jayne Lesley, Robert Hyman, Ron Pellitier, Charles G. Orosz, Karen Jacobsen, and Dennis G. Osmond, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, The Salk Institute, San Diego, Ohio State University, Columbus, and McGill University, Montreal.

Precursors of B lineage lymphocytes, as well as those corresponding to seven other blood cell types, only arise and mature within bone marrow. This implies that the marrow provides a unique microenvironment, which can theoretically be characterized in molecular terms. A long list of regulatory cytokines have been discovered and cloned which influence bone marrow. Many of these appear to be made in trace quantities and *in vitro* studies indicate that at least some of them are critical to replication and differentiation of progenitor cells. However, none of these molecules is exclusively made in the bone marrow. A similar situation exists with cell adhesion molecules, which have only recently been actively studied in that tissue. We obtained evidence that CD44/hyaluronate and VLA-4/VCAM-1 may be particularly important in lympho-hemopoiesis and function as CAM/ligand pairs. The same molecules, or related molecular isoforms, have been implicated in a wide variety of other functions which include embryogenesis, leukocyte homing, inflammation, wound healing, and tumor metastasis. The search continues for cell adhesion and other molecules which might be uniquely expressed within bone marrow. In the meantime, monoclonal antibodies and experimental models which were developed to study lympho-hemopoiesis have proven to be applicable to many other questions. For example, antibodies to murine VCAM-1 interfere with cardiac allograft rejection. It will be important to learn how the ligand binding ability of CAMs is modulated during differentiation to allow mature blood cells to leave the bone marrow and reciprocally, how the same CAMs are utilized later to recruit them into inflammatory sites. There is reason to believe that this may be achieved without changes in CAM biosynthesis. CD44 provides an interesting example of extensive molecular diversity and multiple apparent "activation" states. We first used the BM2 B lineage hybridoma to demonstrate that CD44 can mediate the adhesion of leukocytes to hyaluronate bearing cells. Recognition of soluble, plastic immobilized, or cell associated hyaluronate appears to be maximal with this cell line. Resting B lymphocytes are inactive in these assays, despite their expression of CD44. An "intermediate" condition is typified by AKR1 T lymphoma cells, which display moderate avidity for hyaluronate after transfection with CD44. This is strikingly elevated in the presence of a non-blocking Mab to CD44, IRAWB14. Protein sequence heterogeneity of murine, like human and rat, CD44 appears to result from alternative splicing of 8-10 variable exons. There are several reports that the human "epithelial" isoform of CD44 can not recognize hyaluronate. However, we found that the murine homologue, as well as six other variants and mutants of CD44, all mediated hyaluronate recognition in the AKR1 lymphoma system. Furthermore, all were responsive to the enhancing effects of IRAWB14. We believe that unknown molecules interacting with the cytoplasmic and/or extracellular domains of CD44 must regulate its ability to bind hyaluronate. The membrane proximal domain of CD44, which is strikingly affected by alternate splicing, is presumably important for unique functions and may mediate recognition of other ligands. A new candidate cell interaction molecule will be described which is expressed on a subpopulation of bone marrow stromal cells. Of particular interest is its polarity in marrow with respect to cells of primitive lymphoid morphology.

C 017 THE ROLE OF CD44 IN TUMOR CELL GROWTH AND MIGRATION, Ivan Stamenkovic, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston.

CD44 is a broadly distributed, polymorphic cell surface glycoprotein which appears to fulfill multiple functions. Several CD44 isoforms ranging from 85-250 kD are known to result partly from differential splicing of multiple exons encoding a portion of the extracellular domain, and partly from cell type-specific glycosylation. The amino-terminal portion of the extracellular domain of CD44 contains a lectin domain which displays homology to carilage link proteins and proteoglycan core protein. The 85kD isoform, stably expressed in lymphoid cells which are constitutively CD44-negative, promotes adhesion to hyaluronate coated surfaces. A recombinant soluble form of CD44, containing the extracellular domain of CD44 coupled to immunoglobulin constant region, binds hyaluronate in tissues. This isoform of CD44, provisionally called CD44H is presently thought to be the principal cell surface hyaluronate receptor. A second human isoform CD44E, expressed preferentially in a subset of epithelial cells and carcinomas, fails to promote cell adhesion to hyaluronate substrates, suggesting that a conformational alteration due to the additional exons may regulate binding to substrate. Introduction of CD44H and CD44 E into human lymphoma and melanoma cells revealed that CD44H expression enhanced tumorigenesis in nude and SCID mice, whereas expression of CD44E failed to do so, and in fact, inhibited tumor growth over a period of 3 months. Similarly, CD44H-positive transfectants developed metastases much more rapidly than CD44E expressors and CD44-negative counterparts. Soluble CD44-Ig was observed to block hematogenous dissemination of CD44+ lymphomas, suggesting potential use in blocking of tumor dissemination. Motility of tumor cells on hyaluronate substrates is determined, in part, by expression of CD44H. In melanoma cells, CD44H promoted migration on hyaluronate surfaces whereas CD44E failed to do so. Cytoplasmic deletion mutants of CD44H fail to enhance cell motility but retain substrate binding capacity. Expression of CD4H may play an important role in selectively determining mobility and adhesion of tumor cells in host tissues.

Alternative Roles of CAMs in Disease: A) Metastasis and B) Viral, Bacterial, and Parasitic Diseases

C 018 INTERACTIONS OF MALARIA-INFECTED ERYTHROCYTES WITH ICAM-1 AND VASCULAR ENDOTHELIUM, Anthony R. Berendt[1], Alister G. Craig[1], Gerard Nash[2], Brian Cooke[2], David J. Roberts[1], Robert Pinches[1], Shahid Khan[1], Peter Warn[3,1], Norbert Peshu[3], Kevin Marsh[1,3] and Christopher I. Newbold[1], [1]Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, England, [2]Department of Haematology, University of Birmingham Medical School, Birmingham, B15 2TT, England and [3]Wellcome-KEMRI Research Laboratories, P.O.Box 230, Kilifi, Kenya

Erythrocytes infected with mature forms of the human malarial parasite *Plasmodium falciparum* do not circulate but are sequestered in deep vascular beds by adhesion to post-capillary venular endothelium. This phenomenon is a major virulence factor and on occasions leads to the massive localisation of infected red blood cells (IRBC) in certain organs, such as the brain, with clinically serious consequences. A major goal is therefore to understand the molecular basis of adhesion of IRBC to endothelial cells and the role of this process in pathogenesis.

Adhesion of IRBC has a number of parallels with leukocyte adhesion to endothelium. Both involve specific interactions between receptors and counter-receptors which must occur with sufficiently rapid kinetics and strong enough forces to immobilise a cell under the shear flow conditions prevailing in the post-capillary venule. Furthermore the apparently selective localisation of IRBC to certain vascular beds and the differences in relative distributions of IRBC between organs in different individuals and disease states imply a degree of heterogeneity of adhesion mechanisms with some conceptual similarities to leukocyte homing. We have studied adhesion of IRBC from laboratory and clinical isolates to vascular endothelium, transfected cells and purified proteins under static and shear flow conditions to define novel receptors and assess the clinical relevance of those identified to date. Although two endothelial receptors for adhesion, the secreted glycoprotein thrombospondin and the membrane glycoprotein CD36 (glycoprotein IV) were identified some time ago, doubt remains as to their role in all manifestations of severe disease, in particular cerebral malaria.

We showed that intercellular adhesion molecule-1 (ICAM-1) is an adhesion receptor for some strains of parasite and have more recently mapped the binding site on ICAM-1 for the IRBC using domain expression constructs, monoclonal antibody epitope mapping and mouse-human chimeric ICAM-1 mutants. The IRBC-binding site on ICAM-1 is distinct from the LFA-1 binding site and molecular modelling studies, based on our data and the crystal structures of the immunoglobulin superfamily adhesion molecule CD4 and the REI variable domain, shed further light on these differences. We have constructed a number of ICAM-1-IgG1Fc chimeras which we have used in studies of the interaction between IRBC and ICAM-1 and in adhesion-blocking studies. In addition, we have examined the behaviour of a large number of field and laboratory isolates and find that adhesion to ICAM-1 appears to be a property of certain parasite antigenic strains. This has implications for the likely clinical and biological relevance of adhesion to ICAM-1. I shall discuss these data, together with progress to date on the identification of novel endothelial receptors, the likely *repertoire of potential* receptors and the implications these pose for potential anti-adhesion therapy in severe malaria.

C 019 CD44 AND CD44 SPLICE VARIANTS IN NORMAL DIFFERENTIATION AND IN TUMOR PROGRESSION, P. Herrlich[1], M. Hofmann[1], M. Zöller[2], W. Rudy[1], R. Arch[2], K.'H. Heider[1], C. Tölg[1], and H. Ponta[1], [1]Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, P.O. Box 3640, D-7500 Karlsruhe 1, Germany[1], [2]Krebsforschungszentrum Heidelberg, Institut für Radiologie und Pathophysiologie, Im Neuenheimer Feld 280, D-6900 Heidelberg, Germany

Lymphogenic metastases of rat and human tumors carry one or several specific isoform(s) of the ubiquitously expressed surface glycoprotein CD44. These isoforms represent splice variants. They carry additional exon-encoded sequences in the extracellular portion of the molecule which are recognized by monoclonal and polyclonal antibodies.
Isoforms were individually expressed in several non-metastasizing rat tumor cell lines. Beyond a threshold level these transfectants became fully metastatic in that they spread lymphogenically. The smallest isoform tested carries an 85 amino acid extra domain in CD44. The critical role of these isoforms is further documented by the effective inhibition of metastasis formation by i.v. injection of antibody.
The human and murine CD44 promoter has been isolated. Both contain a binding site for the transcription factor AP-1 around position -110. This AP-1 binding site is the target for activation by the oncoprotein Ras. In rat embryonal carcinoma cells this activation leads to the concomitant expression of metastasis specific isoforms and to an increase of metastatic potential.
The genomic locus spans some 50 kb including at least 10 "variant" exons. Different splice variants are expressed in certain normal tissues, especially in the embryo and in selected epithelial cells of the adult. Interestingly, a small variant is transiently expressed on antigen-stimulated T and B lymphocytes and in macrophages. Antibody or F(ab')2 fragments directed against the CD44 variant domain, block B and T dependent immune responses in vivo. We hypothesize that immune cells need the surface protein after meeting antigen in the periphery and for an interaction within the draining lymphatic tissues that permit activation and expansion of antigen-specific cells. Further we speculate that tumor cells in progression mimic lymphocytes.

C 020 THE ROLE OF CONSTITUTIVELY EXPRESSED ENDOTHELIAL CELL ADHESION MOLECULES IN LUNG METASTASIS FORMATION. Bendicht U. Pauli[1] and Daniel A. Hammer[2], [1]Cancer Biology Laboratories, Department of Pathology, College of Veterinary Medicine and [2]School of Chemical Engineering, Cornell University, Ithaca, NY 14853

Organ-specific endothelial cell adhesion molecules have been implicated in the colonization of select secondary organ sites by blood-borne cancer cells. Two constitutively expressed adhesion molecules of the lung vasculature have recently been purified by our laboratory (PNAS 88:9568-9572,1991; TIGG, in press). The first is the lectin-like, murine B16 melanoma cell-binding endothelial cell adhesion molecule Lu-ECAM-1. The 90 kDa Lu-ECAM-1 is localized preferentially on endothelia of pleural and subpleural capillaries and venules and, to a lesser extent, perivenous and peribronchial venules. The molecule is not upregulated by tumor necrosis factor-α, lipopolysaccharide, recombinant interleukin-1α, or phorbol 12-myrisate 13-acetate. Lu-ECAM-1 promotes the selective, Ca^{++}-dependent attachment of lung-metastatic B16 melanoma cells. Corresponding with their metastatic performance, high lung-metastatic B16-F10 melanoma cells bind in significantly higher numbers to Lu-ECAM-1 than their intermediate and low lung-metastatic counterparts B16-L8-F10 and B16-F0, respectively. B16 melanoma cell binding to Lu-ECAM-1 is blocked by mAb 6D3 and is competitively inhibited by soluble Lu-ECAM-1. Under conditions of flow, B16 melanoma cell adhesion to Lu-ECAM-1-expressing endothelial cells is characterized by an abrupt arrest of tumor cells to the endothelium. This process is significantly different from the rolling that precedes neutrophil arrest on LPS-stimulated endothelial cells of the same type. C57Bl/6 mice passively immunized with anti-Lu-ECAM-1 mAb 6D3 or actively immunized with purified bovine Lu-ECAM-1 exhibit an anti-Lu-ECAM-1 antibody titer-dependent reduction in the number of experimental B16 metastases. Lu-ECAM-1 promotes neither binding nor metastasis of other lung-metastatic tumor cells (e.g., KLN205 squamous carcinoma cells) or tumor cells that metastasize to other organs than the lungs. The B16-F10 ligand has recently been identified as a glycoprotein with an apparent molecular weight of 200 kDa. Binding of Lu-ECAM-1 to this ligand is mediated by lactosamine residues, most effectively by lacto-N-fucopentase (LFN I). Similar to anti-Lu-ECAM-1 mAb 6D3, these carbohydrate derivatives are efficient in inhibiting adhesion and lung colonization by B16-F10 melanoma cells.

The second adhesion molecule is rat dipeptidyl peptidase IV (DPP IV). This exopeptidase also known as a fibronectin-binding protein is expressed on endothelia of distinct vascular branches including the vasa recta of the renal medulla, splenic red pulp venules, and lung capillaries. DPP IV has a high binding affinity for lung-metastatic rat R3230AC-MET breast and RPC-2 prostate carcinoma cells *in vitro*, but not for their non-metastatic counterparts R3230AC-LR and RPC-LR. The DPP IV binding affinity parallels the propensity with which these tumor cells metastasize to the lungs. Tumor cell binding to immobilized DPP IV is inhibited by anti-DPP IV mAb 8.6A3 and by soluble DPP IV, both in a dose-dependent manner. No effect on tumor cell binding to DPP IV is observed with peptide substrates to the enzymatic portion of this exopeptidase, the fibronectin-derived peptide Gly-Arg-Gly-Asp-Ser, as well as several control mAbs. Significant accumulations of fibronectin on the surface of lung metastatic, but not on non-metastatic breast and prostate carcinoma cells suggest that DPP IV tumor cell binding is mediated by its fibronectin-binding domain. Fibronectin serves as a binding intermediary between upregulated tumor cell β_1 and β_3 integrins and endothelial cell DPP IV. This conclusion is consistent with recent reports that associate increased expression of tumor cell surface fibronectin and/or integrins with higher binding affinity for endothelium and higher propensity for metastasis.

Our data reinforce the concept that distinct endothelial cell adhesion molecules constitutively expressed in select vascular beds contribute to organ preference of metastasis.

Banquet Address (Joint)

C 021 **TOYS FOR GLYCOBIOLOGY**, Ole Hindsgaul, Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada

The field of Glycobiology is blowing wide open with ever increasing reports that specific sugar sequences on complex carbohydrates can act as ligands to initiate important recognition events. The active structures are often complex, tetra- to hexasaccharides, and are difficult to isolate from natural sources in quantities sufficient for systematic investigation. As a result, enormous pressure is being exerted on synthetic carbohydrate chemists by glycobiologists to "make me this and make me that", and make them quickly! The requested compounds are usually predicted by the glycobiologist to have an activity, for example: to assay an enzyme activity, to inhibit a selectin or to target a glycoprotein or cell to a carboydrate-recognizing receptor. The importance of the requested compounds is emphasized to the carbohydrate chemist by refering to them as invaluable "**Tools for Glycobiology**".

Both chemical and enzymatic synthesis are used to produce the required synthetic probes but, most often, the compounds require many "post-doc months" to prepare. When they are finally finished the products are sent off to the glycobiologist by courier. Unfortunately, the compounds often end up either in a black hole or in the back of a fridge since by the time they are finished the original problem has either been solved, reformulated, or has lost its importance. The research director or group leader must then explain to the post-docs and students that they did very nice work, but I'm sorry no one is interested in these compounds anymore.

After a recent post-doc and student rebellion in my group over such matters, I was convinced that sometimes the synthetic chemical efforts were not well appreciated and that it was the glycobiologists who were having all the fun. Certainly, organic chemists know very little about experiments with cells or animals and they should not be the ones to design such experiments. However, they can make novel molecules that do not naturally exist (we call these "**Toys for Glycobiology**") and perhaps some component of the research in glycobiology should be guided by this ability, i.e. very long-shot chemical ideas. The trials and tribulations of generating such toys, and convincing people to use them, form the topic of this lecture.

Novel Therapies Based on Modulation of CAM Activity

C 022 CROSSLINKING OF ICAM-1 INDUCES CO-SIGNALING OF AN OXIDATIVE BURST FROM MONONUCLEAR LEUKOCYTES. **Robert Rothlein, Grace Chow, Elizabeth Mainolfi and Takashi Kei Kishimoto. Department of Immunology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT**

Cell adhesion molecules were first described as accessory molecules, simply to bridge one cell to another. More recently, it has been appreciated that the integrins can transmit information from outside of the cell to inside. For example, crosslinking of LFA-1 (CD11a/CD18) is co-stimulatory on T cells with crosslinking of CD3 or the TCR. Crosslinking of Mac-1 (CD11b/CD18) is required for the adhesion-dependent oxidative burst of leukocytes. One ligand for the integrins LFA-1 and Mac-1 is the ICAM-1 molecule. We show that crosslinking of the surface ICAM-1 with anti-ICAM-1 MAb in the presence of sub-optimal levels of fMLP chemoattractant results in co-stimulation of the oxidative burst of mononuclear leukocytes. The onset of the oxidative burst was delayed compared to that induced by CD18-crosslinking. Anti-L selectin MAb did not induce an oxidative burst. The addition of an excess of soluble ICAM-1 to compete for the anti-ICAM-1 MAb, inhibits the oxidative burst in response to ICAM-1 crosslinking but not to CD18 crosslinking. These results suggest, for the first time, that ICAM-1 may also be capable of delivering a co-stimulatory signal.

Late Abstract

EXPRESSION OF LEWIS X AND SIALYL LEWIS X EPITOPES IN AVIAN B LYMPHOCYTE DEVELOPMENT CORRELATES WITH TISSUE SPECIFIC LOCALIZATION OF IMMUNE CELLS. Robert D. Larsen[1], Louise M. Carlson[2], James Pickel[3], Terrie Schultz[1], John B. Lowe[4], Craig B. Thompson[4] and Kelvin P. Lee[5], [1]Glycomed Inc., Alameda, CA 945012 [2]Medimmune Inc., Gaithersburg, MD 20877, [3]NCI-Frederick Cancer Research Program, Frederick, MD 21702, [4]Howard Hughes Medical Institute, Ann Arbor, MI 48109, [5]Henry M. Jackson Foundation, Bethesda, MD 20814.

Among avian species, normal B lymphocyte development requires that pre-B cells home to a specialized organ, the Bursa of Fabricius, to undergo further maturation. This homing occurs between day 8 and day 14 of embryogenesis. During this time period/developmental stage, the bursa becomes populated with cells that express sialyl-Lewis x (sLex) epitopes but do not express Lewis x (Lex) epitopes. Following initial colonization of the bursa of Fabricius the lymphocytes undergo a period of proliferation from day 15 to several days post hatch. From day 15 to day 18 essentially all of the bursal lymphocytes switch their cell surface phenotype from high level expression of sLex epitopes to high level expression of Lex epitopes. This high level expression of Lex epitopes is maintained until one week post hatch. From one week post hatch onward the population of bursal lymphocytes downregulates surface expression of Lex, to the point where, at ten weeks post hatch these Lex-negative cells constitute nearly all of the bursal lymphocytes. Only these (low expressors) of Lex epitope are found in the peripheral lymphoid organs and most likely represent mature B-cells.

We have used a modification of the Stamper-Woodruff assay to demonstrate that adhesion of DT40 cells (a pre-B cell like cell line) to bursal sections is sLex dependent. Neither neuraminidase treated DT40 cells nor DT40 cells coated with an antibody to sLex epitope adhered appreciably to bursal sections, while untreated DT40 cells bound avidly to the periphery of bursal follicles. Additionally, bursal lymphocytes which express only Lex epitopes did not adhere to bursal sections. These data suggest that homing of pre-bursal lymphocytes to the Bursa of Fabricius is mediated by expression of carbohydrate epitopes (sLex) on the cell surface of pre-B cells, and further suggests that the adhesion of bursal lymphocytes may be mediated by interaction of sLex epitopes with putative selectin molecules displayed by bursal epithelial tissues.

Differentiation of the Microvasculature Regulation of CAM Expression: Transcriptional and Post-Transcriptional Controls

C 100 LYMPHOCYTE-VASCULAR ADHESION PROTEIN-2 (L-VAP-2) IS INVOLVED IN LYMPHOCYTE BINDING TO ENDOTHELIUM, Laura Airas, Marko Salmi, Sirpa Jalkanen, National Public Health Institute and BioCity, 20520 Turku, Finland

Extravasation of leukocytes from the blood is essential in normal lymphocyte recirculation as well as in amounting adequate inflammatory responses in different tissues. Leukocyte emigration from the blood is controlled by sophisticated interactions between surface receptors on leukocytes and their ligands on endothelial cells. Here we describe a novel adhesion molecule, L-VAP-2, recognized by a monoclonal antibody 4G4 that was produced after immunizing mice with enriched endothelial cell preparation isolated from inflamed synovium. 4G4 stains a subpopulation of vessels in non-lymphoid tissues and also few high endothelial venules in lymphoid tissues. L-VAP-2 is constitutively expressed on human umbilical vein endothelial cells (HUVEC) and its expression cannot be upregulated by cytokines and mitogens (TNF-alfa, IL-1 and LPS). Approximately 25% of peripheral blood lymphocytes express this antigen, whereas granulocytes and monocytes are negative. On lymphocytes, L-VAP-2 is preferentially expressed on B cells and CD8 positive T cells. The molecular weight of L-VAP-2 is 65-75 kD when determined from iodinated endothelial cell lysates. The involvement of L-VAP-2 in lymphocyte binding to endothelium was tested in vitro using HUVEC. Both the intact antibody and $F(ab)_2$ fragments of it constantly inhibited lymphocyte binding to HUVEC by 20-50%. On the basis of the molecular weight analyses and the stainings of tissue sections, leukocyte populations and ICAM-1 and ICAM-2 transfectants, L-VAP-2 seems to recognize an antigen that has not been reported earlier to be involved in lymphocyte-endothelial cell interaction.

C 101 INTERLEUKIN-10 (IL-10) AND INTERFERONg (IFNg) INDUCED INFLAMMATION IN PANCREATIC TISSUE IN TRANSGENIC MICE. Lise W. Bach, Myung S. Lee, and Nora Sarvetnick. Scripps Clinic and Research Foundation, La Jolla, CA 92037.
Insulin dependent diabetes mellitus (IDDM) is characterized by peri-islet inflammation and insulitis. Interactions between local produced cytokines and the endothelial cells are suggested to play a major role in this process. Transgenic expression of IL-10 or IFNg in pancreatic ß-cells induces inflammation. We examined the mechanisms of homing to the pancreatic tissue by testing the expression of endothelial cell specific antigens, HEV specific antigens and adhesion molecules utilizing immunohisto-chemistry on frozen tissue. In both transgenic lines the first cells appearing are Mac-1 positive cells, later the infiltrate is dominated by T- and B-lymphocytes. In both transgenic models increased expression of ICAM-1 on the endothelial cells precedes induction of the mesenteric specific HEV-antigen, and the peripheral specific HEV-antigen. Both HEV types are only expressed in lymphocyte infiltrates. Expression of the pan-endothelial cell marker MECA 32 is increased in areas of lymphocyte inflammation. Induction of HEV-like structures and increased ICAM-1 expression is also demonstrated in lymphocyte-deprived mice expressing IFNg in the ß-cells (IFNg transgenic x immunodeficient SCID-mice). The initial event in the IL-10 model is an up regulation of ICAM-1 on the endothelial cells leading to homing of Mac-1 positive cells. The later, moderate induction of HEV is likely mediated by as yet unknown, locally produced secondary mediators. A similar mechanism may lead to the inflammation in the IFNg model, although HEV expression is more pronounced.

C 102 THE GENOMIC ORGANIZATION OF THE HUMAN CD44 GENE, Martyn V Bell, Gavin R Screaton, David G Jackson and John I Bell, Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU.

CD44 cell surface glycoprotein has been implicated in cell adhesion functions including lymphocyte homing and tumour metastasis. The ability of this molecule to participate in such diverse functions is thought to be linked to the extensive differential splicing observed in the CD44 gene mRNA, which introduces variation in the membrane proximal extracellular domain of the protein.

As a prerequisite to the study of the mechanisms of alternative splicing of this gene, we have analysed the genomic structure of the human CD44 gene by the isolation and mapping of the corresponding DNA in cosmids and YACs (yeast artificial chromosomes) and inter-exon PCR. This analysis (and the data of Screaton *et al*, this symposium) show that the CD44 gene occupies approximately 50 kb of genomic DNA, and that the alternatively spliced region contains at least ten variable exons distributed within approximately 20 kb. Plasmid subclones of the YAC are currenly being examined for the presence of further exons, and will serve as constructs for the study of alternative splicing. In addition, a comparison with the genomic structure of the murine CD44 locus is being undertaken.

C 103 **Genomic Organization & Cerebral Microvascular Expression of Basigin/GP42**
Frank R. Burns, Xiaobin Li and Yi Cheng, Neurology Department, University of Pennsylvania, Philadelphia, PA 19104
Basigin/GP42 is a mouse Ig superfamily protein previously cloned from mouse teratocarcinoma cells and transformed T cells. Basigin/GP42 is the species homolog of the chicken blood brain barrier endothelial specific protein HT7, the rat activated T cell & endothelial cell marker OX 47 and the human monocyte & activated T cell marker M6. Recently, using a differential screening approach, we found that Basigin/GP42 mRNA is expressed in cultured mouse cerebral microvascular endothelial cells (CMVEC) and is regulated by cytokines (Burns.& Joseph, in preparation). Three isoforms of Basigin RNA, all apparently encoding distinct proteins, have been reported from teratocarcinoma cells. In order to better understand the expression of Basigin/GP42 in mouse CMVECs we used primer extension analysis to determine that two of the reported Basigin/GP42 isoforms are made in CMVECs. In order to uncover the origin of these isoforms we determined the genomic organization of the Basigin/GP42 gene. The gene encoding the major Basigin/GP42 isoform is comprised of eight exons. Isotype two is generated by the splicing of separate upstream exon into a cryptic splice acceptor site in the middle of the third exon of the major isoform.

To determine if the expression of Basigin/GP42 in cultured CMVECs is mirrored in vivo we generated rabbit anti-sera against bacterial recombinant Basigin/GP42 as well as anti-sera against Basigin/GP42 peptides. Immunohistochemical staining with these anti-sera show that Basigin/GP42 is expressed on mouse endothelial cells in vivo and that this expression is, within the CNS, restricted to the micro vasculature, capillaries and small branching vessels (arterioles and/or venules)

C 104 TYROSINE KINASE ACTIVITY REGULATES LPS-INDUCED SYNTHESIS OF IL-6 AND GM-CSF, BUT NOT ADHESION MOLECULES, BY HUVEC.

Theresa A. Deisher, Terry Franklin, Chris B. Wilson and John M. Harlan. Division of Hematology and Department of Pediatrics and Immunology, University of Washington Medical Center, Seattle, WA. 98195.

The activation of monocytes by lipopolysaccharide (LPS) has been shown to be mediated, at least in part, by tyrosine kinase activity. Herbimycin, an inhibitor of tyrosine kinase, blocks induction of tumor necrosis factor-α (TNF-α), interleukin-6 (Il-6) and interleukin-1 expression in monocytes stimulated with LPS. On human umbilical vein endothelial cells (HUVEC), LPS induces the expression of the adhesion molecules endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). The tyrosine kinase inhibitors herbimycin, genistein, tyrphostin, lavendustin A, RCAM lysozyme and methyl 2,5-dihydroxycinnamate do not reduce LPS- or TNF-α-induced adhesion molecule expression on HUVEC. Likewise, tissue factor expression on HUVEC stimulated by LPS is not prevented by inhibitors of tyrosine kinase. In contrast, herbimycin and genistein inhibit LPS-induced Il-6 and GM-CSF production in HUVEC. Half-maximal inhibition was seen at 5µg/ml herbimycin, with complete inhibition at 7.5µg/ml for interleukin-6 production and 10µg/ml for GM-CSF production. These results demonstrate that at least two signal transduction pathways are activated in HUVEC following stimulation by LPS, and that distinct pathways mediate adhesion molecule expression and cytokine production in HUVEC.

C 105 EXPRESSION OF CELL ADHESION MOLECULES AND LYMPHOKINE GENE ACTIVATION IN GLIOBLASTOMA INFILTRATING LYMPHOCYTES.

Marie-Claude Gingras[1], Eugène Roussel[2], Elizabeth A. Grimm[3], Janet M. Bruner[4], and Richard P. Moser[1], Depts of Neurosurgery, Thoracic Surgery, Tumor Biology, and Pathology, MD Anderson Cancer Center, Houston, Texas, 77030.

We have studied if lymphocyte populations that infiltrate brain tumor tissue have a modified expression pattern of cell adhesion molecules (CAM) and specific lymphokine (LK) gene activation in comparison with autologous circulating blood lymphocytes (PBL). Tumor infiltrating lymphocytes (TIL) were extracted from 6 primary glioblastoma multiforme by mechanical disruption without enzyme use and then separated from other cells over a double density gradient of Ficoll. Cell populations were immediately double labelled with αCD3,αCDx and analyzed by flow cytometry. The proportion of T cells expressing CD49a, CD49b, CD54, CD56, CD58, CD15, CD65, or CD25 were several fold increased in TIL over PBL. TIL-T cell population also showed a decrease in expression of CD11a, CD18, and L-Selectin. To assess lymphokine gene activation, we performed mRNA reverse transcriptase PCR on the TIL, the autologous PBL and CD3 activated normal PBL as comparative control (CTR). PCR products were subjected to gel electrophoresis and visualized with EthBr. Bands were compared with image analysis. Individual variations were observed in the type and amount of LK detected. In general, both PBL and TIL products appeared less in amount than the CTR. The average $\log_{(10)}$ of PCR product relative units are presented.

	IL-1ß	IL-2	IL-4	IL-6	IFN-τ	GM-CSF	TNF-ß
TIL	3.00_{6}*	2.34_{5}	2.15_{5}	2.59_{6}	2.08_{4}	2.25_{6}	2.30_{5}
PBL	2.74_{3}	1.35_{2}	1.88_{1}	2.02_{3}	1.41_{2}	1.84_{2}	1.92_{4}
CTR	3.32	3.08	1.97	3.14	3.20	3.12	3.14

*number of patients/6 where LK were detected

TIL unique expression of CAM suggests that a defined T cell population has migrated inside the tumor tissue. Moreover, TIL activation may have occurred at the tumor site since a high proportion of TIL-T cells displayed a CAM phenotype like activated T cells and consistently more different LK gene were expressed in TIL than PBL.

C 106 INDUCIBLE EXPRESSION OF ICAM-1 IN MOUSE L929 CELLS,

Cynthia H. Jurgensen, Brian E. Huber, Cynthia A. Richards, Marie A. Iannone, and Gerald Wolberg, Division of Experimental Therapy, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

Intercellular adhesion molecule-1 (ICAM-1) is involved in leukocyte function and adherence and plays a role in a variety of disease states. To facilitate studies of ICAM-1, this molecule was inducibly expressed in mouse L929 cells. A plasmid was constructed in which the ICAM-1 protein coding sequence was transcriptionally controlled by the metallothionein promoter (MT-1). This promoter is activated in the presence of glucocorticoids or heavy metals to induce expression of ICAM-1. Cells transfected with this plasmid were treated with $ZnCl_2$, and ICAM-1 expression was determined by indirect immunofluorescence. Primary transfectants which expressed ICAM-1 were cloned by cell sorting to obtain single cell clones with inducible ICAM-1 expression. We found that $ZnCl_2$-treatment induced both ICAM-1 expression and adhesiveness for human neutrophils in several of these clones. These characteristics were both dose- and time-dependent, with maximal expression/adhesion observed upon treatment with 200 µM $ZnCl_2$ for 48 h. Thus, single cell clones of L929 cells were constructed which conditionally express ICAM-1 and adhere human neutrophils. These cells will facilitate studies with ICAM-1 and potential antagonists of this molecule.

C 107 REGULATION OF L-SELECTIN EXPRESSION IN JURKAT CELLS: DIVERGENT TRANSCRIPTIONAL RESPONSES TO ACTIVATION STIMULI,

Eric P. Kaldjian, Praveen Reddy and Lloyd M. Stoolman, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109.

Jurkat cells expressing high levels of surface L-selectin were subcloned from a parent cell line. Treatment of these cells with the phorbol ester PMA caused rapid loss of surface protein followed by return to near baseline levels by 24 hours. In contrast, treatment with PMA plus the calcium ionophore ionomycin caused rapid surface loss without subsequent replenishment. Northern hybridization revealed that 24-hour steady-state L-selectin mRNA levels are significantly increased in the PMA-treated cells, while they are decreased in cells treated with PMA + ionomycin, relative to untreated cells. To assess whether the altered level of L-selectin mRNA could be the result of enhanced stability or degradation, cells were cultured for 24 hours as above, treated with actinomycin D to halt transcription, and then RNA was isolated at 30, 90 and 240 minutes thereafter. Northern analysis revealed no change in mRNA levels in control and experimental groups, indicating that changes in stability of transcripts are not likely to account for the difference in steady-state mRNA levels at 24 hours. Thus, the effects of PMA and PMA + ionomycin on L-selectin gene expression are likely the consequence of increased and decreased transcriptional activity, respectively. Treatment with PMA + ionomycin in Jurkat cells induces changes similar to those seen in proliferating T-cells. The current study suggests, therefore, that transcriptional down-regulation contributes to the decrease in surface L-selectin seen in actively proliferating normal T-cells as well. Experiments are in process to further define the mechanisms of transcriptional regulation within these systems.

C 108 REGULATION OF E-SELECTIN EXPRESSION IN ENDOTHELIAL CELLS BY TRANSFORMING GROWTH FACTOR-β, Yeesim Khew-Goodall, Jennifer R. Gamble and Mathew A. Vadas, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Adelaide, SA., Australia.

Transforming growth factor-β (TGF-β) has been shown to decrease the adhesiveness of human umbilical vein endothelial cells (HUVECS) for neutrophils, lymphocytes and tumour cells. We have investigated the mechanism whereby TGF-β inhibits the adhesiveness of HUVECS and found that it inhibited both basal and tumour necrosis factor-α (TNF-α) stimulated cell-surface expression of E-selectin, an endothelial cell-specific adhesion molecule which has previously been shown to mediate several aspects of the inflammatory response involving neutrophils and T-lymphocytes. TGF-β had no effect on the expression of VCAM-1 and ICAM-1, two other adhesion molecules found on the surface of endothelial cells, but was additive with IL-4 in inhibiting the expression of E-selectin. TGF-β also decreased the level of E-selectin mRNA in endothelial cells and this is due, at least in part, to inhibition of transcription of the E-selectin gene. Several putative TGF-β inhibitory elements are present on the E-selectin promoter and currently we are carrying out functional analysis using segments of the promoter fused to the chloramphenicol acetyl-transferase reporter gene to determine sequences in the E-selectin promoter responsible for mediating TGF-β inhibition.

C 109 **HIGH ENDOTHELIAL VENULES IN INFLAMMATION**

Georg Kraal and Toshiyuki Maruyama,
Department of Cell Biology, Vrije Universiteit, Van der Boechorststraat 7; 1085 BT Amsterdam, the Netherlands

Endothelial cells of high endothelial venules in lymphoid organs constitutively express vascular addressins by which lymphocyte trafficking is initially mediated. Inducing a state of activation in the lymph node draining site by injecting FCA leads to alterations in the high endothelial venules after which monocytes can now adhere to HEV. The changed binding patterns after FCA could also be induced by injecting high doses of γ IFN in the draining area of the lymph node. The kinetics of these induced adhesion patterns and the expression of adhesion molecules on HEV were studied. Monocyte binding to activated HEV peaked at day 4 after FCA treatment and was independent of peripheral vascular addressin expression (MECA 79). The HEV in the activated (popliteal) lymph nodes did not show increased expression of ICAM-1 and VCAM, and no expression of the mucosal addressin (MECA 367), as determined by immunohistochemistry. In concordance, no role for these molecules could be demonstrated in the binding of monocytes to HEV. When monocytes were incubated with antibody against C3b1 strong blocking of binding was observed. No role for sialyl-lewis x could be demonstrated sofar. Lymphocyte binding seemed not to be impaired in these lymph nodes. In addition to enhanced binding of monocytic cells we were able to demonstrate that also purified dendritic cells could adhere to these activated HEV.

The results demonstrate that HEV, although highly differentiated, can still undergo changes in function under the influence of local inflammatory stimuli.

C 110 SELECTIVE CYTOKINE CONTROL OF THE ICAM-1 PROMOTER AND LOCALIZATION OF ITS INTERFERON-γ RESPONSE-ELEMENT IN CULTURED EPITHELIAL CELLS, Dwight C. Look, Mark R. Pelletier, and Michael J. Holtzman, Department of Medicine, Washington University School of Medicine, Saint Louis, MO 63110

The basis for selective induction of intercellular adhesion molecule-1 (ICAM-1) by interferon-γ (IFN-γ) in epithelial cells was examined using a transient DNA-transfection system with primary culture human tracheal epithelial cells (HTECs) or SV-40 virus-transformed human bronchial epithelial cells (BEAS-2B) and plasmid constructs composed of the 5'-flanking region of the ICAM-1 gene and a luciferase-reporter gene. Constructs containing the 5'-flanking region of -5.0, -1.3, and -0.176 kb from the translation start site demonstrated equivalent activities in response to IFN-γ, but no significant responsiveness to interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) in either HTEC or BEAS-2B cells. By contrast, human umbilical vein endothelial cell (HUVEC) monolayers exhibited increases in transfection assay activity only after IL-1β or TNF-α but not after incubation with IFN-γ. This pattern of gene activation was accompanied by a parallel profile of expression for ICAM-1 protein and functional activity. An epithelial cell element responsive to IFN-γ was localized to a DNA segment at -130 to -93 bp in the ICAM-1 5'-flanking sequence by comparison of PCR-generated constructs containing this region to ones without it and by demonstrating that this sequence confers IFN-γ-responsiveness. The same segment formed a distinct complex in gel retardation assays with nuclear proteins from IFN-γ stimulated but not unstimulated epithelial cells. The results suggest that the selective responsiveness of the ICAM-1 gene to IFN-γ in pulmonary airway epithelial cells is mediated by a trans-acting factor which binds a DNA segment containing two consensus IFN-γ response motifs (GAAA) located on opposite strands at -109 and -113 bp in the ICAM-1 5'-flanking sequence. The lack of the expression of this nuclear binding protein in other cell types (such as vascular endothelial cells) may be responsible for the difference in ICAM-1 levels generated by cytokine stimulation of epithelial vs endothelial cells. The complementary profile of cytokine-responsiveness of the ICAM-1 gene in different cell types may allow for selective localization of leukocytes in epithelium or endothelium under different inflammatory conditions.

C 111 DIFFERENTIAL EXPRESSION OF ICAM-1, P- AND E-SELECTIN IN CANINE MYOCARDIAL ISCHEMIA-REPERFUSION. A.M.Manning[1], G.L. Kukielka[2], H. Hawkins[2], L.H. Michael[2], C.W. Smith[2], M.L. Entman[2] and D.C. Anderson[1], Discovery Research, Upjohn Laboratories, Kalamazoo, MI[1] and Baylor College of Medicine, Houston, Tx[2].

We have previously demonstrated the induction of ICAM-1 in ischemic myocardium of canine animals following ischemia/ reperfusion injury. To evaluate the role of selectins in the pathogenesis of myocardial inflammation, the expression of P- and E-selectin were assessed in the same canine model. P- and E-selectin gene expression was initially studied in cultured canine jugular vein endothelial cells (CJVEC), where either LPS (10ng/ml) or TNF (20U/ml) treatment elicited a marked, transient increase in P- and E-selectin mRNA levels. Maximal stimulation of E-selectin mRNA occurred earlier than that for P-selectin. E-selectin mRNA levels diminished rapidly after treatment, whereas significant P-selectin mRNA was detectable up to 24h post-TNF or LPS. Animals administered LPS (1 mg/kg, I.V.) displayed similar patterns of tissue specific P- and E-selectin mRNA expression, with elevated levels of mRNA observed in many different tissues, including myocardium. P- and E-selectin mRNA levels were elevated in ischemic myocardial segments following 1h coronary artery occlusion and 3h reperfusion, being inversely related to regional blood flow. Following 24h of reperfusion, elevated levels of P-selectin mRNA were observed in ischemic segments, whereas little or no E-selectin mRNA was detectable. Neutrophil infiltration into tissue was observed to increase after 2h of reperfusion and was directly related to increasing levels of ICAM-1, P- and E-selectin mRNA in myocardial segements and to increasing levels of ICAM-1 observed on cardiac myocytes. Post-ischemic (but not pre-ischemic) cardiac lymph collected at intervals from 1h to 24h following reperfusion elicited P- and E-selectin mRNA expression, E-selectin surface expression and E-selectin-dependent neutrophil adhesion in canine jugular vein endothelial cells *in vitro*. These data demonstrate that P- and E-selectin gene expression is induced in response to multiple inflammatory stimuli, including myocardial ischemia/ reperfusion and that though these molecules display a similar pattern of tissue-specific expression, their regulation is temporally distinct.

C 112 CELL SURFACE ANTIGEN EXPRESSION IN A MURINE ENDOTHELIOMA CELL LINE IN RESPONSE TO CYTOKINES, F. M. Rollwagen, N. D. Pacheco, Y-Y. Li, R. Auerbach, R. Sneed and T. B. Nielsen. Naval Medical Research Institute, Bethesda, MD, GEO-CENTERS, Ft. Washington, MD and Univ. Wisconsin, Madison, WI.

Expression of antigens on the endothelial cell (EC) surface modulate a variety of leukocyte adhesion functions. While the mouse is the species of choice for many immunologic studies, to date no murine model of EC surface expression in response to cytokines (CKs) has been available. We have characterized a murine endothelioma (EOMA) cell line for its ability to modulate surface antigens in response to incubation with CKs. Incubation with crude supernatant from Con A stimulated rat spleen cells (CAS) increased surface expression of ICAM-1. Incubation with recombinant (r) cytokines did not mimic the changes seen with CAS. Incubation with rTNF or rIL-6 alone or together failed to induce increased ICAM-1 surface expression. Recombinant IL-2 and IL-4 similarly failed to modulate cell surface expression.

Lectin binding profiles of this cell line were made with and without culture in CAS. Binding profiles for many lectins were similar to those seen for porcine microvascular (PMVEC) or aortic endothelial cells (PAEC). The GAL-GALNAc binding lectin, soybean agglutinin (SBA), bound strongly to PMVEC and PAEC but weakly to EOMA cells. However, significant increases in SBA binding were seen if the EOMA cells were incubated in CAS for 24 hours. Conversely, Abrus precatorius agglutinin (APA), which has the same nominal specificity, showed no such changes in pattern. The α-D-GAL binding lectins, Bandiera simplifolica agglutinin-1 (BSA-1) and Maclura pomifera agglutinin (MPA), showed no changes in binding to EOMA cells which had been incubated with CAS. These observations suggest that this cell line may be useful in elucidating EC-leukocyte interactions in a murine model.

C 113 CELL ADHESION MOLECULE EXPRESSION AND LYMPHOKINE GENE ACTIVATION IN TUMOR INFILTRATING LYMPHOCYTES FROM LUNG ADENOCARCINOMAS.
Eugène Roussel[1], Marie-Claude Gingras[2], Elizabeth A. Grimm[3], and Jack A. Roth[1], Departments of Thoracic Surgery, Neurosurgery, and Tumor Biology, MD Anderson Cancer Center, Houston, Texas, 77030.

We have investigated if lymphocyte populations that infiltrate lung tumor tissue have a modified expression pattern of cell adhesion molecules (CAM) and specific lymphokine (LK) gene activation in comparison with autologous circulating blood lymphocytes (PBL). Tumor infiltrating lymphocytes (TIL) were extracted from 6 non small cell primary lung adenocarcinomas by mechanical disruption without enzyme use and then separated from other cells over a double density gradient of Ficoll. Cell populations were immediately double labelled with αCD3,αCDx and analyzed by flow cytometry. The proportion of T cells expressing CD49a, CD49b, CD54, CD58, CD15, CD65, or CD25 were several fold increased in TIL over PBL. TIL-T cell population also showed a decrease in expression of CD49e, CD49f, CD26 and L-Selectin. To assess lymphokine gene activation, we performed mRNA reverse transcriptase PCR on the TIL, autologous PBL and CD3 activated normal PBL as comparative control (CTR). LK mRNA were detected in PBL and TIL. Individual variations were observed in the type of LK detected. Band size and intensity of PCR products were compared with image analysis. Both PBL and TIL products consistently appeared 10-100 times less in amount than the CTR. The average $\log_{(10)}$ of PCR product relative units are presented.

	IL-6	IL-1β	TNF-β	GM-CSF	IL-4	IFN-γ	IL-2
TIL	2.24_{6^*}	2.68_5	1.76_5	1.71_4	1.52_4	1.58_3	1.32_2
PBL	2.21_5	3.05_5	1.93_5	0_6	1.29_2	1.63_5	1.57_3
CTR	3.28	3.44	3.26	3.17	1.61	3.26	3.24

***number of patients/6 where LK were detected**

TIL unique expression of CAM suggests that a defined T cell population has migrated inside the tumor tissue. Moreover, TIL activation may have occurred at the tumor site since a high proportion of TIL-T cells displayed a CAM phenotype like activated T cells and activated GM-CSF gene was only detectable in TIL.

C 114 A NOVEL ENDOTHELIAL CELL MOLECULE MEDIATING LYMPHOCYTE BINDING IN HUMANS, Marko Salmi and Sirpa Jalkanen, National Public Health Institute, BioCity, SF-20520 Turku, Finland

Leukocyte extravasation is critically dependent on proper interactions between leukocytes and vascular endothelial cells. In order to characterize novel endothelial cell molecules involved in the leukocyte migration, we have produced monoclonal antibodies (mAbs) against human endothelial cells by immunizing mice with synovial stroma. One of the mAbs obtained, 1B2, stains high endothelial venules (HEV) in frozen sections of synovium, peripheral lymph node and tonsil. VAP-1 is localized preferentially in cytoplasmic granules and at luminal side of endothelium. Staining of mucosal HEV is much weaker. Importantly, the 1B2-defined antigen designated as vascular adhesion protein-1 (VAP-1) is not expressed on normal or cytokine-activated (IL-1, TNF-alfa, lipopolysaccharide) human umbilical vein endothelial cells, nor on any of the leukocyte subsets. However, its expression is upregulated at sites of chronic inflammation in vivo. In an in vitro frozen section assay, 1B2-antibody is capable of inhibiting lymphocyte binding to tonsillar, synovial and peripheral lymph node HEV 50-70%, and 30% to appendix HEV. It does not significantly affect the HEV-binding of neutrophils. Moreover, isolated VAP-1 supports lymphocyte binding, and this binding is inhibited by IB2 antibody. In conclusion, these data together with a unique N-terminal sequence of the 90 kDa species of VAP-1 define VAP-1 as a novel member of endothelial cell molecules involved in lymphocyte migration. More detailed biochemical analyses and molecular cloning are currently in progress. VAP-1 will be relevant to understanding of the physiologic lymphocyte recirculation in man, and it will be especially valuable for dissecting the molecular mechanisms of selective lymphocyte homing.

C 115 PRECISE INTRON-EXON DEFINITION AND SEQUENCE OF ALTERNATIVE SPLICE SITES OF THE ADHESION MOLECULE CD44, Gavin R Screaton, Martyn V Bell, David G Jackson and Andrew J McMichael.

The homing receptor CD44 is known to demonstrate extensive size heterogeneity which has been attributed to both alternative splicing and differential glycosylation. The presence of several alternatively spliced exons has been predicted from cDNA sequencing, but the precise intron exon-exon organisation has not been described to date.

We have analyzed the CD44 gene in both humans and mice, which demonstrate considerable homology. In the human we have sequenced across all the exon boundaries from both genomic PCR products and subclones of a CD44 containing Yeast Artificial Chromosome. We present sequence data for all the 20 exons of human CD44 together with with their flanking splice sites,(the physical map is presented by Bell et al , this symposium). The proximal extracellular domain of the CD44 molecule is coded by at least 10 alternatively spliced exons, all of which are spliced out to generate the basic "Haemopoietic" isoform of the molecule. These variable exons show close sequence homology with the 10 variable exons of the mouse.

The variation previously reported in the length of of the cytoplasmic tail is accounted for by alternative splicing of a further two exons. Very extensive heterogeneity in CD44 isoform expression can be demonstrated by PCR. If all the varible exons together with the alternative splice donor and acceptor sites within exons could be utilised in any combination a total of over 6000 different isoforms could be generated.

C 116 INCREASED INTERCELLULAR ADHESION MOLECULE -1 (ICAM-1) EXPRESSION BY HUMAN SYNOVIAL MICROVASCULAR ENDOTHELIAL CELLS, Shing S. T. To, Peter Newman, Christopher J. Jackson and Leslie Schrieber, Sydney University Department of Rheumatology, Royal North Shore Hospital, St. Leonards, NSW, 2065, Australia.

ICAM-1 plays an important role in the adhesion and transendothelial migration of leukocytes. Most endothelial adhesion studies have been performed using macrovascular human umbilical vein endothelial cells (HUVEC). Whether these are representative of microvascular *endothelium at sites of inflammation* such as rheumatoid synovium is uncertain. The aim of this study was to compare ICAM-1 expression in HUVEC and microvascular endothelium. *Ulex europaeus* agglutinin type I coated Dynabeads (1) were used to isolate human synovial microvascular endothelial cells (HSMEC) and neonatal foreskin microvascular endothelial cells (FMEC). Flow cytometric analysis of the expression of ICAM-1 was performed on HUVEC, FMEC and HSMEC. Under basal conditions, HUVEC expressed low levels of ICAM-1 whereas HSMEC expressed much higher levels of ICAM-1 (Fig. 1).

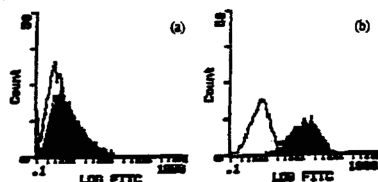

Fig. 1 Flow cytometric analysis of HUVEC (a) & HSMEC (b) labelled with control Ig (outlined) and with anti-ICAM-1 (shaded).

The intensity of ICAM-1 expression by FMEC is between that of HUVEC and HSMEC (HSMEC >> FMEC > HUVEC). Constitutive high levels of ICAM-1 expression by HSMEC may play an important role in the regulation of leukocyte traffic into the inflamed joint.

Reference: (1) Jackson *et al.* (1990) J Cell Sci 96:257.

C 117 EXPRESSION OF ICAM-1 *IN VIVO* IN IMMUNE COMPLEX-MEDIATED ALVEOLITIS IS TNFα DEPENDENT, Ara A. Vaporciyan, Michael S. Mulligan, Masayuki Miyasaka, Takuya Tamatani and Peter A. Ward, Department of Pathology, Univ. of Mich. Medical School, Ann Arbor, MI, 48109

Intraalveolar deposition of IgG immune complexes results in a neutrophil mediated lung injury that is CD18- and TNFα-dependent. On the basis of immunohistochemical analysis of lung tissue, there is increased endothelial expression of ICAM-1 *in vivo*. ICAM-1 expression in lung can also be induced by airway instillation of TNFα. Utilizing a cell based ELISA or ^{125}I labeled antibody specific for rat ICAM-1, the expression of ICAM-1 was quantified *in vitro* and *in vivo* respectively. The time course of ICAM-1 upregulation in IgG immune complex-mediated injury was similar to that achieved by instillation of TNFα into the airway, both being similar to *in vitro* expression of ICAM-1 by TNFα-stimulated endothelial cells. Treatment of immune complex injured rats with antibody to TNFα greatly inhibited morphological evidence of injury (intraalveolar hemorrhage, fibrin deposits and neutrophil accumulation). As well, this protective intervention was accompanied by a 61% reduction in intrapulmonary vascular expression of ICAM-1. These data demonstrate the linkage between *in vivo* generation of TNFα and vascular upregulation of ICAM-1.

C 118 ***C-erb-2/NEU* ONCOGENE-EXPRESSION ENHANCES MULTIPLE STEPS IN THE METASTATIC CASCADE.** Dihua Yu, Jun-ichi Hamada, Hong Zhang, Garth L. Nicolson, and Mien-Chie Hung, Department of Tumor Biology, U. T. M. D. Anderson Cancer Center, Houston, TX 77030, USA

The *neu* oncogene encodes a 185 kDa transmembrane protein with extensive sequence homology to epidermal growth factor receptor. Amplification/overexpression of the HER-2/*neu* gene has been shown to correlate with the number of lymph node metastasis in breast cancer patients and poor prognosis in both breast and ovarian cancers. However, there was no systematic study on the role of *neu* oncogene in metastasis. Here we demonstrate that 3T3 cells transformed by mutation-activated rat *neu* oncogene induced metastatic lung nodules by in vivo experimental metastasis assays, while the parental 3T3 cells did not. Monoclonal antibodies specific for the *neu*-encoded p185 protein abrogated the *neu*-induced metastatic properties. The data provided strong experimental evidence that the *neu* oncogene expression is sufficient in inducing metastasis in 3T3 cells. Important steps in the metastatic event are tumor cell adhesion to blood vessel and invasion of basement membrane. Therefore, we compared the *neu* transformed cells with the parental 3T3 cells the ability to adhere to microvessel endothelial cells, migrate through layer of reconstituted basement membrane and secrete basement membrane-degradative enzymes. The *neu*-transformed 3T3 cells exhibited significantly higher adhesion efficiency, enhanced cell motility and increased gelatinase activity. The results indicate that the *neu* oncogene expression can induce metastasis in 3T3 cells by facilitating multiple steps in the metastatic cascade.

Signal Transduction and Affinity/Avidity State Changes of CAMs
CAM Interactions with the Cytoskeleton/Junctional
Integrity of Endothelium

C 200 TAMB, A CELL SIGNALLING MOLECULE WHICH SELECTIVELY ACTIVATES LFA-1:ICAM1 ADHESION Andrew, D.P., Yoshino,T. and Butcher,E.C. Dept of Pathology, Stanford University, California.

A panel of mabs to molecules on TK1 cells (a CD8 T cell line) were raised, which induced homotypic aggregation of these cells, in the hope of identifying either adhesion or cell signalling molecules. On further characterization of the panel, three mabs were found to recognize CD45, CD11a and Beta7 respectively. One mab recognized a novel molecule, TAMB (T cell Activation and Memory B cell marker).

Mabs to TAMB, induced homotypic aggregation of TK1 cells, which was blocked by cytochalasin B, deoxyglucose or low temperature, while colchcine, staursporin, genestein, actinomycin D and amphicoidin were without effect. Blocking mabs to CD44, B7 and alpha4 did not inhibit mab induced TK1 cell aggregation, but anti-LFA1 and anti-ICAM-1 mabs did block. Therefore, TAMB is a cell signalling molecule which selectively induces LFA-1 dependent adhesion.

Interestingly, TAMB is expressed on all thymocytes, but is absent from mature T cells. However, on T cell activation, expression is induced. Thymocytes are split into subsets by the mab to TAMB. Thymocytes which express high levels of TAMB are $CD4^+CD8^+$ thymocytes, while CD4 and CD8 thymocytes express low levels of TAMB. $CD4^-CD8^-$ thymocytes are split into dull and high subsets by the mab to TAMB.

C 201 A NOVEL SIMULATED PHYSIOLOGICAL SHEAR MODEL SYSTEM FOR EXAMINATION OF SELECTIN FUNCTION ON LEUKOCYTES, PLATELETS, AND ENDOTHELIAL CELLS. Robert F. Bargatze,*+ Sandy Kurk,* Aaron R. Warnock,+ Eugene C. Butcher,*+ and Mark A. Jutila*. Veterinary Molecular Biology*, Montana State University, Bozeman, MT. 59717 and the Department of Pathology+, Stanford University, Stanford, CA. 94305

In recent years, considerable effort has been expended in leukocyte-endothelial cell research to define the receptors responsible for these cell's counter-adhesive properties. Results of these studies indicate that homing associated adhesion receptors, within tissues, have distinct roles in the sequence of events leading to leukocyte localization. Several research groups have hypothesized that a multi-step process is required to achieve successful extravasation. These steps include: a rolling interaction, activation-dependent adhesion strengthening, and permanent adhesion. We have developed a novel *ex vivo* closed loop circulating system to study these interactions under physiological shear. We found that activated endothelial cells and platelets support neutrophil rolling in this system. A mAb, EL246 against a common epitope on E-and L-selectin specifically blocked nearly 100% of an established rolling interaction of human neutrophils on 4 hour PMA activated human umbilical vein endothelial cells (HUVECS). Neutrophils that had undergone permanent arrest under static conditions for periods longer than one minute were resistant to EL246 induced displacement from HUVECS and greater than 60% of these cells remained attached suggesting an additional adhesion interaction. Human platelets activated with thrombin and bound to collagen, supported both rolling and arrest of human neutrophils under shear. The initial rolling interaction and permanent arrest could be blocked by anti-P-selectin mAb 12.2. Human neutrophils that were blocked by 12.2 under shear were also prevented under static conditions from establishing binding to the platelets, suggesting an exclusively P-selectin mediated neutrophil platelet interaction. These two results suggest a distinct difference in the primary interaction of E-selectin and P-selectin. The E-selectin interaction involves rolling whereas P-selectin may support both rolling and permanent arrest. This system provides a powerful means to evaluate binding properties of all members of the selectin family on cells immediately after they are taken from the animal. Under these conditions a more physiologically relevant appraisal of receptor ligand interactions is inevitable. This work is funded by a grant from The American Cancer Society (CD476).

C 202 EXPRESSION OF MEROSIN IN MOUSE THYMUS AND ITS INTERACTION WITH THYMOCYTES. Andrew C. Chang, Scott Wadsworth and John E. Coligan, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Merosin, a laminin-like protein, is a tissue specific basement membrane-associated protein found in placenta, striated muscle, and peripheral nerve. In this report, we demonstrate that merosin is also expressed in the mouse and human thymus. Laminin transcripts, however, were not detected by RT-PCR in the thymus. A partial cDNA sequence of mouse merosin exhibited 88% sequence identity compared to human merosin. 15-25% of unfractionated adult mouse thymocytes were capable of binding to merosin. Magnesium, but not calcium, was essential for binding. Merosin binding was inhibited by anti-VLA $\alpha 6$ or $\beta 1$ antibodies, suggesting that VLA-6 is a merosin receptor. Virtually all thymocytes express VLA-6 (Wadsworth et al. 1992. J. Immunol. 149:421-428), but only nonmature thymocytes (J11d+) were capable of binding to merosin. The addition of phorbol 12-myristate 13-acetate (PMA) in the binding assay slightly increased the binding of J11d+ thymocytes but did not induce binding in the mature J11d- population. Splenic T-lymphocytes and unseparated lymph nodes cells bound to merosin only after stimulation with PMA. The expression of merosin in the thymus and its selective interaction with nonmature thymocytes suggest that merosin may play a role in T cell ontogeny.

C 203 ICAM-1 IS A CO-STIMULATOR OF NK CELL-MEDIATED CYTOTOXICITY, Anita S.-F. Chong, Ian A. Boussy and Lloyd H. Graf. Department of General Surgery and Immunology/ Microbiology, Rush-Presbyterian-St Luke's-Medical Center, Chicago; Department of Biology, Loyola University, Chicago and Center for Research in Molecular Biology of Oral Disease, University of Illinois, Chicago, Illinois.

The molecules regulating the expression of cytotoxic activity in NK cells have not been completely characterized. Antibody inhibition suggest roles for a number of cell adhesion molecules. These include members of the immunoglobulin supergene family (ICAM-1, LFA-2 and LFA-3) and members of the integrin supergene family (LFA-1, VLA-4 and VLA-5). Adhesion molecules may function to stabilize cell-to-cell interaction and to provide co-stimulatory signals that are crucial for the triggering of cytotoxic activity. It has been difficult to experimentally demonstrate that adhesion molecules are co-stimulatory in NK cell-mediated cytotoxicity. We have developed an experimental system using cells transfected with genes encoding adhesion molecules to allow us to investigate the function of adhesion molecules. We report that the expression of ICAM-1 or LFA-3 alone, or both ICAM-1 plus LFA-3 does not convert a cell that is resistant to NK cell-mediated lysis into a susceptible one. Thus LFA-1-ICAM-1 and/or CD2-LFA-3 interactions cannot trigger cytotoxic activity by NK cells. We next tested the ability of these interactions to provide co-stimulation using a "three cell" experimental system comprising human NK cells, ^{51}chromium-labelled targets and transfected cells as a source of co-stimulation. ^{51}Chromium-labelled cells, K562 cells or anti-CD16-coated tumor cells, trigger NK cells to express cytotoxic activity. Addition of ICAM-1-transfected cells significantly enhanced the ability of NK cells to lyse target cells whereas the addition cells transfected with LFA-3 or irrelevant genes did not. Since this three-cell system allows triggering of cytotoxic activity (NK-target cell interaction) at a site spatially separate from LFA-1-ICAM-1 interaction (NK-transfected cell interaction), our data suggest that LFA-1-ICAM-1 interactions can provide remote co-stimulation via signalling events. Unexpectedly, CD2-LFA-3 interactions do not provide significant co-stimulatory activity for NK cells in our experimental system. Our results are in contrast to the observations that LFA-2 (CD2)/LFA-3 interactions are more potent than LFA-1-ICAM-1 interactions in providing remote T cell co-stimulation. We hypothesize that either the signalling pathways for these molecules are different in NK and T cells, or these molecules play different roles in the induction of cytotoxic activity and in the stimulation of cell proliferation.

C 204 REGULATION OF HUMAN NEUTROPHIL AGGREGATION: Mony M. Frojmovic and Yvan P. Rochon, Departments of Physiology and Experimental Medicine, McGill University, Montreal, Quebec, Canada H3G1Y6
We have recently described a flow cytometry technique allowing rapid and direct measurements of aggregation of isolated, stirred neutrophil suspensions (J Leuk Biol, 1991, 54,434). The parameters of neutrophil aggregation, measured for FMLP as activator, included latent times between the addition of activator and the onset of aggregation, initial rates, maximal extents, and reversibility of aggregation. We now compare the effects of three different receptor-active chemoattractants: FMLP, PAF and LTB_4 and two intracellular signal mimetics which yield sustained activation bypassing normal stimulus-response coupling: phorbol ester (PMA) and calcium ionophore (ionomycin). Neutrophil suspensions (480 µl) are stirred at 900 rpm at 37C ± activator, and 30µl subsamples withdrawn at the desired times and fixed in 250µl of 2% glutaraldehyde, and diluted with 1000µl of buffer for flow cytometry. The intracellular signal mimetics (PMA/Ionomycin) caused complete (≥98%) and irreversible neutrophil aggregation. In contrast, we found five properties of neutrophils which regulate and limit aggregation in similar fashions for the three receptor-specific activators (FMLP,PAF,LTB_4): delayed onset of aggregation (4.5s ± 1.5s), independent of activator concentration; similar concentrations for onset of aggregation (≈1nM) which increased over a similar broad range of activator concentration, with one-half maximal rates of aggregation at 10nM-30nM corresponding to reported K_D values; comparable limited recruitment and spontaneous reversibility of aggregation; absence of inter-activator synergism; and similar exponential decays in activated cell stickiness (refractoriness), with t½ = 10 to 20sec. We found no evidence for neutrophil subpopulations in responses to dual activators, nor any "cross-desensitization" of FMLP- or LTB_4-induced aggregation. In vivo, these properties are expected to provide localization of the aggregatory response, minimizing the otherwise detrimental effects of circulating activated neutrophils.

C 205 THE CYTOPLASMIC DOMAIN OF L-SELECTIN IS REQUIRED FOR LYMPHOCYTE ADHESION TO HEV AND LEUKOCYTE ROLLING Geoffrey S. Kansas, Klaus Ley and Thomas F. Tedder, Division of Tumor Immunology, Dana Farber Cancer Institute, Boston, MA; Dept of Physiology, Free University of Berlin, Berlin, Germany.
The selectins are a family of carbohydrate binding adhesion molecules involved in the regulation of leukocyte migration. L-selectin (LAM-1) is expressed on leukocytes and mediates leukocyte rolling, lymphocyte adhesion to high endothelial venules (HEV) of lymph nodes, and leukocyte attachment to cytokine activated endothelium. The strong conservation between human and mouse L-selectin in the cytoplasmic domain (13/17 amino acids identical) suggested an important role for this region in the function of L-selectin. In order to assess the role of the L-selectin cytoplasmic domain in leukocyte adhesion, a stop codon which deleted the 11 carboxyl terminal amino acids of the predicted 17 amino acid cytoplasmic domain of L-selectin was introduced into the pLAM-1 cDNA using PCR based site-directed mutagenesis. Stable transfectants were prepared in the 300.19 murine pre-B cell line using the normal and mutant pLAM-1 cDNAs. The cytoplasmic truncation mutant, termed L∆cyto, expressed each unique epitope defined by a panel of 11 mAb, and exhibited the expected Mr with SDS-PAGE analysis. Importantly, the L∆cyto mutant also retained normal lectin activity, as assessed by binding of PPME-FITC. However, in contrast to the L-selectin parental transfectant, the L∆cyto transfectants failed to bind to HEV in Stamper-Woodruff frozen section assays, and also failed to roll in exteriorized rat mesenteric venules. Pretreatment of L-selectin transfectants with cytochalasin B also abolished adhesion to HEV without affecting PPME binding. These results suggest that an interaction between the cytoplasmic domain of L-selectin and the cytoskeleton is essential for leukocyte adhesion mediated by L-selectin. Supported by NIH grant CA 54464 and Deutsche Forschungsgemeinschaft grant Le 573/3-2.

C 206 ISOLATION AND CHARACTERIZATION OF AN INHIBITOR OF CD18-MEDIATED CELL ADHESION FROM CONOBEA SCOPARIOIDES (SCROPHULARIACEAE), Loran Killar*, Laslo Musza#, Phyllis Speight*, Susan McElhiney* and Colin Barrow#, *Departments of Immunopharmacology* and Natural* Products Chemistry#, Sterling Winthrop Pharmaceuticals Research Division, Malvern, PA 19355
In the course of high volume screening for antagonists of CD18-mediated cell adhesion, an extract with inhibitory activity was identified from the stem and leaves of Conobea scoparioides. With a three step bioassay-guided isolation procedure, a pure compound was obtained and identified by spectroscopy as the known compound cucurbitacin E. Cucurbitacin E is a member of a family of highly oxygenated tetracyclic triterpenoids that are present in a wide variety of plants. Although many biological activities have been reported for cucurbitacins, cell adhesion inhibition has not bee described to date. The IC_{50} for cucurbitacin E in the high volume adhesion assay was less than 1 µM. Although long term exposure to the compound was toxic for the cells in the adhesion assay, viability of the cells was not effected during the time period of the assay (45 minutes). Closely related cucurbitacin analogs had different potencies, pointing to substructural features that are important for the activity. Although the cucurbitacins did not inhibit certain processes that are required for CD18-mediated cell adhesion (activation as defined by CA^{++} flux, protein kinase C activity), they inhibited actin polymerization in activated neutrophils with IC_{50}'s that paralleled adhesion inhibition. This result suggests that the cucurbitacins nonspecifically inhibit cell adhesion by disrupting the cytoskeleton.

C 207 PERTUSSIS TOXIN INHIBITION OF T-CELL HYBRIDOMA INVASION *IN VITRO* IS REVERSED BY MANGANESE, Geertje La Rivière, Jacqueline W.T.M. Klein Gebbinck, Mariëtte H.E. Driessens and Ed Roos, Department of Cell Biology, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands (fax nr. 31-206172625)

Invasiveness of T-cell hybridomas *in vitro* and metastasis *in vivo* is strongly inhibited by pertussis toxin (PT). Since PT blocks G-protein-transduced signals, this indicates that invasion is stimulated by an extracellular factor. In line with this, suramin an agent that dissociates such factors from receptors, similarly inhibited invasion by T-cell hybridoma cells into fibroblast monolayers.

We have tested whether one of the effects of the PT-affected signal was functional activation of LFA-1, an adhesion molecule required for invasion. We show that Mn^{2+}, known to activate LFA-1, stimulated invasion by PT-pretreated T-cell hybridomas, and invasion in the presence of suramin. Mn^{2+} also stimulated invasiveness of untreated cells to a variable extent, but much less. The effect of Mn^{2+} on invasion was inhibited by anti-LFA-1 antibodies and Mn^{2+} did not stimulate invasion by LFA-1-deficient mutants. Thus stimulation was mainly due to an effect on LFA-1. Furthermore, Mn^{2+} induced binding of T-cell hybridoma cells to purified ICAM-1, one of the counterstructures for LFA-1. This shows that LFA-1 was not constitutively active and that it was stimulated by Mn^{2+}.

The PT and suramin effects were serum-independent, excluding serum factors as the involved extracellular factor(s). PT and suramin did not reduce spontaneous migration and therefore probably did not affect putative autocrine motility factors. Therefore we propose that the relevant factor is produced by the fibroblasts.

C 208 LFA-1 AFFINITY FOR ICAM-1 INCREASES UPON PHORBOL ESTER ACTIVATION OF T CELLS, Bridget A. Lollo, Kyle Chan, Vincent T. Moy, Elaine M. Hanson and Adrienne A. Brian, Department of Chemistry, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0063

Activation of T cells by receptor crosslinking or phorbol ester stimulation increases T cell adhesion to ICAM-1-coated surfaces. The mechanism by which adhesion avidity is increased is unclear, but may involve an increase in the affinity of LFA-1 for ICAM-1. We have measured the affinity of the LFA-1/ ICAM-1 interaction on resting and phorbol ester-activated T cells by using a soluble form of murine ICAM-1 to inhibit the binding of radiolabelled, function-blocking, anti-LFA-1 antibody Fab fragments. Measuring the kinetics of the competition of soluble ICAM-1 and anti-LFA-1 Fab fragments and fitting the data to the solution of the simultaneous rate equations gives rate constants for the LFA-1/ ICAM-1 interaction. Results from these and equilibrium competition experiments show that the affinity of ICAM-1 for LFA-1 on unactivated T cells is low, K_D = 5-15 $\times 10^{-5}$ M, apparently due to a very slow on rate, on the order of ~10^4 M^{-1} min^{-1}. We observe a reproducible but modest increase in the average affinity , ~2-fold, upon phorbol ester activation. Whether this 2-fold increase in affinity reflects a small change in affinity for all the LFA-1 molecules or a larger change in a subpopulation of LFA-1 molecules is still unclear. Preliminary experiments suggest that a subpopulation of LFA-1 molecules undergo a substantial affinity change.

C 209 VLA-4 INTEGRIN ON SARCOMA CELL LINES RECOGNIZES ENDOTHELIAL VCAM-1. DIFFERENTIAL REGULATION OF THE VLA-4 AVIDITY Pirkko Mattila, Marja-Leena Majuri and Risto Renkonen, Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland.

Osteosarcomas and rhabdomyosarcomas are vigorously invading tumors. Before they can extravasate to the parenchymal organs and form metastasis they have to adhere to the endothelial cells lining the blood vessels and then penetrate through the endothelium. We show in this paper that several human sarcoma cell lines, osteosarcomas HOS, MG-63, U2-OS, and a rhabdomyosarcoma RD express VLA-4 molecule on their surface and bind to VCAM-1 expressing activated endothelial cell line Ea.hy 926. The increased sarcoma cell adhesion could be abolished by treating the sarcoma cells with monoclonal anti-VLA4 antibodies (α- and β-chain, HP2/1 and 4B4 respectively) or treating endothelial cells with anti-VCAM-1 antibody (4B9). Furthermore we showed that sarcoma cells adhere to recombinant soluble VCAM-1 protein. On the other hand these sarcoma cell lines do not express marked amounts of other ligands (such as CD11/18 or sialyl-Lex) for other endothelial adhesion molecules (ICAM-1, ICAM-2, E- and P-selectin) indicating that the VLA-4 - VCAM-1 dependent pathway might be of major importance in the sarcoma extravasation. VLA-4 is not always in an avid form and therefore the expression of VLA-4 does not directly predict adherence to VCAM-1. The avidity of VLA-4 (measured by adherence to soluble VCAM-1) of MG-63 and U2-OS cells could be increased by a 30 min PMA treatment whereas the avidity of VLA-4 on HOS cells increased only after 48 h PMA-induction. Taken together these results show that sarcoma cell lines adhere to stimulated endothelium via VLA-4 - VCAM-1 adhesion molecules and that the VLA-4 avidity on sarcoma cells can be differentially modulated by PMA.

C 210 FUNCTIONAL ACTIVATION OF THE LEUKOCYTE INTEGRIN, LFA-1, BY DIVALENT CATIONS INDEPENDENTLY OF PROTEIN KINASE C, J E Merritt, J M Budd, D W Westmacott, R F G Booth and T J Hallam, Roche Research Centre, Broadwater Road, Welwyn Garden City, Herts AL7 3AY, UK.

Leukocyte function-associated antigen (LFA-1) is an integrin constitutively expressed in a low affinity "inactive" form on T and B cells. "Activation" of LFA-1 to increase its binding to ICAM-1 has been monitored in two cellular systems, homotypic aggregation of a human B cell line (IM9) which expresses both LFA-1 and ICAM-1, and adhesion of a human T cell line (JM) which expresses only LFA-1 to a human endothelial cell line (ECV304).

The phorbol ester, phorbol dibutyrate (PDBu), promotes concentration-dependent homotypic aggregation of IM9 cells and adhesion of JM cells that is dependent on LFA-1 - ICAM-1; responses are blocked by the neutralising antibodies, BCA1 (anti LFA-1) and 84H10 (anti ICAM-1). The selective protein kinase C (PKC) inhibitor, Ro31-8425, inhibits the PDBu-stimulated responses (IC50 0.2μM) as expected. The response to PDBu is dependent on extracellular Mg. $MgCl_2$ alone (>1mM) also stimulates LFA-1 - ICAM-1 dependent aggregation, but only in the absence of extracellular Ca. $MnCl_2$ (0.06-0.3mM) stimulates LFA-1 - ICAM-1 dependent aggregation of IM9 cells and adhesion of JM cells. The response to Mn is not dependent on Mg or Ca; however, the Mn dose curve is shifted to the right in the presence of Ca, which is consistent with an inhibitory effect of Ca at the divalent cation binding site. As expected, the Mn-stimulated responses are unaffected by the PKC inhibitor, Ro31-8425. In summary, two functional assays for activation of LFA-1 have demonstrated two divalent cation-dependent mechanisms for activation: direct activation by Mn or indirect PKC-dependent activation by phorbol esters.

C 211 A PEPTIDE FROM ICAM-2 RECOGNIZES THE LEUKOCYTE INTEGRIN CD11a/CD18

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Leukocyte interactions with one another, with extracellular matrix and with vascular endothelial cells are crucial to development, tissue organization, migration and immune responses of all leukocytes. This kind of cell adhesion is mediated by a variety of cell adhesion molecules and among the most important ones are the leukocyte specific integrin CD11a/CD18 and its cellular ligands ICAM-1 and ICAM-2. It has been shown that aminoterminal domains of ICAM-1 are involved in binding to CD11a/CD18, but very little is known about the binding of ICAM-2 to its receptor CD11a/CD118.

In the present study we have defined a peptide region from the ICAM-2 molecule which is specifically involved in binding to CD11a/CD18. This peptide binds to purified CD11a/CD18, and inhibits the adhesion of endothelial cells to this integrin. Moreover leukocytes bind to the peptide coated on plastic.

C 212 ACTIVATION OF HUMAN NEUTROPHILS INDUCES AN INTERACTION BETWEEN THE INTEGRIN β_2 SUBUNIT (CD18) AND ACTIN FILAMENTS VIA α-ACTININ, Fredrick M. Pavalko and Suzette LaRoche, Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 46202
Activation of neutrophils with the chemotactic peptide FMLP results in increased binding of Mac-1 (CD11b/CD18) to C3bi, ICAM-1, LPS, fibrinogen and endothelial cells that does not involve increased surface expression of Mac-1. Similarly, phorbol ester stimulation of neutrophils increases LFA-1 (CD11a/CD18)-dependent cell adhesion. We are pursuing the likelihood that interactions between β_2 (CD18) integrins and the actin cytoskeleton play a role in the increased avidity of integrins upon activation. Using *in vitro* binding assays, we and others demonstrated that the F-actin binding protein α-actinin binds to the cytoplasmic domain of β_1 integrin (Otey, Pavalko and Burridge, 1990, *J. Cell Biol.* 111:721-730). A proteolytic fragment of α-actinin was identified which binds integrin *in vitro* but does not contain the actin binding region. This fragment co-localized with β_1 integrins in focal adhesions when fluorescently labeled and microinjected into living fibroblasts suggesting that α-actinin can interact with integrins in cells (Pavalko and Burridge, 1991, J. Cell Biol. 114:481-491). Given the extensive homology between the β_1 and β_2 integrin cytoplasmic domains, we are exploring the possibility that α-actinin may link actin filaments to CD18 integrins in neutrophils. We conjugated a peptide corresponding to a 19 amino acid segment within the cytoplasmic domain of the integrin β_2 subunit (CD18) to sepharose, and found that this peptide bound α-actinin from TX-100 extracts of human neutrophils. A polyclonal antibody directed against this segment of the CD18 cytoplasmic domain blocked binding of purified α-actinin to the cytoplasmic domain in solid phase binding assays. To determine if α-actinin interacts with CD18 integrins in cells, we immunoprecipitated α-actinin from 1% CHAPS extracts of neutrophils and subjected the precipitates to immunoblotting using an antibody (KIM127) against CD18. We found that CD18 does co-precipitate with α-actinin from extracts of neutrophils following activation with 10 nM FMLP, but not from unactivated cells. Activation of cells with 1 nM FMLP caused association of only a small amount of CD18 compared to the association caused by activation with 10 nM FMLP. CD18 did not co-precipitate with either talin or vinculin, both of which participate in linking actin to β_1 integrins at focal adhesions. Our results suggest that induction of actin filament attachment to CD18 integrins via the actin-binding protein α-actinin may play a role in activation-dependent cell adhesion.

C 213 STRUCTURE AND FUNCTIONS OF SOLUBLE MURINE VCAM-1 AND VCAM-1-IgG RECOMBINANT MOLECULES, Mark E. Renz, Henry H. Chiu, Susan Jones, and Sherman Fong, Department of Immunology, Genentech, Inc., South San Francisco, CA 94080-4990
To further characterize the structural requirements of VCAM-1 interaction with VLA-4+ cells in adhesion and co-stimulation of lymphocyte responses, soluble truncated murine VCAM-1 and chimeric VCAM-1-murine IgG1 recombinant molecules were constructed and characterized. Both the immobilized soluble murine VCAM-1 and VCAM-1-IgG moleucles (7 Ig-like domain forms) were capable of binding mouse and rat lymphoid cells and human Ramos (VLA4+, LFA-1-) lymphoblastoid cells in adherence assays and by FACS analysis. Cell adherence was greatly enhanced by the presence of $Mn^{++} >> Ca^{++}Mg^{++} = Mg^{++} = Ca^{++}$. VCAM-1 and VCAM-1-IgG (at 350 nM each) were capable of inhibiting by 70-80% Ramos cell adherence to 24 hour TNF-α activated HUVEC monolayers. VCAM-IgG cross blocked completely Ramos binding to fibronectin. Truncated VCAM-1 recombinant variants with domain deletions were also generated to explore lymphoid adherence and co-stimulatory functions. Structural and functional properties of these molecules will be reported.

C 214 SIGNAL-TRANSDUCTION PATHWAYS INVOLVED IN EOSINOPHIL CHEMOTAXIS, René C. Schweizer, Ruud A.J. Warringa, Tjander Maikoe, Jan-Willem J. Lammers and Leo Koenderman, Dept of Pulmonary Diseases, University Hospital Utrecht, PO Box 85500, 3508 GA Utrecht, the Netherlands.
Cytokines such as GM-CSF, IL-3 and IL-5 are important modulators of eosinophil chemotaxis. Some chemotaxins, such as Platelet-Activating Factor (PAF), act upon unprimed eosinophils, whereas other inflammatory mediators, such as fMet-Leu-Phe (fMLP) and IL-8 are chemotaxins for eosinophils only after priming with cytokines. Apparently, different signaling pathways are involved in eosinophil chemotaxis. Here, we have studied in a Boyden chamber assay several signal-transduction pathways, that can mediate eosinophil chemotaxis.
We have found that changes in $[Ca^{2+}]_i$ are not essential for eosinophil chemotaxis, since responses of unprimed and cytokine primed cells are not inhibited by either buffering intracellular Ca^{2+} with BAPTA (5 μM) or depletion of both intra- and extracellular Ca^{2+}. Interestingly, PAF responses are markedly increased (2 fold) by Ca^{2+}-depletion, whereas fMLP responses are not influenced.
Also several protein kinases might play an important role in signaling for eosinophil chemotaxis. Inhibition of PK-A by the specific inhibitor H-89 potently inhibits fMLP-induced eosinophil chemotaxis (ID_{50}:10 nM), whereas PAF responses are not influenced. On the other hand, inhibition of PK-C by staurosporine does not influence fMLP responses, whereas PAF responses are markedly upregulated (2-3 fold).
In conclusion, at least two signal-transduction pathways mediate eosinophil chemotaxis. One pathway, illustrated by the fMLP response, needs priming by cytokines to be apparent and is mediated by PK-A. The other pathway, illustrated by the PAF response, acts on unprimed cells and is downregulated by signals involving PK-C and Ca^{2+}

C 215 Molecular cloning of ICAM-3, a third ligand for LFA-1, constitutively expressed on resting leukocytes
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The co-ordinated function of effector and accessory cells in the immune system is assisted by cell surface adhesion molecules which stabilise interactions between different cell types. Leucocyte Function-associated Antigen 1 (LFA-1) is expressed on the surface of all white blood cells and is a receptor for Intercellular Adhesion Molecules (ICAM) 1 and 2, members of the immunoglobulin superfamily. The interaction of LFA-1 with ICAMs -1 and -2 provides essential accessory adhesion signals in many immune interactions including those between T and B lymphocytes and cytotoxic T cells and their targets. In addition, ICAMs -1 and -2 are expressed at low levels on resting vascular endothelium; ICAM-1 is strongly upregulated by cytokine stimulation and plays a key role in the arrest of leukocytes in blood vessels at sites of inflammation and injury. Recent work has indicated that resting leukocytes express a third ligand, ICAM-3, for LFA-1. ICAM-3 is potentially the most important ligand for LFA-1 in the initiation of the immune response since ICAM-1 expression on resting leukocytes is low. We report the expression cloning of a cDNA, pICAM-3, encoding a protein constitutively expressed on all leukocytes, which binds LFA-1. ICAM-3 is closely related to ICAM-1, consisting of 5 immunoiglobulin (Ig) domains and binds LFA-1 through its 2 N-terminal domains.

C 216 DIFFERENT PHOSPHOLIPASE D (PLD)-ASSOCIATED SIGNALS REGULATE CD18-MEDIATED PHAGOCYTOSIS AND OXIDATIVE ACTIVATION IN HUMAN NEUTROPHILS, Olle Stendahl, Lena Larsson, Maria Fällman & Tommy Andersson. Departments of Medical Microbiology and Cell Biology, Faculty of Healh Science, Linköping University, S-581 85 Linköping, Sweden.
The three ß2-integrins CD11a/CD18, CD11b/CD18 and CD11c/CD18 participate in multiple functions in neutrophils, such as adhesion, phagocytosis and oxidative activation. It has recently been suggested that these events are associated with PLD-mediated hydrolysis of phosphatidylcholine and subsequent protein kinase C-mediated phosphorylation of specific proteins. To evaluated the regulatory role of PLD in CD18-induced phagocytosis and oxidative activation, we utilized two selective stimuli for CD18; C3bi-opsonized yeast particles and *Staphylococcus aureus* Cowan 1 coated with anti CD18 antibodies.
The opsonized yeast particles are easily ingested while the anti-CD18 antibody-coated bacteria remain bound to the cell surface. Both particles activate PLD as measured by phosphatidylethanol (PEt) formation, indicating that ingestion is not necessary for CD18-induced PLD activation. When PLD activation was inhibited by 50%, using the protease inhibitor zLYCK, yeast phagocytosis was reduced with 50% as well, whereas the concomitant oxidative activation was reduced with >90%. When zLYCK inhibited PLD activation in cells stimulated with the non-ingested but surface-bound S. aureus-CD18 antibody particles with 50%, oxidative activation was reduced with >90%. The fact that pretreatment of the cells with phorbol myristate acetate (PMA), which potentiates CD18-induced PEt formation, eliminated the inhibitory effect of zLYCK, suggests that PLD and PKC play a central role in CD18-mediated activation in neutrophils.
The results also suggest a direct link between PLD and PKC activation and CD18-induced phagocytosis, whereas the CD18-mediated signalling for oxidative activation requires an additional link downstream to PLD activation.

Carbohydrate Recognition: Selectins and Their Ligands

C 300 GENERATION OF MONOCLONAL ANTIBODIES TO MOUSE E-SELECTIN AND THEIR USE TO INVESTIGATE THE ROLE OF E-SELECTIN IN INFLAMMATION, Daniel K. Burns, Christine R. Norton, John M. Rumberger, Margaret L. Harbison*, Polly A. Knaack*, and Barry A. Wolitzky, Dept. of Inflammation and Autoimmune Diseases and *Dept. of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, N.J., 07110

We have isolated and characterized a recombinant phage containing the structural gene for the murine homolog of E-selectin. Using PCR techniques, the exons encoding the mouse lectin and egf domains were fused to create an artificial cDNA for expression in eukaryotic cells. Transient expression in COS cells indicated that the fused mouse exons were sufficient to mediate the binding of human and mouse neutrophils. None of the MAbs directed against human or rabbit E-selectin cross reacted to the mouse lec/egf domains. Several of our previously characterized MAbs directed against human E-selectin were found to recognize determinants in cr1 and cr2. To produce mouse E-selectin for use as an immunogen for the generation of MAbs the first and second cr elements of human E-selectin were fused to the mouse lec/egf domains. Transient expression in COS cells of the murine/human E-selectin chimera was performed to generate large quantities of material that could be purified by immunoaffinity chromatography with MAbs directed against the human portion of the chimera. Rats immunized with fusion protein were used to generate eight anti-mouse E-selectin MAbs, including five that inhibit the adhesion of HL60 cells or mouse neutrophils to COS cells expressing the mouse lec/egf domains. These five MAbs crossreact and inhibit adhesion of HL60 cells to human E-selectin, but do not bind the rabbit homolog. All eight MAbs bind to the lec/egf domains as determined by immunofluorescence on COS cells transfected with membrane anchored mouse lec/egf and will immunoprecipitate the mouse/human chimeric E-selectin. These MAbs have been used to study E-selectin expression in cytokine treated mice and to evaluate the role of E-selectin in murine models of inflammation.

C 301 CHARACTERISATION OF SIALOADHESIN AS A STROMAL MACROPHAGE-RESTRICTED CELL ADHESION MOLECULE FOR DEVELOPING AND MATURE MYELOID CELLS.
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Sialoadhesin is a stromal macrophage-restricted receptor of 185 kD which can mediate specific adhesion of cells through recognition of Neu5Acα2,3Galß1,3GalNAc in cell surface glycoconjugates The distribution and properties of the receptor indicate that it is involved in cellular interactions with developing myelomonocytic cells in the haemopoietic compartments of the bone marrow and with lymphocyte subpopulations in the subcapsular sinus and cortex of peripheral lymph nodes. To investigate these possibilities further, we have developed cell binding assays with sialoadhesin, using the receptor either in a purified, iodinated form or following expression by macrophages in culture. In both assays, sialoadhesin exhibited differential binding to various murine cell populations in a sialic acid and monoclonal antibody-inhibitable manner. In rank order, sialoadhesin bound neutrophils > bone marrow cells > blood leukocytes > lymphocytes = thymocytes > RBC. These results are consistent with the notion that sialoadhesin on tissue macrophages plays a role in cellular interactions with granulocytes at different stages of their life history.

C 302 INHIBITION OF SELECTIN MEDIATED ADHESION IN VIVO AND IN VITRO, Barbara J. Dalton*, John A. Feild*, Janice R. Connor*, Kyung Johanson", Patrick McDevitt", Daniel J. O'Shannessy", Raj Parekh', Colin Campion', Len Martin+, Don E. Griswold+, Departments of Cellular Biochemistry*, Respiratory and Inflammation Pharmacology+, and Protein Biochemistry", SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, and OxfordGlyco Systems', Abingdon, Oxon, UK.

Selectins are a family of cell adhesion molecules whose expression on the surface of endothelial cells, platelets and leukocytes, is specifically regulated. The three known members of this protein family, E-, L-, and P-Selectin, share a common extracellular domain structure that is composed of an N-terminal C-type lectin domain followed by an EGF domain and a varying number of complement regulatory protein domains attached to transmembrane and cytoplasmic domains. All three members of this family can recognize the sialyl-LewisX (sLeX) tetrasaccharide. These molecules facilitate the initial interaction of leukocytes with inflammed endothelium leading to the extravasation of leukocytes into the surrounding tissues. The regulation, and specifically the inhibition of selectin mediated adhesion, is of interest for the therapeutic manipulation of inflammatory responses. In vitro we demonstrate that monoclonal antibodies to E-selectin or its carbohydrate ligand inhibit leukoctye binding to cytokine stimulated human umbilical vein endothelial cells or CHO cells transfected with E-selectin. Similar inhibition can be demonstrated with soluble E-Selectin (produced without the transmembrane and cytoplasmic domains), or as others have demonstrated, with very high concentrations of the free sLeX tetrasaccharide. The in vivo inhibitory activity of these agents was explored in a mouse model of inflammation. The topical application of aracadonic acid to a mouse ear elicits an inflammatory response characterized by both fluid (edema) and inflammatory cell infiltration. The inflammatory cell infiltration, as measured by tissue myeloperoxidase, is significantly inhibited, in a dose dependent manner, by soluble E-selectin or sLeX. These data indicate that inhibition of selectin mediated adhesion in vivo results in reduced leukocyte tissue infiltration.

C 303 THE ROLE OF SIALIC ACIDS AND THEIR SIDE CHAINS IN RECOGNITION OF THE MYELOID CELL LIGAND BY P-SELECTIN, Sandra Diaz[1], Kevin Moore[2], Karin E. Norgard[1], Dave Smith[3], Nancy Stults[3], Rodger McEver[2], Richard Cummings[2] and Ajit Varki[1]. [1]Glycobiology Program, UCSD Cancer Center and Division of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, [2]Department of Medicine and Biochemistry, University of Oklahoma, Oklahoma City, OK 73104 and [3]Department of Biochemistry, University of Georgia, Athens, Georgia 30602
Lectin-carbohydrate interactions between the selectins and their ligands are the earliest recognition events which lead to lymphocyte recirculation, normal recruitment of leucocytes into areas of inflammation and possibly abnormal recruitment of leucocytes in disease states. All members of the selectin family have been shown to require sialylation of their natural ligands for recognition. Previously, we isolated a novel 120 kDa sialylglycoprotein from myeloid cells which specifically recognizes P-Selectin. Here, we demonstrate that this 120 kDa glycoprotein contains the tetrasaccharide sequence Sialyl Lewis^x which has been found to be required for binding of other selectin ligands. We further find that the predominant form of sialic acid in this ligand is N-acetylneuraminic acid. Remarkably, the glycoprotein represents <1% of the total sialic acids and is only a minor component of the total cellular Sialyl Lewis^x structures. We demonstrate that the side-chain of the sialic acids on this glycoprotein are not required for binding. This allows us to selectively remove the side-chain of the sialic acid and introduce a tritium label into the truncated sugar. The ability to introduce a radioactive label has facilitated initial characterization of this ligand. We find that the chemically labelled ligand, as well as its metabolically labelled counterpart, are susceptible to cleavage by the enzyme O-glycoprotease. O-glycoprotease is a recently described enzyme, purified from *Pasteurella hemolytica*, whose proteolytic activity is directed towards sialylated, O-linked glycoproteins such as glycophorin, and some well known antigenic determinants (e.g. CD43, CD34 and CD45). Review of the types of glycoproteins cleaved by this enzyme suggests that it recognizes clusters of sialylated, O-linked oligosaccharides. In fact, we found that it does not cleave glycoproteins with few or widely spaced O-linked oligosaccharides. We are currently exploring O-glycoprotease treatment of HL-60 cells to see if it selectively destroys their ability to bind to P-Selectin while maintaining the integrity of other cell surface molecules. These results suggest that specificity of this ligand may be induced by specific clustering and/or orientation of common sialylated O-linked oligosaccharide structures found on this glycoprotein. However, the possibility of a rare or unique oligosaccharide structure also needs to be explored.

C 304 EXPRESSION OF SELECTIN LIGANDS BY CUTANEOUS NEOPLASIA. Richard W Groves, Michael H Allen, Jonathan NWN Barker, Elizabeth L Ross, Donald M MacDonald. Laboratory of Applied Dermatopathology, St John's Institute of Dermatology, UMDS, Guy's Hospital, London, UK.

Metastasis is a complex process involving interactions between tumour cells and endothelium during initial vascular invasion and in deposition of tumour cells at distant sites. Recent evidence suggests that endothelial E-selectin and tumour expression of its carbohydrate ligands sialyl-Lewis-X (SLex) and sialyl-Lewis-A (SLea) may be important in these processes.

To investigate the role of such mechanisms in cutaneous tumours, whole skin biopsies were examined immunohistochemically using specific monoclonal antibodies; in squamous cell carcinoma (SCC, n=12) there was expression of SLex in all tumours, but SLea was not expressed. These E-selectin ligands were absent on keratinocytes in both basal cell carcinoma (BCC, n=8) and normal skin (n=8), with the exception of one BCC which expressed SLea. E-selectin was strongly expressed by dermal endothelium in both SCC and BCC. Keratinocyte cell lines A431, HaCat and SVK14 were investigated by flow cytometry, which demonstrated SLex and SLea expression by all three, whereas normal human keratinocytes did not express these molecules.

Dermal vessels in cutaneous tumours express E-selectin, and SCC, BCC and transformed keratinocytes express its cognate ligands. These results suggest a role for selectin mediated events in early and late metastatic events. E- selectin/carbohydrate interactions may provide a future therapeutic target for anti-metastatic chemotherapy.

C 305 MULTIPLE BINDING SITE INVOLVEMENT IN NEUTROPHIL-SELECTIN ADHESION: IMPLICATIONS FOR DESIGN OF PEPTIDE AND CARBOHYDRATE INHIBITORS, George A. Heavner, Leon Epps, Miljenko Mervic, Margaret Falcone, Douglas Riexinger and Marian Kruszynski, Centocor, Inc., 200 Great Valley Parkway, Malvern, PA 19355
Acute inflammation involves the recruitment of neutrophils from postcapillary venules, their migration into tissues and accumulation at sites of injury, infection or immune complex deposition. The selectins, a family of structurally related, Ca^{++}-dependent membrane glycoproteins that recognize complex carbohydrate ligands, are involved in the initial phases of neutrophil emigration. We were interested in establishing the location and extent of the binding domains of the selectins, in determining whether more potent analogs could be designed and in defining the extent of the inhibitory activity of the resulting compounds. Synthetic studies identified several non-contiguous regions within the lectin domain of P-selectin that could independently modulate the binding of human neutrophils to P-selectin (IC_{50} 0.1-1.5 mM). No inhibitory sequences were found in the EGF domain. Collectively the active peptides define a surface that recognizes the P-selectin counter molecule. The definition of a large binding surface on the selectins has implications for the design of both peptide and carbohydrate inhibitors. Homologous sequences of some of these peptides within E- and L-selectin also inhibit P-selectin dependent adhesion. The synthesis of peptides within these regions has led to the identification of smaller active peptides. Structure-activity studies have led to analogs that are significantly more potent than their parent structures (IC_{50} 1-10 μM), that inhibit both P- and E-selectin dependent adhesion and that are resistant to enzymatic hydrolysis. Several of the peptides from one structural class are potent inhibitors (IC_{50} 1-30 μM) of neutrophil adhesion to HUVEC cells that have been stimulated with IL-1 for 19 hours, suggesting that this family of peptides may inhibit not only selectin but also ICAM-dependent adhesion as well. The broad specificity of analogs of the selectins in inhibiting leukocyte adhesion makes them attractive candidates for the modulation of both acute and chronic inflammatory conditions.

C 306 Cloning of a cDNA Encoding a Selectin Homolog that is Expressed on Undifferentiated Murine Hemopoietic Cells.

Robert Kay, Graeme Dougherty, Vivienne Rebel, Peter Lansdorp and R. Keith Humphries, Terry Fox Laboratory, British Columbia Cancer Agency, and the Departments of Medical Genetics, Pathology and Medicine, University of British Columbia, Vancouver, BC, Canada.

The 11F6 antigen is expressed on a rare class of undifferentiated murine hemopoietic cells that includes pluripotent stem cells and their immediate progeny. The 11F6 antigen is also expressed at low levels on the pluripotent hemopoietic cell line B6SUtA$_1$. A library of cDNAs from B6SUtA$_1$ cells was screened by immunoselection, and one 2500 bp clone that directed expression of 11F6 on transfected COS cells was cloned. The sequence of the cDNA revealed that 11F6 is a large cell surface protein of 820 amino acids. It contains a single "complement-like" domain immediately N-terminal to its transmembrane segment, which is most similar to the equivalent domain in L-selectin. Unlike the selectins, the N-terminus of the 11F6 peptide lacks a potential lectin domain. Instead, it is homologous to the N-terminus of the PC-1 extracellular kinase, particularly in their precise conservation of cysteine spacing. However, 11F6 does not appear to have a nucleotide binding site needed for kinase activity and so its structural similarity to PC-1 kinase may reflect homologous ligands rather than equivalent functions. It remains to be determined what the 11F6 ligand is, and what role this protein plays in regulating the initial stages of hemopoietic cell development and lineage commitment.

C 307 HISTIDINE-PROLINE-RICH GLYCOPROTEIN AND MITOGENIC LECTINS STIMULATE T-LYMPHOCYTE ADHESION AND MORPHOLOGIC CHANGES,

Rebecca J. Lamb and William T. Morgan, Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri 64110.

Histidine-proline-rich glycoprotein (HPRG) is a negative acute-phase plasma protein which binds to a specific receptor on T-lymphocytes and represses T-cell activation and proliferation. HPRG also inhibits the formation of auto-rosettes between murine red blood cells and T-lymphocytes. Therefore, HPRG may function as a natural regulator of human T-lymphocytes. A human acute lymphoblastic T-cell leukemia cell line (MOLT-3) is being used in our laboratory to study HPRG receptor function on T-cells. Interestingly, when MOLT-3 cells are placed in serum-free medium and incubated with low levels of HPRG and a T-cell mitogenic lectin (either concanavalin A (Con A) or phytohemagglutinin (PHA)), the spherical cells in suspension rapidly become attached, spread-out cells with many processes. Importantly, a substantial fraction of human peripheral blood lymphocytes incubated under the same conditions also undergoes attachment, but cell morphology varies. The attachment of MOLT-3 cells appears to be mediated through a high affinity receptor for HPRG since increasing concentrations of HPRG produce a saturable dose-response curve, and low concentrations (5nM) of HPRG are effective. The transformation is nearly complete within two hours but almost completely reverses by 72 hours, suggesting that HPRG is involved in a transient lymphocyte adhesion process rather than terminal differentiation of T-cells. Fibrinogen, a plasma protein which binds to $\beta 3$ integrin adhesion receptors, was found to inhibit MOLT-3 cell attachment, and total recovery of this inhibition is not achieved with the addition of either excess HPRG, Con A, or PHA. We propose that HPRG influences lymphocyte activity through selectins, which are naturally expressed leukocyte lectins involved in adhesion. The HPRG-mediated attachment phenomenon may participate in either lymphocyte migration through the endothelium wall or in cell-cell interactions at inflammatory sites. (Supported by USPHS grant HL-37570)

C 308 L-SELECTIN TRANSFECTED CELLS ROLL IN RAT MESENTERIC VENULES IN VIVO,

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Leukocyte rolling in vivo is inhibited by antibodies to the leukocyte adhesion receptor L-selectin (LAM-1) and by soluble recombinant L-selectin. This suggests that functional L-selectin is necessary for rolling, but it is unknown whether it alone is sufficient. An L-selectin negative murine pre-B cell line (300.19) was transfected with cDNA encoding for human L-selectin, which led to stable cell surface expression of L-selectin on the cell surface as seen by flow cytometry. Cells were labeled with carboxyfluorescein diacetate and introduced into the rat mesenteric microcirculation via a catheter inserted into a side branch of the superior mesenteric artery. All applications were performed between 30 and 80 min after surgery, during which time 'spontaneous' leukocyte rolling remained approximately constant in rat mesenteric venules. Rolling was investigated in venules (diameter 42 ± 2 μm, mean$\pm$SEM) in which 30-50% of the rats' own leukocytes rolled at a mean velocity of 49 ± 5 μm/s. On average, 22 ± 6% of L-selectin transfectants rolled at a mean velocity of 451 ± 53 μm/s (7 venules in 5 rats), while freely flowing cells traveled at 2587 ± 199 μm/s. Mock-transfected cells did not roll in the same venules under similar flow conditions (2152 ± 161 mm/s). Rolling of transfected cells was completely abolished by in vitro pretreatment with monoclonal antibodies to human L-selectin (LAM1-3 or LAM1-6, 1:400 dilution of ascites), while a binding control antibody (LAM1-11) had no effect. We conclude that transfection of L-selectin cDNA enables 300.19 cells to roll in venules. The higher velocity of transfected cells compared to the rats' own granulocytes may be due to an imperfect match between human L-selectin and its rat ligand, to the larger size and limited deformability of 300.19 cells, or to additional factors modulating leukocyte rolling.

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C 309 L- and E-SELECTIN IgG-CHIMERAS CAN RECOGNIZE THE SAME NATURALLY OCCURRING LIGAND ON HEV,

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The Selectin family of cell adhesion molecules consists of three members, E-Selectin (ELAM-1), L-Selectin (LECAM-1), and P-Selectin (CD62, GMP140, or PADGEM), which are all involved in binding of leukocytes to endothelial cells. All the selectins have similar overall organisation with an N-terminal calcium-dependent lectin domain followed by an epidermal growth factor-like (EGF) domain, and then a number of short consensus repeat (SCRs) domains similar to those found in members of complement regulatory proteins.

The discovery that all of these molecules contained a calcium dependent lectin suggested that they may bind to carbohydrate-containing ligands. Earlier observations have shown that binding of lymphocytes to high endothelial venules (HEV) of peripheral lymph node is inhibitable by particular carbohydrates, such as mannose-6-phosphate, fucoidan, and PPME. More recently it has been demonstrated that the ligand for E-Selectin and possibly P-Selectin is sialyl Lewis X. There is also evidence *in vitro* that L-Selectin can recognize sialyl Lewis X.

To study cross-reactivity of the selectins in more detail we have used immunoglobulin chimeras of L- and E-Selectin . Both chimeras bound to the natural ligand of L-Selectin on specialized high endothelial venules (HEV) in peripheral lymph nodes as determined by immunohistochemistry and were able to precipitate 50 and 90 kD sulfated ligands from organ cultures of mesenteric lymph nodes. They both could inhibit lymphocyte trafficking to lymph nodes equally well. Since only L-Selectin would be expected to bind to high endothelial venules in this way, we propose that cross-recognition of ligands by selectins occurs. Although all the selectins appear able to recognize a common carbohydrate, sialyl Lewis X, it is likely that specificity is achieved by additional unique elements.

C 310 COVALENT CROSS-LINKING OF L-SELECTIN TO THE HIGH ENDOTHELIAL VENULE LIGAND VIA SELECTIVELY OXIDIZED SIALIC ACIDS, Karin E. Norgard, Huiling Han, Leland Powell, Michael Kriegler*, Ajit Varki and Nissi M. Varki, Glycobiology Program, UCSD Cancer Center and Division of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093 and * Cytel Corp., 3525 John Hopkins Court, San Diego, CA 92121

The selectins are a family of adhesion receptors which play key roles in the trafficking of leukocytes into lymphoid tissues and areas of inflammation. Three members of this family have been described thus far: E-, P- and L-selectin which were initially found on endothelial cells, platelets and leucocytes respectively. Each has an N-terminal C-type lectin domain, which binds to specific carbohydrates in a calcium-dependent manner. As shown by sequence analysis, each lectin domain also contains many lysine residues (ranging from 10 for E-selectin to 14 for the P-selectin). While much information is known about the structure of the selectin molecules, relatively little is known about the detailed structure of the ligands recognized by the selectins. Interference with this initial binding event has great therapeutic potential for abnormal situations where leucocytes can cause tissue damage. Thus, there is a major effort underway to elucidate the structure of these ligands and their mode of interaction. L-selectin recognizes sulfated, sialylated ligands on the high endothelial venules of lymph nodes. This recognition is abolished by strong periodate oxidation, under conditions which completely destroy oligosaccharides. However, mild periodate oxidation, which selectively oxidizes the side chain of sialic acid residues without affecting the underlying oligosaccharide, markedly enhances this interaction. The enhancement is calcium-dependent, indicating that the lectin recognition is still responsible for the interaction. Reduction of the sialic acid aldehydes generated by mild periodate to alcohol groups abolishes this effect. We can demonstrate covalent cross-linking of the oxidized ligand to the L-selectin after cyanoborohydride reduction. This indicates the likelihood of Schiff base formation, presumably between lysine residues of the selectin and the newly formed aldehydes of the sialic acids. Such selectively oxidized sialylated ligands could be used to probe the lectin domains of the selectins, to identify lysine residues at or close to the binding site. Also, this approach could be used to design drugs which effectively interfere with the leukocyte-endothelial interactions that lead to pathological inflammation and post-perfusion injury.

C 311 SELECTIVE EXPRESSION OF SIALYL-LEWIS A AND SIALYL-LEWIS X, PUTATIVE LIGANDS FOR L-SELECTIN, ON PERIFERAL LYMPH NODE HIGH ENDOTHELIAL VENULES

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High endothelial venules (HEV) lined by the high endothelium are the sites where leukocytes enter into the lymph nodes from the blood. Lymphocyte homing into lymph nodes is organ-selective i.e. different molecules are involved in the lymphocyte homing to peripheral nodes compared to mucosa associated lymphoid tissue. The traffic into peripheral nodes is regulated by the expression of L-selectin on leukocytes and its ligand on HEVs. The ligand for L-selectin is suggested to be a 50, 90 or 105 kD glycoprotein, which is sulfated, fucosylated and sialylated. We have inititated a study to identify the expression of sialylated ligands for L-selectin on endothelial surfaces. Here we present data demonstrating that HEVs in peripheral nodes, but not in the mucosa associated lymphoid tissue, express large quantities of sialyl-Lewis^x (sLex) and sialyl-Lewis^a (sLea) identified by mAbs in immunohistology. Endothelium in capillaries or larger vessels in non-lymphoid tissues does not react with anti-sLex or-Lea mAbs. We provide evidence for the first time of the very restricted pattern of sLex and sLea expression on peripheral lymph node HEVs and propose that these carbohydrates (or closely related structures) are components of the endothelial ligands for L-selectin.

C 312 SIALYL LEWIS^X AND NEW CARBOHYDRATE ANALOGS; THE NEXT GENERATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS. Richard H. Smith, Nandi Wang, Pandurang Nikrad, Wasimul Haque, Andre Venot and Robert Ippolito, ALBERTA RESEARCH COUNCIL, *Carbohydrate Research Program,* Department of Biotechnology, Edmonton, Alberta, Canada.

Several different research groups have indicated that Sialyl Lewis^x and other carbohydrates are the ligands for the selectin family of adhesion molecules The selectins are believed to initiate the rolling of leukocytes which leads to activation, adherence and migration through the vascular endothelium. It has been previously shown that monoclonal antibodies to the selectins and soluble recombinant selectins can block this event. It has also been suggested that these monoclonal antibodies and recombinant selectins may be the next generation of non-steroidal anti-inflammatory drugs. We believe that soluble low molecular weight carbohydrates will be the next generation of anti-inflammatory drugs. Evidence will be presented from our research laboratories which demonstrate that the soluble carbohydrates Sialyl Lewis^x, Sialyl Lewis^a, as well as other analogs inhibit experimentally induced inflammation in a mouse model system.

C 313 CHARACTERIZATION OF PORCINE E-SELECTIN, Yvonne T. M. Tsang, Dorian O. Haskard*, and Martyn K. Robinson, Celltech, Ltd., 216 Bath Road, Slough, Berkshire UK SL1 4EN; *Rheumatology Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London UK W12 ONN.

E-selectin (also known as ELAM-1) is a member of the selectin or LEC-CAM family of cell adhesion molecules believed to be important in mediating the initial interactions between leukocytes and the endothelium during an inflammatory process. 1.2B6, a monoclonal antibody against human E-selectin, shows cross-reactivity to a protein on the surface of porcine endothelium that has an apparent molecular weight of 92 kD and is inducible by hrTNFα and hrIL-1α. Activation of porcine aortic or venous endothelium with hrTNFα gives a biphasic response while stimulus with either human or porcine IL-1α results in a protracted expression of the molecule which is unlike that observed on activated human umbilical vein endothelium. Cloning and transient expression of this protein shows that is has homology with human E-selectin and like its human counterpart, plays a role in mediating neutophil adhesion.

C 314 IDENTIFICATION OF A GLYCOPROTEIN LIGAND FOR MOUSE E-SELECTIN, Dietmar Vestweber, Jens Mühlhoff and Agneta Levinovitz, Hans Spemann-Laboratory at the Max Planck Institute for Immunology, 7800 Freiburg, Germany

E-selectin (ELAM-1) is a cytokine inducible endothelial adhesion molecule for PMNs and monocytes. We have searched for glycoprotein ligands of mouse E-selectin on mouse myeloid cells. These studies employed a soluble chimeric fusion protein consisting of the first four domains of mouse E-selectin (lectin-EGF-CB1-CB2 domains) fused to the Fc-part of the heavy chain of human IgG1. Coating of this protein to plastic specifically supports the binding of mouse PMNs and a mouse neutrophilic cell line, in a Ca^{++} dependent manner. Binding of the mouse PMNs and the neutrophilic cell line could be inhibited by the monoclonal antibody 21KC10 which we have produced against mouse E-selectin. A control mAb against the Fc-part of human IgG1 did not block cell binding and no cell binding was observed with a CD4-IgG1 chimeric protein. In immunoprecipitations from detergent extracts of these cells with mouse E-selectin-IgG1 a 160 kD protein was detected after metabolic labeling with ^{35}S-methionine/^{35}S-cysteine. This protein only binds to E-selectin-IgG1 in the presence of Ca^{++}, the binding can be inhibited by the mAb 21KC10, the protein does not bind to CD4-IgG1 and is not identical with L-selectin. The E-selectin ligand has been partially purified, using an affinity matrix with the E-selectin-IgG1 chimeric protein covalently conjugated to protein-A sepharose.

C 315 GAMMA/DELTA T-CELLS RECOGNIZE E-SELECTIN VIA A NOVEL GLYCOPROTEIN. Bruce Walcheck, Gayle Watts, Sandy Kurk, Kathy Jutila, and Mark Jutila. Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717.

Lymphocyte traffic from the blood into lymphoid or nonlymphoid tissues is mediated by adhesion proteins on the leukocyte and counter-receptors on the vascular endothelium. Both gamma/delta T-cells and conventional lymphocytes recirculate; however, gamma/delta T-cells preferentially "home" to extralymphoid epithelial-associated tissues and conventional lymphocytes to secondary lymphoid compartments. Recently, the inducible endothelial cell adhesion protein, E-selectin (ELAM-1), has been proposed as a vascular addressin for a subset of memory T-cells (Shimizu et al., or CLA^+ lymphocytes Picker et al.) in inflamed skin. Furthermore, low level constitutive expression of E-selectin has been demonstrated within the epithelial-associated tissues of the skin and gut. Consistent with E-selectin expression and gamma/delta T-cell localization, we have found that bovine gamma/delta T-cells, which represent up to 70% of the peripheral blood T-cells, avidly adhere to E-selectin transfected L-cells in an anti-E-selectin mAb inhibitable manner (CL-2 and EL-246). Neuraminidase or EDTA treatment of the gamma/delta T-cells abrogates adhesion, but antibodies that recognize two carbohydrate ligands for E-selectin, sLex and HECA-452/CLA, do not react with these cells. A glycoprotein appears to mediate adhesion to E-selectin because protease treatment of the gamma/delta T-cells inhibits binding by over 90%. Previously characterized adhesion-associated proteins, such as L-selectin, CD44, and CD18 on gamma/delta T-cells are not involved, since antibodies against these molecules do not affect binding. In summary, we propose that gamma/delta T-cells recognize E-selectin as a vascular addressin to access epithelial compartments via a novel glycoprotein. This work is funded by grants from the USDA (CRGO-90-01666) and the Pardee Research Foundation.

C 316 CHARACTERIZATION OF E-SELECTIN EXPRESSION IN A RABBIT MODEL OF ACUTE CUTANEOUS INFLAMMATION Barry A. Wolitzky, Margaret L. Harbison*, Daniel K. Burns, Robert J. Ramos, Christine R. Norton, Ann L. Goldstein, and Diana F. Gaizband, Dept. of Inflammation and Autoimmune Diseases, and * Dept. of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ 07110

E-selectin is a cytokine inducible endothelial cell surface protein that participates in the adhesion of neutrophils, monocytes and specific subsets of T-cells. We have generated anti-rabbit E-selectin monoclonal antibodies (MAb) as tools for studying the role of E-selectin in leukocyte migration in rabbit models of inflammation. Anti-rabbit E-selectin MAb 9H9 binds to the lectin-egf domain of E-selectin and inhibits the adhesion of HL60 cells to COS cells transfected with rabbit E-selectin cDNA or stable CHO cell lines expressing rabbit E-selectin on their cell surface. E-selectin is expressed on endothelium of veins and capillaries of rabbit skin in response to intradermal injection of IL-1α or LPS and correlates with neutrophil infiltration and margination. Neutralizing anti-CD18 and anti-rabbit E-selectin MAbs significantly inhibit the influx of neutrophils into these sites. In contrast, intradermal injection of the chemotaxins LTB_4 or fMLF did not induce endothelial cell expression of E-selectin and anti-rabbit E-selectin MAb was ineffective in blocking neutrophil influx induced by these agents. These results suggest that E-selectin may play a fundamental role in regulating neutrophil trafficking in response to IL-1 and LPS in vivo.

C 317 SULFATED POLYSACCHARIDES AS NOVEL LIGANDS FOR L-SELECTIN, Tomoaki Yoshida[1], Yuan Chuan Lee[1] and Laurence A. Lasky[2], [1]Dept. of Biology, The Johns Hopkins Univ., Baltimore MD 21218,; [2]Dept. of Immunology, Genentech Inc., South San Francisco, CA 94080

In peripheral lymphnodes, lymphocytes traffic from blood vessels to lymphatic flow through high endothelial venule (HEV) to which L-selectin, a murine lymphocyte homing receptor, binds specificcally. The chemical nature of endogenous ligand for L-selectin is vigorously pursued by many groups. We present here a method for an quantitative analysis of ligand-binding by L-selectin-Fc chimera (J. Cell Biol. 110:2221) by inhibition. Fucoidan conjugated to tyrosine at the reducing end and radioiodinated was employed as a reference ligand. The reference ligand is allowed to bind with the L-selectin-Fc chimera, and after incubation, the reaction mixture is treated with anti-human IgG1 monoclonal antibody and Staphylococcus bacteria (protein G), and centrifuged through a layer of silicon oil-mineral oil mixture. Various anionic polysaccharides containing sulfates, phosphates or carboxyl acids were examined. Amylose-sulfate, fucoidan and carageenans were most potent, whereas yeast phosphomannan, previously reported to be an effective inhibitor in a non-equilibrium system, was much less effective than the sulfated poly-saccharides. Under the assay conditions, ID_{50} values of amylose-sulfate, unmodified fucoidan, carrageenan-λ, and phosphomannan were 10μM, 13μM, 7μM, and >10mM, respectively. Furthermore, the affinity of sulfated polysaccharides was proved to be dependent both on the degree of sulfation and molecular size, which may indicate that a particular arrangement of clustered sulfate groups is crucial for the affinity. Although these sulfated polysaccharides are structurally different from natural ligands of L-selec-tin, they may provide potential therapeutic applications.

Dynamics of Transendothelial Migration; Hemopoiesis, Immunologic Memory and Specialized Adhesion/Traffic Mechanisms; Novel Therapies Based on Modulation of CAM Activity

C 400 ADHESIVE INTERACTIONS OF CD34+ PRECURSORS TO HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs), Camille N. Abboud, Karen E. Frediani, Abigail L. Harbol, Jane L. Liesveld, Department of Medicine, University of Rochester School of Medicine, Rochester, NY 14642

Hematopoietic stem cells are able to home to the bone marrow sinusoidal sacs when administered intravenously for marrow transplantation. Hemopoietins and inflammatory cytokines are able to promote the recruitment of CD34+ progenitors into the circulation. Precise modeling of this complex regulatory process is difficult, but one can begin by delineating the various adhesive pathways that participate in either stem cell homing or egress. We hereby focus on the binding of normal CD34+ cells to HUVECs (characterized by Factor VIII Antigen and acetylated LDL receptors). HUVECs constitutively express CD44 and CD54. The expression of both ELAM and VCAM-1 can be induced by TNF-α, IL-1α and IL-1ß. CD34+ cells obtained by affinity selection using avidin-biotin immonoadsorption columns (CellPro, Inc), ranged in purity from 80 to 95% by FACS analysis. Baseline adhesion of these normal CD34+ cells to untreated HUVECs was 33±4% vs 16±4% to plastic (n=7). This adhesion process was significantly increased after treatment with TNF-α or IL-1ß. The increased adhesion in the presence of TNF-α could not be decreased by blocking antibodies to ELAM and ICAM-1. Similarly, the CD34+ leukemic cell line KG1a bound to HUVECs (33±5% vs. plastic 8±1%, n=14). KG1a baseline adhesion was not blocked by antibodies to CD18, CD49d or ICAM-1. Adhesion was however increased by the 4B4 antibody to CD29. KG1a binding to HUVECs was also increased in the presence of TNF-α and IL-1ß. This process however was decreased by antibodies to ELAM but not VCAM-1 or ICAM-1 (28%, n=3). These results underscore the adhesive interactions normal and leukemic CD34+ cells to endothelial cells and detail the upregulation of CD34 cell adhesion by inflammatory cytokines such as IL-1ß and TNF-α. This increased adhesion to endothelial cells may affect stem cell homing after marrow transplantation. Finally, since CD34+ leukemias have a poor prognosis one may postulate that these leukemic blasts migrate to sanctuary sites more efficiently than CD34 negative leukemic blasts.

C 401 FETAL HEMATOPOIETIC STEM CELLS EMPLOY DISTINCT ADHESION STRATEGIES TO INTERACT WITH MARROW 'STROMAL' AND UMBILICAL VEIN ENDOTHELIAL CELLS, **Pervin Anklesaria and Tünde Zsido**, Department of Radiation Oncology, University of Massachusetts Medical Center, Worcester, MA 01655.

During development, hematopoietic stem cells migrate from yolk sac to fetal liver and spleen before finally colonizing within the developing marrow. The mechanism for this migration is unknown. We have analyzed receptors utilized by fetal cord blood CD34+ cells to adhere to human umbilical vein endothelial cells (HUVECs) and to adult marrow 'stromal' cells. Circulating cord blood CD34+ stem cells express L-selectin and β1 integrins. Both anti-L-selectin and anti-VLA-4 antibodies inhibited adhesion of cord blood CD34+ cells to cytokine-activated marrow 'stroma' by 37% and 38% respectively, but failed to inhibit adhesion of these fetal cells to activated HUVECs. Under similar conditions, adhesion of peripheral blood lymphocytes to activated HUVECs was inhibited by anti-L-selectin and anti-VLA-4 antibodies. Further, a cord blood derived hematopoietic cell line KMT2 expresses L-selectin and binds to cytokine-activated HUVECs and marrow stroma. Adhesion of KMT2 cell line to adult marrow 'stromal' cells was inhibited 46% by anti-L-selectin antibodies and 40% by anti-VLA-4 antibodies, while, that to HUVECs was marginally inhibited (7%) by antibodies to L-selectin. These studies demonstrate that fetal hematopoietic CD34+ cells utilize distinct adhesion pathways to adhere to adult marrow 'stromal' cells and venous endothelial.

C 402 FUNCTIONAL ANALYSIS OF THE EARLY HAEMATOPOIETIC SURFACE MARKERS CD33 AND CD34, Elizabeth K. Barber and D.L. Simmons, Cell Adhesion Laboratory, Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom

CD34 is the best currently available marker of human multipotential stem cells and CD33 is the earliest marker of commitment to the myeloid lineage which is down-regulated as myeloid cells mature. CD34 is a mucosialin and CD33 a member of the immunoglobulin superfamily. To determine the functions of CD34 and CD33 in early haematopoiesis, soluble forms of CD34 and CD33 have been synthesised by PCR based construction of extracellular domain-IgGFc fusion plasmids. The chimaeric proteins (ECD-Fc fusions) have been used to define the functions of CD33 and CD34 in a range of assay systems including 1) screening cell lines by immunofluorescence analysis 2) screening human tissue sections using biotinylated ECD-Fc protein and 3) direct screening of cDNA libraries transiently expressed in COS cells, panning using anti-human Fc dishes and rescue of selected plasmids. This allows for direct molecular cloning of the ligand(s) for CD34 and CD33 if they are cell surface molecules. Candidate clones for both CD34 and CD33 have been isolated from a direct expression screen of a number of different cDNA libraries (placenta, bone marrow, K562, HUVEC) and further molecular characterization of these clones is in progress.

C 403 ADHESION MOLECULE EXPRESSION BY EPIDERMAL DENDRITIC LANGERHANS CELLS Jonathan N.W.N. Barker, Elizabeth L. Ross, Michael H Allen, Richard W. Groves, Donald M. MacDonald, Laboratory of Applied Dermatopathology, UMDS, Guy's Hospital, London, England.

Langerhans' cells (LC) are the primary antigen presenting cells of human epidermis. Following epicutaneous exposure to antigen LC migrate through dermal lymphatics to regional nodes where processed antigen is presented to T lymphocytes. Since cellular adhesion molecules play a critical role both in leukocyte trafficking and in antigen presentation, expression of these molecules by LC has been examined in human skin. By quantitative immunohistochemistry in normal skin LC expressed the E-selectin ligand, sialyl Lewis X (SLX) but not LFA-1. In inflamed skin characterised by a T cell infiltrate (allergic contact dermatitis (n = 4), psoriasis (n = 6), atopic dermatitis (n = 4), LC expressed SLX and LFA-1. Concomitantly, E-selectin and ICAM-1 were induced on dermal vascular endothelium in inflamed skin. Expression of these ligands by LC was confirmed by double immunofluorescence labelling of isolated, purified epidermal LC. In contrast other adhesion ligands including non sialylated Lewis X (CD15), sialyl Lewis A and VCAM-1 were not expressed under any experimental conditions. These studies suggest that selectin (SLX/ELAM-1) mediated mechanisms are involved in LC migration. LFA-1 is also likely to act as an accessory signal in LC presentation of antigen to T cells, although in contrast to other APC VLA-4/VCAM-1 pathways appeared not to be involved in LC function.

C 404 CD31 AND LYMPHOCYTE TRANSMIGRATION ACROSS ENDOTHELIAL MONOLAYERS, Ian N. Bird, Julia H.Spragg and N. Matthews, Yamanouchi Research Institute, Littlemore Hospital, Oxford OX4 4XN, U.K.

CD31 is a 130kDa transmembrane glycoprotein found on the surface of endothelial cells, PMNL, monocytes and about 50% of peripheral blood lymphocytes. CD31 localises to cell-cell contact zones of endothelial cells when they form a monolayer. In view of its cellular distribution and its ability to bind homotypically, we have investigated the role of CD31 in lymphocyte transmigration across endothelia. We found that $CD31^-$ lymphocytes were able to cross Human Umbilical Vein Endothelial Cell (HUVEC) monolayers *in vitro* and $CD31^+$ lymphocytes were, in fact, depleted in transmigrated lymphocyte populations. If anything, CD31 expression was inversely correlated with ability to transmigrate. CD31 expression was, however, correlated with CD45RA expression on lymphocytes, but no comparable association was seen with CD45RO expression. Lymphocytes that had crossed HUVEC monolayers *in vitro* were enriched in $CD31^-CD45RO^+$ cells and depleted of $CD31^+CD45RA^+$ cells. When HUVEC monolayers were treated with IL1-β the difference in the transmigration of these two subpopulations of lymphocytes was exaggerated. These results suggest that co-expression of CD31 with CD45 variants may account for the negative correlation between CD31 expression on lymphocytes and transmigration that we have seen. We are investigating the transmigration of these lymphocyte subpopulations further as CD45RA and CD45RO are recognised as markers for lymphocytes with different recirculation pathways.

C 405 MICROCHEMOTAXIS CHAMBERASSAY TO STUDY EOSINOPHIL TRANSMIGRATION ACROSS A MONOLAYER OF HUMAN ENDOTHELIAL CELLS, M. Blom, T.W. Kuijpers, E.F. Mul, A.T.J. Tool, J.H.W. Leusen and D. Roos, Centr. Lab. of the Neth. Red Cross Blood Transf. Service and Lab. of Exp. and Clin. Immunol, Amsterdam, the Netherlands.

Of the integrin family of adhesion molecules, the ß2 subfamily of CD11/CD18 molecules as well as the Very Late Antigen (VLA-4) (CD49d/CD29) member of the ß1 subfamily have been reported to contribute to the eosinophil adherence to endothelium ligands, such as ICAM-1 and VCAM-1. To study the influence of these molecules on eosinophil diapadesis and chemotaxis, we developed a novel migration assay. The 'classical' chemotaxis assay employing a 48-well microchamber with two filters placed between the upper and lower compartment (a lower 'stop' filter -0.45 μm pore size- and an upper 'migration' filter -8.0 μm pore size-) was expanded with a third filter. This third filter was covered with a monolayer of human umbilical vein endothelial cells (EC) subcultured for 6 days until confluency was reached. The filter carrying the EC monolayer was placed on top of the 'migration' filter. Eosinophils isolated from normal blood donors were added to the upper compartment. After 2.5 h at 37°C, the migration filters were removed, fixed and stained with Weigert's solution. The number of cells that had completely passed the 150 μM-thick migration filter was determined with light microscopy in 10 high power fields (hpf) per filter. We tested the migratory capacity of eosinophils in this microchemotaxis chamberassay with the chemoattractants recombinant complement fragment C5a (rC5a), or the lipid mediator platelet-activating factor (PAF). When the filters were covered by a monolayer of endothelial cells, diapedesis was inhibited by CD18 mAb CLB LFA1/1 for 60%, and by the CD49d mAb HP1/2 for 30%. Eosinophil diapedesis was completely inhibited by a combination of CD18 and CD49d mAbs. In contrast, eosinophil chemotaxis through untreated, bare filters was only inhibited by CD18 mAb. Because the endothelial cells had been subcultured on fibronectine (FN)-precoated filters, we preincubated the filters with FN before eosinophil chemotaxis was determined. Under these conditions CD49d mAb reduced migration by about 60%, i.e. to a similar extent as did CD18 mAb. The difference in inhibition by CD49d mAb of eosinophil migration through bare untreated filters (less 10%), filters with cultured endothelium (about 30%), and FN-pretreated filters (60%) can be explained by the involvement of alternative integrin receptors and matrix proteins other than FN.

C 406 METASTATIC POTENTIAL VIA CELL ADHESION USING A HUMAN MICROVASCULAR ENDOTHELIAL CELL LINE; HMEC-1, Diane Bosse, Keri McFarlane, Velma George, Luna Chen, Edwin W. Ades, Biological Products Branch, Centers for Disease Control, Atlanta, GA 30333

One of the properties of malignant tumors is that they will metastasize, releasing malignant cells into the blood stream which then adhere to normal cells setting up new foci of tumor. Endothelial cells, as the major barrier between the blood and extravascular tissue, are a prime target for this adherence. However, if it were possible to change the adhesion properties of endothelial cells, one could perhaps reduce the likelihood of metastatic cells from adhering. As the first step toward this end, we utilized two cell lines--CDC/EU.HMEC-1 (HMEC-1), a continuous human microvascular endothelial cells line; and HLF, a human lung fibroblast cell line and measured the effects of five cytokines (IL-1, IL-2, IL-4, IL-6, and TNF-α) on the adhesion properties of HLF to HMEC-1. While the five cytokines had no effect on the growth properties of HMEC-1, it was found that pre-incubation of HMEC-1 cells with IL-1, IL-2, IL-6 or TNF-α increased the rate of HLF adherence to HMEC-1. The pre-incubation of IL-2 with HLF also increased adherence while pre-incubation with IL-6 decreased it. HMEC-1 was also examined for the effect of the cytokines on expression of a battery of adhesion molecules. FACScan analysis showed that expression of Alpha-V was decreased by IL-4 or IL-6, but increased by TNF-α; TNF-α also caused an increase in VCAM. No change in the expression of the adhesion molecules in the presence of the cytokines on HLF cells was observed.

C 407 $\alpha^E\beta_7$ MEDIATES ADHESION OF INTRAEPITHELIAL LYMPHOCYTES TO EPITHELIAL CELLS, Karyn L Cepek, Christina M. Parker, Sunil K. Shaw, and Michael B. Brenner, Department of Rheumatology and Immunology, Lymphocyte Biology Section, Brigham and Women's Hospital, Boston, MA 02115

Intestinal intraepithelial lymphocytes (iIEL) are a distinct subpopulation of T lymphocytes diffusely distributed in the epithelium. Because >95% of these iIEL express an integrin which is not expressed on circulating T lymphocytes, we reasoned that this complex might mediate the localization of iIEL to the epithelium. This epithelial lymphocyte associated integrin is composed of β_7 in association with a biochemically distinct subunit, presumed to be an α subunit, which we designated α^E. Recently we determined the amino-terminal amino acid sequence of this α^E subunit and confirmed that it is a novel integrin α subunit. Utilizing degenerate oligonucleotide screening, cDNA clones which are candidates to encode the α^E subunit have been identified.

In functional studies, we found that cultured iIEL bound to mucosal epithelial cell lines. This binding was blocked by mAb specific for two integrins, $\alpha^E\beta_7$ and $\alpha^L\beta_2$. After TGF-β1 treatment, cultured iIEL cell lines expressed $\alpha^E\beta_7$ at levels similar to those found on iIEL *in vivo*. Under these conditions this integrin appeared to predominate in mediating the adhesion of iIEL to epithelial cells. While mAb against $\alpha^L\beta_2$ blocked the adhesion of iIEL to both endothelial and epithelial cells, anti-$\alpha^E\beta_7$ mAb blocked binding only to epithelial cells suggesting that the ligand for $\alpha^E\beta_7$ is not expressed on IL-1β treated cultured endothelial cells. iIEL did not readily bind to the apical surface of confluent polarized epithelial cell monolayers. However, iIEL did bind to these cells via $\alpha^E\beta_7$ when tight junctions were disrupted, a treatment which allows redistribution of proteins compartmentalized to the basolateral surface. Thus, it is likely that expression of the $\alpha^E\beta_7$ ligand is restricted to the basolateral membrane of polarized epithelial cells. Because $\alpha^E\beta_7$ appears to be a predominant integrin on freshly isolated iIEL, we hypothesized that $\alpha^E\beta_7$ mediated adhesion may be particularly important in cell-to-cell interactions between T cells and epithelial cells *in vivo*.

C 408 MOTILITY OF LYMPHOCYTES MEDIATED BY VLA-4/ VCAM-1 INTERACTION. Po-Ying Chan and Alejandro Aruffo. Department of Cellular Interactions, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

The exit of lymphocytes from blood circulation is a prerequisite to their migration to peripheral non-lymphoid tissues or lymph organs where immune responses are elicited. Lymphocyte migration across cultured cytokine-activated human umbilical cord vein endothelial cell monolayer *in vitro* which express the adhesion molecules ICAM-1 and VCAM-1 has recently been described. Lymphocytes bind ICAM-1 via the ß2 integrin LFA-1 and bind VCAM-1 via the ß1 integrin VLA-4. Antibody blocking studies have suggested a significant role for the LFA-1/ICAM-1 adhesion pathway in the transmigration process. The LFA-1-mediated lymphocyte locomotion on purified ICAM-1 incorporated in planar membrane has also been reported. We have now extended the attribution of lymphocyte motility to the VLA-4/VCAM-1 adhesion pathway using purified recombinant VCAM-1 in an *in vitro* migration model. The seven extracellular immunoglobulin (Ig)-like domains of VCAM-1 were genetically recombined with the Fc fragment of Ig and the VCAM-1/Fc fusion protein was purified from COS cell transfectant supernatant. An EBV-transformed B cell line SLA lacking LFA-1 expression was found to migrate through 8.0 µm pores in the nucleopore membranes in a two-chamber assembly (Transwell, Costar), when both sides of the membrane were coated with VCAM-1/Fc but not when they were uncoated or coated with an irrelevant CD7/Fc protein. The migration was specifically blocked by pretreating SLA cells with anti-VLA-4 or pretreating the membranes with anti-VCAM-1. SLA cells were also shown to migrate through membranes coated with fibronectin, an alternate extracellular matrix ligand for VLA-4. However, the migration on fibronectin was up to 12-fold less than that on VCAM-1/Fc. The lower efficicency of SLA migration on fibronectin was consistent with the lower adhesion efficiency of SLA cells to fibronectin compared to VCAM-1/Fc when both were immobilized on plastic at similar site densities. Similar observations were made when phytohaemagglutinin or liposaccharides-induced peripheral blood T or B lymphoblasts were used in the above assays. These results suggest that lymphocytes are able to use the VLA-4/VCAM-1 adhesion pathway efficiently for cell locomotion.

C 409 GENERATION OF NK ACTIVITY FROM INACTIVE PRECURSORS IN MOUSE NK-LONG TERM BONE MARROW CULTURES (NK-LTBMC) DEPENDS ON PGP-1 (CD44)/ HYALURONIC ACID INTERACTION, Domenico V. Delfino, Kenneth D. Patrene, Albert B. DeLeo, Lina Lu, Ronald B. Herberman, and Sallie S. Boggs, Departments of Radiation Oncology, Pathology and Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA and Pittsburgh Cancer Institute, Pittsburgh, PA, 15261
Bone marrow, cultured for 4-5 weeks (NK-LTBMC), is enriched for precursor cells capable of responding to rhIL-2 by proliferation and development of cells highly enriched for NK-1.1$^+$, large granular lymphocytes, and cells that kill YAC-1 cells in 4 hours. This IL-2 response is dependent on the presence of stromal cells in the culture. To investigate the role of the adhesion molecule Pgp-1 and its ligand (hyaluronic acid) in this stromal-precursor interaction, hyaluronidase (5000 U/ml) and rhIL-2 (5000 U/ml) were added to 4-5 week cultures. Addition of the hyaluronidase resulted in reduced proliferation and lack of development of cytotoxic activity measured after 7-9 days. This was not due to cellular toxicity of the hyaluronidase, since a similar result was observed when free hyaluronic acid (1 mg/ml or 0.1 mg/ml) was added to compete for the binding of Pgp-1 to cellular hyaluronic acid. With a very small (0.01 mg/ml) dose of hyaluronic acid, no inhibition was seen. The addition of antibody anti-Pgp-1 to 4-5 week cultures when rhIL-2 was added, inhibited both the proliferation and development of NK activity, confirming the specificity of the above results. About 75% of the cells in the 4-5 week NK-LTBMC were positive for Pgp-1 antigen.

These data suggest that Pgp-1 might be a good marker for IL-2-responsive precursors and that binding to hyaluronic acid is necessary for the stromal dependent response to IL-2.

C 410 INVOLVEMENT OF ICAM-1 IN LYMPHOMA INVASION AND METASTASIS, **Mariëtte H.E. Driessens, Felix Rodriguez Erena and Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands**
In a mouse T cell hybridoma model for lymphoma invasion and metastasis we obtained evidence for an important role for LFA-1. Anti-LFA-1 antibodies inhibited invasion of tumor cells into monolayers of fibroblasts and hepatocytes, and invasive and metastatic potential of LFA-1 deficient mutants was much reduced. The preference of T cell hybridomas and lymphomas to form metastases in liver and kidney might be related to the presence of LFA-1 counterstructures. Counterstructure ICAM-1 was recently shown to be constitutively expressed along microvessels in the liver, on hepatocytes and in kidney glomeruli. We observed that ICAM-1 is expressed by isolated rat hepatocytes and that invasion of T cell hybridoma cells into hepatocyte cultures could be inhibited by anti-ICAM-1 antibodies, almost to the same extent as by anti-LFA-1 antibody. However, invasion of the cells into fibroblast monolayers, a model for more widespread metastasis, could not be inhibited by anti-ICAM-1 antibodies. This suggests that the preference of lymphomas for metastasis to organs like liver and kidney is determined by this constitutive ICAM-1 expression. To investigate this further, the effect of blocking ICAM-1 and LFA-1 antibodies on metastasis formation in vivo is being tested and the results will be presented.
VLA-4 is not expressed on T cell hybrids but is present on the metastatic lymphomas ESb and MDAY-D2, that express relatively low levels of LFA-1. The role of VLA-4 in invasion and metastasis is currently being investigated.

C 411 THE ROLE OF T-CELL/MATRIX PROTEIN AND T-CELL/ENDOTHELIAL INTERACTIONS IN ANTIGEN SPECIFIC IMMUNTIY IN VIVO. Thomas A. Ferguson and Thomas S. Kupper. Washington University School of Medicine, St. Louis, MO. 63110 and Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115
The interaction of T-cells with matrix proteins and endothelium are important events in T-cell migration and activation. While most studies have dealt with these mechanisms in vitro, little is known about the function these interactions in vivo. We have been exploring the mechanisms by which antigen specific T-cells migrate from the vasculature to sites of antigen during cutaneous immune responses. Recently we demonstrated that peptides derived from integrin-binding sequences in fibronectin (FN) abrogate the T-cell-mediated contact hypersensitivity (CHS) reactions in vivo suggesting that T-cell interaction with fibronectin (FN) is an important step in T-cell migration. In order to further explore the importance of matrix proteins in T-cell migration we have studied the interaction between antigen specific T-cells and matrix proteins. Incubation of lymphoid cells from TNCB (Trinitro- chlorobenzene) immune mice with FN or type I collagen coated plates prior to adoptive transfer removes the ability of the cells to transfer contact hypersensitivity (CHS). The cells which transfer this immunity can be recovered from the plates. These cells are not detectable in conventional in vitro adhesion assays and represent less than 1% of the total cells. We have also examined the role of T-cell/endothelium interactions in immunity. Preincubation of lymphoid cells from sensitized mice with activated endothelium removes the antigen specific cells from the population. The immune T-cells are contained in the recoverable adherent population. Antibody blocking studies show that both ICAM-1 and VCAM-1 are important in this interaction. To further support the role of integrins in vivo, antibody blocking studies were performed. Antibody to VLA-4, LFA-1 and ICAM-1 abrogate the effector phase of CHS when administered in vivo. Our data demonstrate that a very small number of antigen specific T-cells which are not detectable in conventional binding assays but bind to fibronectin, collagen, and activated endothelium mediate immunity in vivo. These interactions are likely mediated by several T-cell integrins. This work is supported by NIH grants EY06765 and AI25082.

C 412 GENETIC MARKERS AND PERSISTENT MEMBRANE STAINS: TWO SYSTEMS FOR THE STUDY OF ADHESION OF HAEMOPOIETIC STEM CELLS AND LEUKEMIC CELLS.

P. Jan Hendrikx, Anton C. M. Martens, Anton Hagenbeek and Jan W. M. Visser. TNO Institute of Applied Radiobiology and Immunology, Rijswijk, The Netherlands.

In order to be able to study the homing and lodging behaviour of leukemic cells we developed a subline of the BNML rat leukemia model which expresses high levels of ß-galactosidase and set up a suspension assay for these cells with a sensitivity of 1 leukemic cell per 10^8 normal cells. Using this assay, we have characterized in a quantitative way the homing of leukemic cells and their redistribution after therapy. In situ staining of these cells enables us to specifically detect and characterize homing sites for leukemia in the bone marrow. In the murine system we showed that staining normal bone marrow with the fluorescent membrane stain PKH-26 does not influence formation of CFU-S day 8 or CFU-S day 12. We are currently using flow cytometry to study the homing behaviour of PKH-26 labeled purified haematopoietic stem cells quantitatively, and in situ detection of these cells in frozen sections will allow for a comparison between stem cell and leukemic cell homing. Both these systems will also be used to study the effect of known and as yet unknown adhesion factors and their antibodies on the growth characteristics of stem cells and leukemic cells.

C 413 THREE DIMENSIONAL MIGRATION OF FETAL THYMOCYTES INTO CLUSTERS OF THYMIC EPITHELIAL CELLS IN VITRO - A MODEL FOR INTRATHYMIC MIGRATION DURING T LYMPHOPOIESIS-,

Akira Imaizumi, Irving Goldschneider and Takeshi Yoshida, Tokyo Institute for Immunopharmacology, Inc., Tokyo 171, Japan and Department of Pathology, University of Connecticut Health Center, Farmington, CT 06030-3105

Several sequential stages of lymphoid cell-stromal cell interactions may be required at defined developmental steps of intrathymic T cell differentiation. As pro-T cells differentiate along these steps, they may sequentially "walk" from one microenvironmental site in the outer cortex to other microenvironmental site in deeper cortex. Despite the relatively simple architecture of the thymus, a precise structure-function definition of the thymic microenvironment has not been possible due to the lack of appropriate thymic stromal cell lines and systems.

In this study, we have established a panel of mouse thymic epithelial cell lines from fetal thymus. These epithelial cell lines could be separated into two populations: (1) highly NCAM-positive (NCAM^{high}), which also express relatively high intensity of I-A antigen; and(2) low NCAM-positive (NCAM^{low}). Although most day 14 fetal thymocytes could bind around the clusters of all thymic epithelial cell lines tested, large number of the fetal thymocytes could infiltrate into MTSC-0420-1.4, MTSC-0420-1.5 and MTSC-0613-1.2 clusters only (NCAM^{high} TEC population). In contrast, very few day 14 fetal thymocytes could infiltrated into MTSC-0531-5.1 and MTSC-0531-5.2 (NCAM^{low} TEC population). On membrane culture, some of the infiltrated fetal thymocytes showed clonal growth inside NCAM^{high} TEC clusters. The neural cell adhesion molecule (NCAM), which is thought to play a central role in histogenesis has been reported on murine thymocytes in thymic cortex, rare in the medulla and absent from the sub-capsular area. NCAM-mediated adhesion is believed to be homophilic, i. e. NCAM on one cell binds to NCAM on another cell. NCAM has been reported to play a role in regulation of cell growth by cell-cell contact in nervous tissues. These observations suggest that NCAM^{high} TEC populations might be located in the thymic cortex and correlate to the intrathymic migration and contact-dependent growth regulation of pro-T cells in developing thymus. Hence, these NCAM-positive epithelial cell lines may play an important role in intrathymic migration and the clonal growth of pro-T cells in fetal thymus. We anticipate that the three-dimensional migration assay using these thymic epithelial cell clusters will be a useful with which analyze functional molecules that regulate the "intrathymic-walking" of developing pro-T cells. (Partially supported by NIH grant GM-38306 and ACS grant IM-645)

C 414 ENDOTOXIN ACTIVATION OF ENDOTHELIUM FOR POLYMORPHONUCLEAR LEUKOCYTE (PMNL) TRANSENDOTHELIAL MIGRATION AND MODULATION BY INTERFERON γ,

Andrew C. Issekutz, Nancy Lopes, Department of Pediatrics and Microbiology - Immunology, Dalhousie University, Halifax, Canada B3J 3G9.

Endotoxin (LPS) is a potent inflammatory stimulus and can activate human umbilical vein endothelium (HUVE) for leukocyte adhesiveness and transendothelial migration. Here we investigated the role of endogenously HUVE produced cytokines in this process. LPS activated HUVE monolayers grown on filters supported ^{51}Cr labelled PMNL trans-endothelial migration in a dose and time dependent manner. Preincubation of the HUVE for 3 hours with LPS (1ng/ml) resulted in transmigration of 26±3% of added PMNL in 75 min. while the unstimulated value was 1±0.2%. Neutralizing antibodies to IL-1α&β, TNFα, IL-8 or recombinant IL-1 receptor antagonist had no effect on the activation, by LPS of the HUVE for migration of PMNL. The HUVE "activated state" declined with prolonged (22 hrs.) exposure to LPS as reflected by a decrease in PMNL transendothelial migration to 5.5±1% and in E-selectin expression as compared to 3 hrs. stimulation. However, simultaneous exposure to IFNγ (200U/ml) and LPS, but not to IFNα/β+LPS maintained maximal PMNL transendothelial migration (28±4%) for at least 24 hrs., prolonged E-selectin expression and superinduced ICAM-1 expression. IFNγ also enhanced HUVE responses to a suboptimal concentration of LPS (0.1ng/ml), without inducing PMNL migration itself. The PMNL transendothelial migration was blocked by >90% by mAb to CD18 with either 3 hrs. LPS or 22 hrs. LPS + IFNγ stimulation. Migration was also partially and comparably inhibited by mAb to E-selectin (25-35%) or to ICAM-1 (35-45%) and by a combination of both reagents(50-60%) under both stimulation conditions. Thus, LPS activation of HUVE for PMNL transendothelial migration: a) does not require secretion of IL-1, TNFα or IL-8 by the endothelium, b) IFNγ enhances and prolongs endothelial activation by LPS and may increase leukocyte infiltration in LPS or bacterial inflammatory reactions, and c) CD18 dependent mechanisms are equally important under both acute (3 hr.) and prolonged (22 hr. LPS+IFNγ) activation conditions. (Supported by MRC Canada.)

C 415 THE STRUCTURE OF THE GENE ENCODING THE ALTERNATIVELY SPLICED CD44 GLYCOPROTEIN,

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The CD44 cell surface glycoprotein has been implicated in a number of important cell adhesion events including the adhesion of lymphocytes to lymph node HEV and components of the extracellular matrix such as hyaluronic acid and fibronectin. The expression of CD44 is not restricted to lymphoid cells and many cell types including fibroblasts and epithelial cells express variant CD44 molecules ranging in size from 85 to 200kD . We and others have shown recently that these variant CD44 molecules are generated by a mechanism of alternative splicing in which different exons are inserted at a site within the membrane proximal extracellular domain. We have determined the intron-exon organization of the CD44 gene isolated from a human YAC library by means of PCR and restriction mapping. We show that the gene contains nine alternative exons within a 20kb region coding for different membrane proximal extracellular domains and that these exons are spliced either singly or in different combinations according to cell type.

C 416 REGULATION OF CD11b (MAC-1) EXPRESSION IN HUMAN NEUTROPHILS BY PHOSPHOLIPASE A_2, Peer B. Jacobson and Denis J. Schrier, Department of Immunopathology, Warner-Lambert / Parke-Davis, Ann Arbor, MI 48105.
In response to a wide variety of physiological stimuli, neutrophil recruitment and diapedesis to inflammatory sites is to a large extent dependent upon the upregulation of the integrin CD11b (MAC-1), a process believed to result from the mobilization of intracellular granules to the cell surface. Signal transduction mechanisms involved in stimulus-secretion coupling have included activation of phospholipase A_2 as a primary effector of arachidonic acid release, platelet activating factor generation, calcium mobilization and other less well defined lipid remodeling events. In view of the potential role of PLA_2 in secretion and cellular adhesion, our laboratory has examined the effects of the selective PLA_2 inhibitors manoalide (MLD) and scalaradial (SLD) on neutrophil function, and demonstrate that these compounds not only have profound effects on ^{3}H-arachidonic acid release and LTB_4 production, but also on degranulation events such as myeloperoxidase and lactoferrin release. Additional studies demonstrate that MLD, SLD, and other less specific PLA_2 inhibitors such as 4-bromophenacylbromide (BPB) and nordihydroguiaretic acid (NDGA), inhibit the expression of MAC-1 (IC_{50}s: MLD, 500 nM; SLD, 150 nM; BPB, 5 µM; NDGA, 5 µM) at concentrations similar to those which inhibit ^{3}H-arachidonic acid release, LTB_4 production, and degranulation. Similar effects were not seen with cyclooxygenase, 5-lipoxygenase, protein kinase C inhibitors, or calcium channel antagonists. In addition, none of the PLA_2 inhibitors had any effect on the shedding of the extracellular domain of neutrophil LCAM in response to 10-minute incubations with 10 nM fMLP, 100 nM A23187, 100 nM PMA, 100 ng / ml IL-8, 10 units / ml TNFß, or 100 nM PAF. Functional assays further demonstrate that the selective inhibition of PLA_2 by MLD and SLD prevents MAC-1-dependent adhesion of fMLP-stimulated neutrophils to keyhole limpet hemocyanin-coated substrates at the same concentrations which block MAC-1 expression. Attempts to circumvent the inhibition of MAC-1 upregulation by the addition of exogenous arachidonic acid (from 1-100 µM) were unsuccessful, but did demonstrate that arachidonic acid had stimulatory effects of its own which were reversible by PLA_2 inhibition. These studies therefore emphasize the importance of phospholipase A_2 as a key regulator of cellular adhesion.

C 417 ABERRANT LYMPHOCYTE BINDING TO VASCULAR ENDOTHELIUM IN INFLAMMATORY BOWEL DISEASES, Sirpa Jalkanen, Kaisa Granfors and Marko Salmi, National Public Health Institute, and BioCity, SF-20520 Turku, Finland
Lymphocyte recirculation between the blood and lymphoid organs as well as lymphocyte migration into sites of inflammation is controlled by lymphocyte interaction with vascular endothelium. Normally, small virgin lymphocytes home to all lymphoid tissues, while immunoblasts preferentially migrate back into tissues where they have become activated. In the present work, binding of lamina propria lymphocytes (LPL) from normal and inflamed (colitis ulcerosa and Crohn's disease) bowel to high endothelial venules (HEV) was studied. Also, expression of adhesion molecules on LPL (lymphocyte-function associated antigen-1, LFA-1; very late activation antigen-4, VLA-4; CD44; and L-selectin) and on endothelial cells (E-selectin; intercellular adhesion molecule-1, ICAM-1; vascular cell adhesion molecule-1, VCAM-1; and MECA-79-defined peripheral lymph node-specific addressin) was analyzed. The most important new findings were as follows. i) The differentiation stage of LPL critically determined the efficiency and selectivity of HEV-binding. Small lymphocytes from the normal gut bound to all HEV-specificities tested, while immunoblasts bound efficiently to mucosal and synovial HEV, but not at all to peripheral lymph node HEV. ii) In inflammatory bowel diseases, the selectivity of lymphocyte-endothelial interaction was lost, i.e. immunoblasts bound well also to lymph node HEV. iii) The set of homing receptors expressed on mucosal immunoblasts did not directly predict the HEV-binding properties of these cells. For example, blasts from the inflamed bowel were L-selectin negative but still adhered well to lymph node HEV. iv) Unexpectedly, MECA-79, but not VCAM-1, was induced on the endothelial cells in the inflamed mucosa. These findings may be important in understanding the physiology of lymphocyte homing in man and the pathogenesis of intestinal and extraintestinal manifestations of inflammatory bowel diseases.

C 418 INTERLEUKIN-2 ENHANCES T LYMPHOCYTE MIGRATION TO THE TUMOR SITE
Richard Janssen, Bart Jan Kroesen, Wijnand Helfrich, Henk Kal and Lou de Leij, Department of Clinical Immunology, University Hospital, 9713 EZ Groningen, and *TNO Institute of Applied Radiobiology, Rijswijk, The Netherlands.*
Human renal cell carcinoma (RCC) is a tumor sensitive to recombinant interleukin-2 (rIL-2) treatment. Since it is becoming more and more clear that T cells are involved in rIL-2 induced anti tumor activity, a prerequisite for successful rIL-2 therapy is the induction of T cell migration to the site of the tumor.
To study the influence of rIL-2 on homing in an experimental system, we set up a rat tumor model.
In this model the rat lung adeno carcinoma L37 was injected sc in WAG/RIJ rats. The L37 tumor is weakly immunogenic and tumor cells can be identified by the monoclonal antibody MOC-153.
IL-2 treatment of tumor bearing rats consisted of human rIL-2 (EuroCetus, $1x10^6$ IU) administered in a 5-day cycle subcutaneously every week for 4 consecutive weeks. rIl-2 treatment decreased tumor growth rate. Staining of tumor sections with mAb R73 (anti-TCR) showed T cell infiltration, both in treated and untreated rats. However, the number of T cell infiltrates in rIL-2 treated rats was >4 times higher than in untreated rats. IL-2 treated rats showed an increased expression of the tumor associated antigen, recognized by the mAb MOC-153, compared to untreated rats.
Data on the expression of adhesion molecules like VLA-4, VCAM-1, ICAM-1, CD18, and LFA1-α in this tumor model will be presented.

C 419 EXPRESSION, REGULATION, & MEDIATION OF TIL-TUMOR INTERACTIONS BY CD44 & CD54 IN RENAL CELL CARCINOMA. Eric Klein, Kazu Tenabe, Jeanine Alexander, Ray Tubbs, & James Finke, Department of Urology, Cleveland Clinic, Cleveland, OH, 44195
Cell surface adhesion molecules are essential for antigen specific binding between T cells & tumor targets. We have investigated the expression & regulation of the adhesion molecules CD44 & CD54 (ICAM-1) in renal cell carcinoma (RCC) & examined their role in mediating autologous tumor - tumor infiltrating lymphocyte (TIL) interactions. Immunohistochemical (IH) staining of 4 RCC cell lines with antiCD54 Mab showed high intensity cell membrane staining in all. Two-color FACScan immunocytometry (IC) showed greater than 90% positivity in 3 lines derived from primary tumors but only 30% positivity in one derived from a metastasis. CD44 expression was detected by IH in all lines but was more variably expressed (40 - 50% positivity) by IC. Similar results were seen by IC in 5 primary RCC. IH of 10 primary RCC & normal kidneys demonstrated that normal proximal tubular epithelium expressed CD44 or CD54 rarely while all 10 tumors expressed both with high intensity. TNF upregulated expression of both CD54 & CD44, IFNa & IFNg upregulated expression of CD54 only, & IL1 & GM-CSF had no effect. Northern analysis demonstrated increased CD54 mRNA expression after treatment with IFNg. AntiCD54 Mabs inhibited tumor lysis by autologous TIL 24 - 53% in 6/7 cases by 4hr ^{51}Chromium release; antiCD44 Mabs did so by only 20% in 2/7. AntiCD54 inhibited TIL proliferation in response to autologous tumor 14 - 31% in 5/7 cases, while antiCD44 showed no inhibition. In conclusion, CD44 & CD54 are expressed as part of the malignant phenotype in RCC; CD44 & CD54 cell surface & mRNA expression by RCC can be modified by cytokines; & CD54 is an important mediator of autologous tumor - TIL interaction as measured by tumor lysis & TIL proliferation.

C 420 LYMPHOCYTE MIGRATION ACROSS BRAIN ENDOTHELIUM: ROLE OF LFA-1/ICAM-1, VLA-4/VCAM AND SURFACE CHARGE, David Male, Jameel Rahman, Washington Santos and Gareth Pryce, Department of Neuropathology, Institute of Psychiatry, London, UK.

We have examined lymphocyte adhesion to rat cerebral endothelium in vitro, and the ability of the cells to migrate across endothelial monolayers. In these systems, lymphocyte adhesion is dependent on the state of lymphocyte activation and is increased when the endothelium is pulsed with cytokines including TNF-α and IFN-γ. Adhesion is maximal 1-2 days after lymphocyte activation and this is coincident with the expression of VLA-4 and LFA-1 by the cells. However the ability to migrate continues to increase over 1-8 days after activation.
Following activation of the endothelium ICAM-1 expression increases over 8 - 96 hours with a profile which directly mirrors the enhanced lymphocyte adhesion seen over this period. For this reason we attempted to block adhesion with antibodies to LFA-1 or ICAM-1. Anti-LFA-1 (CD11a or CD18) partially block adhesion of activated lymphocytes but not of control cells to the endothelium. Anti-ICAM-1 does not block, but paradoxically partly reverses the effect of anti-LFA-1. Since depletion of endothelial ICAM-1 by continuous culture in anti-ICAM-1-containing medium has no effect on lymphocyte adhesion, we consider that the levels of lymphocyte LFA-1 are limiting in this system, but endothelial ICAM-1 is not. Anti-VLA-4 also blocks adhesion weakly and enhances blocking caused by anti-LFA-1. Since anti-VLA-4 causes some homotypic aggregation of the adherent lymphocytes, it may act by interfering with normal lymphocyte motility or by blockage of VLA-4/VCAM-1 interactions. A peptide containing the CS-1 binding site of fibronectin failed to block adhesion significantly suggesting that VLA-4/fibronectin binding is of limited significance in this system.
We have also measured surface charge on the brain endothelium using poly-l-lysine conjugates. Charge is highly dependent on the state of growth of the endothelium, but charge levels do not change with cytokine activation of this type of endothelium or correlate with areas of lymphocyte adhesion.

C 421 **DIFFERING REQUIREMENTS FOR CYTOKINES AND ADHESION MOLECULES IN NEUTROPHIL-MEDIATED INJURY,** Michael S. Mulligan and Peter A. Ward, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, 48109
IgG immune complex deposition has been induced in rat lung and skin (BSA-anti-BSA immune complexes) and in the kidney (nephrotoxic nephritis). By the use of blocking antibodies to E-selectin, ICAM-1, TNFα and IL-1 or by the use of natural antagonists (soluble TNFα receptor-1 and IL-1 receptor antagonist), the roles of these factors in neutrophil recruitment and in tissue injury (defined by permeability change and hemorrhage or proteinuria) have been defined. In all three models of immune complex-induced injury (alveolitis, dermal vasculitis and glomerulonephritis), ICAM-1 has been found to be an essential factor for neutrophil recruitment and tissue injury. In the lung injury models, E-selectin also plays a vital role, as do TNFα and IL-1. In the skin, E-selectin is an absolute requirement while IL-1 but not TNFα is requisite for neutrophil requirement and tissue injury. In the kidney, damage (as assessed morphologically and by proteinuria) is independent of a requirement for either E-selectin or IL-1 but absolutely depends on the participation of TNFα. These data suggest that neutrophil-dependent tissue injury triggered by deposition of IgG immune complexes has a common requirement for ICAM-1 but variable requirements for E-selectin, TNFα and IL-1, depending on the vascular bed under study.

C 422 SELECTIVE CYTOKINE REGULATION OF T LYMPHOCYTE ADHESION TO EPITHELIAL AND ENDOTHELIAL CELLS, Shin Nakajima, Dwight C. Look, and Michael J. Holtzman, Departments of Medicine and Surgery, Washington University, Saint Louis, MO 63110
The basis for T lymphocyte adherence to epithelial and endothelial cell monolayers was studied using a newly-developed adherence assay which utilizes flow cytometry to identify and quantify adherent leukocytes, thereby avoiding extensive leukocyte purification and/or labeling which may alter leukocyte function and may eliminate cell-cell interactions between different classes of leukocytes. Initial experiments performed with peripheral blood leukocytes isolated by single-step centrifugation over Ficoll-Hypaque and a FITC-conjugated anti-CD3 mAb indicated little basal T lymphocyte adherence under control conditions (< 0.1% of T cells added to the monolayer) and markedly stimulated adherence after leukocyte treatment with phorbol dibutyrate (to 8-12%) with three types of cell monolayers: human vascular endothelial cells (HUVECs), human tracheal epithelial cells (HTECs), and SV-40 virus-transformed human bronchial epithelial cells (BEAS-2B cells). Superimposed cytokine treatment of monolayers indicated that T cell adherence to HUVECs was further increased (to 30%) by IL-1β or TNF-α and adherence to HTEC or BEAS-2B cells was increased (to 15%) by monolayer treatment with interferon-γ (IFN-γ). These results were parallel to the profile for cytokine induction of ICAM-1 on these cell types, and accordingly, the increased T cell adherence could be inhibited 70% by anti-CD18 or anti-ICAM-1 blocking mAb and 30% by anti-VLA-4 mAb. Interestingly, in the case of cytokine treatment of monolayers alone (without phorbol dibutyrate), the only condition which increased T cell adherence (to 8%) was treatment of HUVEC monolayers with TNF-α, an effect that was inhibited 70% by anti-CD18 or anti-ICAM-1 mAbs but not significantly by anti-VLA-4 mAb. The TNF-induced increase in HUVEC-T cell adherence was not accompanied by significant increases in the level of ICAM-1 over other stimulation conditions. The results indicate that even T cells with no evidence of exogenous activation may exhibit increased adherence under selective conditions most likely on the basis of binding to cells with both increased expression and altered avidity of ICAM-1. In addition, the selective responsiveness of the ICAM-1 gene to IFN-γ in airway epithelial cells and to IL-1β and TNF-α in vascular endothelial cells may provide a basis for coordinated influx of T lymphocytes to the airway lumen by sequential cytokine action.

C 423 DIFFERENT SPATIAL DISTRIBUTIONS OF THE L-SELECTIN (LECAM-1) AND BETA2 INTEGRIN (Mac-1) ADHERENCE RECEPTORS ON HUMAN NEUTROPHILS, Robert D. Nelson, Sharon R. Hasslen and Stanley L. Erlandsen, Departments of Dermatology, and Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455

We have developed a method utilizing high resolution field emission scanning electron microscopy and backscatter electron imaging of immunogold for the detection of cell adherence receptors on the surface of unfixed human neutrophils. This combination of technologies has allowed us to discover unique spatial distributions of LECAM-1 and Mac-1 on the neutrophil surface. The distributions of these receptor species appear to fit with current knowledge of the separate roles played by the respective receptors in the attachment of circulating neutrophils to vascular endothelium and emigration of neutrophils to extravascular sites of inflammation. Immunogold labeling of LECAM-1 and Mac-1 involved the use of a primary murine monoclonal antibody specific for each receptor species (DREG-56/IgG2a, Dr. T.K. Kishimoto, and Mo-1/IgM, Coulter, respectively) and affinity purified goat anti-mouse IgG and IgM antibodies conjugated to colloidal gold (Jackson ImmunoResearch Labs). Ultrastructural analysis of the neutrophils involved examination of only rounded cells that had attached to the substratum without any sign of spreading. The surfaces of these cells were seen to consist of two major membrane domains including: membrane projections in the form of microvilli and ruffles, and the membrane between adjacent projections, or cell body. Discrimination of these cell surface features was enhanced by stereoscopic examination of paired SEM micrographs. LECAM-1 was observed to label as small clusters of gold particles on the tips of microvilli or ruffles, and was seldom observed on the cell body. Mac-1, in contrast, was observed to label as single or small clusters of gold particles on the cell body, and was rarely seen on microvilli or ruffles. Thus, LECAM-1 appears to be expressed on the neutrophil surface in a location ideal to mediate rolling and initial stopping of the neutrophil on the endothelial surface. Flattening of the stopped cell against the endothelium would then bring Mac-1 into contact with its co-receptors to strengthen cell adhesion and facilitate migration of the neutrophil through the endothelium.

C 424 IDENTIFICATION OF A UNIQUE HYALURONIC ACID BINDING SITE IN THE EXTRACELLULAR DOMAIN OF CD44, Robert J. Peach and Alejandro Aruffo, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

CD44 is a polymorphic transmembrane glycoprotein that has been implicated in a number of important cellular functions including lymph node homing, leukocyte activation, lymphopoiesis, and tumor metastisis. The diverse range of biological functions can be attributed to CD44 acting as a cell adhesion molecule. Of the 250 amino acids encoding the extracellular domain, the first 135 share approximately 30% sequence homology to regions of cartilage link protein, the proteoglycans versican and aggrecan and to a TNF-inducible protein TSG-6, each of which bind the extracellular matrix glycosaminoglycan hyaluronic acid (HA). CD44 has been identified as the principal cell surface receptor for HA but structural requirements for HA binding are not well defined.

To locate the HA binding site we genetically constructed truncation mutants of the CD44 extracellular domain fused to part of the heavy chain of a human IgG molecule. The resulting fusion proteins were tested for their ability to bind HA immobilized on ELISA plates. Data suggested a short sequence of amino acids contained a HA binding site and site-directed mutagenesis of specific residues in this region dramatically reduced the ability of the mutants to bind HA. This establishes a unique HA-binding site in the extracellular domain of CD44 that is not conserved in other HA-binding proteins.

Manipulation of HA binding to CD44 will provide a means to more closely study the role CD44 plays in diverse biological activities.

C 425 ENDOTHELIAL CELL CA^{2+} MODULATES TUMOR CELL ADHESION. Roberto Pili, Stefano Corda, Antonino Passaniti, Roy C. Ziegelstein and Maurizio C. Capogrossi. Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224.

The signal transduction mechanisms involved in tumor cell adhesion to endothelial cells are still largely undefined. The effect of metastatic murine melanoma cell (B16/G3.26) adhesion on bovine pulmonary artery endothelial cell (BPAEC) cytosolic $[Ca^{2+}]$ (Ca_i) was examined in indo-1 loaded BPAEC. A rapid increase in BPAEC Ca_i occurred on contact with melanoma cells; BPAEC contact with 8 μm inert beads did not elicit an increase in Ca_i. The increase in BPAEC Ca_i was not inhibited by extracellular Ca^{2+} removal. In contrast, BPAEC pre-treatment with thapsigargin which releases endoplasmic reticulum (ER) Ca^{2+} into the cytosol abolished Ca_i rise upon tumor cell contact. Furthermore, BPAEC pre-treatment with the intracellular Ca^{2+} chelator BAPTA-AM blocked the rapid increase in BPAEC Ca_i. Under static and dynamic flow conditions (0.47 dynes/cm^2), BAPTA-AM pre-treatement of BPAEC inhibited initial tumor cell adhesion by ~50 %. Thus tumor cell adhesion induces a rapid Ca^{2+} release from intracellular stores in BPAEC, which appears to have functional significance in enhancing cell-cell adhesion.

C 426 ENDOTHELIAL CELL BINDING OF NAP-1/IL-8: ROLE IN NEUTROPHIL EMIGRATION, Antal Rot, Sandoz Research Institute, Vienna, A-1235 Austria

Experimental evidence indicates that neutrophil emigration induced in vivo by intradermal injections of various attractants, including NAP-1/IL-8, is regulated by mechanisms involving hypothetical attractant receptors present in skin sites. Using an in situ binding assay on human and animal skin samples, we identified NAP-1/IL-8 binding sites on the surface of venular endothelial cells. We argue that these binding sites are instrumental for NAP-1/IL-8-induced neutrophil emigration. Under the conditions of venous blood flow only the endothelial cell surface-bound NAP-1/IL-8 can effectively stimulate the second, integrin-mediated step of neutrophil adhesion to the endothelium. Conversely, soluble NAP-1/IL-8 should inhibit neutrophil-endothelial cell adhesion. Thus the endothelial binding sites for NAP-1/IL-8 could be involved in regulation of neutrophil-endothelial cell adhesion.

In addition, we show that NAP-1/IL-8, but not formyl peptide, induces the in vitro neutrophil migration in Boyden-type chamber when bound to the surface of the polycarbonate filter. By analogy with in vitro haptotaxis we also suggest that neutrophil transmigration across the endothelial cell barrier can be induced by NAP-1/IL-8 bound to the endothelial cell surface.

C 427 ENHANCEMENT OF NATURAL KILLER ACTIVITY BY AN ANTIBODY TO CD44, Brenda M. Sandmaier, Philip H.S. Tan, and Erlinda B. Santos, Transplantation Biology Program, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

In the canine model, radioresistant host-derived large granular lymphocytes with natural killer (NK) activity are associated with rejection of DLA-nonidentical marrow grafts. However, pre-treatment of recipients with an anti-CD44 monoclonal antibody (MAb), S5, before total body irradiation (TBI) and marrow transplant abrogates this rejection. All NK cells express CD44, though the function of this molecule on NK activity is unknown. The in vitro effect of MAb S5 on NK function was examined using canine peripheral blood mononuclear cells (PBMC) as effectors in a canine thyroid adenocarcinoma cell line (CTAC) as targets. CTAC does not express class II and has minimal class I MHC antigen expression. S5 enhances NK activity in a dose dependent and rapid fashion while being temperature independent. The activation of increased killing by S5 occurred only when NK sensitive targets were used, suggesting that lymphokine activated killer (LAK) cells were not being induced. Enhanced killing of CTAC could not be attributed to ADCC as S5 incubation of target cells did not lead to increased cell lysis. Also, when $F(ab)_2$ fragments of S5 were used, enhanced NK activity comparable to what was seen with intact MAb was seen, indicating that the Fc portion of the molecule was not important. F(ab') fragments of S5 were equivalent to intact S5 in their ability to stimulate NK activity, demonstrating that neither crosslinking of CD44 was a component of stimulation, nor was nonspecific agglutination of target to effector cells occurring via the two F(ab) arms. Pretreatment of cells with radiation, which enriches for NK cells that are known to be relatively radioresistant, further enhances the S5-induced killing. In addition, monocyte depleted PBMC also respond to MAb treatment, suggesting S5 mediates its effect directly on the NK cell. Other MAbs that recognize the same (IM7) and different (S3 and Hermes-1) epitopes of CD44 were compared to S5, and while IM7 was comparable in its ability to enhance NK activity, the others had a more variable effect.

Activation of NK activity in vivo by treatment of marrow graft recipients with an anti-CD44 MAb peritransplant may cause NK cells to have an increased susceptibility to TBI, thereby enhancing marrow engraftment.

C 428 KINETIC EXPRESSION OF ENDOTHELIAL ADHESION MOLECULES IN EVOLVING ACUTE AND CHRONIC INFLAMMATORY MODELS, A Silber, K Reimann, E Hendricks, M Mercier, W Newman, D Ringler, Depts of Pathology and Immunology, Harvard Medical School, Southboro, MA 01772, Otsuka America Pharmaceutical, Inc., Rockville, MD 20850

We examined and quantified the kinetic expression of E-selectin, VCAM-1, P-selectin, and ICAM-1 in the skin of rhesus monkeys after endotoxin (LPS)-induced injury and during a delayed-type hypersensitivity (DTH) reaction against tuberculin. We then compared these parameters to relative numbers and types of leukocytes in the tissue, cytokine localization, and expression of relevant adhesion ligands on circulating leukocytes.

In endotoxin-induced skin injury, E-selectin was maximally expressed by 8 hrs after intradermal injection but sustained expression was seen during the entire 72 hrs of study. VCAM-1 expression was minimal, while P-selectin expression was reduced to negligible levels between 8-24 hrs post-injection of endotoxin. Neutrophil numbers peaked at 24 hrs but were relatively reduced in proportions thereafter because of mononuclear cell infiltration. TNF-α and IL-1 were expressed predominantly on epithelium and dermal mononuclear cells respectively by 2-4 hrs.

In contrast, the DTH model was characterized by dual expression of both E-selectin and VCAM-1. E-selectin was maximally expressed by 8 hrs at half the level seen in LPS-treated skin and remained expressed for 7-10 days. VCAM-1 expression paralleled E-selectin's with a 2 day lag phase. P-selectin and cytokine expression were unchanged from the LPS model. Low numbers of neutrophils were seen in all animals during E-selectin expression. However, T cell and macrophage localization was maximal at 48-72 hrs, just after peak VCAM-1 immunoreactivity. Analysis of peripheral granulocyte and mononuclear cells by flow cytometry showed no measurable change to CD11a/CD18, L-selectin, and VLA-4 density.

In conclusion, E-selectin can be persistently expressed (>24 hrs) *in vivo* and is kinetically associated with neutrophil extravasation in acute injury, while both E-selectin and VCAM-1 are associated with T cell/monocyte recruitment in DTH reactions.

C 429 PLATELET ACTIVATING FACTOR (PAF) OR TUMOR NECROSIS FACTOR-α (TNF) INDUCED ELEMENTAL CHEMICAL MODIFICATIONS, EVALUATED BY ESCA (ELECTRON SPECTROSCOPY CHEMICAL ANALYSIS) MICROANALYSIS, IN ENDOTHELIAL CELLS (EC), L. Silvestro[1], F. Sommer[2], L. Biancone[3], Tran Minh Duc[2], G. Camussi[3], R. Da Col[1], I. Viano[4], [1]Res Pharma Pharm. Res. s.r.l., Via Belfiore 57, 10125 Torino Italy [2]CENATS, Univ. C. Bernard, Villeurbanne France [3]Lab. Immunopatologia Cattedra di Nefrologia. Univ. di Torino e Cattedra di Nefrologia Sperimentale Dip. Biochimica Biofisica, I Fac. di Medicina, Napoli Italy. [4]Ist. di Farmacologia, Univ. di Torino Italy.

PAF end TNF enhance the polymorphonuclear leukocytes (PMNs)-EC adhesion. No current data on modifications of the elemental chemical composition of EC membranes are available. Few techniques gives informations on the chemical composition of surfaces, without interferences from the underlying structures, as ESCA microscopy.

In our study we used this technique to analyze human EC treated in vitro with PAF or TNF in comparison with untreated controls. The contents of S,C,P,O,N were evaluated on air dried cultures. Radio-labeled PMNs adherence to EC was correlated to the modifications of the elemental chemical composition.

A significant dose-dependent reduction of S content, but not of C,P,O,N, was observed with concentrations of TNF and PAF increasing the PMNs-EC adhesion. Our current studies are aimed to evaluate the modifications of specific chemical groups. Further studies are needed to define if the modifications of the elemental chemical composition correlate with the known mechanisms of cell adhesion.

C 430 ICAM-1 DEFICIENT MICE. James E. Sligh, Jr.[1], Christie M. Ballantyne[2], Allan Bradley[1], Hal K. Hawkins[3], C. Wayne Smith[4,5], and Arthur L. Beaudet[1,4,6]. Institute for Molecular Genetics[1] and Departments of Medicine[2], Pathology[3], Pediatrics[4], and Microbiology and Immunology[5], Baylor College of Medicine and Howard Hughes Medical Institute[6], Houston, Texas 77030.

Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin (Ig) superfamily, and exhibits heterophilic binding to members of the β_2 integrin family of proteins. In order to assess the role of ICAM-1 *in vivo*, we produced a mutation in the *Icam-1* gene by targeted recombination in AB1 and AB2.1 murine embryonic stem (ES) cells. A 5.5 kb segment of the murine *Icam-1* gene was subcloned into pBluescript, and a neomycin resistance gene under the control of the RNA polymerase II promoter was inserted within exon 5 which encodes the fourth extracellular Ig domain. This mutation is expected to produce a premature stop codon and a truncated protein. After electroporation of the mutagenic construct and 9 days of G418 selection, individual ES cell colonies were picked and screened for homologous recombination by PCR, using a primer within the neo cassette paired with a primer lying in the *Icam-1* gene, but outside of the region contained within the electroporated plasmid. Using the plasmid cut as a replacement vector, several targeted clone were generated at a frequency of 1 in 70. Germline transmission was achieved and heterozygous progeny were mated to produce mice homozygous for the *Icam-1* mutation. Homozygous mutant animals are generally healthy and fertile, and routine autopsy showed normal tissue architecture. Immunohistochemical staining of lung tissue with an anti-ICAM-1 monoclonal antibody produced intense staining in the normal animals and no staining in the mutant animals. FACS was performed on cultured splenocytes and confirmed the total lack of detectable ICAM-1 protein on the cell surface. Chemical peritonitis was induced by intraperitoneal injection of thioglycollate and the exudate was collected at three hours for analysis. The mutant animals had significantly fewer granulocytes in the peritoneal exudate when compared to the normal group of mice. These mutants will be valuable resources for the study of a variety of inflammatory processes in the mouse.

C 431 T LYMPHOCYTE ADHESION, PENETRATION AND PERTURBATION OF TWO- AND THREE-DIMENSIONAL COLLAGEN SUBSTRATA, Karl-G. Sundqvist, Dan Hauzenberger, Kjell Hultenby and Sten-E. Bergström, Department of Clinical Immunology, The Clinical Research Center and Department of Pulmonary Medicine, Huddinge Hospital, Stockholm, Sweden

Lymphocytes were plated on two-dimensional (2D) and three-dimensional (3D) collagen substrata and their distribution and behaviour determined using light and scanning electron microscopy. When allowed to settle on 2D collagen substrata T-lymphocytes "rapidly" attached and penetrated and thus virtually never remained attached on top of the collagen. As a consequence of this penetration the cells appeared below the collagen. In contrast to lymphocytes fibroblasts and macrophages allowed to settle on 2D collagen did not penetrate but underwent cytoplasmic spreading on top of the collagen. Lymphocyte attachment and penetration of 2D collagen was specifically inhibited by monoclonal antibodies to β1-integrins (Mab 13) indicating that the process depends on molecular adhesion to the collagen. Noteworthy, chloroquine also acted inhibitory. The penetration of 2D collagen appeared to consist of tight binding of collagen fibres to the cells, local dissolution of the collagen carpet and redistribution of the cell-attached collagen fibres from the "lower" to the "upper" pole/surface of the lymphocyte. Lymphocyte infiltration of 3D collagen substrata was also specifically inhibited by Mab 13. During the infiltration of 3D collagen lymphocytes exhibited collagen fibres attached to their surface, and seemed to provoke perturbation of the collagen. These results show that T lymphocytes are programmed to penetrate and perturb 2D and 3D collagen substrata by a mechanism dependent on adhesive interaction. However, deattachment tends to counteract persistent binding of the cells to 2 D collagen and is thus a distinguishing feature between lymphocyte interaction with 2D and 3D collagen substrata.

C 432 LYMPHOCYTE ACTIVATION STATUS IN CORRELATION TO THEIR BINDING TO ENDOTHELIUM AND THE ROLE OF CD11a, CD18 AND CD49d. Turunen JP# and Renkonen R##. #Transplantation Lab. and ##Dept. of Bacteriology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, FINLAND.

We report here the importance of activation status of lymphocytes and endothelial cells (EC) in lymphocyte adherence to endothelium, and the role of CD11a/CD18 (LFA-1) and CD49d (VLA-4 a-chain) in their binding. When EC was stimulated with IL-1 and/or lymphocytes with rIL-2 or PHA, the binding of lymphocytes to EC was increased. PMA treatment of lymphocytes alone did not increase their binding to EC, but when EC was additionally induced by IL-1 the binding was increased. Both resting and stimulated lymphocytes expressed similarly high levels of LFA-1. The expression of CD49d was increased after rIL-2 and PHA stimulus. The PMA- and rIL-2-induced lymphocyte binding to IL-1 induced EC was inhibited by anti-CD11a, CD18 and CD49d mAbs; on the other hand, the enhanced binding of PHA-stimulated lymphocytes to EC could not be blocked by these mAbs. These results show that activation of lymphocytes by various stimuli leads to different usage of adhesion pathways in their binding to inflammatory EC.

C 500 INHIBITION OF ICAM-1, VCAM-1 AND E-SELECTIN EXPRESSION WITH ANTISENSE OLIGONUCLEOTIDES, C. Frank Bennett, Susan Grimm, and Hedy Chan, Department of Molecular Pharmacology, ISIS Pharmaceuticals, 2280 Faraday Ave., Carlsbad, CA 92008

Increased expression of the endothelial-leukocyte adhesion molecules, ICAM-1, VCAM-1, and E-selectin occurs in a variety of inflammatory disorders. We have designed a series of phosphorothioate oligonucleotides 18 to 21 bases in length which selectively hybridize to either human ICAM-1, VCAM-1 or E-selectin mRNA. The oligonucleotides were tested for their ability to inhibit tumor necrosis factor induced expression of the respective endothelial adhesion molecules in human umbilical vein endothelial cells by ELISA. In each case oligonucleotides were identified which selectively inhibited the expression of either ICAM-1, VCAM-1, or E-selectin. The most active oligonucleotides were found to cause a reduction in the cellular content of the target mRNA, however, a reduction in target mRNA was not obligatory for inhibition of protein expression, suggesting that oligonucleotides may inhibit gene expression by multiple mechanisms. Antisense oligonucleotides which targeted E-selectin and ICAM-1 were found to inhibit adhesion of HL-60 cells or Jurkat cells to cytokine activated endothelial cells and keratinocytes, respectively. Inhibition of cell adhesion correlated with inhibition of protein expression. These data demonstrate that antisense oligonucleotides can selectively inhibit the expression of endothelial-leukocyte adhesion molecule expression. Furthermore inhibition of ICAM-1 and E-selectin expression correlated with a loss in adhesiveness of the endothelial cells or keratinocytes to leukocytes.

C 501 NEUTROPHIL MEDIATED, CD18 DEPENDENT, PULMONARY INJURY IN A CANINE MODEL OF CARDIOPULMONARY BYPASS. William J. Dreyer, Lloyd H. Michael, Glenn A. Laine*, Gary Liedtke, Peggy Jackson, Mark L. Entman, C. Wayne Smith. Baylor College of Medicine, Houston, TX 77030; *Texas A&M University, College Station, TX 77843.

Previous studies have documented a complement-mediated inflammatory reaction to cardiopulmonary bypass (CPB). Neutrophil (PMN) sequestration in the lungs is thought to contribute to microvascular injury and pulmonary dysfunction. This study explored the hypothesis that the leukocyte integrin CD18 mediates this response. Six adult, mixed-breed dogs underwent CPB (Cobe VP-CML membrane oxygenator) with the aorta cross-clamped 60 min and the animals cooled to 24-28°C. After rewarming, dogs were recovered off CPB for 3 hrs. Analysis of arterial blood samples by flow cytometry demonstrated that PMN CD18 expression increased 59 $\pm$ 10% above the pre-thoracotomy baseline 20 min after the onset of CPB, and reached a maximum of 95 $\pm$ 6% above baseline by 3 hrs of recovery. CD18 failed to increase over a similar time course in 4 "sham bypass" controls. By 3 hrs of recovery, CPB dogs demonstrated markedly decreased oxygenation on 100% O_2, with arterial pO_2 reduced to 62 $\pm$ 7 mmHg (controls 380 $\pm$ 45 mmHg, $p<0.01$). Extravascular lung water (EVLW) increased significantly in CPB dogs compared to sham controls (lung wet/dry weight ratio (W/D), 4.88 $\pm$ 0.19 vs 3.89 $\pm$ 0.25, $p=0.01$). Four additional animals were treated prior to CPB with a 1mg/kg iv bolus of R15.7 IgG, a monoclonal antibody to CD18. When compared to the CPB group, the R15.7 dogs demonstrated a marked improvement in arterial pO_2 at 3 hrs (338 $\pm$ 106, $p=0.02$) as well as a decrease in EVLW (W/D 4.12 $\pm$ 0.21, $p=0.03$). These data imply that pulmonary injury after CPB is caused in part by a neutrophil-mediated, CD18-dependent mechanism.

C 502 DIFFERING REQUIREMENT FOR CD18 IN NEUTROPHIL ACCUMULATION IN LUNG AND SKIN INFLAMMATION

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Antibodies to the ß2 integrin CD18 abrogate neutrophil accumulation and tissue injury in a number of in vivo models of inflammation. The exception appears to be pulmonary inflammation where protective effects of anti-CD18 mAbs are variable, depending on the model. In this study the effect of the anti-CD18 mAb 60.3 on accumulation of ^{111}In-labelled neutrophils in the lung and skin of the same rabbits induced by C5a or IL-1 was studied. Rabbit neutrophils (>95% purity) were isolated from peripheral blood using discontinuous plasma-Percoll gradients, labelled with ^{111}In and infused intravenously. C5a and IL-1 were selectively instilled into the left and right cranial lobes, respectively, using a bronchoscope and were also injected intradermally into the shaved dorsal skin of the same animals. Scintigraphy scans were taken for up to 4h at which time neutrophils in bronchoalveolar lavage (BAL) fluid and in skin sites were quantified. Scintigraphic scans showed that in control animals C5a and IL-1 induced retention of ^{111}In-neutrophils in the lungs with similar kinetics, with most sequestration occurring over 0-60 min. 60.3 significantly ($p<0.05$) reduced retention in response to IL-1 (eg. by 50-70% at 1-4h) but not C5a at any time point. Total neutrophils recovered by BAL at 4h in response to IL-1 and C5a were 11.1 ± 3.6 and $9.9\pm7.2\times10^6$, respectively. In animals treated with 60.3 the numbers found were 5.4 ± 1.5 and $7.2\pm2.4\times10^6$ for IL-1 and C5a. Recovery of ^{111}In-neutrophils in BAL induced by IL-1 was 0.34 ± 0.09% of infused counts which was reduced ($p<0.05$) to 0.07 ± 0.03% by 60.3. Accumulation of labelled cells in response to C5a was reduced from 0.27 ± 0.07% to 0.11 ± 0.03% in animals treated with 60.3 although this did not reach statistical significance. However, in the skin of the same animals, accumulation of ^{111}In-neutrophils induced by C5a (0.5-5μg) and IL-1 (1-100pg) at 4h was inhibited by >95% by 60.3 treatment. These data suggest that neutrophil accumulation in the cutaneous circulation is totally dependent on CD18 while in the pulmonary circulation dependency on CD18 is dictated by the stimulus; IL-1 is not totally CD18 dependent but is more CD18-dependent than C5a. The mechanisms by which C5a and IL-1 induce CD18-independent neutrophil accumulation in the lung is under study.

C 503 ADHESION MOLECULES AND CYTOKINES IN ACUTE GLOMERULONEPHRITIS, Johnson K.J. Mulligan, M.S. and Peter A. Ward, Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109-0602

Using the model of neutrophil dependent acute nephrotoxic (anti-GBM) nephritis in the rat the role of leukocyte adhesion molecules and cytokines was examined. The upregulation of the adhesion molecules CD11a, CD11b, VLA-4, ICAM-1, and ELAM-1 in the glomeruli was assessed immunohistochemically and the role of these molecules in the development of the glomerular injury and proteinuria was assessed by the use of specific antibodies to these molecules. Immunohistochemically, infusion of the anti-GBM antibody induced glomerular upregulation of ICAM-1, ELAM-1, and VCAM-1. A similar pattern of adhesion molecule upregulation was observed in normal rats given renal artery infusions of TNFα but not IL-1B. Infusion of specific antibodies to LVA-4 and ICAM-1 as well as the leukocyte adhesion molecules CD11b and CD18 prevented the upregulation of the glomerular adhesion molecules as well as providing protection against the resulting glomerular injury and proteinuria. Treatment of rats with anti-TNF or soluble recombinant human TNF receptor-1 (sTNFr-1) produced similar protective effects. In summary, these studies suggest this neutrophil dependent model of acute glomerular injury is Mac-1 and ICAM-1 but not E-selectin dependent, as well as requiring VLA-4 and TNFα but not IL-1. These studies also suggest that a primary role for TNF in vivo in acute inflammation is upregulation of adhesive molecules.

C 504 INCREASED ICAM-1 EXPRESSION ON HUMAN TUMOR CELL LINES TREATED WITH LEVAMISOLE.

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ICAM-1 expression on human umbilical vein endothelial cells (HUVEC) and on a panel of human colon, breast and melanoma tumor cell lines was studied as a function of 48 hours in vitro treatment with levamisole, a drug which is able to modulate various aspects of the host immune response and which is used in adjuvant therapy of Duke's C colon cancer . ICAM-1 expression was quantitated in situ using a solid phase ELISA. Levamisole had no effect on ICAM-1 expression by HUVEC, whereas tumor necrosis factor (10 ng/ml) was capable of inducing ICAM-1 expression by as much as 25-fold over unstimulated cells. Increased ICAM-1 was observed, though, on HT-29 colon tumor cells cultured with levamisole at concentrations ranging between .001 and 10 ug/ml. The ICAM-1 increases ranged from 40% to greater than 200% above unstimulated control levels for these cells, while TNF-induced ICAM-1 increases were as high as 10-fold. In contrast, SW-620 colon tumor cells failed to respond to levamisole, but responded to TNF with 200 % increases in ICAM-1, while LoVo colon tumor cells failed to respond to levamisole and responded only poorly to TNF, if at all. Increases of 50% to 200% in ICAM-1 expression were also observed after levamisole treatment of MCF-7, but not for MDA MB-231 breast tumor cells. Up to 200% increases in ICAM-1 were observed for Hs294t melanoma cells treated with levamisole, but none were observed with A-375 melanoma. These last 4 cell lines had greater than 200% increases in ICAM-1 in response to TNF. We are studying the relationship between ICAM-1 induction by levamisole in vitro and the in vivo effects of levamisole on these same tumor cell lines when transplanted into nude mice.

C 505 ODIFICATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS BY ADMINISTRATION OF ANTIBODIES TO LFA-1 AND ICAM-1, Michael K. Racke, Dorothy Scott*, Laura Quigley, and Dale E. McFarlin Neuroimmunology Branch, NINDS and *NIAMS, NIH, Bethesda, MD 20892

The expression of adhesion molecules in the central nervous system (CNS) during inflammation in experimental allergic encephalomyelitis (EAE) implicates these molecules in the disease pathogenesis. The role of one such receptor/ligand pair in EAE was studied by administering antibodies specific for either LFA-1 or ICAM-1 for days 4-10 after the transfer of encephalitogenic T cells. Lower doses of these antibodies (.2-.4 mg/mouse/day) resulted in more severe disease. Higher doses (.8-1 mg/mouse/day) caused a marked delay in disease onset and when antibodies to both LFA-1 and ICAM-1 were given, a significant suppression of clinical signs was observed.

These same antibodies were then used in combination with antibodies directed at CD3 and CD4 molecules to stimulate MBP-specific lymph node cells in vitro and enhance their encephalitogenic potential. PCR analysis of T cells receiving additional stimulation through LFA-1 or ICAM-1 demonstrated increased production of inflammatory cytokines implicated in the pathogenesis of EAE. These results suggest that LFA-1 and ICAM-1 not only play a role in the adhesion between lymphocytes and the target organ (CNS), but also provide important costimulatory signals leading to the production of different cytokines.

C 506 A CRITICAL ROLE FOR INTEGRIN α_4 AND CD44 IN THE INDUCTION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN MICE, Timo Veromaa[1], Irving L. Weissman[1,3], Lawrence Steinman[2], and Stefan Brocke[2], Departments of [1]Pathology and [2]Neurological Sciences, and [3]Howard Hughes Medical Institute, Stanford University Medical Center, Stanford, CA 94305

EAE is an autoimmune disease model for human MS-disease characterized by chronic relapsing paralysis with histopathology demonstrating perivascular inflammatory infiltrates in the central nervous system. We are characterizing the mechanisms of entry of the inflammatory cells into the brain parenchyma through the blood-brain-barrier. To this effect, myelin basic protein-specific CD4+ T cell clones were used to adoptively induce EAE in (PJxSJL)F_1 mice. *In vivo* treatment of these mice with rat monoclonal antibodies (mAb) against integrin α_4-chain (R1-2) and CD44 (IM781) prevented the disease induction whereas mAb against L-selectin (MEL-14) had a marginal effect and mAb against integrin α_M (Mac-1) had no effect. These results will be discussed in the context of specific tissue localization of the T cells injected.

Late Abstracts

PATIENTS IDENTIFIED FOR AN INCREASED RISK OF POST-OPERATIVE SEPSIS MAY HAVE ALTERED LEUKOCYTE HOMING MECHANISMS. Gordon, J., Hassan-Zahraee, M. and Lacey, C. Department of Surgery (Div. of Surgical Research), McGill University, 740 Dr. Penfield Ave., Montreal, Quebec, H3A 1A4.

Anergic surgical patients identified by a lack of delayed type hypersensitivy (DTH) to a battery of 5 recall antigens have an increased risk of post-operative sepsis and death. These patients have also a reduced ability to make anti-protein but not anti-polysaccharide antibodies, have Ig-secreting cells in their circulation, but show no apparent T cell or B cell abnormality in vitro. To investigate whether the lack of mononuclear cell (MNC) infiltrate at DTH sites may be linked to adhesion receptor expression in skin vascular endothelium, 24 or 48 hour biopsies of skin injected with irrelevant or specific antigen were analysed for E-selectin and VCAM-1 by immunocytochemistry. Among 15 DTH reactive patients none injected with irrelevant antigen expressed E-selectin, whereas all skin sites injected with specific antigen were positive. Seven anergic patients injected with 1 antigen were negative for E-selectin; among 5 injected with 2 antigens (mumps and varidase each with an incidence of sensitivity >90% in the patient population) 3 failed to express E-selectin at both skin sites and none expressed VCAM-1 detected in 5/5 reactive patients at least at one test site. In an additional study evidence was obtained to suggest that lymphocyte recirculation may also be modified in anergy. RNA extracted from biopsies of uninjected skin from 20 DTH reactive donors was analysed for mRNA for the CD3 molecule of T cells following amplification by PCR. Whereas all 20 skins gave a signal for CD3, biopsies from 6 anergics were uniformly negative.

ANTI-TUMOR EFFECTS OF GBS TOXIN ARE CAUSED BY INDUCTION OF A TARGETED INFLAMMATORY REACTION. C.G. Hellerqvist, G.B. Thurman, D. L. Page, Y.-F. Wang, T.J. Gensler, T.D. Mitchell and H.W. Sundell Vanderbilt University School of Medicine, Nashville, TN 37232.

Group B Streptococcus (GBS) isolated from human neonates with respiratory distress produces a polysaccharide exotoxin (GBS Toxin). We have associated GBS Toxin with the pathophysiology seen in developing lung capillary endothelium until four days post partum. We also found by immunohistochemistry that GBS Toxin can bind to developing endothelium associated with human neoplasia. In nude mice bearing human tumor xenografts, i.v. administered GBS Toxin caused tumor necrosis and hemorrhagic lesions, and inhibited the rate of growth of the tumors. We now report that in immunocompetent BALB/c mice bearing Madison Lung Tumors, GBS Toxin induced an inflammatory response resulting in marked changes in tumor morphology, including vasodilation, necrosis, and infiltration by inflammatory cells. Data also support the notion of a receptor mediated induction of the inflammatory response by GBS Toxin and strongly implicates TNFα in a time and dose-dependent manner in the overall response. In both tumor models, no evidence of toxicity to the vasculature of other tissues was observed. Lack of pathogenicity of GBS in adults and these results suggest that GBS Toxin has potential as a non-toxic anti-tumor agent in human therapy. (Supported by CarboMed, Inc.)

LYMPHOCYTE/ENDOTHELIAL CELL RECOGNITIONS : INVOLVEMENT OF A NEW FUCOSE-BINDING PROTEIN. C. Kieda, N. Bizouarne, V. Denis and M. Monsigny. Dpt de biochimie des glycoconjugués et lectines endogènes. C B M, CNRS, 1, rue haute. 45071 Orléans Cédex 02. France.

Molecules involved in leukocyte/endothelial cell recognitions include cell surface receptors named selectins, the specifity of which reflects involvement of carbohydrate recognition in their functions. Cell surface lectins were identified on mouse lymphocytes using neoglycoproteins. One cell surface lectin on leukocytes (L-selectin) has been shown to contain a sequence homology with calcium-dependent sugar-binding protein, as well as two cell surface lectins in endothelial cells : E-selectin and P-selectin. These lectins recognize fucosylated oligosaccharides namely Sialyl Lewis^x and Lewis^x.

Here we report the characterization of an immortalized mouse peripheral lymph node endothelial cell line. Endothelial cells, harvested from mice treated according to Ager, were transfected with a plasmid containing SV40 T antigen. Selected clones expressed : SV40 T antigen, Factor VIII, angiotensin converting enzyme, addressin MECA 79 and grow with a characteristic epithelioïd morphology. These clones are also labelled with anti-E-selectin and bind preferentially neoglycoproteins bearing fucose. The expression of the fucose-binding protein is up-regulated by GVH activated lymphocyte-conditionned medium. Fucose-specific lectin was isolated and it is shown not to be E-selectin.

One of these characterized endothelial cell clones grows in monolayers and allows efficient binding of lymph node lymphocytes as well as lymphoma cells. We have selected two lymphoma cell lines : the EL4 lymphoma which was known to be domiciliation-deficient. These cells are unable to adhere on the endothelial cells, they have low lectin and fucosylated glycoconjugate expression on their surface ; in contrast, EL4-IL2 lymphoma cells express lectins and fucosylated glycoconjugates on their membrane. Fucosyl-glycopeptides isolated from EL4-IL2 lymphoma cells are able to inhibit the adhesion between lymphoma and endothelial cells.

In conclusion, the immortalized endothelial cell line is suitable for an *in vitro* model to study the involvement of lectins and glycoconjugates in the interactions between lymphoma and endothelial cells.

CYTOKINE-ACTIVATED ENDOTHELIAL CELLS INTERNALIZE E-SELECTIN (ELAM-1) INTO A LYSOSOMAL COMPARTMENT OF TUBULAR SHAPE: A TUBULIN-DRIVEN PROCESS.

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E-selectin is an adhesion molecule that will only be expressed on activated endothelial cells. It has been suggested to regulate the influx of inflammatory cells into inflamed tissues, because of its ability to bind neutrophils (and also monocytes and CD4+ lymphocytes). Binding of these cells to E-selectin depends on the recognition of SLe^x moieties presented on glycosylated surface-expressed structures on these leukocytes.

In the present study, we investigated the expression of E-selectin on TNF-activated monolayers of human umbilical vein-derived endothelial cells (HUVEC) by confocal laser scanning microscopy. The activated endothelial cells internalized MoAb to E-selectin in a very rapid, energy-dependent fashion. When excess of non-bound MoAb was washed away, *surface* expression of E-selectin was not detected after 20 min of incubation at 37°C. The E-selectin MoAb was recovered in *intracellular* compartments with a clear tubular morphology, some of which seemed to be connected with each other. Cathepsin B was found to co-localize in these elongated structures, which suggests a lysosomal nature of (some of) these compartments.

To investigate the mechanism responsible for the internalization of E-selectin, we tried to inhibit this process with several chemical reagents. Internalization was unaffected by inhibition of Protein Kinase-C, cAMP-dependent Protein Kinase-A, or Protein Tyrosine Kinase activity. Also, when actin polimerization was prevented by preincubation of HUVEC with cytochalasin B, the internalization process was not blocked -even though the cells had obtained a more rounded appearance under these conditions. On the other hand, reagents that are known to interfere with the metabolism of tubulin completely prevented the formation of the elongated structures in which E-selectin would normally be internalized.

NF-κB TRANSCRIPTION FACTOR AND CELL ADHESION: IMPLICATIONS ON INFLAMMATION AND TUMORIGENICITY,

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The NF-κB transcription factor complex is a pleiotropic activator that participates in the induction of a wide variety of cellular and viral genes. The active complex is composed of two subunits designated p50 and p65. Binding sites for NF-κB are present in many cell adhesion molecules (CAMs), cytokines, and growth-factor receptors. CAMs play an important role in diverse cellular responses including inflammation, transformation, and tumor cell metastasis. Antisense techniques were used to ascertain the role of NF-κB in cell growth. Inhibiting the individual subunits of NF-κB exerted differential effects on cell adhesion. Antisense oligos to p65 caused a rapid and profound inhibition of cell adhesion in diverse cell types. Individual extracellular matrix components were unable to overcome the requirement for CAMs that are regulated by NF-κB function. The inhibition of p50 expression, on the other hand, blocked adhesion only in certain cell types, dependent on the differentiative status of cells. In differentiated leukemic cells, antisense p65 blocked the adhesion and expression of integrins as well as tyrosine phosphatase. Antisense p65 exerted antiproliferative effects on diverse transformed cells *in vitro*. These results show that inhibitors of NF-κB function can be useful in anti-inflammation and antitumor therapy.

ENDOTHELIAL-SPECIFIC EXPRESSION OF AN E-SELECTIN GENE IN TRANSGENIC MICE

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E-selectin, also known as endothelial leukocyte adhesion molecule (ELAM-1), is expressed on activated endothelial cells *in vivo* and *in vitro*. Although E-selectin is known to be induced by a variety of stimuli, little is known about the mechanisms which restrict its expression to endothelial cells. We have examined the expression of the rabbit E-selectin gene in transfection assays in primary endothelial cells in culture and in transgenic animals. A CAT gene under the control of the rabbit E-selectin promoter was highly inducible by IL-1 and TNFα in tissue culture but failed to show any tissue specificity, being equally active and inducible upon transfection into endothelial or nonendothelial cell types. In addition, this construct was not expressed at significant levels in transgenic mice. Transgenic mice were also created which carried a rabbit E-selectin minigene, consisting of the entire E-selectin coding region including all exons (with the exception of 3' untranslated sequences) and introns. This construct produced low constitutive levels of E-selectin transcripts in a variety of tissues and high levels of transcripts upon induction with IL-1α or β. The tissue distribution of the E-selectin transgene was similar to that seen for the endogenous murine E-selectin gene except that the transgene was significantly overexpressed relative to the endogenous gene *in situ* hybridization demonstrated that expression of the E-selectin transgene was restricted to endothelial cells and was ubiquitously expressed throughout the microvasculature. Thus, sequences capable of efficiently driving E-selectin expression to endothelial cells *in vivo* are contained within the the rabbit E-selectin transgene.