THE ENDOTHELIAL CELL

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The Endothelial Cell

Keynote Address

CN 001 THE ENDOTHELIUM: A CELL OF MANY TALENTS, Guido Majno, Department of Pathology, University of Massachusetts Medical School, Worcester, MA 01655

Despite their bland morphology, endothelial cells are attracting ever more interest because of their central role in biology and medicine. Besides their function as a barrier, they are recognized as an endocrine organ of a volume exceeding that of the endocrine and exocrine pancreas combined. Furthermore, because of their many properties, endothelial cells are - directly and indirectly - of immediate concern to virtually all medical specialists. The pathologist sees them, for example, (a) as the keystone of inflammation: the endothelial membrane regulates the exudation of both fluid and cells; (b) as a target as well as a cause of ischemia; (c) as the neoplastic cell in many tumors. To the neurologist they are important because they constitute the blood-brain-barrier, which represents an obstacle to the delivery of drugs to the brain, and can also break down under pathologic conditions. To the vascular surgeon they are important in relation to wound healing, to the formation of adhesions, and currently also in relation to the endothelial seeding of vascular prostheses. To the cardiologist they represent the surfaces that initiate the processes of atherosclerosis and thrombosis. To the pediatrician they are of interest in several respects: they participate in neonatal retinopathy, and in the delayed detachment of the umbilical cord, which may be the symptom of a severe defect of adhesion between leukocytes and microvascular endothelium. To the oncologist they play an important role in the several phases of the metastatic cascade. To the nephrologist they represent a filter which may malfunction. To the radiologist they represent an intentional or accidental target of radiation therapy. To the immunologist they represent an essential step in graft rejection, because endothelial cells are the first to come in contact with host blood. To the obstetrician they are of concern in relation to the pathogenesis of eclampsia. To the gynecologist they are of concern in relation to carcinoma in situ of the cervix. To the infectious disease expert they represent selected targets of certain parasites. To the cell biologist they offer countless challenges, ranging from phagocytosis and transcytosis to the more complex process of "activation" to applications of molecular biology, and to the vast field of angiogenesis. Still unsolved is the problem of endothelial diversity between different organs and between different segments of the microcirculation: when that phenomenon will be understood, it might become possible to target drugs to given organs. The only cinderella in this story is the lymphatic endothelium, which appears to have been forgotten.

Regulation of Endothelial Cell Growth

CN 002 KERATINOCYTE GROWTH FACTOR, A NEW FGF FAMILY MEMBER, Stuart A. Aaronson, Donald Bottaro, Dina Ron, Christopher Molloy and Jeffrey Rubin, LCMB, NCI, Bethesda, MD 20892

A heparin binding growth factor specific for epithelial cells and designated keratinocyte growth factor was initially identified and purified from conditioned medium of human embryonic fibroblasts. Its complementary DNA sequence demonstrates around 40% predicted amino acid sequence homology in conserved regions with the FGFs, establishing KGF as a new member of this growth factor family. The KGF transcript is expressed in stromal cells derived from most epithelial tissues. By comparison with other epithelial cell mitogens, only KGF has properties of a stromal mediator of epithelial cell proliferation

Prokaryotic expression of recombinant KGF has made it possible to produce large quantities of this biologically active mitogen. This system is being utilized for structure-function analysis of KGF mutants as well as recombinants with other FGF family members. Efforts to isolate and characterize the KGF receptor as well as investigate the involvement of KGF in autocrine or paracrine stimulation of human tumors will be presented. Finally, comparisons of mitogenic signalling by FGF family members and growth factors such as PDGF and EGF have revealed common as well as distinct substrates of their activated receptor tyrosine kinases. Efforts to dissect the role of specific substrates in different signalling pathways triggered by such growth factors will be discussed.

CN 003 STRUCTURE-FUNCTION STUDIES OF HEPARIN BINDING (ACIDIC FIBROBLAST) GROWTH FACTOR USING SITE-DIRECTED MUTAGENESIS AND cDNA TRANSFECTION ANALYSIS,
Wilson H. Burgess and Jeffrey A. Winkles, Molecular Biology Laboratory, Jerome H. Holland Laboratory, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.
The potential importance of the heparin binding or fibroblast growth factors (HBGFs) as regulators of cell growth and migration, angiogenesis, wound repair, neurite extension and mesoderm induction has become apparent over the last few years. As many as five newly identified and related proteins have been described, three of which are the products of cellular oncogenes. At least two receptors for the HBGFs have been identified. The receptor tyrosine kinase activity has been established and specific substrates involved in signal transduction have been identified. The long term goal of our research is to provide a rigorous structural basis for the mechanisms of action of the HBGF family of polypeptides. A better understanding of the structural basis for the different functions of these proteins should facilitate the development of agonists and antagonists of specific HBGF activities.

Chemical modification studies of Harper and Lobb (1) implicated lysine 132 in HBGF-1 as being important to the heparin binding, receptor binding and mitogenesis activities of the protein. We have changed lysine 132 to a glutamic acid by site-directed mutagenesis of the human cDNA and expressed the mutant protein in E coli to obtain sufficient protein for functional studies. Replacement of this lysine with glutamic acid reduces the apparent affinity of HBGF-1 for immobilized heparin (elutes at ~.45 M NaCl vs. 1.0-1.1 M for wild type). Mitogenic assays establish two points: 1) human recombinant HBGF-1 is highly dependent on the presence of heparin for optimal mitogenic activity and 2) the change of lysine 132 to glutamic acid lowers the specific mitogenic activity of HBGF-1 by at least 30-fold. Interestingly, we cannot detect any significant differences in the receptor binding properties of the mutant HBGF-1. Despite these observations, the mutant HBGF-1 displays no antagonist activity when assayed with wild type HBGF-1. Differences in the transforming potentials and subcellular localization of the wild type and mutant HBGF-1 also were observed.

1) Harper, J.W. and Lobb, R.R. 1988. Biochemistry 27:671-678.

CN 004 FIBROBLAST GROWTH FACTOR-5 BIOSYNTEHSIS: COMPLEX TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL SUGGESTS MULTIPLE FUNCTIONS IN DEVELOPING AND ADULT MICE, Olivia Haub, Brian Bates, and Mitchell Goldfarb, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Fibroblast growth factors (FGFs) constitute a homologous set of mitogenic proteins. Each FGF, of which seven are now known, is encoded by a distinct gene. We are exploring the possible functions of FGF-5, a factor discovered as the product of a human oncogene.

Western blot analysis demonstrates that human FGF-5 is secreted from producer cells as glycoprotein molecules of heterogenous molecular weight. Inefficient translation of FGF-5 mRNA adversely affects levels of FGF-5 secreted. An additional open reading frame (ORF-1) in the mRNA upstream and overlapping the FGF-5 coding sequence is responsible for a 20-fold inhibition of translation assayed in vitro. Analysis of point mutations shows that each of two AUG codons in ORF-1 contributes to translational inhibition, while the ORF-1 terminator codon subtly promotes FGF-5 synthesis. Hence, FGF-5 is translated by ribosome scanning past upstream AUG codons and, perhaps, by reinitiation following ORF-1 translation. The conservation of ORF-1 in the murine FGF-5 transcript suggests that ORF-1 exists to provide regulation of FGF-5 translation.

Northern blot and in situ hybridizations have been used to detect FGF-5 mRNA in adult mice and mouse embryos. In the adult, FGF-5 expression is restricted to regions of the central nervous system. Areas of expression include the piriform cortex, olfactory tubule, the dentate gyrus, and the pyramidal cell layer in the CA3 region of the hippocampus, all areas of high neuron cell density. Such a profile suggests that much of FGF-5 is synthesized by a subset of neurons in the brain. During development, FGF-5 expression has been detected in cells which are progenitors to the nervous system as well as to other tissues. This expression profile shows that FGF-5 performs many distinct functions in the developing and mature animal. Experimental methods being employed to determine these functions shall be discussed.

Mechanisms of Endothelial Cell Activation and Homing-I

CN 005 ENDOTHELIAL CELL SURFACE ADHESION MOLECULES IN INFLAMMATION AND METASTASIS, Michael P. Bevilacqua, Department of Pathology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115.

Adhesion of blood leukocytes to the vessel wall appears to depend in part on the de novo expression of endothelial cell surface adhesion molecules. Endothelial leukocyte adhesion molecule 1 (ELAM-1) is a cell surface glycoprotein expressed by cytokine-activated endothelium that mediates neutrophil binding.[1] A full-length complementary DNA for ELAM-1 was isolated by transient expression in COS cells.[2] The primary sequence of ELAM-1 predicts an unusual mosaic structure with an amino-terminal lectin-like domain, an EGF domain, and 6 tandem repeats (~60 amino acids each) related to those found in complement regulatory proteins. A similar domain structure is also found in the MEL-14 lymphocyte cell surface homing receptor and in granule membrane protein 140 (GMP-140), a membrane glycoprotein of platelet and endothelial secretory granules that can be rapidly mobilized (less than 5 minutes) to the cell surface by thrombin and other stimuli. Endothelial GMP-140 also appears to function as a neutrophil adhesion molecule.[3] Together ELAM-1, GMP-140, and MEL-14 define a new family of structurally related molecules, designated SELECTINS, in which the initial members all function in endothelial-leukocyte adhesion.

Hematogenous metastasis requires the arrest and extravasation of blood borne tumor cells, often involving direct adhesive interactions with vascular endothelium. Cytokine activation of endothelial cells also increases the adhesion of malignant melanoma and carcinoma cell lines. An inducible 110 kD endothelial cell surface glycoprotein (INCAM-110) was found to support melanoma cell adhesion.[4] INCAM-110 was also shown to mediate the adhesion of lymphocytes and monocytes suggesting that tumor cells can recognize endothelial molecules that function normally in leukocyte adhesion.[5] This concept was further supported by the observation that ELAM-1 mediates the binding of a colon carcinoma. Thus, activation of endothelium results in the expression of cell surface molecules that support the adhesion of blood leukocytes. In some cases, circulating tumor cells may utilize these molecules to accomplish one of the essential steps of metastasis.

1. Bevilacqua, MP, et al., Proc. Natl. Acad. Sci. USA. 84:9238, 1987.
2. Bevilacqua, MP, et al., Science 243:1160, 1989.
3. Geng, J-G, et al., Nature 1990, in press.
4. Rice, GE and Bevilacqua MP, Science 246:1303, 1989.
5. Rice, GE, et al., J. Exp. Med. 1990, in press.

CN 006 MOLECULAR AND CELL BIOLOGY OF GMP-140, Rodger P. McEver, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

GMP-140 (granule membrane protein-140) is a membrane glycoprotein of apparent Mr 140,000 located in α-granules of human platelets and Weibel-Palade bodies of human endothelial cells. When these cells are activated by agonists such as thrombin, GMP-140 is rapidly redistributed to the cell surface during fusion of granule membranes with the plasma membrane. In endothelial cells, maximal surface expression of GMP-140 occurs 5 minutes after activation, declining to basal values over the next 20 minutes because of apparent endocytosis. The cDNA-derived primary structure of GMP-140 predicts a cysteine-rich protein containing several domains. These include an N-terminal domain homologous to Ca^{2+}-dependent lectins, followed by an EGF-type module, nine consensus repeats similar to those in complement-binding proteins, a transmembrane domain, and a short cytoplasmic tail. The gene for human GMP-140 has been mapped by in situ hybridization to bands q21-24 of human chromosome 1; it spans 40 kb and consists of 17 exons. Individual exons encode the lectin domain, the EGF domain, and each of the nine consensus repeats. Some cDNAs contain an in-frame deletion of the seventh consensus repeat, predicting an 8-repeat variant of GMP-140. Other cDNAs have an in-frame deletion of the transmembrane domain, predicting a soluble form of the molecule. Both deletions are encoded by specific exons, suggesting that the variants arise by alternative splicing of mRNA. GMP-140 shares sequence similarity and domain organization with ELAM-1, a cytokine-inducible endothelial cell receptor for neutrophils, and the MEL 14 lymphocyte homing receptor which is also found on neutrophils and monocytes. These three molecules define a new gene family termed selectins. Like ELAM-1 and MEL 14, GMP-140 mediates interactions of leukocytes with other vascular cells. COS cells transfected with GMP-140 cDNA support adhesion of neutrophils and HL-60 cells that is inhibited by monclonal antibodies (mAb) to GMP-140. Neutrophils and HL-60 cells also bind specifically to purified platelet GMP-140 immobilized on microtiter wells. Adhesion requires Ca^{2+} but not active neutrophil metabolism, and is inhibited by mAb to GMP-140 and by purified GMP-140. Within minutes after stimulation with phorbol esters or histamine, cultured human endothelium becomes adhesive for neutrophils; the neutrophil binding is blocked by mAb to GMP-140. Similarly, thrombin-stimulated platelets form Ca^{2+}-dependent rosettes around neutrophils, HL-60 cells, and U937 cells; this adhesive interaction is blocked by mAb to GMP-140 and by purified GMP-140. By mediating rapid interaction of leukocytes with both activated platelets and endothelium, GMP-140 may provide an important link between the hemostatic and inflammatory responses to tissue injury.

CN 007 MOLECULAR CLONING OF PECAM-1 (CD31): A NOVEL MEMBER OF THE IMMUNOGLOBULIN GENE SUPERFAMILY EXPRESSED ON HUMAN PLATELETS AND AT ENDOTHELIAL CELL INTERCELLULAR JUNCTIONS. Peter J. Newman and William A. Muller, The Blood Center of Southeastern Wisconsin, Milwaukee, WI 53233 and Rockefeller University, New York, NY. A new component of the platelet/endothelial cell surface, termed PECAM-1, has been identified. Primary sequence analysis of this 130,000 MW glycoprotein revealed the presence of 6 extracellular immunoglobulin (Ig)-like homology domains, each domain stabilized by characteristic disulfide-bonded cysteines approximately 50 residues apart. PECAM-1 has a single putative membrane-spanning region connected to a 118 amino acid cytoplasmic domain that contains one potential tyrosine phosphorylation site near the carboxyl-terminus. Alignment of the immunoglobulin-like domains with other members of the Ig-superfamily showed that PECAM-1 is structurally most related to the cell adhesion molecule (CAM) family of receptors. In order to more precisely determine a functional role for PECAM-1, a series of monoclonal antibodies previously raised against platelet and endothelial cell surface glycoproteins of similar size and pI were tested for their ability to react with PECAM-1. Two of these monoclonal antibodies, anti-CD31 and anti-hec7, reacted strongly with a platelet protein that co-migrated on SDS-PAGE gels with PECAM-1. CD31 is a leukocyte differentiation antigen of uncharacterized structure and function that has been reported to be present on human platelets, endothelial cells, granulocytes, and monocytes, whereas the hec7 antigen has recently been localized to the intercellular junctions of human endothelium. In order to confirm the relatedness of these three components, the hec7 and CD31 antigens were immunoprecipitated from human endothelial cell lysates by their respective monoclonal antibodies and analyzed by immunoblotting with an anti-PECAM-1 antiserum. Our results demonstrate that PECAM-1 is immunologically related or identical to the previously described CD31 and hec7 antigens. Studies to more precisely define the immunologic epitopes on PECAM-1 recognized by these monoclonal antibodies are in progress. In the meantime, the availability of the primary sequence of PECAM-1 should prove valuable in studying differentiation and lineage relationships of CD31 positive cell types, and will also make possible further definition of the role of hec7 in cellular adhesive interactions. Finally, it has recently been shown that several members of the Ig superfamily can serve as receptors for a number of clinically important viruses. Thus, the major rhinovirus receptor is ICAM-1, the receptor for HIV is CD4, and the poliovirus receptor is a cell surface glycoprotein containing 3 immunoglobulin-like loop domains. Although it is clearly not the function, but rather the misfortune of these Ig-like molecules to act as viral receptors, the possibility remains that other glycoproteins comprised of Ig homology units may also serve as a means for viruses to escape normal host immune surveillance, leading to enhanced viral survival and subsequent infection. Whether PECAM-1 plays a role in the entry of viruses into platelets and endothelial cells remains an intriguing avenue of future investigation.

CN 008 PLATELET-DERIVED GROWTH FACTOR, Russell Ross, Department of Pathology, School of Medicine, University of Washington, Seattle, WA 98195

Platelet-derived growth factor (PDGF) is a ubiquitous growth-regulatory molecule formed by many cells. It is found in storage granules in platelets and endothelial cells, and can be synthesized by activated macrophages, endothelial cells, smooth muscle cells, fibroblasts, astrocytes, and numerous tumor cells. Three dimeric forms of PDGF are derived from the A- and B-chains. These disulfide-bonded dimers bind in a specific manner to three dimeric forms of the receptor derived from alpha and beta PDGF receptor subunits. Different numbers of alpha and beta receptor subunit numbers are present on different cell types. These differences determine the relative mitogenic effects of the different dimeric forms of PDGF on each given cell. PDGF exerts many functions, ranging from chemotaxis and mitogenesis to augmented antigen presentation, protein synthesis, collagen degradation, and numerous other activities. It is a key molecule in numerous disease entities, such as neoplasia and atherosclerosis, as well as in the augmentation of normal activities such as wound healing and embryogenesis. Newer aspects of the molecule, its receptors, and the role of this molecule in health and disease will be discussed.

Mechanisms of Endothelial Cell Activation and Homing-II

CN 009 PRIMATE HOMOLOGUES OF MEL-14 AND CD44: ROLE IN ADHESION TO ENDOTHELIUM AND LYMPHOCYTE TRAFFIC, W. Michael Gallatin, Patricia Hoffman, Dennis Willerford, Stephen Rosenman, Joey Meyer, and Thomas St. John, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Many cells of hemopoietic origin, particularly lymphocytes, move continuously between the various lymphoid tissues to reach specialized microenvironments presented by each organ. Transmigration across post-capillary venules in organized lymphoid organs and at sites of inflammation is a multistep process that can be divided conceptually into (1) attachment to endothelial cells (EC), (2) lateral movement on the surface of EC, (3) transmigration between EC, (4) penetration of the basement membrane, (5) release, and (6) intraorgan sorting into appropriate microenvironments (e.g. T vs. B). The initial phase of this movement, attachment, is orchestrated by organ-specific adhesion of the trafficking cells to the endothelial lining of the microvasculature at each site. In lymph nodes, tonsils etc. microvascular EC support this process constitutively while at sites of inflammation pro-inflammatory cytokines induce a "traffic" phenotype. For example, we have found that lymphocyte adhesion to microvascular EC is markedly enhance by pretreatment of the EC with IL-4. Traffic is also modulated as a function of lymphocyte activation by varied expression of at least three distinct classes of lymphocyte cell adhesion molecules (CAMS): integrins (e.g. LFA-1), the CD44 glycoproteins, and selectins (e.g. Mel-14, LAM-1). For example, lack of Mel-14/LAM-1 on IL-2 dependent, CD8+ cytotoxic T cells propagated in vitro, may contribute to poor recirculation and limit therapeutic utility in vivo. We have used a primate model to analyze the structure and function of both CD44 and Mel-14 (LAM-1) type CAMs. In macaques expression of these structures by normal PBL correlated with their ability recirculate in vivo and to adhere to lymphokine induced cultured EC in vitro. T cells could be placed in a maturational sequence of noncycling virgin, actively cycling, and resting memory cells according to expression of these receptors. These stages of activation/maturation were anatomically partitioned in vivo. High-level CD44 expression was also correlated with an increased responsiveness to mitogenic stimuli transmitted via CD3. This may involve a direct contribution of CD44 to the activation cascade. To facilitate structure/function analyses of CD44 in each of these processes and to test the relative contributions of CD44 and Mel-14/LAM-1 type CAMs to EC binding, we isolated cDNAs encoding the macaque homologues of each. In both cases cross-species comparison of primate, human, and murine genes reveals a high degree of conservation. Functional analyses of cells expressing CD44 or macaque LAM-1 (LAM-1_{mac}) de novo following plasmid and retrovirus mediated gene transfer indicate that CD44 expression per se is insufficient to mediate lymphocyte adhesion to EC. Nonetheless, CD44 can promote cell-cell adhesion in other contexts and may serve as a facilitating CAM for lymphocyte-EC attachment. In contrast, a more direct functional correlation with LAM-1_{mac} expression was observed. Constitutive expression of the latter in IL-2 dependent T cell clones may provide a means to enhance recirculation and effector function in a clinical setting.

CN 010 STRUCTURE-FUNCTION ASPECTS OF THE PERIPHERAL LYMPH NODE HOMING RECEPTOR TYPE LECTIN-CELL ADHESION MOLECULE, Laurence Lasky, Susan Watson, Benjamin Bowen, Christopher Fennie, Donald Dowbenko, Joyce Geoffrey, Mark Singer, and Steven Rosen, Dept. of Cardiovascular Research, Genentech, Inc., 460 Pt. San Bruno Blvd., S.S.F., CA 94080, and Dept of Anatomy, University of California, Sanfrancisco, CA 94143
Lymphocytes traffic to peripheral lymph nodes (PLN) by utilizing a lectin-like adhesion molecule termed the PLN homing receptor. cDNA cloning of the murine and human forms of these molecules has revealed that they are constructed from three different protein motifs which include a type C lectin domain, and egf-like domain, and two copies of a complement regulatory motif. Gene structure analysis has revealed that the different protein motifs are encoded by seperate exons localized on chromosome 1 of the mouse. Analysis of homing receptor reactivity with the adhesion blocking monoclonal antibody, MEL 14, has shown that this antibody recognizes an epitope within the N-terminal 53 amino acids of the lectin domain whose conformation appears to be dependant upon the presence of the adjacent egf-like domain, suggesting that the lectin domain is involved in adhesion to and recognition of the PLN endothelium. Production of chimeric molecules containing either all, or a subset, of these protein domains and the human IgG1 constant region were used in sugar binding, cell blocking, and immunohistochemical experiments. These results showed that these chimeric molecules could be utilized as reagents for the analysis of the roles of each of these domains in PLN homing receptor function.

Endothelial Cell Homeostatic Functions

CN 011 STRUCTURE, FUNCTION AND REGULATION OF THE PROTEIN C ANTICOAGULANT PATHWAY. Charles T. Esmon, Howard Hughes Medical Institute, Oklahoma Medical Research Foundation, and Departments of Pathology and Biochemistry, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

The endothelial cell possesses a receptor, thrombomodulin (TM), that binds thrombin and alters its function. When bound to TM, thrombin activates the anticoagulant, protein C, but no longer possesses the procoagulant functions. With the cloning of TM, it became possible to examine structure function correlates in TM in an attempt to delineate the mechanism of these changes. TM is an integral membrane protein with several apparent domains. Limited proteolysis revealed that the thrombin binding domain is located in the growth factor repeats and that an 80 residue region corresponding to the 5th and 6th growth factor repeats was all that was required for thrombin binding. Though this region did not accelerate protein C activation, it retained the ability to inhibit fibrinogen clotting. A domain composed of 4.5 of the 6 growth factor repeats was all that was required for protein C activation. Thrombin bound to TM is located about 70Å from the membrane surface.

For activated protein C to function, it must interact with cell surfaces. Platelets and endothelial cells can provide these sites, but the binding interaction is dependent on protein S, a second vitamin K-dependent protein in this pathway. The protein S circulates both free and in complex with C4b-binding protein (C4bBP). The latter behaves as an acute phase protein. Only the free form of protein S supports activated protein C anticoagulant activity. Cytokines and endotoxin inhibit both the expression of TM and protein S activity. This led us to investigate the role of activated protein C, C4bBP and coagulation in processes thought to involve inflammatory mediators such as gram negative septic shock. Activated protein C protects the animals and prevents the consumptive coagulation in response to bacterial infusion. Elevation of C4b-binding protein to the high levels obtained in such disease processes increases sensitivity to challenge. The protection obtained with activated protein C does not appear to be due to its anticoagulant response, since active site blocked factor Xa, which is a better inhibitor of coagulation *in vivo* than activated protein C, prevents the coagulation response without altering the shock response. Thus, this pathway appears to modulate both coagulation and inflammation.

CN 012 THE BIOCHEMISTRY AND MOLECULAR BIOLOGY OF TYPE 1 PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1), A MAJOR BIOSYNTHETIC PRODUCT OF CULTURED ENDOTHELIAL CELLS. David J. Loskutoff, Committee for Vascular Biology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Plasminogen activation provides an important source of localized proteolytic activity during fibrinolysis, ovulation, cell migration, epithelial cell differentiation, and tumor cell invasion. Precise regulation of plasminogen activator (PA) activity thus constitutes a critical feature of many biological processes. This control is achieved in large part through the action of PAI-1, an efficient, endothelial cell (EC)-derived inhibitor of both urokinase and tissue-type PA. PAI-1 accounts for as much as 12% of the total protein released by ECs, and is also a major component of their extracellular matrix (ECM). Little PAI-1 can be detected on the cell surface using immunogold electron microscopy. Although newly synthesized, intracellular PAI-1 is rapidly glycosylated, glycosylation is not required for PAI-1 secretion or activity. PAI-1 is produced by ECs in an active form, but is rapidly converted into an inactive (latent) form upon secretion. The specific binding of PAI-1 to ECM, or to binding proteins in blood, protects it from this inactivation. One binding protein has been purified using affinity chromatography on PAI-1 Sepharose and shown to be the adhesive glycoprotein vitronectin. The production of PAI-1, and its deposition into ECM, is stimulated by endotoxin, interleukin-1, tumor necrosis factor, transforming growth factor β, and dexamethasone. These increases are relatively specific for PAI-1, and result primarily from an increased rate of transcription of the PAI-1 gene rather than from increased stability of PAI-1 mRNA. The PAI-1 gene itself is 12.2 kilo-base pairs in length and is organized into nine exons and eight introns. Transfection experiments indicate that the 5'-flanking region of the PAI-1 gene contains the DNA sequences necessary for stimulation by the above agents, and for the tissue-specific expression of PAI-1 in vitro. We are continuing to characterize this region by linker scan analysis, DNA footprinting and gel mobility shift experiments, and through the use of transgenic mice.

CN 013 REGULATION OF TISSUE FACTOR, James H. Morrissey, Cardiovascular Biology Research, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.
Tissue factor is the cell surface receptor for coagulation factor VII and is the major cellular trigger of the coagulation protease cascade. Tissue factor has a complex tissue distribution but is normally absent from vascular cells. However, it can be induced to appear on vascular and endothelial cells and monocytes in response to specific cytokines and other inflammatory stimuli. In monocytes, induced expression of the tissue factor gene is primarily controlled at the level of transcription. Recent determination of the sequence of the human tissue factor gene has now permitted initial characterization of promoter elements responsible for induced expression of tissue factor by growth factors.

Endothelial Cell Metabolic Functions

CN 014 ORGANSPECIFIC DIFFERENTIATION IN VITRO: INTERACTIONS BETWEEN ENDOTHELIAL CELLS AND NEURAL CREST-DERIVED SECRETORY CELLS. P.I. Lelkes, Laboratory of Cell Biology, Dept. Medicine, University of Wisconsin Med.School, Milwaukee, WI. 53201, U.S.A.
The regulatory mechanisms for organspecific differentiation of vascular endothelial cells (EC) are not known. Well known is the outcome: an endothelial cell is not an endothelial cell! Besides obvious morphological and probably also functional differences, there are variations between EC of the same phenotype, as manifested for example in the occurrence of organspecific cell-surface antigens. Our working hypothesis predicts, that organspecificity is a manifestation of interactions between the various cell types, e.g. EC and parenchymal cells, within a given organ. Since at this point we do not have reliable markers for EC differentiation, we have primarily focussed on the role of endothelial cells in the organspecific differentiation of parenchymal cells. Our model system is the adrenal medulla. The final fate and differentiation of the neural crest-derived chromaffin cells in this gland are modulated by microenvironmental stimuli, e.g. signals contained in the extracellular matrix and by humoral factors such as growth and trophic factors. Part of these stimuli might be derived from capillary EC which, during early stages of development, are closely juxtaposed to the secretory cells. To test our hypothesis, we have developed an *in vitro* model system by coculturing adrenal medullary pheochromocytoma (PC12) and endothelial cells. PC12 cells are exceptionally suitable for these studies, since, depending on environmental stimuli, such as NGF or dexamethasone, PC12 cells can differentiate either into sympathetic neurons or into a chromaffin cell like phenotype. When cocultured with EC derived from the adrenal medulla, PC12 cells will differentiate into a chromaffin cell-like phenotype, as assessed by morphological, biochemical and molecular biological criteria. The signals involved are complex and are at least in part residing in the cell membranes of the two cell types. Under these coculture conditions the endothelial cells will undergo significant alterations in their morphology and in protein expression, suggesting that stimuli derived from parenchymal cells will contribute to their organspecific differentiation e.g. into fenestrated capillaries. By contrast, when cocultured with endocardium-derived EC, PC12 cells were found to extend neurites; concomitantly, the endocardial EC changed their morphology to a more epithelial cell-like. The factor(s) causing neurite outgrowth in PC12 cells are humoral in nature, since the sames effect can be observed when growing PC12 cells physically separated from the endocardial EC in a Millicell system or in the presence of conditioned medium from endocardial EC. We propose that during development vascular endothelial cells and parenchymal cells might communicate via membrane-membrane contacts and/or humoral signals and that these cues reciprocally modulate organspecific differentiation.

CN 015 BIOLOGICAL ACTIVITY OF ENDOTHELIN, Tomoh Masaki, Masashi Yanagisawa, Akihiro Inoue, Yoh Takuwa*, Katsutoshi Goto and Sadao Kimura, Institute of Basic Medical Sciences and Institute of Clinical Medicine*, University of Tsukuba, Tsukuba, Ibaraki 305, JAPAN

Endothelin (ET) is a potent vasoconstrictor peptide produced by vascular endothelial cell. This peptide has attracted attention of many investigators, because of its unique structural and pharmacological properties. ET comprises 21 amino acid residues including four cystein residues forming two disulfide bonds. We could not detect similar sequence in the known peptides of mammalian origin. Analysis of human genomic gene of ET revealed the existence of three distinct endothelin genes, encoding three distinct ET-like peptides. These were designated as ET-1. ET-2 and ET-3. ET-1 is the original ET isolated from the conditioned medium of the cultured porcine aortic endothelial cell. In addition, sarafotoxin S6b, a snake venom of Israeli burrowing asp, comprises also 21 amino acid residues and 14 out of 21 residues are the same as that of ET. The three distinct mRNAs of ET are expressed in various tissues in different proportions. ET elicits slow developing and long-lasting vasoconstriction in almost all arteries and veins. An intravenous bolus injection of ET into rat causes initial transient depressor response followed by a sustained long-lasting pressor response. ET is a potent mitogen which is demonstrated in several cell types including cultured vascular smooth muscle cells, fibroblasts, glomerular mesangial cells and osteoblasts. ET stimulated incorporation of thymidine into DNA of these cells. ET exerts synergistic effects with other growth factors including insulin like growth factor, epidermal growth factor and transforming growth factor α. The mitotic effect is likely mediated by stimulation of phospholipase C via a receptor coupled with G-protein, and subsequent activation of protein kinase C. Analysis of ET gene detected the TPA-responsive element, mRNA stabilizing sequence, motifs for binding site of nuclear factor 1 and acute phase reactant regulatory elements in preproform of ET-1. Indeed, northern blot analysis in cultured endothelial cells from human umbilical veins showed that prepro ET-1 mRNA was rapidly induced by the active phorbolester 12-o-tetradecanoylphorbol 13-acetate within 10 min. Incubation of the serum-derived confluent porcine aortic endothelial cell with transforming growth factor β1, resulted in a several fold increased in ET-mRNA, and induced the release of ET from the cells. The effect of endothelin may contribute to the cell-proliferative response under various physiological and pathological conditions such as wound healing and development of atherosclerosis and glomerulonephritis.

CN 016 ENDOTHELIUM-DERIVED VASOACTIVE FACTORS, Gabor M. Rubanyi, Berlex Laboratories, Inc., 110 East Hanover Avenue, Cedar Knolls, NJ 07927

The discovery of endothelium-derived relaxing factor (EDRF) by Furchgott and Zawadski in 1980 focused attention on the role of endothelial cells (EC) in the control of vascular tone. EDRF can be released from EC by a variety of drugs, endogenous mediators and also by increases in shear stress. The existence of more than one, chemically distinct, EDRFs has been proposed. Accumulating evidence suggests that at least one of the EDRFs is identical with nitric oxide (NO) or a labile nitroso compound, which is produced from L-arginine by an NADPH-dependent enzyme. Signal transduction pathway(s) in EC leading to synthesis/release of EDRF have not been fully identified yet, but a role of G-proteins, Ca^{2+} and changes in membrane potential have been proposed. The target of EDRF (NO) is soluble guanylate cyclase, the stimulation of which results in an increase in cyclic GMP. EDRF (NO) affects not only the underlying vascular smooth muscle, but also platelets, inhibiting their aggregation and adhesion to EC. The antiaggregatory effect of EDRF is synergistic with prostacyclin, so their combined release from EC may represent a physiological mechanism aimed at preventing thrombus formation. An additional proposed biological function of EDRF (NO) is cytoprotection by virtue of scavenging superoxide radicals. Production of NO has been reported to occur in cells other than EC (e.g., neutrophils, monocytes and neurons), suggesting a more widespread role of this mediator. The endothelium can also mediate vasoconstriction by the release of a variety of endothelium-derived contracting factors (EDCF). Drugs, endogenous mediators, physical forces (pressure) and hypoxia can evoke endothelium-dependent vasoconstriction in a variety of blood vessels. Other than the polypeptide endothelin, the nature of EDCFs has not yet been firmly established. Autoregulation of cerebral and renal blood flow and hypoxic pulmonary vasoconstriction may represent the *physiological* role of endothelium-dependent vasoconstriction. In physiological states, a delicate balance exists between endothelium-derived vasodilators and vasoconstrictors. Alterations in this balance can result in local (vasospasm) and generalized (hypertension) increase in vascular tone and also in facilitated thrombus formation. Endothelial dysfunction (decreased EDRF and PGI_2 release and maintained or enhanced production of EDCFs) may also contribute to the pathophysiology of angiopathies associated with hypercholesterolemia, atherosclerosis and diabetes.

Endothelial Cell Pathology-I

CN 017 IMMUNE AND INFLAMMATORY FUNCTIONS OF VASCULAR WALL CELLS Peter Libby, Harald Loppnow, Robert N. Salomon. Departments of Medicine, Pathology, and Cellular and Molecular Physiology, Tufts University, School of Medicine and New England Medical Center, Boston, MA 02111

Many aspects of the pathogenesis of inflammation and immune-mediated injury involve blood vessels. The intrinsic cells of the blood vessel wall, endothelium and smooth muscle, have long been regarded as targets for immune and inflammatory responses. Recent studies have established that these cells can also play active rôles in immune and inflammatory responses. For example, endothelial and smooth muscle cells can produce mediators whose elaboration was previously believed limited to leukocytes. Both vascular cell types can express genes that encode both isoforms of interleukin (IL) 1, a cytokine that stimulates T- and B-lymphocytes among many other important biological actions. Smooth muscle cells under some circumstances can also express the gene for tumor necrosis factor (TNF), an inflammatory mediator functionally related to IL1. Both vascular cell types can also elaborate IL6, a B- and T- cell stimulator. Endothelial cells can produce IL8 and IL9, factors that attract polymorphonuclear leukocytes and mononuclear phagocytes respectively. Vascular endothelial cells respond to cytokines such as IL1 and TNF by increasing adhesivity for leukocytes and altering their hemostatic balance to favor blood clot formation and stabilization. Thus, local production of these mediators by intrinsic vascular wall cells and/or infiltrating leukocytes may alter important homeostatic functions within blood vessels. Both vascular endothelial and smooth muscle cells can express transplantation antigens, notably Class II major histocompatibility molecules which are obligatorily involved in recognition events involving helper T cells. In fact, vascular endothelial and smooth muscle cells can effectively present antigens to helper T cells. In summary, vascular wall cells are not only targets of immune and inflammatory mediators, but may participate actively in regional inflammation and immunity by presenting antigens and elaborating multipotent molecules that modulate these important host defense mechanisms.

CN 018 DEVELOPMENTAL MECHANISMS IN VASCULAR PATHOLOGY, Stephen M. Schwartz, Ronald Heimark, Mark Majesky and John Medina, Department of Pathology, University of Washington, Seattle, WA 98195.

Mechanisms that are important in the development of blood vessels may also underlie major parts of the pathological processes involved in atherosclerosis, angiogenesis, and hypertension.

Studies in the chicken, as well as our recent work, show that isolated endothelial cells line up in a pattern that presages the future pattern of the dorsal aortae. Recent work from our laboratory as well as others has identified a likely candidate involved in endothelial assembly: a calcium dependent interendothelial cell adherence molecule we call v-cadherin (v-cad). V-cad is of particular interest because similar molecules are important in the aggregation of other epithelial precursors into primitive organ masses. Treatment of endothelial cells with heparin binding growth factor is sufficient to cause the loss of v-cadherin. FGF treated endothelial cells are unable to form a confluent monolayer. Furthermore, under these conditions, FGF is able to induce the synthesis of its own mRNA. Since, in our cells, cells derived from the bovine aortic endothelium without exposure to fibroblast growth factor, there is no evidence of pituitive synthesis of FGF-mRNA, it is tempting to believe that the induction of FGF from RNA by conditions which disrupt the monolayer is part of a localized autocrine loop that maintains endothelial cell proliferation at wound edges.

Much less is known about the developmental origins of smooth muscle cells. Work done in our laboratory over the last several years, has suggested that at least two different stable phenotypes exist in the vessel wall. One of these, which we will call "pup-like", is characterized by an epithelial growth pattern in culture, and in a similar morphology which can be seen at the surface of neointima formed in vivo. A series of genes specific in one or the other phenotype have been studied. Cells isolated from the neointima produced after balloon injury show the same pattern. Finally, cells with these peculiar properties show greatly diminished growth factor acclimatization in culture, and perhaps correspondingly, can be shown to have a dramatic over-response when the intact vessel is exposed to added growth factors. In summary, it is conceivable that much of the special proliferation seen in pathological vessels is due to the presence of a "proliferagenic subset" of vessel wall smooth muscle cells. In this view, the proliferagenic subset might well account for the important role of cell replication in hypertension during the early developmental stages, as well as for the focality and clonality of atherosclerotic lesions.

CN 019 ENDOTHELIAL CELLS, GROWTH FACTORS AND INFLAMMATORY ARTHRITIS, Ronald L. Wilder, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892.

Endothelial cells play a fundamental role in the pathogenesis of inflammatory arthritides in humans, as well as experimental animals. The aim of this presentation is to review data in support of this concept. The earliest apparent abnormalities in the synovial tissues of patients with rheumatoid arthritis and Lewis rats with streptococcal cell wall-induced arthritis appear to reflect microvascular endothelial cell injury. These abnormalities include: (1) gaps between and swelling of the endothelial cells, (2) platelet aggregation and fibrin thrombus formation, and (3) perivascular accumulation of polymorphonuclear and mononuclear inflammatory cells. These early synovial microscopic endothelial abnormalies are also relected in enhanced expression of various biochemical markers including class II major histocompatibility complex antigens, adhesion molecules, cyclooxygenase and metalloproteinases such collagenase and stromelysin/transin. Cytokines such as interleukin-1 (IL-1), platelet derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), and fibroblast growth factors (aFGF and bFGF) are also present during these early events, and probably play an important role.

The development of severe, chronic, destructive arthritis is dependent upon thymic-derived lymphocytes of the immune system. This phase of disease is accompanied by massive proliferation of cells of the synovial connective tissue stroma (blood vessels and fibroblastlike cells), which results in resorptive destruction of bone and cartilage at the margins of the joint where the proliferative and invasive synovium is joined to bone. Angiogenesis is a prominent microscopic feature of development of these marginal erosions. Biochemically, the tumorlike proliferative and erosive process is accompanied by markedly increased expression of protooncogenes such as FOS and MYC, metalloproteinases such as collagenase and stromelysin/transin, and cytokines such as FGF, PDGF and TGF-beta.

Finally, in addition to the important role of autocrine and paracrine regulatory factors in development of the inflammatory process, recent data in experimental animals demonstrate that neuroendocrine regulatory mechanisms operating through the hypothalamic-pituitary-adrenal axis play a critical counterregulatory role in the development of the disease process. The counterregulatory effects are mediated, in part, by inhibition of endothelial cell activation.

Endothelial Cell Growth and Differentiation

CN 100 **VASCULAR ENDOTHELIAL GROWTH FACTOR: A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR GENE FAMILY**, Judith Abraham, Edmund Tischer, Richard Mitchell, Maria Silva, Denis Gospodarowicz*, and John Fiddes, California Biotechnology Inc., 2450 Bayshore Parkway, Mountain View, CA 94043, and *Cancer Research Institute, University of California, San Francisco, CA 94143.

Cultured bovine pituitary folliculo stellate cells secrete a mitogen with a target cell specificity apparently limited to cells of vascular endothelial origin. Structural analyses indicate that this approximately 45 kD mitogen, provisionally named vascular endothelial growth factor (VEGF), is a homodimer of two 23 kD disulfide-bridged subunits. Amino-terminal sequence information has shown that this factor is identical to vascular permeability factor [Connolly et al., J. Clin. Invest. 84, 1470 (1989)], a protein secreted from tumor cells that transiently increases the fluid permeability of blood vessels. We have obtained bovine and human clones for VEGF, and find that the factor is structurally related to the A and B chains of platelet-derived growth factor (PDGF). This result was surprising, given that the cell types responsive to VEGF are distinct from those responding to PDGF. Comparison of VEGF genomic and cDNA clones indicates that several forms of the coding region exist, due to alternative RNA splicing. Normal cell types expressing VEGF will also be discussed.

CN 101 SYNTHESIS AND PURIFICATION OF HUMAN RECOMBINANT PRO-ENDOTHELIN-1 (PRO-ET-1) FROM E. COLI. Luca Benatti, Lucia Monaco, Paolo Caccia and Paolo Sarmientos. Department of Biotechnology, Farmitalia Carlo Erba, Viale Bezzi 24, 20144 Milano, Italy. Endothelin (ET) is a 21-amino acid peptide isolated from the supernatant of cultured porcine aortic endothelial cells. As many peptide hormones ET-1 is synthesized as a long precursor of 212 aa (prepro-ET-1). Very little is known on the proteolitic pathway of pro-ET. As an initial attempt in studying the secretion and maturation of human ET-1 we synthesized pro-ET-1 in E. coli. Human endothelin cDNA (ET-1) was isolated from a placenta cDNA library using synthetic oligonucleotides. High level of expression of pro-ET1 in E. coli was obtained using an expression vector based on the tryptophan promoter and the Shine-Dalgarno region of the cII protein of phage lambda. Recombinant pro-ET-1 could be easily detected by Coomassie Blue staining and its authenticity was confirmed by protein sequencing and Western blot analysis with antiserum against human mature ET-1. Recombinant pro-ET-1 is insoluble within the bacterial cell and could be solubilized by denaturation in 6M guanidine HCl followed by controlled renaturation. The crude renaturation mixture was further purified using standard chromatographyc techniques. The availability of recombinant pro-ET-1 will allow us to study the mechanism of processing to mature ET-1. Mutants of pro-ET-1 possibly resistant to processing are also being investigated.

CN 102 THE LECTIN WHEAT GERM AGGLUTININ (WGA) INCREASES LEVELS OF DECAY-ACCELERATING FACTOR (DAF), A PHOSPHATIDYLINOSITOL-ANCHORED SURFACE PROTEIN, ON HUMAN ENDOTHELIAL CELLS. R. W. Bryant, C. A. Granzow, M. I. Siegel, R. W. Egan and M. M. Billah. Schering-Plough Research., Bloomfield, NJ. 07003.

A number of cell-surface proteins are anchored by a phosphatidylinositol (PI)-glycan moiety. These proteins can be released by PI-specific phospholipases C . Decay-accelerating factor (DAF) is such a cell-surface protein that protects the cell from inadvertent complement attack by binding to and inactivating C3 and C5 convertases. Endothelial cells have the highest levels of surface DAF among a variety of human cells tested and are a major contributor to the total cell surface area in the vascular compartment. Thus endothelial DAF is likely to be a significant regulator of intravascular complement activation. We have previously found that DAF on human umbilical vein endothelial cells (HUVEC) is predominantly PI-anchored and that the level of endothelial cell DAF is increased greatly (3- to 4-fold) by overnignt treatment of cultures with phorbol myristate acetate (PMA). The induction of DAF by PMA is evident only after 8 hours, requires RNA and protein synthesis and appears to involve activation of protein kinase C. A number of other endothelial cell activators such as endotoxin, tumor necrosis factor and γ-interferon were inactive in stimulating DAF synthesis. WGA (5 to 50 ug/ml), a lectin specific for sialic acid and N-acetyl glucosamine residues, did increase DAF levels 2 to 6 fold when incubated with cultures for at least 8 hours. Succinylated WGA, which has unaltered affinity for N-acetyl glucosamine but which does not bind to sialic acid residues was inactive. Furthermore, a number of other lectins including Ulex europaeus which is known to bind to endothelial cells were much less active. The data suggest that lectin binding to sialic acid-rich glycoproteins on the endothelial cell surface triggers events leading to increased expression of anti-complement DAF.

CN 103 EFFECT OF SEX STEROID HORMONES ON PROLIFERATION OF CULTURED ENDOTHELIAL CELLS, *Jane E. Dahlstrom, Andrew J. Hapel and Neville G. Ardlie, Division* of Clinical Sciences, John Curtin School of Medical Research, Canberra, A.C.T., Australia.
We studied the effect of sex hormones on cultured human umbilical vein endothelial cell proliferation. Cells were incubated in physiological (nM) and pharmacological (μM) concentrations of sex hormones. Proliferation was determined by measuring tritiated thymidine incorporation. It was found that 17β-estradiol, estrone, estriol, tetosterone and progesterone in pharmacological concentrations inhibited proliferation by 30-50% while physiological concentrations of the estrogens enhanced endothelial cell growth up to 140%. Physiological concentrations of testosterone and progesterone had no effect. Pharmacological doses of estrogen combined with testosterone or progesterone further depressed proliferation while the addition of physiological concentrations of testosterone or progesterone to estrogens inhibited the enhanced proliferation of endothelial cells observed with estrogens alone. The maximal effects of the steroids on proliferation were seen with cells grown in the absence of fetal calf serum. The presence of phenol-red in the media did not alter the effects of estrogen on endothelial cell proliferation. Preliminary observations show that mRNAs encoding PDGF A and B chains are both elevated in estradiol treated cell cultures, and our cell cultures proliferate in response to PDGF. Estradiol may play a role in maintaining the endothelium in females and this could explain the reported delay in atherogenesis in females.

The EA.hy926 cell line was established by hybridization of human umbilical vein endothelial (HUVE) cells with A549 cells, a human tumor derived line. It is a clonally pure, continuous cell line that replicates vigorously. EA.hy926 cells have been shown to maintain a wide range of endothelium-specific properties including von Willebrand factor, Weibel Palade bodies, prostacyclin, and many others. We have estimated the fraction of mRNA in EA.hy926 cells that is endothelium-specific by testing randomly selected cDNAs from a library representing all the genes expressed in this cell line. Two clones out of twenty, 10%, represented mRNAs found on blots of RNA from EA.hy926 cells and HUVE cells but not A549 cells or other human cells we tested. Since more than 10 different mRNAs are thought to be expressed in the typical mammalian cell, our data indicate that 10^3 endothelium-specific gene products are expressed in the EA.hy926 cell line (assuming that the tissue-specific mRNAs are not generally present at higher copy numbers than mRNAs for non-differentiated functions). Therefore, many of the gene products that contribute to the specialized functions of endothelium have yet to be defined.

CN 105 REGULATION OF INSULIN-LIKE GROWTH FACTOR-I AND BINDING PROTEIN SECRETION BY ENDOTHELIUM, Corinne M. Gajdusek, Depts. Surgery and Pathology, Children's Hospital, Seattle, WA 98105.
Endothelial cells elaborate growth promoting activities into culture medium that support limited growth of smooth muscle cells and fibroblasts. One of these activites is identified as a member of the insulin-like growth factor family, IGF-I. In this study, we compared the IGF-I and binding protein levels and binding protein species secreted by fetal and adult bovine aortic endothelium. IGF-I was detected by a specific radioimmunoassay using antibodies obtained from the NIDDK and binding proteins by both a polyethylene glycol precipitation assay and ligand blotting. An IGF-I having an apparent M_r of 10,000 was detected in media from both cells after chromatography on a sizing column under acidic conditions. Confluent cultures of fetal cells secreted approximately 3-fold more IGF-I than adult cells while adult cells secreted 3-fold higher levels of binding proteins. The binding protein data were confirmed in ligand blots and indicated secretion of similar species of binding proteins. When subconfluent fetal cells were compared with confluent monolayers for IGF-I secretion, a five-fold increase in detectable IGF-I was found. These data suggest both developmental and density-dependent regulation of IGF-I and binding protein secretion by aortic endothelium. (Supported by NIH Grant 34387).

CN 106 HGBF-1 STIMULATION OF HUMAN ENDOTHELIAL CELLS INDUCES PDGF A-CHAIN GENE EXPRESSION, Cyril G. Gay and Jeffrey A. Winkles, Laboratory of Molecular Biology, American Red Cross, Rockville, MD 20855.

Heparin-binding growth factor 1 (HBGF-1), also known as acidic fibroblast growth factor, is a potent mitogen for vascular endothelial cells. The addition of HBGF-1 to quiescent human umbilical vein endothelial (HUVE) cells rapidly and transiently increases the level of platelet-derived growth factor (PDGF) A-chain mRNA, but has no effect on PDGF B-chain mRNA levels. HBGF-1 induction of PDGF A-chain mRNA occurs in the presence of the protein synthesis inhibitor cycloheximide. Actinomycin D and nuclear run-on experiments indicate that the increase in PDGF A-chain mRNA expression is due, at least in part, to transcriptional activation. Furthermore, immunoprecipitation analysis using PDGF A-chain specific antibodies demonstrates that HBGF-1-stimulated HUVE cells secrete more PDGF than unstimulated cells. Since both HBGF-1 and PDGF-AA are mitogenic for human smooth muscle cells, HBGF-1 may play an important role in the regulation of smooth muscle cell proliferation in vivo.

CN 107 MECA-32, A DIFFERENTIATION MARKER FOR BRAIN ENDOTHELIUM

R. Hallmann; D. Mayer; E.L. Berg and E.C. Butcher.
Dep.Pathology,StanfordMedical School, Stanford CA94305 and the VA Hospital, Palo Alto CA94304.The embryonic brain is avascular early in development. It is then vascularized by ingrowth of blood vessels from the primary vascular plexus surrounding the brain. The vasculature of the brain is thought to differentiate upon receiving inductive signals from the microenvironment to form the blood brain barrier (BBB). This differentiation occurs at embryonic day E16 in the mouse, as the formation of tight junctions between the endothelial cells inhibits the diffusion of proteins from the blood into the brain tissue from that day on. We describe here a monoclonal antibody, MECA-32, that specifically detects endothelium in the mouse. The MECA-32 antigen is a heterodimer with 58kDa and 60kDa subunits. It is expressed on most endothelial cells in the mouse with the exception of adult brain endothelium. Interestingly, the antigen is expressed during early development until E15, and disappears from most brain endothelium at E16. No MECA-32 antigen is found on brain endothelium at E17 or later. Loss of MECA-32 expression thus correlates with the development of the BBB. The antibody MECA-32 should be useful for studying the differentiation of brain endothelium.

CN 108 GROWTH FACTORS INDUCE EXPRESSION of SPARC in BOVINE AORTIC ENDOTHELIAL CELLS. Paula Hasselaar, and Helene Sage, Dept. of Biological Structure, Univ. of Washington, Seattle, WA 98195.

Cellular proliferation, migration, and differentiation are regulated by the coordinate action of several growth factors. Since SPARC (Secreted Protein Acidic and Rich in Cysteine), a 43 kD Ca^{+2}-binding protein, has also been described in association with these processes, we asked whether the expression of SPARC is influenced by growth factors shown to be relevant to vascular endothelium. Cultured bovine aortic endothelial (BAE) cells secreted increased levels of SPARC protein upon incubation with transforming growth factor-ß (TGF-ß). This upregulation was accompanied by cell rounding, a morphology which has been attributed to the action of SPARC. Prolonged incubation of BAE cells with basic fibroblast growth factor (bFGF) resulted in modest increases in SPARC protein levels, but no cell rounding was observed. Platelet-derived growth factor had no effect on the levels of SPARC protein. SPARC mRNA increased shortly after addition of TGF-ß and did not change significantly after 24 h. bFGF also caused an increase in SPARC mRNA 1-3 d after addition. These data suggest a role for SPARC in growth factor-mediated cell responses. Since SPARC induces changes in cell shape, it may act coordinately with TGF-ß and bFGF to alter endothelial cell behaviour.

CN 109 ENDOTHELIAL CELL GROWTH IS STIMULATED BY ANGIOTENSIN II.
Fiona Lyall, MRC Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT, Scotland, UK.

In addition to its rapid vasoconstrictor effect Angiotensin II (Ang II) also raises blood pressure by a slow gradual process. Ang II could have a slow effect by promoting vascular smooth muscle cell growth leading to a gradual onset of hypertrophy with a resulting slow rise in arterial pressure involving a vascular amplifier as described by Folkow and co-workers. We have already shown that Ang II stimulates the growth of vascular smooth muscle cells in culture in a dose dependent manner (1). Under certain pathological conditions such as hyperlipidaemia and hypertension endothelial cell turnover is markedly accelerated.

The present work was designed to examine the effect of Ang II on pig aortic endothelial cell growth. Ang II (maximum dose 10^{-5}M) added to endothelial cell cultures over a period of 6 days, resulted in a 20% increase in cell number. This effect was blocked by the Ang II receptor antagonist saralasin.Because of breakdown of the peptide (measured by radioimmunoassay) in the culture medium the likely effective concentration was probably much higher. The effects of Ang II on cellular proto-oncogene expression (*myc, fos & jun*) will also be presented.

References:
1) Lyall *et al* (1988) *J. Hypertension* **6**: 438-441

CN 110 CHARACTERIZATION OF THE RECEPTOR TO VASCULOTROPIN ON BOVINE CAPILLARY CELLS.
Jean Plouët, Hafida Moukadiri, U 86 INSERM, PARIS 75006

An endothelial cell growth factor has been purified from the conditioned medium of the AtT 20 pituitary cell line. It has been characterized as a homodimer composed of two subunits with M_r of 23 kDa whose amino terminal sequence revealed no significant homology to any known protein. Since it was angiogenic in vivo, and had so far an unique specificity for vascular endothelial cells in vitro, it was named vasculotropin (J. Plouët et al., EMBO J., 1989, 8, 3801). ^{125}I labeled vasculotropin retained its full bioactivity after iodination and bound in a saturable, specific and reversible manner to cell membranes. Scatchard analysis of the equilibrium binding gave apparent Kd of 6 and 50 pM with 900 and 4600 binding sites per cell. Cross-linking experiments induced the formation of a major complex of 220 kDa. These data establish the existence of a receptor for vasculotropin and give an estimation of the size at 175 kDa.

CN 111 TRANSCRIPTIONAL REGULATION OF THE GENE OF BASIC FIBROBLAST GROWTH FACTOR IN CAPILLARY ENDOTHELIAL CELLS,
Herbert A. Weich, Niggi Iberg, Michael Klagsbrun[1] and Judah Folkman[2], Department of Surgery, Children's Hospital and Departments of [1] Biological Chemistry and of [2]Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.
The growth of capillary endothelial cells (BCE) is an important regulatory step towards the formation of capillary blood vessels. The proliferation of these cells can be highly controlled by exogenous polypeptide growth factors such as acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF). It has been shown in earlier experiments that bFGF molecules can be synthesized and stored by vascular endothelial cells, suggesting an important role of this mitogen for autocrine growth stimulation during capillary vessel formation. Here we present evidence for a transient increase of the mRNA encoding bFGF as an early event in the prereplicative phase of BCE exposed to exogenous bFGF (autoinduction), in response to phorbol ester and after treatment with thrombin. We found that stimulation of confluent, quiescent BCE with 2.5 ng/ml recombinant bFGF transiently increased the level of all three bFGF transcripts (7kb, 4kb, and 1.9kb) more than 2-fold within 2 hours. Treatment with 5U/ml thrombin increases the level of bFGF mRNA 2.4-fold within 2 hours, whereas 100 ng/ml TPA increases the level of mRNA 2.6 fold within the first 4 hours. Treatment with 10 ug/ml cycloheximide before and during bFGF induction leads to a non-additive effect of bFGF gene expression after 2 hours, compared to treatment with cycloheximide alone. These results demonstrate the existence of a positive autocrine feedback loop in BCE that may serve as an amplification mechanism of the mitogenic response under various physiological conditions, e.g. wound healing and capillary tissue formation.

CN 112 PURIFICATION AND CULTURE OF THE ENDOTHELIAL CELL COMPONENT OF MURINE BONE MARROW STROMA. Norman S. Wolf, Rui-Gao Fei, Phillip E. Penn, Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195
The stromal cells of the murine bone marrow, as grown out in medium term liquid culture, consist of fibroblasts, macrophages and endothelial cells. The endothelial cell component is of particular interest, since it may be vital in both the arrest of circulating hematopoietic stem cells (as after bone marrow transplantation) and in the production of factors which control the growth and differentiation of the stem cells and their differentiating descendants. We have devised a simple method for purification and growth of the endothelial cells from the stroma, using a magnetic bead/phagocytosis technic. The endothelial cells will not grow in standard liquid culture conditions (MEM-FBS 15%) and require the addition of at least one growth factor. Addition of 10 to 25 units of GM-CSF allows rapid growth of these cells. Other cytokine stimulants are under study. The endothelial cells have be identified by positivity for acid phosphatase, factor VIII associated antigen (VW factor) and Bandeiraea simplicifolia lectin. Studies are underway for adherence of stem cells to the purified endothelial cells.

CN 113 CHICKEN PLATELET-DERIVED GROWTH FACTOR: CLONING AND EXPRESSION IN DEVELOPING EMBRYONIC TISSUES
Hans-Günter Zerwes* and Werner Risau Max-Planck-Institut für Psychiatrie, Abt. Neurochemie D-8033 Martinsried, FRG; *present address: Sandoz AG, CH-4002 Basel, Switzerland

Our previous work had suggested a role for platelet-derived growth factor (PDGF) in the differentiation of the vascular wall. To investigate the pattern of its expression during development we used the chick embryo as a model system. Using the coding region of human PDGF A and B-chain genes as hybridisation probes we cloned fragments of cicken genomic DNA with high homology to the human probes. The nucleotide sequences of the homologous fragments are shown. The chicken A and B-chain sequences seem to correspond to the fourth exon of human A and B chain genes since the putative splice-sites are conserved. These genomic fragments were used as probes in northern blots with RNA from chick embryonic tissues. The A-chain probe recognizes multiple transcripts of different intensities in the range from 2.1 to 5.5 kb whereas the B-chain probe recognizes mRNAs of 2.8 and 3.7 kb.Both chains are expressed as early as day 2.5 of development in the yolk sac and in the embryo proper. Northern blots with RNA from tissues at different stages of development are shown. Experiments to localize the sites of expression by *in situ* hybridization are under way.

Endothelial Cell Activation and Homeostatic Functions

CN 200 SECRETION OF ENDOTHELIAL-LEUKOCYTE ADHESION MOLECULE (ELAM-1) UPON DELETION OF PORTIONS OF ITS CARBOXYL TERMINUS. Tim J. Ahern, Mary A. Shaffer, Dianne S. Sako, Glenn R. Larsen, Genetics Institute, 87 CambridgePark Drive, Cambridge, MA. 02140. The carboxyl terminus of endothelial-leukocyte adhesion molecule (ELAM-1) consists of a 22-amino acid, hydrophobic domain followed by a 32-amino acid, hydrophilic domain. In an effort to identify the portions of the carboxyl terminus necessary for efficient subcellular transport and anchoring of ELAM-1 in the endothelial cell membrane, we have altered these domains by site-directed mutagenesis of the molecularly cloned sequence of ELAM-1. Expression of mature ELAM-1 in mammalian cells produced no detectable ELAM-1 in the conditioned media, as determined by pulse-chase labeling with 35S-methionine. Modifications within the carboxyl terminus of ELAM-1 resulted in expression and secretion of the variant forms. Unlike cells expressing full-length ELAM-1, none of the cells expressing secreted mutant forms bound either neutrophils or HL-60 cells (a promyelocytic precursor of neutrophils), indicating that functional ELAM-1 was not associated with the cell. We conclude that the carboxyl terminal region is required for anchoring ELAM-1 into the cell membrane.

CN 201 ENDOTHELIAL CELL GROWTH FACTOR AND HEPARIN DIMINISHES TISSUE FACTOR ANTIGEN AND ACTIVITY IN ENDOTHELIAL CELLS. Fanny E. Almus, L. Vijaya Mohan Rao and Samuel I. Rapaport, Departments of Medicine and Pathology, UC San Diego, La Jolla, CA 92093
We observed (Thromb Res 50: 339, 1988) that growing primary cultures of human umbilical vein endothelial cells (HUVEC) with endothelial cell growth factor (ECGF), 20 ug/mL and heparin (hep), 90 ug/mL inhibits expression of surface tissue factor (TF) activity after exposing HUVEC to thrombin or phorbol. We have now measured total TF antigen levels after perturbation with thrombin or phorbol in HUVEC grown with or without ECGF-hep. TF antigen in cell extracts was measured by an enzyme-linked immunosorbant assay (ELISA) in a double antibody sandwich technique utilizing rabbit and goat antibodies to human TF. Dilutions of purified human brain apoprotein were used for the calibration curve. Mean ± SD for 3 different primary cultures were: with ECGF-hep, 0.7 ± 0.3 ng/10^6 cells; without ECGF-hep, 4.7 ± 0.9 ng/10^6 cells. Surface TF activity and TF activity after cell lysis were measured as the ability of cells to support F.VIIa-catalyzed activation peptide release from ^{3}H-F.X. Cells grown with ECGF-hep had decreased surface and total TF activity compared to cells from the same culture grown without ECGF-hep. However, the difference was more striking for surface TF activity than for lysed cell TF activity. Growing cells with or without ECGF-hep had no effect upon cell surface phospholipid-like procoagulant activity as evaluated by the ability of monolayers to support activation of prothrombin in the presence of factor Xa and Va. All of our data are compatible with the hypothesis that growing HUVEC with ECGF-hep impairs the ability of HUVEC to synthesize TF apoprotein after perturbation.

CN 202 ISOLATION AND SEQUENCING OF A cDNA CLONE ENCODING FOR THE BOVINE HOMOLOGUE OF CD44. Brad T. Bosworth*, Tom St. John†, W. Michael Gallatin†, and James A. Harp*, *National Animal Disease Center, USDA-Agricultural Research Service, P.O. Box 70, Ames, IA 50010 USA and †Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98104 USA
Circulating lymphocytes in ruminant species exit the bloodstream and enter secondary lymphoid organs by attachment to and migration through post-capillary venules (PCV). Unlike PCV in lymph node paracortex of humans and rodents, paracortical PCV in lymph nodes of ruminants do not have high-walled endothelium. Despite this, it is reasonable to assume that the molecular mechanisms regulating adhesion between circulating lymphocytes and endothelial cells are similar in ruminant and nonruminant species. Using a baboon cDNA clone that encodes for a lymphocyte homing receptor for high-walled endothelium, CD44, we have isolated a clone from a bovine lymphocyte cDNA library. This bovine cDNA clone has a high degree of sequence identity with the primate CD44.

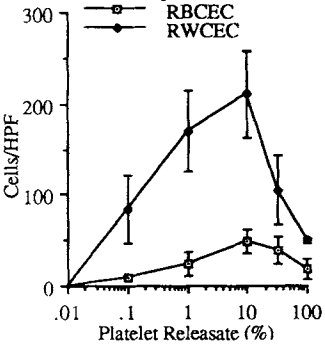
CN 203 THE MEL-14 ANTIBODY BINDS TO THE LECTIN DOMAIN OF THE MURINE PERIPHERAL LYMPH NODE HOMING RECEPTOR, Benjamin R. Bowen, Christopher W. Fennie, and Laurence A. Lasky, Department of Immunobiology, Genentech, Inc., So. San Francisco, CA 94080
We constructed a variety of mutants of the murine peripheral node homing receptor, gp90^{mel}, in an effort to determine which portions of the molecule comprise the binding epitope for the mAb MEL-14. While a truncated form of the HR containing only the lectin and egf domains is recognized by MEL-14, the lectin domain alone is not. Next we constructed chimeric molecules in which portions of the MEL-14 non-reactive human pln HR (Leu-8) are replaced by the homologous regions of the murine HR. Analysis of these chimeras by FACS and by radioimmune precipitation indicates that the first 52 amino acid residues of the murine HR are required for recognition by MEL-14. These results suggest that MEL-14 recognizes a conformational epitope in the N-terminal half of the murine HR lectin domain. Because MEL-14 blocks the binding of either lymphocytes or soluble homing receptor-immunoglobulin chimeras to the pln, these results support the hypothesis that the N-terminal portion of the lectin domain is involved in the binding of lymphocytes to pln postcapillary venule endothelium. A similar mapping strategy for the human HR indicates that the Leu-8 mAb recognizes an epitope within the first 52 amino acid residues of the human HR.

CN 204 **ONCOSTATIN M STIMULATES THE RELEASE OF INTERLEUKIN-6 FROM VASCULAR ENDOTHELIAL CELLS,** Thomas Brown, John Rowe, Jill Rillema and Mohammed Shoyab. Oncogen, Seattle, WA 98121. Oncostatin M and TGF-b selectively inhibit the growth of certain tumor cells. We observed that an Oncostatin M/TGF-b resistant breast carcinoma cell line, ZR-75-30, was growth arrested when exposed to conditioned medium from Oncostatin M-treated human umbilical vein endothelial cells (HUVEC). Immunochemical analysis of HUVEC-conditioned medium indicated the inhibitor of ZR-75-30 growth to be IL-6. The stimulation of IL-6 release from HUVEC was rapid, reaching maximal levels within the first 24 hours of exposure to Oncostatin M (100 ng/ml/10^6 cells). Oncostatin M was effective over a dose range of 50-500 nM. By contrast, TGF-b did not upregulate IL-6 concentrations. In addition, Oncostatin M inhibited the response of HUVEC to mitogenic stimulation by heparin/ECGS or bFGF at doses 10-fold below those required to stimulate IL-6 release. Therefore, Oncostatin M probably acts directly to inhibit the growth of HUVEC. Radioreceptor assays detect the presence of high numbers of cell surface receptors for Oncostatin M on HUVEC (300,000 sites/cell). These findings suggest that utility of Oncostatin M in controlling tumorigenesis may be greatly expanded through its actions on vascular endothelial cells by (1) stimulating the release of tumor suppressor molecules and (2) inhibiting endothelial cell proliferation/neovascularization.

CN 205 **ENDOTHELIAL CELL ACTIVATION CAUSES RELEASE OF AN ENDOTHELIAL CELL-SPECIFIC PROTEIN,** Craig W. Carson, Gene G.Hunder, Karen L. Kaplan, and Christopher M. Johnson, Hematology Research, Mayo Clinic/Foundation, Rochester, MN 55905 and Mount Sinai Services City Hospital at Elmhurst, Elmhurst, NY 10032 We recently described an ELISA for the detection of an endothelial cell-specific antigen, E92, in the sera of patients with rheumatic disease. Circulating E92 was high in patients with active disease and undetectable in normal controls. This finding suggested to us that detection of E92 may be an important marker of endothelial cell activation, thus providing an important tool for evaluating and following patients with diseases that affect the endothelial cell. We used cultured endothelial cells to test this hypothesis *in vitro.* Porcine aortic endothelial cells, which we had previously found to express the E92 antigen, were stimulated with thrombin (0.01-10 U/ml), endotoxin (0.01-10 μg/ml), or interleukin-1 (0.01-3 U/ml). The culture supernatants were then assayed for E92 by ELISA as we have previously described. Without stimulation, porcine aortic endothelial cells released little or no E92 antigen into the culture supernatant. Activation with all of the above mediators caused a significant increase in the E92 detected in the culture supernatant. E92 levels rose from a baseline of 35% to 259% for cells treated with thrombin, 213% for cells treated with endotoxin, and 138% for cells treated with interleukin-1. The peak effect on E92 release was achieved with 0.01 U/ml thrombin, 10 μg/ml endotoxin, and 1 U/ml interleukin-1 and the maximal effect was seen at 24 hours for all three mediators. This occurred in the absence of disruption of the monolayer or endothelial injury detectable by ^{51}Cr release. These results suggest that detection of E92 in the supernatant of cultured endothelial cells and in the sera of patients with rheumatic disease is a marker for endothelial cell activation.

CN 206 CHIMERIC HOMING RECEPTOR -HIGH ENDOTHELIAL VENULE INTERACTIONS, Christopher Fennie, Susan Watson, Laurence Lasky, Steven Rosen, Yasuyuki Imai, and Joyce Geoffrey, Dept. of Immuno-Biology, Genentech, Inc., 460 Pt. San Bruno Blvd., S.S.F., CA 94080, and Dept of Anatomy, University of California, San Francisco, CA 94143
The binding of lymphocytes to high endothelial venules (HEV) within peripheral lymph nodes (pln) is thought to be mediated by adhesion molecules termed homing receptors. Previous work has demonstrated that the adhesive interaction between lymphocytes and pln high endothelium appears to involve a lectin-like activity, since lymphocyte binding to pln HEV could be inhibited by sugars such as mannose-6-phosphate. In agreement with this possibility, the cloning and sequencing of cDNAs encoding both murine and human homing receptors revealed that these adhesion molecules contain protein motifs that are homologous to lectin, or carbohydrate-binding, domains as well as to epidermal growth factor (egf) and complement regulatory protein domains. In order to begin to examine the nature of the interactions between the murine homing receptor and its ligand(s) localized on the pln HEV, we have constructed soluble murine homing receptor-human immunoglobulin constant region chimeras containing the lectin, lectin-egf, and lectin-complement regulatory motifs. We are examining the ability of each of these antibody-like molecules to inhibit the homing receptor-dependant interaction of lymphocytes to pln HEV. We have also used one of these IgG chimeras (mHR LEC-IgG) as an imminohistochemical reagent. These studies have demonstrated that this chimera detects a ligand(s) specifically on pln HEV as well as the HEV of Peyer's patch.

CN 207 IN VITRO ANGIOGENIC RESPONSES OF WOUND CAPILLARY ENDOTHELIAL CELLS, Vance D. Fiegel, Barbara G. Penner, and David R. Knighton, Dept. of Surgery, University of Minnesota, Minneapolis, MN 55455.



Endothelial cell (EC) migration and proliferation are two distinct cellular responses which occur during angiogenesis. These studies were designed to examine the chemotactic and proliferative responses of rabbit wound capillary endothelial cells (RWCEC) isolated from capillaries undergoing active angiogenesis and rabbit brain capillary endothelial cells (RBCEC) isolated from quiescent capillaries. RWCEC, obtained from sponge implants containing granulation tissue, and RBCEC were isolated by enzyme dissociation and centrifugation on Percoll gradients. Uptake of fluorescent acetylated-LDL was used to confirm endothelial origin. When tested in a modified Boyden chemotaxis assay, both types of EC exhibited a similar pattern of dose dependent chemotaxis to a platelet releasate containing platelet-derived angiogenesis factor. However, the magnitude of the RWCEC response was significantly greater than that of the RBCEC at all doses tested ($p<0.02$). Basic fibroblast growth factor (bFGF, 0.01-300 ng/ml) was unable to induce either chemotaxis or proliferation in these cells, though it did induce proliferation in a control endothelial cell line. These results indicate that EC from granulation tissue respond more vigorously to an angiogenic stimulus than EC from quiescent endothelium and further suggests that bFGF is not a chemoattractant or mitogen for all types of EC.

CN 208 SHEAR-INDUCED MEMBRANE PERTURBATIONS IN ENDOTHELIAL CELLS, J. A. Frangos and F. Berthiaume, Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802.

It is known that endothelial cells respond to fluid flow, but the mechanism by which a physical force is transduced into a chemical signal across the cell membrane is still unknown. Merocyanine 540 (MC540), a fluorescent dye that intercalates between the phospholipids of the upper leaflet of the cell membrane, was used to monitor lipid packing in confluent HUVECs subjected to flow. HUVECs were stained with MC540 for 5 min while being sheared in a parallel plate flow chamber. Cells stained in non-flow conditions were used as controls. There was a shear-dependent increase in the amount of dye bound by the cells. This suggests that the membrane phospholipids become more loosely packed when endothelial cells are subjected to fluid flow.

Shear stress (dy/cm^2)	Number of samples	Dye bound (ratio to control)
(steady)	6	1.13 ± 0.08
(steady)	13	1.4 ± 0.1 ($p<0.05$)
(steady)	25	1.6 ± 0.1 ($p<0.01$)
(pulsatile)	15	1.6 ± 0.2 ($p<0.05$)

CN 209 ADHESION PROTEIN GMP140 PREVENTS THE ADHESION OF ACTIVATED NEUTROPHILS TO ENDOTHELIUM, Jennifer R. Gamble[+], Michael P. Skinner, Michael C. Berndt and Mathew A. Vadas[+], [+]Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, 5000, and Research Centre for Thrombosis and Cardiovascular Disease, Department of Medicine, Westmead Hospital, New South Wales, 2145, Australia.

The adhesion of blood cells is an essential component of inflammatory responses. However, strict control over the process of adhesion is required in order to prevent or limit vascular damage, suggesting the need for active anti-adhesive mechanisms. We report here on the effect of the LEC-CAM glycoprotein, GMP140, on the adhesion of blood cells to endothelium and to plastic surfaces. GMP140 when coated onto plastic surfaces supported the adhesion of neutrophils and monocytes but not lymphocytes.

In contrast, soluble GMP140 strongly inhibited the adhesion of neutrophils to endothelial cells. Since this inhibition of adhesion is selective, it is likely that it results from an anti-adhesive mechanism rather than purely a competition of the soluble GMP140 for its receptor on neutrophils.

Our findings therefore suggest that GMP140 may serve both adhesive and anti-adhesive roles and may have therapeutic uses *in vivo*.

CN 210 EFFECT OF MUTATION OF ASP71 ON HUMAN PROTEIN C ACTIVATION & FUNCTION. Bruce Gerlitz, John F.Parkinson, Nils U. Bang and Brian W. Grinnell. Dept.of Molecular Genetics, Lilly Research Laboratories and Dept.of Medicine, Indiana University, Indianapolis, Indiana, 46285. Human protein C (HPC) is a serine protease involved in hemostasis. HPC activation by the thrombin-thrombomodulin (T-TM) complex requires a calcium-dependent conformational change near the thrombin cleavage site and formation of a calcium bridge between the Gla-domain of HPC and TM. A calcium-binding site is associated with the EGF domain of HPC and probably involves the beta-hydroxylated Asp71 residue (bOH-Asp). The role of bOH-Asp in HPC activation was studied by mutation of Asp71 to Glu (EO71) or Gly (GO71). Cell lines expressing each mutant were isolated and the fully gamma-carboxylated proteins purified. Activation parameters were determined with thrombin in complex with two forms of soluble recombinant TM designated TMD1-105 and TMD1-75, which exhibit maximal HPC activation rates at 3 and 0.3 mM calcium, respectively. With TMD1-105, activation rates for HPC, EO71 and GO71 by thrombin were similar, exhibiting the same calcium-dependency and increasing to an optimum at 3 mM calcium. The activation rate was half-maximal at ~ 0.3 mM calcium for HPC and both mutants. With TMD1-75, HPC activation by thrombin was bimodal: activation rates increased to a maximum at 0.3 mM calcium, and decreased at higher calcium concentrations. The bimodal calcium-dependencies for EO71 and GO71 were similar, but shifted to higher calcium concentrations. The bimodal calcium-dependency of TMD1-75 is similar to that described for an elastase cleavage fragment of rabbit TM (el-TM) and is likely due to formation of a high-affinity calcium bridge between the Gla-domain of HPC and el-TM. The shift in calcium-dependency observed for EO71 and GO71 suggested a decrease in the affinity of this interaction. These activation studies suggest that bOH-Asp in the EGF domain of HPC has little influence on the calcium-dependent thrombin cleavage site in HPC, but may influence the conformational state of the Gla-domain and thus its interaction with TM. These minor effects of bOH-Asp on calcium-dependent HPC activation were in contrast to its critical role in the anticoagulant function of activated HPC. The anticoagulant activity of EO71 and GO71 was only 10-30% of HPC as assessed by clotting assays.

CN 211 ROLE OF PROTEIN KINASE C IN THE TRANSDUCTION OF SHEAR STRESS TO ALTERATIONS IN ENDOTHELIAL CELL MORPHOLOGY. P.R. Girard and R.M.Nerem, Bioengineering Center, Georgia Institute of Technology, Atlanta, Georgia 30332-0405. Endothelial cells exposed to a fluid-imposed shear stress both in vivo and in vitro show an alignment with the direction of flow and an elongation of cells with a concomitant reorganization of the cytoskeletal network. The phosphorylation of cytoskeletal proteins is thought to play a role in the regulation of cytoskeletal structure. We have implicated protein kinase C (PKC) in shear stress-induced morphological alterations based on several criteria. In control, static cultures of EC, approximately 75% of PKC was localized in the cytosol. In EC subjected to a shear stress of 30 dynes/cm^2 for 60 min, there was a 45% decrease in cytosolic levels concomitant with a 47% increase in membrane levels of PKC. Flow-induced morphological changes were inhibited when EC were preincubated with the PKC inhibitor, sphingosine (25 μm), for 90 min prior to exposing the cells to a shear stress of 30 dynes/cm^2 for 24 hr. Immunofluorescent studies of the intermediate filament protein, vimentin, indicated a reorganization of this network in response to shear stress. EC preincubated with ^{32}P and subsequently exposed to shear stress or treated with the PKC activating phorbol ester, TPA, showed an increased level of phosphorylation of vimentin compared to controls. This suggests that PKC-dependent phosphorylation of specific cytoskeletal proteins may be responsible, at least in part, for the dynamic changes in cytoskeletal structure and cell morphology which occur during exposure to a flow environment.

CN 212 A CONSTITUTIVE CYTOPLASMIC ANTIGEN IS DISPLAYED ON THE CELL SURFACE OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS UPON IL-1 ACTIVATION. T. Venkat Gopal, Tom Polte, Diane Wilson, Norma Graber, L. Dawson Beall, and Walter Newman. Departments of Molecular Biology and Immunology, Otsuka America Pharmaceutical, Inc., Rockville, MD 20850. A monoclonal antibody, 3A2, developed against IL-1 activated human umbilical vein endothelial cells (HUVEC) binds to IL-1 activated but not to normal HUVEC. Despite its presence in the cytoplasm the appearance of this antigen on the IL-1 activated HUVEC surface is dependent upon denovo RNA and protein synthesis. Immunoprecipitation with 3A2 from a metabolically labeled detergent extract showed bands corresponding to 170 Kd, and 170 Kd plus 110-120 Kd proteins in normal and IL-1 activated HUVECs, respectively. The 110-120 Kd protein band was shown to be ELAM-1 by immunoprecipitation of extracts from metabolically labeled COS cells transfected with ELAM-1 cDNA, obtained by PCR technology, and two newly developed anti-ELAM-1 monoclonal antibodies, 7A9 and 3B7. These results were confirmed by analysis of 3A2 immunoprecipitates in 2D gels. The appearance of the 170 Kd 3A2 protein on the cell surface is, therefore, possibly dependent upon the synthesis of ELAM-1. 3A2 may interact non-covalently with ELAM-1 preceding its appearance on the cell surface. The 3A2 antigen is distinct from von Willebrand factor and GMP-140 (PADGEM). Its association with ELAM-1 suggests it may play a role in the proinflammatory events triggered in endothelial cells by IL-1.

CN 213 IDENTIFICATION OF A NOVEL ENDOTHELIAL CELL ADHESION PROTEIN, Norma Graber, T. Venkat Gopal, Diane Wilson, L. Dawson Beall, Tom Polte, and Walter Newman, Departments of Immunology and Molecular Biology, Otsuka America Pharmaceutical Co., Rockville, MD 20850. Indirect evidence suggests the existence of an endothelial membrane protein distinct from ELAM-1 and ICAM-1 which mediates the adhesion of mononuclear leucocytes to cytokine-activated endothelial cells. A 2D gel analysis of the membrane proteins of 6 hour IL-1 stimulated human umbilical vein endothelial cells (HUVEC) shows that only three new major glycoproteins are induced from among 652 detectable spots. Two of these are ELAM-1 and ICAM-1. We have developed two monoclonal antibodies, 1E7 and 2G7 to distinct epitopes of this third and novel 114 Kd sialglycoprotein. The 1E7/2G7 protein is inducible on human umbilical vein endothelial cells (HUVEC) by the short term action of either IL-1 or TNF. Resting HUVEC and resting and/or IL-1 stimulated mononuclear cells, platelets, fibroblasts and keratinocytes do not express the 1E7/2G7 antigen. PCR technology was used to reconstruct the ELAM gene from published sequence data. The use of ELAM transfected COS cells allowed us to characterize two additional antibodies, 3B7 and 7A9, as anti-ELAM. The 1E7 and 2G7 antibodies do not react with ELAM transfected COS cells and, in addition, show a distinct profile from ELAM-1 and ICAM-1 by immunoprecipitation criteria in 1 and 2D gels. Binding inhibition studies with $F(ab')_2$ fragments of 1E7 and 2G7 suggest that the 1E7/2G7 protein is involved in the adhesion of some types of mononuclear cells to cytokine activated HUVEC.

CN 214 ROLE OF GLYCOSYLATION IN THE PROCESSING AND FUNCTION OF RECOMBINANT HUMAN PROTEIN C. Brian W. Grinnell, Jenna D. Walls, Bruce Gerlitz, and S. Betty Yan, Departments of Molecular Genetics and Biochemistry, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis IN 46285. Human protein C (HPC) is a vitamin K-dependent plasma protease involved in the regulation of hemostasis. This protein has potent anticoagulant activity and may be useful in the treatment of thrombotic disease. HPC derived from plasma is heterogeneous, consisting of from 10 to 15% single chain material, three forms of the heavy chain (serine protease domain) and a light chain component.(Gla and EGF domains) The cDNA for human protein C has been expressed to high levels in several cell lines and the fully carboxylated protein has been purified. Carbohydrate analysis of the rHPC from each cell line revealed differences in the content of several glycosyl residues, and high sialic acid content correlated with reduced functional activity. Thus, differences in glycosylation may affect the properties of HPC. To determine the role of glycosylation in the secretion, activation efficiency, and functionality of the protein, each of the potential glycosylation sites in rHPC were individually eliminated by site directed mutagenesis, and stable recombinant 293 cell lines secreting each of the variants were developed. From an analysis of these mutants, we find that the previously described subunits of the heavy chain are due to variation in the number of sites glycosylated. This includes partial glycosylation of Asn329, which has the unusual recognition sequence Asn-X-Cys-Ser. In addition, we find that glycosylation at Asn 248 is critical to the efficient secretion of the protein and full glycosylation of Asn313 and 329 is only required for secretion *in the absence of glycosylation at Asn248. Further, the amount of single chain material increases approximately* three-fold in the absence of glycosylation at Asn248, suggesting that carbohydrate at this site in HPC is important for the processing of the internal KR dipeptide. The effect of each glycosylation site on the anticoagulant activity of the protein and on its rate of activation by thrombin will be presented. Through these structure/function studies, it is hoped that a molecule with improved therapeutic characteristics can be obtained.

CN 215 INTERLEUKIN 6 MODULATES c-sis GENE EXPRESSION IN CULTURED HUMAN ENDOTHELIAL CELLS, Victor B. Hatcher, Tina M. Calderon, Jeffrey Sherman, Helen Wilkerson and Joan W. Berman, Department of Pathology, Microbiology and Immunology and Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461
Human vascular endothelial cells secrete platelet derived growth factor (PDGF)-like polypeptides which mediate some of the vascular effects in the inflammatory process. We have demonstrated that early passaged human endothelial cells incubated with human recombinant IL-6 exhibit a substantial increase in c-sis mRNA transcripts. Optimal induction of message was observed with 50 units/ml IL-6 following a five to twenty four hour incubation period. We initially studied c-sis expression in response to IL-1, since many of the biological activities assigned to IL-6 were originally believed to be IL-1 induced. IL-1 alpha and IL-1 beta increased c-sis mRNA transcripts after a twenty four hour incubation period. Both cytokines acted synergistically with IL-6 in increasing c-sis expression. Tumor necrosis factor enhanced the accumulation of c-sis mRNA while interferon gamma decreased its level. In the inflammatory process specific cytokines modulate c-sis expression in human endothelial cells. The production by endothelial cells of PDGF-like polypeptides stimulates cell migration, proliferation, and causes the release of vascular inflammatory mediators.

CN 216 CELL-TYPE SPECIFIC REGULATION OF VON WILLEBRAND FACTOR GENE EXPRESSION, Nadia Jahroudi, Zamiul Haque and D.C. Lynch, Division of Neoplastic Disease Mechanism, Dana-Farber Cancer Institute and Departments of Medicine and Pathology, Harvard Medical School, Boston, MA 02115

Von Willebrand factor (vWF) is a large, adhesive plasma glycoprotein which mediates the interaction of platelets with damaged endothelial surfaces. Deficiencies in vWF result in von Willebrand disease, the most common inherited bleeding disorder. Epidemiologic studies suggest that as much as 1% of the general population may carry one defective gene for vWF. The gene coding for this protein spans about 200 kilobases and appears to be expressed only in endothelial cells and megakaryocytes. We have previously cloned the gene and determined the transcriptional initiation site. We are now interested in determining the elements which regulate the expression of the vWF gene and the mechanism controlling its cell-type specific expression. Transfection studies were carried out with a series of plasmids in which fragments of the vWF gene containing 150 base pairs of 5' untranslated and increasing segments of 5' flanking regions were fused to the ß globin structural gene. The results of these analyses indicate that fragments containing between 1.4 and 0.1 kilobases of 5' flanking region immmediately upstream of the transcription start site can activate the expression of the fused ß globin gene. The promoter activities of these fragments were not cell-type specific since the activation occured when the plasmids were transfected into HeLa cells, as well as into endothelial cells. In addition the transcription initiation site was ~50 base pairs downstream of that observed for endogenous vWF mRNA in endothelial cells. We are currently investigating the possible role of chromatin structure in initiating the transcription at the expected site, by analyzing the RNA from cell lines stably transfected with these constructs. We are also analyzing differences in the structure of the chromatin containing the vWF gene and modifictions of the DNA, such as methylation, in expressing and nonexpressing cells. Preliminary analysis comparing the methylation pattern of the vWF gene in endothelial and HeLa cells has indicated that the 5' flanking region of this gene is hypomethylated in endothelial cells.

CN 217 RAPID METABOLISM OF CYSTEINYL-CONTAINING LEUKOTRIENES BY CEREBRAL ENDOTHELIAL CELLS, Irina Karnushina*, Jan-Ake Lindgren** and Hans-Eric Claesson**, *Department of Biology, University of Colorado, Colorado Springs, CO 80933-7150; **Department of Physiological Chemistry, Karolinska Institute, Stockholm, Sweden. Leukotrienes (LTC_4, LTD_4, LTE_4) are glutathion-linked derivatives of arachidonic acid via 5-lipoxygenase pathway. They are mainly released by leukocytes and some other cells causing constriction of smooth muscles in bronchi and blood vessels. Metabolism of leukotrienes by isolated brain microvessels was studied using a very sensitive method for detection of leukotrienes - reverse phase HPLC, on-line UV spectroscopy and radiolabeled leukotrienes. In comparative studies we found that brain tissue homogenate showed approximately 100 times less ability to convert exogenously given LTC_4 into LTD_4 and LTE_4 by gamma-glutamyltranspeptidase and dipeptidase consecutively than isolated brain microvessels from the same homogenate. Cerebral endothelial cell primary cultures gradually lost their ability to convert LTC_4 and LTD_4 during 4-6 weeks of growth. Cloned endothelial cells derived from bovine brain microvessels and bovine aorta did not metabolize LTC_4. (Cells were generously supplied by D. Gospodarowicz). In conclusion, this report shows that mammalian brain is actively protected from exposure to potentially harmful leukotrienes (released from blood cells) by enzymes present in the functionally developed cerebral endothelial cells.

CN 218 THROMBIN INDUCES C-FOS EXPRESSION IN CULTURED HUMAN ENDOTHELIAL CELLS BY A CA^{2+}-DEPENDENT MECHANISM, Maria Grazia Lampugnani, Francesco Colotta, Nadia Polentarutti, Maria Pedenovi, Alberto Mantovani, Elisabetta Dejana, Mario Negri Institute for Pharmacological Reserch, Via Eritrea, 62, Milano 20157, Italy. c-fos proto-oncogene has been implicated in the modulation of various cell functions. We have found that thrombin,a pleiotropic activator of endothelial cells, induced c-fos mRNA in human umbilical vein endothelial cells (HEC). This effect was dose-related (0.05-1.0 U/ml) and transient (maximal after 1 h and negligible within 4 h). Pertussis toxin (PT) inhibited thrombin's effect on c-fos expression,but had no effect on c-fos expression by PMA. As thrombin activates PI hydrolysis and Ca^{2+}movement in HEC, we investigated the role of protein kinase C and intracellular free Ca^{2+} (Ca_i^{2+}) rises in c-fos induction by thrombin. Down regulation of protein kinase C by prolonged exposure to PMA had no effect on thrombin and ionomycin stimulation of c-fos,but inhibited PMA activation of this gene. Quenching of the Ca_i^{2+}rise in response to quin2 loading inhibited or suppressed thrombin activity on c-fos expression. Under the same conditions PMA activity was not inhibited or only partially inhibited. IL-1b and bFGF stimulation of c-fos mRNA level was not inhibited by quin2; on the contrary ionomycin effect was suppressed by quin2. These results suggest that thrombin-induced c-fos expression in HEC does not require a fully active protein kinase C but is closely dependent on normal intracellular Ca^{2+} availability.

CN 219 THE ROLE OF FIBRONECTIN RECEPTOR IN TUMOR CELL ADHESION TO THE ENDOTHELIUM Davide Lauri, Guendalina Rossi, Ines Martin-Padura, Raffaella Giavazzi, Sergio Bernasconi, Tiziana Biondelli and Elisabetta Dejana. Laboratory of Vascular Biology, Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milano, Italy. An active role for endothelial cells (EC) in promoting circulating tumor cell (TC) adhesion has been postulated. Recent data show that, following EC activation by cytokines, TC adhesion significantly increases. This effect seems to be mediated, for some TC, by an RGD dependent mechanism. Therefore we have investigated the role of fibronectin receptor ($\alpha_5\beta_1$)(FNR) on adhesion of 3 human TC lines to resting and interleukin-1 activated human cultured umbilical EC in an in vitro assay. A significant increase in adhesion of radiolabelled A549(lung carcinoma), MG63 (osteosarcoma) and HT29(colon carcinoma) (151±15%, 242±11% and 376±12% respectively; data expressed as %of control, mean ±SD, n=10) to EC pretreated for 4h with 20ng/ml of human recombinant interleukin-1β (IL1) was observed. When a FNR-antiserum was added to the assay the enhanced A549 and MG63 but not HT29 adhesion to IL1 activated EC was blocked. A comparable activity was observed when a mAb to β_1 or to α_5 chain were used, while an antiserum to β_3 chain was inactive. The FNR-antiserum did not significantly inhibit TC adhesion to non activated EC. The effect of FNR-antiserum was maintained in the presence of extracellular matrix (fibronectin and laminin) antibodies suggesting a direct activity on TC/EC interaction. Selective incubation of the FNR antiserum with TC but not EC resulted in inhibition of adhesion, suggesting that TC-FNR is responsible of this effect. Our data show that FNR is important for the adhesion of some TC lines to IL1 activated EC, while alternative mechanisms can operate in others.

CN 220 VCAM1 and VLA4: A NEW ENDOTHELIAL CELL/LEUKOCYTE ADHESION PAIR. Roy R. Lobb, Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142
Cytokine-activated human umbilical vein endothelial cells express vascular cell adhesion molecule 1 (VCAM1), which binds lymphocytes. Using a novel method which requires neither monoclonal antibody nor purified protein, VCAM1 was cloned by direct expression, using cell adhesion to human lymphoid cell lines to detect expressing clones. Cells transfected with VCAM1 bind a variety of human leukemic cell lines, but not human neutrophils. VCAM1, which is a member of the immunoglobulin superfamily, binds to VLA4 on lymphocytes. VLA4 surface expression on K562 cells following transfection of the VLA alpha4 subunit results in specific cell adhesion to VCAM1, and antibodies directed to VLA4 completely inhibit VCAM1-dependent cell-cell adhesion. In addition, the interaction of VLA4 with VCAM1 can be distinguished from its interaction with fibronectin. The VCAM1/VLA4 ligand-receptor pair may play a critical role in the recruitment of mononuclear leukocytes to inflammatory sites in vivo.

CN 221 THE ESTABLISHMENT OF MURINE ENDOTHELIAL CELL (EC) LINES IN VITRO. M.J. MacPhee, T.J. Sayers*, K.L. McCormick*, A.M. Pilaro, and R.H. Wiltrout. Biological Response Modifiers Program and *BCDP, Program Resources, Inc., NCI-FCRF, Frederick, MD. 21701
Previous studies of murine EC-leukocyte interactions have been limited by difficulties in obtaining adequate numbers of mouse EC of sufficient purity. To obtain enriched EC for such studies, we used the method of Thompson et al (Science 241:1349, 1988). This technique leads to the neovascularization of collagen-gelatin sponge implants that have been coated with acidic-fibroblast growth factor(aFGF). Sponges were implanted subcutaneously or affixed to the peritoneal wall or liver of Balb/c mice, and removed on day 14. Following digestion with collagenase (Types II and IV, 100U/ml each), approximately $1x10^6$ viable cells were obtained per sponge, of which about 30% were characterized using flow cytometry as EC by their uptake of fluorescent acetylated low density lipoprotein(Ac-LDL). Culture of these cells on fibronectin-coated plates in the presence of aFGF resulted in overgrowth of EC by non-EC after 13 days. However, when the cells were grown on mitomycin-C treated L929 feeder cells in the presence of aFGF, the cells were greater than 90% EC by day 14. These enriched EC were then trypsinized and cloned either by limiting dilution, or using cloning cylinders, with subsequent expansion on L929 feeder cells. Using this technique, EC derived from skin, peritoneal wall or liver implants continued to proliferate in vitro, with virtually all cells incorporating Ac-LDL on day 60. These cultures have been maintained for longer than 90 days. The ability to obtain purified, expanded populations of mouse EC from various sites will facilitate studies of murine EC-leukocyte interactions.

CN 222 IMMUNOLOGICAL PROPERTIES OF CEREBRAL ENDOTHELIUM.

David Male, Gareth Pryce and Jameel Rahman.
Department of Neuropathology, Institute of Psychiatry, London.

We have directly compared cerebral and extracerebral endothelium in a number of assays in vitro. MHC class I expression and its induction by interferon-γ (IFNγ) is similar on both cell types: class II is induced to a much greater extent on cerebral endothelium (3x) by equivalent levels of IFNγ.

The basal level of lymphocyte adhesion to cerebral endothelium is much lower than to extracerebral endothelium (~25%) which may partly explain why lymphocyte traffic into the central nervous system is normally so limited. Nevertheless cerebral endothelium responds rapidly to very low levels of tumour necrosis factor (TNFα) and IFNγ by increased expression of adhesion molecules and lymphocyte adhesion. For example 1 unit/ml of IFNγ increases lymphocyte adhesion to brain endothelium within 1 hour whereas 15 units/ml are required for 2 days to induce MHC class II synthesis. Activation of lymphocytes further enhances their binding to cerebral endothelium, but not to extracerebral endothelium. Adhesion is maximal during the early stages of the cell cycle (G1).

Analysis of the subsets of lymphocytes which adhere to cerebral endothelium showed that T and B cells adhere equally to resting endothelium, but following endothelial activation with IFNγ, the relative proportion of adherent B cells increases. Similarly, following activation the relative proportion of CD4+ cell falls and CD8+ cells rises.

CN 223 CONTRIBUTION OF ICAM-1 AND LFA-1 IN BINDING OF LYMPHOCYTES TO HIGH ENDOTHELIAL CELLS, Masayuki Miyasaka, Takuya Tamatani and Noriko Toyama, Department of Immunology, Tokyo Metropolitan Institute of Medical Science, Bunkyo, Tokyo 113, Japan.

Involvement of ICAM-1 and LFA-1 molecules in lymphocyte binding to high endothelial (HE) cells, the vital step in lymphocyte extravasation in lymph nodes, was examined by the use of a rat HEV-derived cell line (Europ.J.Immunol. 18:1235, '88) and monoclonal antibodies specifically reactive with the rat ICAM-1 and LFA-1, respectively. Adhesion of Con A-stimulated lymphocytes to the cultured HE cell line was inhibited by 30 to 40% by either anti-ICAM-1 or anti-LFA-1 (3 μg/ml; final concentration), whereas isotype-matched antibodies did not inhibit the binding at all. Adhesion of phorbol ester-stimulated lymphocytes was inhibited by anti-LFA-1, but not by anti-ICAM-1, indicating that a molecule different from ICAM-1 is used as a ligand for LFA-1 in this situation. In contrast, adhesion of unstimulated thoracic duct lymphocytes (TDL) was not inhibited to any extent by either anti-LFA-1 or anti-ICAM-1 even at highest concentrations tested (30 μg/ml), suggesting that neither of these molecules is involved in binding of resting lymphocytes to HE cells. Expression levels of LFA-1 on TDL was, however, quite similar to those of phorbol ester-stimulated cells, although LFA-1 dependent adhesion was detected in stimulated cells but not in TDL, suggesting that LFA-1 on activated cells is in a high-avidity state, whereas LFA-1 on resting cells is in a low-avidity state.

CN 224 EFFECT OF SALMONELLA BACTERIA ON THE INTERACTION OF HUMAN NK CELLS WITH ENDOTHELIAL CELLS. Mari Pinola, Eero Saksela. Department of Pathology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland.

Leukocyte - endothelial cell adhesion and its regulation are essential but complex initial aspects of lymphocyte migration. Various factors (IL-1, TNF, IFN-gamma, etc.) have been shown to enhance the endothelial adhesiveness for human lymphocytes, including natural killer cells (NK-cells). We have demonstrated that pretreatment of either the endothelial cell monolayers or LGL-cells with mR595-Salmonella Minnesota bacteria or its lipopolysaccharide results in a substantial increase in the adhesiveness of LGL-cells to endothelial cells. The increase is more prominent when the endothelial cells are treated than when the adhering LGL-cells are similarly pretreated. The adhering cell population is enriched in NK-cells as verified by Facscan analysis with anti CD-56 (Leu19) and CD16 antibodies.

CN 225 Cyclic-AMP IS A SECOND MESSENGER FOR IL-1 IN ENDOTHELIAL CELLS,
Risto Renkonen, Pirkko Mattila and Jarkko Ustinov, Transplantation Laboratory, University of Helsinki, Haartmaninkatu 3, Helsinki, Finland.
We analysed the signal transduction during IL-1-induced lymphocyte binding. Dibutyryl-cyclic-AMP as well as direct activation of adenylate cyclase increased lymphocyte binding to a similar extent as did IL-1. IL-1 increased the level of cytosolic cAMP in a time and dose-dependent manner measured with radioimmunoassay. 2',5'-dideoxyadenosine, which is an inhibitor of adenylate cyclase, decreased both the IL-1 induced lymphocyte binding to endothelial cells and elevation in cytosolic cAMP levels. Other second messenger pathways, such as increased levels of cytosolic Ca^{2+} (induced by calsium ionophore A23187), or dibutyryl-cGMP, had no effect on IL-1-induced lymphocyte binding. These results suggest that cAMP is essential in signal transduction during IL-1-induced lymphcoyte binding to cultured endothelial cells.

CN 226 EFFECT OF TRANSFORMING GROWTH FACTOR-ß ON HUMAN VASCULAR ENDOTHELIAL CELL PROSTACYCLIN PRODUCTION
Ari Ristimäki* , Olavi Ylikorkala** , Jaakko Perheentupa* , and Lasse Viinikka*. Children's Hospital*, and the Department of Obstetrics and Gynecology**, University of Helsinki, 00290 Helsinki, Finland.
Prostacyclin (PGI_2) is an antithrombotic factor, which may prevent the initiation and the complications of atherosclerosis. Vascular endothelium is the most important producer of PGI_2, but the regulation of its production is largely unknown. The role of transforming growth factor-ß (TGF–ß) released from platelets, macrophages,and endothelial cells themselves is of special interest in this respect. We investigated the effect of TGF-ß on cultured human umbilical vein endothelial cell PGI_2 production by measuring the stable metabolite of PGI_2, 6-keto-prostaglandin $F_{1\alpha}$, by radioimmunoassay. TGF-ß induced dose- and time-dependent stimulation of PGI_2 production. The lowest stimulatory concentration of TGF-ß was 0.1 ng/ml and the maximal response, a 2.1-fold rise, was obtained with 1.0 ng/ml. The effect of TGF-ß lasted 48 h and was blocked by transcription, translation, and cyclo-oxygenase inhibitors. The maximal stimulation by TGF-ß was enhanced by epidermal growth factor. This is the first demonstration that TGF-ß enhances PGI_2 production by human vascular cells, and this phenomenon may be involved with the prevention of thrombosis and arteriosclerosis.

CN 227 A GLYCOPHORIN-LIKE SIALOGLYCOPROTEIN ON THE SURFACE OF ENDOTHELIAL CELLS,
J.E. Schnitzer, J.B. Ulmer & G.E. Palade, Dept. Cell Biol., Yale Univ. School of Med., New Haven, CT
Glycophorins are the major sialoglycoproteins of red blood cells in many species and, until recently, been considered to be specific to erythroid cells. Using polyclonal antibodies directed against mouse glycophorin (αgp), we have identified a glycophorin-like molecule on the surface of bovine and rat cultured endothelial cells. Immunoblotting with αgp identified a single 60 kDa polypeptide on transfers of SDS-PAGE of solubilized confluent endothelial monolayers. In addition, a 60 kDa polypeptide was immunoprecipitated by αgp from lysates of ^{125}I-labeled intact endothelial cells. Controls with pre-immune sera were negative. Our past work identified a 60 kDa glycoprotein (gp60) that interacts with albumin and binds *Limax flavus* (LFA), *Ricinus communis* (RCA) & *Triticum vulgare* (WGA) agglutinins but not other lectins including *Glycine max* (SBA). In this study, gp60 was sequentially precipitated from ^{125}I-labeled cell lysates using RCA followed by WGA. By immunoblotting of such lectin precipitates with αgp, a single 60 kDa glycoprotein was detected. Sialidase digestion of ^{125}I-labeled glycoproteins purified by RCA affinity chromatography resulted in: i) a reduction of the apparent molecular weight of gp 60 to 55,000, ii) elimination of WGA binding to gp60, and iii) promotion of SBA binding to gp60. Succinylated WGA did not precipitate native gp60. These results suggest that gp60 is a glycophorin-like, endothelial surface glycoprotein containing sialic acid, galactose and N-acetylgalactosamine residues on O-linked oligosaccharides. We are currently investigating the location of the epitope(s) in common between gp60 and mouse glycophorin.

CN 228 ENDOTHELIAL CELL EXPRESSION OF FCγ RECEPTORS: AN IMMUNOHISTOCHEMICAL STUDY.

Daniel D. Sedmak, Daniel H. Davis, Clark L. Anderson, Departments of Pathology and Medicine, The Ohio State University College of Medicine, Columbus, OH, 43210.

IgG Fc receptors (FcR) are a heterogeneous group of integral membrane glycoproteins that are members of the immunoglobulin gene superfamily and are expressed on the surfaces of human leukocytes. The expression of the three known leukocyte FcR classes by normal tissue endothelium and by cultured human umbilical vein endothelial cells (HUVECS) was studied immunohistochemically using anti-FcR monoclonal antibodies (MoAbs). Only FcR II MoAb B1D6 reacted with the vasculature endothelium of all normal tissues studied. However, FcR II MoAbs IV3, B1D6, KU79, CIKM5, 2E1, KB61, and 41H16 intensely stained the capillary endothelium of placental villi. FcR I and III MoAbs did not react with placental capillaries. FcR II MoAbs B1D6 and CIKM5, and FcR III MoAb 3G8 immunostained HUVECS. Staining was restricted to the cytoplasm, particularly the Golgi region. All other antibodies failed to react with HUVECS prior to and following stimulation. Binding of FcR II MoAb CIKM5 by HUVECS was increased in response to interferon-γ and endotoxin but not in response to tumor necrosis factor-α . With the exception of the placenta, there is limited expression of FcR by the endothelium of normal tissue. FcRs may be upregulated in response to mediators of inflammation.

CN 229 INTEGRIN MATRIX RECEPTOR EXPRESSION IN THE BLOOD VESSELS OF HEALING CUTANEOUS WOUNDS, Marcia G. Tonnesen, James Gailit, Erkki Ruoslahti and Richard A.F. Clark, Departments of Pediatrics and Medicine, National Jewish Center, Denver, CO 80206; La Jolla Cancer Research Foundation, LaJolla, CA 92037

Angiogenesis is crucial for new tissue formation during cutaneous wound healing. Neovascular cells, surrounded first by a provisional matrix and later by a basement membrane, presumably interact with these substrata through specific extracellular matrix (ECM) receptors. Integrin αβ heterodimeric transmembrane glycoproteins containing a β1 or a β3 subunit combined with a specific α subunit form such ECM receptors. Porcine cutaneous wound repair was chosen as a model for the investigation of angiogenesis, and microvascular maturation, organization, and subsequent regression. Immunofluoresence probing was performed at 5, 7, 10, and 14 days after full thickness wounding. Anti-laminin was used to delineate vascular integrity and maturation. Rabbit polyclonal antibodies specific for the β1 (laminin, collagen, fibronectin) or β3 (vitronectin) integrin subunits were used to examine ECM receptor expression. In nascent vasculature of 5 day wounds, laminin appeared as diffuse granules in cells and as a broken stitch-work in the basement membrane zone (BMZ). By 7 days laminin formed bright continuous lines in the BMZ which began to fade in 10 and 14 day regressing vessels. In 5 day wounds β1 and β3 integrin receptors appeared as diffuse granules in neovascular cells; at 7 days, as bright continous lines along the BMZ; at 10 days, as faded broken stitch-work along the BMZ; at 14 days, as dim granules when present. Thus integrin β1 and β3 ECM receptors appear to play a dynamic role in neovascularization.

CN 230 IS A SULPHATED N-LINKED GLYCAN ON LFA-3 INVOLVED IN LEUKOCYTE FUNCTION?

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Rosetting of human T cells with sheep red blood cells is mediated by the interaction of CD2 and the sheep homologue of LFA3. This rosetting is strongly inhibited by sulphated carbohydrates like Dextransulphate (500 KD DexS500), fucoidan and sulphatide in the μg/ml range whereas other sulphated glycans like heparin or Dextransulphate (8KD) are without effect even at concentrations up to 1000 μg/ml. We confirmed observations of Parish et al (1988) that low concentrations of Dextransulphate - 500 (5 μg/ml) were inhibitory to the binding of several CD2 directed monoclonal antibodies to human T cells. FACS analysis showed that binding of T082, CLB-TLL.2/1 (classification according to 4th International Workshop and Conference on Human Leukocyte Differentiation Antigens) to mononuclear cells was strongly depressed by 100 μg/ml Dex S-500 while Dex S-8 was without effect. Binding of other CD2 directed monoclonal antibodies (from the Workshop) were either moderately or not at all affected by DexS-500. To assess for the possibility that CD2 recognizes a sulphated glycan, JY cells ($LFA3^+$) were biosynthetically labeled with inorganic $^{35}SO_4$. After immunoprecipitation a sulphated form of LFA3 was detected on SDS-PAGE autoradiograms. Sulphation of LFA-3 was completely suppressed if dMM (deoxymannojirimycin), an inhibitor of N-linked glycan processing, was present during biosynthesis. Surface iodination showed that LFA-3 was normally expressed at the cell surface in the presence dMM. We will furthermore show that several human leukocyte functions of established CD2-LFA3 mediation are inhibited by dMM, indicating that a properly processed N-linked glycan is involved.
We propose that a sulphated N-linked glycan is most likely involved in CD2-LFA3 interactions.

CN 231 THE ROLE OF FACTOR IX IN FIBRIN DEPOSITION AND AGGREGATE FORMATION IN FLOWING BLOOD, Pim N. Tijburg, J. Ryan, B. Wollitzky. S. Rimon, B. Rimon, D. Stern and Ph. de Groot. University Hospital Utrecht, Utrecht, the Netherlands.
The role of factor IX in fibrin deposition and aggregate formation was investigated in perfusion studies. Whole blood, anticoagulated with low molecular weight heparin was exposed to extracellular matrices (ECM) derived from tumor necrosis factor (TNF)-treated cultured human endothelial cells (EC), for 5 min at a wall shear rate of 300 s^{-1}. Tissue factor (TF) activity on the ECM was dependent on the dose of TNF added to the endothelium (5-600 pM TNF). Fibrin deposition and aggregate formation occured on the ECM in a dose-dependent manner, depending on the TNF concentration. Addition of dansyl-glu-gly-arg Factor Xa (DEGR-Xa) induced a total inhibition of fibrin deposition and aggregate formation at all TNF concentrations tested. In contrast, addition of DEGR-Factor IXa inhibited fibrin deposition and aggregate formation only after stimulation with low concentrations TNF (20-50 pM TNF). These results suggest that Factor IX contributes to the activation of the coagulation in flowing blood principally at lower concentrations of TNF where small amounts of TF are involved in the initiation of the coagulation.

CN 232 **A GLYCOPROTEIN EXPRESSED ON BLOOD VESSELS AND LYMPHATICS OF HUMAN MUCOSAL LYMPHOID TISSUES**, Jaakko Uksila, Marko Salmi, Riitta Aho* and Sirpa Jalkanen, Departments of Medical Microbiology, and *Pathology, University of Turku, Turku, Finland.

We have generated a monoclonal antibody 1H3 against human endothelial cells lining the small veins, arteries and lymphatics of mucosal lymphoid tissues. In tonsils, most of the positive vessels locate at septal areas, and in the appendix 1H3 mainly stains vessels of the submucosa. The organ specificity of 1H3 reactivity is remarcable as it does not stain vascular endothelium of peripheral lymph nodes. A limited number of nonlymphoid tissues studied so far indicate that 1H3 does not recognize vascular structures in other tissues with the exception of the kidney where the endothelium of some vessels are positive for 1H3. There is, however, variation in the number of positive vessels and the intensity of staining detectable in appendices or tonsils from different individuals. In western blotting, 1H3 antibody detects a band of 58 kDa under nonreducing conditions. This band can bind several lectins suggesting a glycoprotein nature of this antigen. We are currently studying the association of 1H3 antigen to the inflammatory status of mucosal lymphoid organs and its possible role in leukocyte adhesion to vascular endothelium.

CN 233 NEUTROPHIL PROTEASES CLEAVE ENDOTHELIAL PROTEOGLYCAN CORE PROTEIN: POTENTIAL ROLE IN INFLAMMATORY THROMBOSIS, Gregory M. Vercellotti, Nigel S. Key, Jeffrey L. Platt, Harry S. Jacob. Departments of Medicine and Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455. Endothelial cell (EC) associated heparan sulfate proteoglycan (HSPG) is critical in the maintenance of vascular integrity, and in the local inhibition of thrombin by activation of antithrombin III (AT-III). Since inflammation is commonly associated with capillary leak and intravascular coagulation, we tested whether the interaction of neutrophils (PMN) with EC might alter EC-HSPG. PG of cultured human umbilical vein EC and porcine aortic EC were biosynthetically radiolabeled with [^{35}S] sulfate and the cells then exposed to freshly isolated human PMN. This treatment caused remarkable release of up to 60 percent of EC PG into the incubation media. PG release increased with time, with the PMN:EC ratio (up to 20:1), and with stimulation of PMN by phorbol ester. Cell-free supernatant of PMN incubated for 60 min at 37°C also released ^{35}S-labeled macromolecules. PG release was not accompanied by EC detachment or lysis assayed with ^{51}Cr. Release was inhibited by PMSF, alpha-1-protease inhibitor, AAPCVK, but not by SOD/catalase or heparin. PMN-released radiolabeled molecules (isolated and purified in the presence of protease inhibitors) were shown to have HS chains and were of lower MW (Sepharose CL-4B run in 4M guanidine) than control. The size of these fragments suggests that glycopeptides or smaller PG (rather than digested glycosaminoglycan chains) were released. The PG remaining EC-associated after PMN treatment were of such size as to suggest substantial processing had occurred. This cell-associated PG binds to ATIII, suggesting heparan-like anticoagulant activity. We conclude that serine proteases from PMN can cleave and cause release of EC PG. Such deficiency of HSPG might be important in the pathogenesis of the increased vascular permeability and thrombosis associated with inflammation.

CN 234 INFLUENCE OF ENDOTOXIN AND NAFTIDROFURYL ON ADHERENCE PLAQUE FORMATION AND PERFORMANCE OF HUVEC IN VITRO UNDER SHEAR STRESS.
Nicolas Wiernsperger*, Ralph P.Franke**,R.Fuhrmann**. *LIPHA Labs,Lyon(F) and**Dept of Anatomy and Cell Biology,University of Marburg,FRG.

HUVEC(P2) cells cultured on extracellular matrix revealed in immunofluorescence a cytoskeletal organization like endothelial cells in situ. Incubation at rest with E.Coli endotoxin (4ug/ml;str.B111) was practically without effect, whereas incubation with endotoxin for 24h and subsequent exposure to arterial levels of shear stress for 3h in a cone-plate rheometer resulted in a progressive decrease of cell adherence. Concomitantly, a reduction in the junctional actin filament system was observed. This was paralleled by a significant diminution of vinculin-containing focal plaques (VCFPs) in the abluminal cell membrane. While there was a numerical reduction in VCFPs, an increase in spotsize of the residual plaques was observed. These impairments were accompanied by a disturbance in PGE2 discharge.

Incubation with the drug Naftidrofuryl (10-7M) after the incubation with endotoxin restored endothelial cell adherence almost completely when cells were exposed to shear stress, and thus exhibited a marked cytoprotective effect. This was accompanied by a significant increase in the number of VCFPs.

CN 235 INFLUENCE OF HYPERTHERMIA ON THE FIBRINOLYTIC POTENTIAL OF HUMAN ENDOTHELIAL CELLS,
Johann Wojta, Günter Christ, Michael Holzer, Richard L. Hoover and Bernd R. Binder, Lab. Clin. Exp. Physiol., Dept. Med. Physiology, Univ. Vienna, Austria, and Dept. Pathol., Vanderbilt Univ., Nashville, TN., USA

It has been shown recently that heat treatment of endothelial cells inhibits angiogenesis in vivo. This effect may at least partially contribute to the fact that hyperthermia inhibits tumor growth. Since changes in angiogenesis are often correlated with changes in the fibrinolytic capacity of the respective endothelial cells, we investigated the possible influence of heat treatment on the production of tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) of human umbilical vein endothelial cells (HUVEC) in vitro. HUVEC were exposed to 42°C for 4, 8, 12 and 24 hours and levels of PAI-1 and t-PA in the respective conditioned media (CM) determined by a specific ELISA, respectively, were compared to t-PA and PAI-1 levels found in CM of HUVEC incubated for the same time periods at 37°C. Whereas t-PA levels were decreased after heat treatment, PAI-1 levels increased significantly under hyperthermia (Table 1).

hours		4	8	12	24
t-PA ($ng/10^5$cells)	37°C	0.24± 0.04	0.50± 0.06	0.87± 0.09	1.63± 0.19
	42°C	0.19± 0.05	0.39± 0.06	0.56± 0.08	1.18± 0.16
PAI-1 ($ng/10^5$cells)	37°C	59.6 ± 5.1	99.1 ± 8.5	153.3 ± 5.2	227.9 ±27.7
	42°C	84.2 ±12.8	127.1 ±12.9	162.6 ±16.3	349.8 ±33.5

The effect of hyperthermia on PAI-1 release was not reversible even when cells were exposed to 37°C for 24 hours after 12 hours at 42°C (335.8±23.1ng/10^5cells as compared to 249.6±33.4ng/10^5cells of control). The effect on t-PA release, however, was completely reversed. Thus, HUVEC released more t-PA when exposed to 42°C for 12 hours and to 37°C for the following 24 hours than when exposed to 37°C for 36 hours (2.76±0.37ng/10^5cells and 1.95±0.16ng/10^5cells, respectively). These effects were not due to cell damage as verified by ^{51}Cr release assays. That HUVEC responded to hyperthermic treatment was proven by the increase in specific heat shock protein 70 RNA as determined by Northern blotting.

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CN 300 VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE INTEGRINS ARE BOTH DIFFERENTIALLY MODULATED BY SOLUBLE FACTORS AND EXTRACELLULAR MATRIX DURING ATHEROGENESIS, Craig Todd Basson, Michael A. Reidy and Joseph A. Madri, Department of Pathology, Yale Univ. School of Medicine, New Haven, CT 06510

Endothelial and smooth muscle phenotypes have been shown to be reciprocally modulated by both soluble factors and extracellular matrix proteins. We have probed the effects of TGF-ß1 and PDGF (AB) on integrin expression by these cell types. In vitro, both factors upregulated bovine aortic endothelial cell surface pools of ß1 and ß3 integrin classes without modulating their cellular organization. Conversely, matrix protein substrates (fibronectin, laminin, and fibrinogen) organized integrins without affecting the size of surface pools for either endothelial or smooth muscle cells. However, both TGF-ß1 and PDGF increased bovine aortic smooth muscle surface ß3 integrins without significantly altering ß1 integrins. In an in vivo model of atherogenesis, re-endothelialization and intimal thickening after balloon catheter injury of the rat carotid artery, immunohistochemical analysis revealed neointimal smooth muscle ß3 integrins were also upregulated in the presence of unchanged ß1 integrins. Moreover, in the rat carotid, ß1 but not ß3 endothelial integrins exhibited a polarized basal localization. Endothelial and smooth muscle migration in such atherosclerotic models may be modulated through a combination of soluble factor and extracellular matrix protein regulation of integrin expression and organization.

CN 301 ENDOTHELIAL CELL TUMORS IN POLYOMA TRANSGENIC MICE, Victoria Bautch[1], Rong Wang[1], Irving Seidman[3], Linda Kolpack[2], and Rich Azizkhan[2], Depts. of [1]Biology and [2]Surgery, U. of North Carolina at Chapel Hill, Chapel Hill, N.C. 27599, and [3]Dept. of Pathology, New York University, New York 10016. Transgenic mice that carry a polyoma (Py) middle T antigen gene linked to the Py early region promoter develop vascular endothelial cell tumors (Bautch et al., Cell 51, 529-538, 1987). We have generated transgenic mice that carry an intact early region capable of expressing all three Py early region proteins. These mice all develop both vascular tumors and bone tumors, but mice of different lineages show different latency of tumor formation. Analysis of expression patterns by RNA protection and PCR indicates that Py middle T and small T antigens are co-expressed in tumors and testes; large T antigen RNA is co-expressed in testes, but it is present at very low levels relative to the other Py RNAs in vascular and bone tumors. In general the spectrum of tissues expressing Py RNA correlates with the lifespan of the mice in each lineage, and one lineage exhibiting widespread transgene expression has a very short lifespan (2.3 mos.) characterized by the development of numerous vascular tumors in the skin and testes. We have recently established cell cultures from the vascular tumors of Py mice, and we are selecting for propagation and characterization the endothelial cells of these cultures.

CN 302 STUDIES ON THE ENDOTHELIAL CYTOADHERENCE RECEPTORS FOR SEQUESTRATION IN *PLASMODIUM FALCIPARUM* MALARIA. A. R. Berendt, D. Simmons, J. Tansey, C. Newbold, K. Marsh and *N. White, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK and *Wellcome Unit for Tropical Medicine, Mahidol University, Bangkok, Thailand. *Plasmodium falciparum*- infected erythrocytes adhere *in vivo* to post-capillary venular endothelium. This process can be modelled using cultured human umbilical vein endothelial cells (HUVEC). We have recently identified intercellular adhesion molecule 1 (ICAM-1, CD54) as a cytoadherence receptor on HUVEC. We have also established that HUVEC do not express another important putative receptor, CD36 (platelet glycoprotein IV). To clarify the *in vivo* importance of CD36-dependent and -independent pathways of adhesion, we are studying the distribution of potential receptors and other endothelial proteins by immunohistochemistry of tissues from patients dying of falciparum malaria. Identifying such receptors may provide crucial insights into the pathogenesis and potential treatment of this condition.

CN 303 LONG-TERM CULTURE OF AIDS-KAPOSI'S SARCOMA ENDOTHELIUM IN THE ABSENCE OF RETROVIRALLY CONDITIONED MEDIUM. Michael Bernas, Marlys Witte, Dennis Way, Milan Fiala, Charles Witte, Ray Nagle, Michael Stuntz, Geronimo Ramirez, and Michael Milligan, Department of Surgery and Pathology, The University of Arizona College of Medicine, Tucson, AZ 85724, and Departments of Medicine and Pathology, Eisenhower Medical Center, Palm Springs, CA 92270.

Understanding the pathogenesis of Kaposi's sarcoma (KS) has been hampered by inability to culture and characterize the specific "KS cell." Based upon our previous successful in vitro isolation of endothelium from benign lymphatic and blood vascular tumors, (i.e., lymphangiomas and hemangiomas), we prepared enzyme isolate cultures of excised KS skin lesions from four patients with AIDS in standard tissue culture medium. Over 5-9 months, these primary cultures were subjected to selective manipulations including modified enzymatic dissociation, medium depletion, short pulse trypsinization, and increased $C0_2$ concentration to select an endothelial cell subpopulation. Inverted light, transmission and scanning electron microscopy as well as fluorescent microscopy (FM) and flow cytometric analysis (FCM) of endothelial marker Ulex europaeaus ligand (UL) and anti-Factor VIII-related antigen antibody (F8) staining were used to characterize endothelial phenotypic patterns which were then compared with cultured bovine mesenteric, artery, and lymphatic endothelial standards and original tissue sections. The initial outgrowth of all cultures yielded a heterogeneous population with focal endothelial cells, which were followed through successive generations. These "KS cells" were spindle to stellate and flattened in appearance. Although varying from patient to patient, monolayer preparations exhibited substantial F8 (range 67-71%) and UL (41-45%) positivity on both FM and FCM. In conclusion, endothelial cell populations derived from AIDS-KS were successfully isolated in vitro and maintained over successive generations while retaining morphologic characteristics found in both lymphatic and blood vascular endothelium in vivo and in vitro. Moreover, the maintenance of the AIDS-KS cells profiled here required no special retrovirally conditioned medium. This in vitro model should allow delineation of the structural and functional features of the AIDS-KS cell while providing a test system to examine therapeutic agents against KS.

CN 304 EFFECTS OF CATECHOLAMINES ON ARTERIAL ENDOTHELIAL INTEGRITY - SMOKING AND NON-DENUDING ENDOTHELIAL INJURY. Göran Bondjers and Knut Pettersson,
Wallenberg Laboratory for Cardiovascular Research, University of Göteborg, S-413 45 Göteborg, Sweden.
Increased plasma levels of catecholamines were induced in rabbits with chloralose anaesthesia. To control for non-catecholamine mediated effects of chloralose, a $beta_1$-selective adrenoceptor antagonist metoprolol was given to control chloralose anaesthesized rabbits. Non-denuding endothelial injury was quantified through the presence of intracellular IgG in injured cells. Compared with untreated control siblings the frequency of injured endothelial cells increased almost tenfold in the experimental animals. This increase was totally abolished in the metoprolol + chloralose control sibling, indicating that the effect was catecholamine mediated probably via effects on heart rate and/or blood pressure. Similar effects were seen both in unbranched and in branched aorta. A positive correlation between frequencies of injured endothelial cells between siblings in various treatment groups ($r=0.83$) was observed indicating an effect of genetic factors on aorterial endothelial integrity. An increased frequency of injured endothelial cells in animals exposed to tobacco smoking was observed. This increase could also be inhibeted by $beta_1$ adrenoceptor blockade. These observations indicate that variations in adrenergic activation may affect endothelial integrity, with possible implications for atherogenesis and thrombogenesis.

CN 305 TISSUE FACTOR INDUCTION BY INFLAMMATORY AGENTS : REFRACTORY PERIOD PHENOMENON. Nathalie Busso, Edwige Nicodème, Stéphane Huet, Jacques Hiernaux and François Hyafil, Laboratoires Glaxo - 25 avenue du Québec-91951 Les Ulis Cédex France.

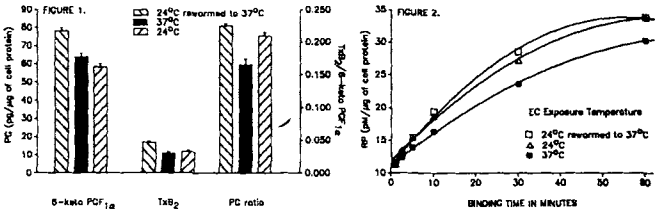
Incubations of human umbilical vein endothelial cells (HUVECs) with inflammatory agents [IL1-β, TNF-α, LPS and phorbol myristate acetate (PMA)], lead to dramatic increases (5-30 fold) in total cellular procoagulant activity (PCA) due to tissue factor (TF) activity. This activity peaks between 4 and 8h and returns to basal levels by 20 to 30h. The modulation of TF activity reflects the marked and transient induction of TF mRNA content (peaks between 1-4h).

Continuous incubations of HUVEC monolayers for 24-48h with each agent induce a hyporesponsiveness state with respect to TF reinduction (refractory period) by the same agent : total unresponsiveness with IL1-β and PMA, partial suppression of reinduction with TNF-α and LPS. By contrast, incubation with one agent does not affect dramatically reinduction by any of the three other compounds. Molecular mechanisms underlying this hyporesponsiveness state will be discussed.

CN 306 EFFECTS OF HIGH GLUCOSE,PHORBOL ESTERS,AND cAMP ON THE EXPRESSION OF BASEMENT MEMBRANE COMPONENTS IN ENDOTHELIAL CELLS, Enrico Cagliero,Tim Roth and Mara Lorenzi,Eye Research Institute and Harvard Medical School,Boston MA 02114. To identify agents and mechanisms responsible for the increased basement membrane thickness characteristic of diabetic microangiopathy,we studied the effects of high glucose (HG,30 mM) and other perturbations on the expression of fibronectin (FN) and collagen IV (COL IV) in cultured human umbilical vein endothelial cells (HUVEC). Cells exposed to HG for 12±1 days (mean±SD) exhibited increased levels of FN and COL IV mRNAs (183±150% of control and 184±137% respectively,n=43),while the actin,c-myc,and collagen I transcripts remained unchanged. In nuclear runoff experiments HG increased the transcription of both FN and COL IV by 2-3 fold;the changes in FN and COL IV proteins (immunoprecipitation) paralleled the changes in the respective mRNAs (FN:$r=0.82$,COL IV:$r=0.99$,$p<0.02$). Since diabetes and HG activate protein kinase C (PKC),and a cAMP-responsive element is present in the human FN and mouse COL IV promoters,we studied whether activation of the PKC or cAMP pathway mimics the effects of HG. Exposure of cells to PMA for 6 h increased COL IV mRNA to 226±72% but failed to change the FN mRNA levels (92±49%). After 48 h of exposure to PMA,COL IV mRNA was still elevated at 315±190% and FN mRNA was decreased (32±42%). Forskolin+IBMX increased both COL IV and FN mRNAs (216±173% and 309±200% respectively). We conclude that HG alters the expression of FN and COL IV in HUVEC by increasing transcription of the genes without affecting the translational activity of the cognate mRNAs. The effect of HG on mRNAs are mimicked by activation of protein kinase A but only partially by activation of PKC;whether these pathways contribute to the effects of HG is being investigated.

CN 307 GROWTH PATTERNS OF HUMAN CYTOMEGALOVIRUS STRAIN AD169 IN HUMAN FIBROBLASTS VERSUS HUMAN ENDOTHELIAL CELLS. Daniel H. Davis, William J. Waldman, Daniel D. Sedmak; Dept of Pathology, The Ohio State University, Columbus, OH 43210 Human cytomegalovirus (CMV) is a common infectious agent associated with increased mortality in immunosuppressed individuals. Endothelial cells have been proposed as a site of active infection in vivo. In this study we have compared the growth of CMV strain AD169 in neonatal dermal fibroblasts (FB) and umbilical vein endothelial cells (EC) using monoclonal antibodies to early and late CMV antigens. When infected with the same multiplicity of infection, 3% of ECs exhibited early nuclear protein as compared to 40% of FBs at 24 hours postinfection (PI). Infection was confirmed by in situ hybridization. The percent increase of cells infected with CMV between 24 and 144 hours PI was greater for FBs. FBs demonstrated a distinct biphasic pattern of replication with a second phase occurring at 72 hours. At 24 hours 0.75% of ECs demonstrated LNP as compared to 29% of FBs. ECs demonstrated simultaneous nuclear and cytoplasmic staining with LNP while in FBs nuclear staining occurred first. Both patterns of CMV antigen expression were seen in ECs infected with a clinical isolate which had been previously propogated in ECs. CMV AD169 infects and replicates more readily in FBs than ECs. In addition, the temporal and cellular location of CMV antigens varies between ECs and FBs.

CN 308 HYPOTHERMIC EFFECTS ON ENDOTHELIAL CELL (EC) PROSTAGLANDIN (PG) SYNTHESIS AND F-ACTIN, D.A. DuBose, U.S. Army Research Institute of Environmental Medicine, Natick MA 01760. Impaired blood flow and enhanced vessel permeability confound the rewarming of human hypothermic victims. Cold exposure of the endothelium may account, in part, for these vascular disturbances. To characterize the hypothermic effects on bovine aortic EC production of vasoactive PGs, bradykinin (BK)-stimulated (10^{-6}M; 5 min) prostacyclin and thromboxane metabolism were determined by radioimmunoassay of their stable metabolites, 6-keto $PGF_{1\alpha}$ and TxB_2, respectively. Prior to BK stimulation, rhodamine phalloidin (RP) binding kinetics were studied to evaluate temperature influences on F-actin, a cytoskeletal protein associated with EC barrier function. ECs exposed (2h) to 37°C or 24°C generated similar PG levels, while rewarming increased ($p<.05$) synthesis (Fig.1). Rewarmed and 24°C-exposed ECs had PG ratios that were more ($p<.05$) prothrombogenic. Such treatments elevated ($p<.05$) RP binding (Fig.2), suggesting EC stress fiber disruption and reduced junctional integrity. Thus, cold exposure alters EC factors that perhaps impact blood flow and vessel permeability.



CN 309 ROLE OF 12(S)-HETE IN ENDOTHELIAL CELL RETRACTION AND ENHANCED TUMOR CELL ADHESION TO EXPOSED SUBENDOTHELIAL MATRIX, Irma M. Grossi and Kenneth V. Honn, Departments of Radiation Oncology, and Chemistry, Wayne State University, Detroit MI 48202. Preveously we've demonstrated that 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] is the principal lipoxygenase metabolite of arachidonic acid in the Lewis Lung carcinoma and B16 amelanotic melanoma. During the hematogenous phase of tumor cell metastasis, attachment of tumor cells to endothelium and subsequent induction of endothelial cell retraction preceeding tumor cell migration to the subendothelial matrix may be rate limiting. We demonstrated that 12(S)-HETE may play a critical role in three events occurring during tumor cell arrest: 1) enhanced receptor expression on tumor cells, 2) enhanced receptor expression on endothelial cells, and 3) induction of endothelial cell retraction. Recently we reported that both endogenous and exogenous 12(S)-HETE increases surface expression of an integrin receptor (i.e., IRGpIIb/IIIa) on several tumor cell lines resulting in their enhanced adhesion to endothelial cells, subendothelial matrix, and its components (i.e., fibronectin). Tumor cells also induce endothelial cell retraction which can be inhibited by lipoxygenase inhibitors but not by cylooxygenase inhibitors. Previous studies from our laboratory have demonstrated that homologous platelets can enhance tumor cell adhesion to endothelial cells and subendothelial matrix. 12(S)-HETE is the principal lipoxygenase metabolite of arachidonic acid synthesized during platelet-tumor cell interaction. We propose that 12(S)-HETE originating from both the tumor cell and the platelet may enhance receptor expression on tumor cells and endothelial cells and induce endothelial cell retraction. This hypothesis suggests that the generation of 12(S)-HETE may be a rate limiting parameter regulating key events occurring during tumor cell arrest and that platelets serve as a rate regulator for receptor expression and endothelial cell retraction.

CN 310 **Molecules involved in homing of hemopoietic precursors to the thymus**
B.A. Imhof, Basel Institute for Immunology, Grenzacherstr.487, Basel, Switzerland

We are intersested in cell-cell interaction of hemopoietic precursors with the thymic or perithymic endothelium. In order to carry out biochemical studies we decided to establish an *in vitro* model using both hemopoietic precursor and endothelial cell lines. A pro-T cell line (FT-F1, obtained from R. Palacios) bound to frozen sections from newborn mouse blood vessels in the thymus, to the thymic capsule, and to liver. No binding was obtained to control tissues like kidney, lung and heart. FT-F1 cells were subsequently used to search for an endothelial cell line that binds these pro-T cells. Out of a tested series, two lines, eEND-2 and t-End (both obtained from E. Wagner) bound FT-F1 to a reasonable extend.
A set of molecules, already found to be implicated in adhesion of mature lymphocytes to endothelium (LFA-1, Pgp-1), seem to play a role as accessory molecules in adhesion of lymphoid precursors. Functional antibodies to these molecules blocked adhesion to a minor percentage of control binding values. Upregulation of accessory molecules on the endothelium did not increase precursor binding. On the other hand co-culture of endothelial cells with thymic epithelium doubled the capacity of endothelial cells for precursor binding. Therefore new molecules may be involved in this cell-cell interaction. Monoclonal antibodies against the endothelial cell line eEnd-2 were prepared in our laboratory and have been screened for recognition of surface antigens. In order to test if positive clones may inhibit cell-cell adhesion we developed a method that quantifies precursor binding to endothelium. Using this assay, several antibodies were found functionally active.

CN 311 BASIC FIBROBLAST GROWTH FACTOR-SAPORIN MITOTOXIN: AN ENDOTHELIAL CELL GROWTH INHIBITOR, *Douglas A. Lappi, Darlene G. Martineau, Shuji Nakamura*, Marino Buscaglia, and Andrew Baird, the Department of Molecular and Cellular Growth Biology, The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92014 and *the Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.*

We have examined the effect on endothelial cells in culture of a mitotoxin (bFGF-SAP) synthesized by the chemical conjugation of saporin, a ribosome-inactivating protein, and basic fibroblast growth factor (bFGF). The mitotoxin enters target cells via the bFGF receptor, inhibits protein synthesis and elicits cell death. Cytotoxicity is observed at picomolar to nanomolar concentrations when tested on corneal endothelial cells, aortic arch endothelial cells and aortic capillary endothelial cells, which have as few as 2500 bFGF receptors per cell. Minimal to no toxicity was observed with saporin alone or with a mixture of bFGF and saporin (10 nM). AIDS-KS-3 cells, isolated from a lung biopsy of Kaposi's sarcoma, are approximately 40-fold more sensitive to the cytotoxic effects of bFGF-SAP than the non-conjugated mixture of bFGF and saporin.

The efficacy of the mitotoxin also has been examined *in vivo*. Mortality to mice occurred at a dose of 0.5 mg/kg, which is more than ten-fold lower than the LD_{50} of saporin, indicating delivery to specific targets. With animals injected with 1 mg/kg there was extensive hemorrhaging, often in the intestinal tract. This may indicate targeting of vascular endothelial cells *in vivo*. bFGF-SAP may be potentially useful as a therapeutic agent for the treatment of endothelial cell pathologies such as Kaposi's sarcoma or diabetic retinopathy.

CN 312 **TOWARDS SOMATIC CELL GENE THERAPY USING ENDOTHELIAL CELLS,** **Carmel M. Lynch, David I. Israel*, and A. Dusty Miller, Program in Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, and *Genetics Institute, Cambridge, MA 02104**

Somatic cell gene therapy may offer an alternative treatment of haemophilia A, a bleeding disorder resulting from a deficiency of factor VIII (FVIII). A truncated FVIII cDNA (B-domain deleted) was inserted into a retroviral vector such that FVIII gene transcription was from the viral 5' LTR, and neo gene transcription was from an internal SV40 promoter. The B-domain of FVIII is not required for *in vitro* or *in vivo* coagulant activity and was deleted to accommodate packaging of the recombinant retroviral mRNA. Virus produced by PA317 amphotropic retrovirus packaging cells had a titer of 10^3 - 10^4 $G418^r$ cfu/ml. The amount of active FVIII protein secreted ranged from 4.5 - 21 mU/10^6 cells/day. The levels of full length viral mRNA in the FVIII PA317 clones was low. The low levels of RNA are probably due to the reported instability of FVIII mRNA and account for the low virus titer and FVIII production observed. Similar results were obtained upon infection of NIH3T3 TK^- cells. However, human primary fibroblasts infected with FVIII produced high levels of mRNA. There was a concomitant increase in the amount of FVIII protein secreted, up to 250 mU/10^6 cells/day. These results demonstrate that retroviral vector-mediated transfer of the FVIII gene to human primary fibroblasts is possible. Endothelial cells are an alternative target tissue for somatic cell gene therapy, offering potential advantages for FVIII gene therapy. In particular, they produce von Willebrand (vW) factor which protects FVIII from proteolysis. Infection of human umbilical vein endothelial (HUVE) cells with FVIII virus is presently underway. The instability of FVIII mRNA in the PA317 amphotropic packaging cells is also being investigated. Deletion analysis of the FVIII cDNA is being performed in an attempt to improve FVIII virus titer and protein production.

CN 313 REGULATION OF GENE EXPRESSION IN ENDOTHELIA CELLS EXPOSED TO SHEAR STRESS: IMPLICATIONS FOR THROMBOSIS, ATHEROSCLEROSIS, AND INTIMAL HYPERPLASIA, Larry V. McIntire, Scott L. Diamond and John D. Sharefkin, Biomedical Engineering Laboratory, Rice University, Houston, Texas 77251
Fluid shear stress can stimulate secretion of tissue plasminogen activator (tPA) by cultured human endothelial cells, while plasminogen activator inhibitor type-1 secretion remains unstimulated. To determine whether hemodynamically-induced changes in tPA messenger RNA (mRNA) levels also occur, cells from the same primary culture of human umbilical vein endothelial cells (HUVEC) were maintained in stationary culture or exposed to arterial levels of shear stress (25 dynes/cm2) for 24 hours. Total cellular RNA was isolated from the shear stressed and stationary cultures and the relative levels of tPA mRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were determined using a coupled reverse transcriptase/polymerase chain reaction (PCR) method. As indicated by the amount of amplification product, tPA-mRNA levels were many fold higher (>10) in endothelial cells subjected to shear stress for 24 hours than in stationary controls. In contrast, mRNA level for GAPDH were similar in control and shear stressed cells. The constancy of the measured GAPDH signal indicated that the tPA response was a selective effect of fluid shear stress. Using the PCR method, the mRNA levels of basic fibroblast growth factor (bFGF) were found not to vary in comparison to GAPDH mRNA after 24 hours of shear stress. HUVEC secretion of endothelin, a potent vasoconstrictor and smooth muscle cell mitogen, was down regulated (by 90%) after 2 to 4 hours of exposure to shear stress. The level of endothelin mRNA was almost undetectable in cells exposed to shear stress for 24 hours. These results indicate that some of the enhancement of the fibrinolytic potential of endothelial cells in response to hemodynamic forces could involve transduction of shear stress to initiate transcriptional events. Also, the thickening of the vessel wall in low shear stress zones near arterial bifurcations could be due to overexpression of endothelin. Intimal thickening is not found in high stress zones and this absense may be due to reduced production of endothelin by the shear stressed endothelium.

CN 114 H_2O_2 AND ENDOTHELIUM: A CONTINUUM FROM LYSIS TO ACTIVATION, C.F.Moldow, S.P.Severson and G.V.Vercellotti, VA Medical Center, Minneapolis, MN 55417
Neutrophil hydrogen peroxide (H_2O_2) causes a diversity of effects in endothelial cells (EC), ranging from alterations in viability, permeability, eicosanoid production (PGI_2) and stimulation of metabolic events (glutathione turnover). We asked (1) would H_2O_2 stimulate signal transduction ? and (2) would H_2O_2 pretreatment interfere with agonist-mediated transduction? Mediators (thrombin and histamine) cause EC activation via a transduction mechanism involving calcium flux, and hydrolysis of phosphatidyl inositol 4, 5, bisphosphate (IP) to yield inositol polyphosphates (IP_3). Brief exposure of EC to H_2O_2 induces a dose-dependent, transient increase in calcium flux, but H_2O_2 (10^{-4}M,1hr) pretreatment of EC blocks thrombin induced calcium flux completely. In addition, pretreatment of EC with H_2O_2 prevents production of IP_3 in response to thrombin and histamine. Inhibition of IP_3 is time and concentration-dependent (65% inhibition after 1 hr). These EC are viable and produce tissue factor in response to endotoxin. Catalase and deferoxamine pretreatment of EC prevents H_2O_2 inhibition of IP hydrolysis. Thrombin-induced PGI_2 and platelet activating factor (PAF) are linked to IP hydrolysis; EC pretreatment with H_2O_2 (10^{-4}M) reduces PGI_2 and PAF production by thrombin at least 80%. We hypothesize a continuum of H_2O_2-mediated EC events ranging from cell activation with release of EC products to inhibition of cellular signal transduction, metabolic function, and finally lysis. H_2O_2 concentration, duration of exposure and EC antioxidant potential modify EC response to this inflammatory stimulus.

CN 115 GLUCOCORTICOID ENHANCED GROWTH OF AIDS-ASSOCIATED KAPOSI'S SARCOMA-DERIVED SPINDLE CELLS. Shuji Nakamura[1], Parkash Gill[2], Shinsaku Sakurada[1], Barbara Ensoli[1], Robert C. Gallo[1], and S. Zaki Salahuddin[1]. [1]Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892 and [2]Norris Hospital, University of Southern California, Los Angeles, CA 90033
Corticoids are well known for inducing and stimulating the production of various proteins which can regulate the important biological functions. Recently, in case of transplantation, immunosuppressive therapy and even AIDS, it has been reported that patients might develop Kaposi's sarcoma (KS) with increased risk when treated with corticosteroids. We had reported earlier the development of long-term cell culture using a novel culture system. Isolates from a number of patients with KS have been established from lung, oral and orbital cavity, skin and pleural effusion. These cultured cells show properties of vascular smooth muscle cells. These cells show properties of vascular smooth muscle cells. These cells are apparently functionally abnormal and respond well to factors released by cells infected with human retroviruses, e.g., culture supernatant from HIV-1 infected cells. Recent studies have also shown that HIV-1 tat gene production can also stimulate the growth of these cells (Ensoli et al.). We report here the effect of the corticosteroids on AIDS-KS cells under standard culture condition in our laboratory. The growth of all the cultured AIDS-KS cell was enhanced by several glucocorticoids and the observed augmentation was inhibited by a cortisone antagonist, RU 486. Our data indicates a direct effect of corticosteroid on the cultured AIDS-KS cells possible through corticosteroid receptor. It also emphasizes the need for judicious use of these compounds in patients at risk for developing KS.

CN 116 CHARACTERIZATION OF VASCULAR PERMEABILITY FACTOR RECEPTORS, Jitka V. Olander, Daniel T. Connolly, Deborah M. Heuvelman, Cheryl E. Hotchkiss, Judith A. Sligar, Joseph Feder, and Joseph E. DeLarco, Cell Culture and Biochemistry Department, Monsanto Co., St. Louis, MO 63167 Vascular permeability factor (VPF) is an approximately 40 kDa glycoprotein, produced by tumor cells and pituitary follicular cells, which induces, at 10^{-9} M, vascular permeability to fluid and proteins. This homodimer also stimulates endothelial cell specific growth at 10^{-12} M and promotes angiogenesis in vivo. The function of VPF is presently unknown but its bioactivities suggest it could act during inflammation, wound healing and tumor angiogenesis. A high affinity (10^{-11}M) VPF receptor has been identified on endothelial cells and its initial characterization is presented in this poster.

CN 117 INHIBITION OF PROTIEN SYNTHESIS IN CEREBRAL MICROVASCULAR ENDOTHELIUM BY PENTOBARBITAL, Jeffrey J.Olson, Charles Schnee, Nancy Edwards and Marsha Merrill, Surgical Neurology Branch, NINDS, National Institutes of Health, Bethesda, MD 20892

Pentobarbital produces profound neuronal depression as used in cases of increased intracranial pressure and during temporary occlusion of major cerebral vessels. Reduction in cerebral energy requirements is proposed as the primary protective mechanism. As an agonist of GABAergic activity, pentobarbital decreases overall neuronal electrical activity and energy requirements. This study evaluated the effect of pentobarbital on another important component of the cerebral parenchyma, the microvascular endothelia. Microvascular endothelia were isolated from adult bovine brain utilizing mechanical dissociation, enzymatic digestion, and separation from other cellular components by gradient centrifugation. The resultant cells stained positively for Factor VIII and displayed autoflourescence with Di-Ac-LDL. Twenty-four hours after confluence, cells of passage four or less on multi-well plates were washed and half the wells were incubated two hours in maintainence media with 10% fetal calf serum. The other half were similarly incubated with the addition of 200 micrograms/ml of pentobarbital. Leucine incorporation was then measured by scintillation counting after incubation with ^{3}H-leucine for 2 hours.The leucine incorporation in the pentobarbital treated group was 15.10×10^{4} +/- 1.18×10^{3} cpm cpm (mean +/- SD). This is significantly less ($p < 0.01$) than the control values of $316\,20 \times 10^{5}$ +/- 4.32×10^{3} cpm, reflecting a clear decrease in the metabolism of leucine. Besides depressing synaptically active tissue, pentobarbital decreases the metabolism of cerebral microvascular endothelia. This is predictable as GABA receptors occur on endothelia elsewhere in the body. This decrease in amino acid metabolism in this important component of the blood-brain-barrier may include a decrease in overall transport, further explaining the decrease in cerebral metabolism induced by pentobarbital.

CN 318 CULTURED KAPOSI'S SARCOMA CELLS EXPRESS VASCULAR PERMEABILITY INDUCING ACTIVITY, Shinsaku Sakurada, Shuji Nakamura, Robert C. Gallo and S. Zaki Salahuddin, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. We reported earlier a novel system for long-term culture of AIDS-associated Kaposi's sarcoma cells (AIDS-KS). This was accomplished with the aid of factor(s) derived from human retrovirus infected and/or transformed CD4 positive cultured T-cells. The same effect was obtained with supernatant from mitogen stimulated normal CD4 positive cells as well. The spindle cells were recovered from various organs, e.g., skin, lung, oral and orbital cavity and pleural fluid of AIDS patients with KS. These AIDS-KS cells share some of the characteristics of vascular smooth muscle cells. They express strong angiogenic activity which was detected in chicken chorioallantoic membrane assay and athymic nude mice after 5 days of transplantation of AIDS-KS cells. In nude mice the cells induced the lesion entirely composed of murine cells with histological features similar to KS lesions in man. In this report we would like to show that, in addition to angiogenesis, the metabolically active AIDS-KS cells induced a strong vascular permeability response at the site of transplantation in nude mice. The highest level of permeability is attained between 9 and 12 hrs, tapering off by 24 hrs post-transplantation. In contrast, fixed AIDS-KS cells did not induce vascular permeability. It is known that KS patients often have severe edema surrounding the lesion as an important clinical symptom. Our data suggest that the edema associated with KS lesions may not only be due to simple mechanical obstruction of the venous and/or lymphatic channel but also due to vascular permeability induced by the KS spindle cells. We suggest that KS spindle cells release factor(s) which support their own growth, the secreted characteristic and chemoinvasive factors causing growth and accumulation of other cells and causing secretion of other factors that mediate the development of KS.

CN 319 THE HIV tat GENE INDIRECTLY INDUCES BOTH DERMAL LESIONS RESEMBLING KAPOSI'S SARCOMA AND HEPATIC TUMORS IN TRANSGENIC MICE, Vogel, J.[1], Hinrichs, S.[2], Napolitano, L.[1] and Jay, G.[1], [1]Laboratory of Virology, Holland Laboratories, American Red Cross, Rockville, MD 20855, [2]Department of Human Pathology, University of California School of Medicine, Davis, CA 95616.

The HIV tat gene is critical for both viral gene expression and viral replication. When placed in transgenic mice under its own regulatory region, both skin lesions that resemble Kaposi's sarcoma (KS) and liver tumors are induced in male mice. These tumors are also present in patients infected with HIV. Expression of the tat gene is only present in the epidermal portion of the skin, and expression is not seen in the dermal tumors or in the hepatic tumors. This would suggest that the KS-like skin lesions and the hepatic tumors are indirectly induced by the tat gene, possibly by the induction of growth factors. These data imply that the HIV tat gene plays a role in the development of malignancies associated with HIV infection.

CN 320 ENDOTHELIAL CYTOPATHOGENICITY OF CYTOMEGALOVIRUS: AN IN VITRO MODEL EMPLOYING A CMV STRAIN PROPAGATED IN ENDOTHELIAL CELLS. WJ Waldman, DH Davis, MV Williams, WH Roberts*, DD Sedmak, RE Stephens; Depts of Pathology & Med Micro, Ohio State University, Columbus, Ohio, & VAMC, Dayton, Ohio*. Cytomegalovirus (CMV) is a source of major complications in immunosuppressed individuals and is associated with atherogenesis. Endothelial involvement in CMV infection is well documented, prompting a need for an accurate in vitro model. Traditionally the virus is propagated in fibroblasts; however this may alter its characteristics. Thus, we have propagated a recent isolate (CMV VHL) in human umbilical vein endothelial cells (HUVE) and, separately, in neonatal human dermal fibroblasts (NHDF). Infection of HUVE inoculated with either sub-strain was confirmed by CMV-specific in situ hybridization and by immunocytochemical staining for CMV antigens. Whereas infection of HUVE by VHL/E (endothelial-raised) was accompanied by dramatic cytopathology resembling that observed in vivo, the endothelial cytopathic potential of VHL/F (fibroblast-raised) was lost by its 20th passage in NHDF. Similarly, the ability of VHL/F to initiate sustained productive infection in HUVE was lost; plaque assay of culture supernatants and cell preparations, as well as virus-specific polymerase assay of cell lysates, demonstrated progressive viral reproductive activity in VHL/E-inoculated HUVE, whereas VHL/F production was barely detectable. Since properties of VHL/F strongly resemble those of other fibroblast-raised CMV strains (AD169, OSU CMV 3), these studies suggest that the process of "fibroblast-adaption" commonly employed in the propagation of CMV restricts the host range of the virus while attenuating its spectrum of cytopathic potential. We demonstrate that endothelial-based propagation preserves the natural cytopathogenicity of the original isolate.

CN 321 PLASMA TUMOUR NECROSIS FACTOR (TNF) AND PHOSPHOLIPASE A_2 (PLA_2) AS PROGNOSTIC MARKERS IN ADULT RESPIRATORY DISTRESS SYNDROME, Paul Walker and Alex Romaschin, University of Toronto and Toronto General Hospital, 200 Elizabeth Street, Toronto, ON M5G 2C4. Recent studies have suggested that cytokines, particularly IL-1 and TNF-α have a pivotal role in the etiology of shock and endotoxemia. TNF-α has potent effects on monocyte and polymorph respiratory burst activity, induction of endothelial white cell adhesive receptors and endothelial permeability. Elevated plasma phospholipase A_2 levels have been documented in patients with hypovolemic shock and ARDS. Phospholipase A_2 is the rate limiting enzyme in the liberation of arachidonic acid (AA) from phospholipid. Liberated AA can be subsequently metabolised via the cyclooxygenase or lipoxygenase pathways to potent mediators of pulmonary hypertension (thromboxanes) and edema (leukotriene C_4, D_4). We studied patients from our Surgical Intensive Care Unit who were mechanically ventillated. The control group consisted of patients who were recovering from orthopoedic and lung surgery or minor trauma. The experimental group consisted of patients who were at risk of developing ARDS and included patients with major abdominal surgery, aspiration, hemodynamic and septic shock. 80% of the patients in the experimental group developed ARDS and showed significant serial elevations in systemic TNF levels (ARDS group $\bar{x}$ = 517±713, n=9, control group $\bar{x}$ = 35±40 n=12, $p<0.03$ unpaired t test, levels given in pg/ml). Similarly patients in the ARDS group had profound elevations in plasma PLA_2 levels ($\bar{x}$=944±766, n=9) compared to control patients ($\bar{x}$ = 54±47, n=12, $p<0.002$ unpaired t test, enzyme activity expressed as nmoles phosphatidyl choline hydrolyzed/10 ul plasma/hr). ARDS patients who died showed a progressive increase in either PLA_2 or TNF levels prior to their demise.

CN 322 ACUTE ETHYL ALCOHOL ADMINISTRATION ALTERS DNA CELL-CYCLE KINETICS OF CULTURED HUMAN OMENTAL MICROVASCULAR ENDOTHELIUM. Dennis Way, Marlys Witte, Michael Bernas, and Charles Witte, Department of Surgery, The University of Arizona College of Medicine, Tucson, AZ 85724.

The mechanism of alcohol injury to cells is unclear including whether the damage is direct or through metabolic or physiologic intermediaries. As microvascular endothelium, particularly in the hepatoportal bed, is considered a prime target cell for ethanol injury, we examined acute effects of ethyl alcohol on DNA cell-cycle kinetics of human omental microvascular endothelium. Endothelial cells were isolated, cultured to confluence in F12:DMEM medium with 25mM HEPES and 20% FBS and exposed to increasing concentrations of ethanol ranging from 0.01-2 vol% for 1 hr. Cell-cycle alterations reflected in relative changes of $\%G_{1/0}$, S-phase, and $G_{2/m}$ were evaluated on a custom built CYTOMUTT flow cytometer (FCM) using propidium iodide-labeling of DNA. Results: Mean±SD (based on 20,000 cell count)

Ethanol (vol%)	0.0%	0.01%	0.1%	1.0%	2.0%
% S-phase	8.5±0.5	15.0±1.0	12.5±0.5	3.0±0.0	2.5±0.5
p value		<.01	<.01	<.01	<.01
% G_{2m}	6.5±0.5	8.5±0.5	9.0±0.0	7.0±0.0	7.0±0.0
p value		<.05	<.01	NS	NS

Twenty-four hours after removal of alcohol, endothelial cell-cycle kinetics returned to control values.

Acute ethanol exposure has a reversible biphasic effect on DNA synthesis: whereas concentrations between 0.01 vol% and 0.1 vol% increased both S-phase of the $G_{2/m}$ fraction (mitotic stimulation), 1 to 2 vol% reduced S-phase activity (mitotic suppression). Altered DNA synthetic rate after acute exposure to alcohol at levels found in circulating systemic and particularly portal blood of "binge-drinkers" provides a plausible basis for cell damage, abnormal tissue metabolism including collagen deposition, neovascularization, and oncogenesis primarily involving the liver and gastrointestinal tract in chronic alcoholism.