THE MOLECULAR BIOLOGY OF THE ENDOTHELIAL CELL

Organizers: Laurence Lasky and Thomas Maciag

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Plenary Sessions Page

Poster Sessions

Vascular Pathogenetic Mechanisms (Joint)

CA 001 MOLECULAR DETERMINANTS OF PULMONARY INFLAMMATION, Peter A. Ward and Michael S. Mulligan, The University of Michigan Medical School, Ann Arbor, Michigan 48109.

The use of animal models in rat lung following deposition of IgG immune complexes has provided important new insights into molecular determinants of inflammation and injury. These reactions feature the participation of both neutrophils and pulmonary macrophages and their products. Within one hr. (and peaking by 3.5 hrs.) after deposition of immune complexes, there is demonstrable appearance in the pulmonary venules and microvasculature of ELAM-1. Tissue upregulation of ELAM-1 is requisite for the recruitment of neutrophils and is associated with the presence of TNFα, which appears in lung to be a major determinant for ELAM-1 expression. Together with the role of ELAM-1, CD11b and CD18 are required for the accumulation of neutrophils and alveolar compartments. Following accumulation of neutrophils in the alveolar compartment, platelet activating factor accentuates the generation and/or release of toxic products from neutrophils and macrophages. It is now apparent that injury is the result of oxygen products from these phagocytic cells and has an L-arginine requirement. It seems that generation of nitric oxide ($^{\cdot}NO$) reacting with $O_2^{\cdot-}$ generates the reactive product, peroxynitrite anion ($ONNO^-$) and, ultimately, the highly reactive and toxic hydroxyl radical ($HO^{\cdot}$). These data demonstrate a complex matrix of mediators and products of different types of phagocytic cells involved in the process leading to tissue injury following deposition of IgG immune complexes.

Endothelial Cell Migration and Differentiation

CA 002 TNFα INDUCED GENES, Vishva M. Dixit, The University of Michigan Medical School, Ann Arbor, Michigan 48109.

Tumor Necrosis Factor α (TNF) is a catabolic proinflammatory cytokine secreted predominantly by cells of the monocyte lineage following activation. Of the many cell types responsive to TNF, the vascular endothelium has a fundamental role in mediating the proinflammatory activities of TNF. Positioned at the interface between the blood stream and body tissues, the endothelium actively regulates the flux of water, metabolites and leukocytes into tissues. When the endothelium is activated by TNF a number of proinflammatory alterations occur, including the induction of procoagulant activity and the expression of leukocyte receptors such as ELAM-1 and VCAM-1 which bind leukocytes and promote their transendothelial migration. In an attempt to characterize regulatory factors which function to initiate the cascade of endothelial responses to TNF, we previously used a strategy involving differential hybridization to clone TNF induced primary response genes. Time permitting, I will discuss progress made on the characterization of 3 such novel gene products.

1. A20: A 4.0kb transcript that is rapidly and transiently induced following TNF treatment. Open reading frame encodes for a protein composed of 790 amino acid residues; the carboxy terminus has seven novel zinc finger motifs that define a new class of zinc finger proteins. Antiserum raised against recombinant A20 protein identifies a protein of the predicted size in a variety of cells treated with TNF. The A20 protein is highly conserved; the amino acid sequence of the mouse homolog is over 90% identical. The over expression of A20 in transfected NIH3T3 cells while not influencing cell growth leads to a remarkable resistance to TNF cytotoxicity. Thus A20 may play an important role in mediating resistance to the cytotoxic effects of TNF. Characterization of the A20 promoter reveals that induction of transcription is mediated via NF-KB.

2. B94: A 3.5 kb transcript that is dramatically induced by TNF in endothelial and other cells including fibroblasts and keratinocytes. Encodes a 70kD protein with limited homology to ser/thr kinases. Mouse homolog is highly conserved and developmental northern blots reveal the presence of 2 related transcripts; the expression of each of which demonstrates temporal and organ specificity. These results suggest that beside mediating some of TNF's effects, B94, may play an important role in development. Additionally, this work raises the possibility that proinflammatory cytokines such as TNF and IL-1 may play a role in tissue remodeling both in inflammation and during embryogenesis.

3. B61: A 1.6kb transcript that is highly induced by TNF in endothelial cells and encodes a 21kD secreted protein; potentially a new paracrine factor. Developmental studies reveal it to be highly expressed in the mouse lung during the perinatal period arguing for a potential role in embryogenesis.

CA 003 PMA-INDUCIBLE IMMEDIATE EARLY GENES IN ANGIOGENESIS AND INFLAMMATION, Timothy Hla, Laboratory of Molecular Biology, Jerome Holland Laboratory for the Biomedical Sciences, The American Red Cross, Rockville, MD 20855.

Cultured human umbilical vein endothelial cells (HUVEC) proliferate in response to heparin-binding growth factor-1 (HBGF-1) and are growth inhibited and differentiate in response to phorbol myristic acetate (PMA). We have isolated and characterized novel PMA-inducible immediate-early (IE) genes in HUVEC by differential hybridization as well as by subtractive hybridization. Growth factor-inducible IE genes such as c-fos and c-jun mediate critical, regulatory events in the cell-cycle traverse. Analogously, PMA-inducible IE genes may be involved in key, decision-making events of endothelial cell growth control and/or differentiation. Sequence determination of two novel IE genes, edg-1 and -2, indicated that they encode a G-protein-coupled receptor (GPR) and a Zinc-finger-containing protein, respectively. Both edg-1 and -2 mRNA levels are induced 4-10 fold following the addition of PMA. While HUVEC growth inhibitors IL-1, TNF and PMA induced the edg-1 transcript in a biphasic fashion, HBGF-1 induced it in a sustained, monophasic manner. Another novel cDNA clone, termed edg-3 was induced strongly but transiently by PMA (50-100 fold). Sequence determination of edg-3 is currently ongoing. Of the three cDNA clones only the edg-1 mRNA was expressed in an endothelial cell-specific manner, suggesting that signal transduction events controlled by edg-1 may play an important role in angiogenesis. We have also cloned and characterized a novel PMA-inducible IE gene (hCox-2) that shares 60% amino acid sequence identity with the cyclooxygenase (Cox), a rate-limiting enzyme in the biosynthesis of prostaglandins (PG). The hCox-2 mRNA was induced 30 fold by PMA whereas the hCox-1 was induced only 2 fold in HUVEC, suggesting that the *de novo* induction of hCox-2 may account for the PMA-induced PG synthesis. In contrast IL-1, induced the hCox-1 gene in a sustained manner. HBGF-1 suppressed the hCox-1 mRNA levels but induced the hCox-2 mRNA transiently. Since PGE_2 is angiogenic and $PGF_{2\alpha}$ and PGI_2 can modulate the activities of the ras signalling pathway, the endogenous Cox-1 and -2 activity may modulate the angiogenic behavior of endothelial cells. The Cox polypeptides are induced *in vivo* in chronic inflammatory diseases such as rheumatoid arthritis (RA). In humans as well as in animal models of RA, the extent and intensity of Cox protein expression correlates with the intensity of the disease. Since angiogenesis is an important feature of RA, upregulation of Cox expression and hence increased PG synthesis may be an important event in the progression of the disease. The study of PMA-inducible IE genes in HUVEC may allow a better understanding of mechanisms involved in angiogenesis and inflammation.

CA 004 QUANTITATIVE STUDIES OF VASCULAR CELL MIGRATION BEHAVIOR, Paul A. DiMilla[1], Steven Albelda[1], John A. Quinn[1], Cynthia L. Stokes[2], Stuart K. Williams[3], and Douglas A. Lauffenburger[4]; [1]University of Pennsylvania, [2]University of Houston, [3]Thomas Jefferson Medical College, and [4]University of Illinois at Urbana-Champaign.

Migration of vascular cells, including endothelial cells and smooth muscle cells, is an important process in a variety of physiological functions. While many molecular components have been identified as influencing agents on vascular cell migration behavior – especially through mediation of cell surface receptors, quantitative information on relationships between receptor/ligand binding properties and migration parameters such as speed, directional persistence, and directional bias have been less forthcoming. Our work has emphasized the combination of engineering analysis and modeling approaches with cell motility assays to obtain truly quantitative data on the dependence of migration parameters on receptor and ligand properties. Initial work focused on migration responses of microvessel endothelial cells to aFGF, including a rigorous determination of chemotaxis in this system. We have found that the chemotactic sensitivity of individual endothelial cells to aFGF is quantitatively comparable to that of the well-characterized neutrophil leukocyte response to bacterial peptides, but in typical cell population assays this sensitivity is masked by the relatively slow movement speed of endothelial cells. More recently, we have studied the effects of extracellular matrix proteins on smooth muscle cell locomotion. In particular, we have developed a model capable of relating cell migration speed to substratum adhesiveness, allowing interpretation of a variety of experimental observations by ourselves and other investigators. An especially significant finding is that cell migration speed may be modulated by the combination of substratum adhesion ligand density and the receptor/ligand binding affinity.

Early Effector Changes - Vascular Cell Gene Regulation (Joint)

CA 005 TRANSCRIPTIONAL CONTROL OF THE ELAM-1 AND VCAM-1 GENES, Tucker Collins, Amy J. Williams, Andrew S. Neish, Jochen W.U. Fries, David M. Dorfman, Michael A. Gimbrone, Jr., and Myron I. Cybulsky, Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Boston, MA.

Endothelial-leukocyte adhesion molecule-1 (ELAM-1) is an endothelial-restricted cell surface glycoprotein expressed by cytokine-activated endothelium that mediates the adhesion of blood neutrophils. Vascular cell adhesion molecule-1 (VCAM-1) is a mononuclear leukocyte-selective adhesion molecule expressed on vascular endothelium following activation by IL-1 or TNF. To characterize the mechanisms regulating expression of the ELAM-1 and VCAM-1, the genes for these leukocyte adhesion molecules were isolated and their organization determined. Overlapping phage clones spanning the ELAM-1 and VCAM-1 genes were obtained from a human genomic library by plaque hybridization. Exons and their corresponding intron boundaries were located and sequenced. The ELAM-1 gene contains 14 exons spanning about 13 kb of DNA. The positions of the exon-intron boundaries correlate with the functional subdivisions of the protein. The human VCAM-1 gene is organized into at least 9 exons which correlate with the functional subdivisions of the cell surface protein.

The ELAM- and VCAM-1 promoters share several features which may be important in the regulation of expression: First, the major transcriptional start sites are located downstream of consensus TATAA sequence elements. Second, both VCAM-1 and ELAM-1 have consensus AP1 binding sites. Third, sequences are present in both promoters which fit the NF-kB binding site consensus (GGGR(C/A/T)TYYCC). These elements may play a role in the cytokine-mediated increased expression of this gene. Mobility shift analysis indicates that NF-kB-like binding factors are present in nuclear extracts of IL-1 treated endothelial cells which bind to this region of both the ELAM-1 and VCAM-1 promoters. Additionally, deletion analysis reveals that the NF-kB site is a functional element in the ELAM-1 promoter. Preliminary data from in vivo footprinting techniques confirms that the NF-kB site is a functional element in intact endothelial cells. To characterize the cytokine inducible proteins interacting with the ELAM-1 promoter, we have obtained a partial cDNA for a protein capable of binding to the NF-kB region of the ELAM-1 promoter. Briefly, an endothelial cDNA library in gt11 was screened with a radiolabeled, concatemerized recognition site DNA probe. Sequence analysis of a 1200 bp partial cDNA clone revealed an open reading frame containing a C2H2 zinc finger motif. Northern blot analysis revealed that the cDNA corresponded to a large (about 10 kb) transcript which is present in several cell types. This protein probably corresponds to the recently described PRDII-BF1 that binds specifically to a positive regulatory domain (PRDII) of the human ß-interferon promoter. The time course of induction of this factor by IL-1 in endothelial cells is consistent with this factor being a negative regulator of ELAM-1 gene expression. We are testing the model that IL-1 activation of the ELAM-1 gene is mediated by NF-kB and that a postinduction repression of ELAM-1 gene expression is controlled in part by a cytokine induced repressor capable of binding to this region of the ELAM-1 gene.

Comparison of the ELAM-1 and VCAM-1 promoter structures may provide insights into the mechanism(s) of cytokine activation as well as the basis for the endothelial-restricted pattern of ELAM-1 gene expression. Understanding the molecular basis of endothelial-specific gene regulation may provide a way of targeting gene expression to specific types of endothelial cells (e.g. postcapillary venular or large vessel aortic) in the germ line of a mouse. Using these elements it should be possible to selectively test what role specific proteins (e.g. growth factors or cytokines) have as contributing factors in vascular disease in defined transgenic models.

CA 006 DIFFERENTIAL REGULATION OF EXPRESSION OF THE TGF-BETA ISOFORMS, Seong-Jin Kim, Andrew G. Geiser, Robert Lafyatis, Paul B. Robbins*, Michael A. O'Reilly, Michael B. Sporn, and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892 and *Department of Molecular Genetics and Biochemistry, University Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Transforming growth factor-ßs (TGF-ß) are potent regulators of cell growth and differentiation. Evidence obtained from several species so far suggests that expression of each of the five TGF-ßs is differently regulated during development and in a tissue-specific manner. TGF-ß's 1, 2, and 3 have been demonstrated to be expressed in mammalian tissues, whereas TGF-ß4 has been described only in chicken and TGF-ß5 only in frog. Examination of the relative expression of TGF-ß's 1, 2, and 3 in a variety of cell lines reveals that some cells, such as human lung WI-38 secrete predominantly TGF-ß1, whereas monkey BSC-1 cells secrete principally TGF-ß2 and mouse myeloid cells (32D123) TGF-ß3. To investigate the mechanistic basis for the differential regulation of expression of the TGF-ß isoforms, the 5' flanking regions of the human genes for TGF-ß1, 2, and 3 were analysed. Significant differences have been found; unlike the TGF-ß2 and 3 genes in which classic TATAA boxes are found in the upstream of the transcriptional start site, the TGF-ß1 gene lacks a TATAA box and is characterized by the presence of a very GC-rich region containing several Sp1 binding sites. The TGF-ß1 gene has two distinct transcriptional start sites and each of these is preceded by one or more AP-1 binding sites which mediate TGF-ß1 autoinduction. Recent evidence indicates that AP-1 sites may also be the targets for activation of TGF-ß1 gene expression by v-src and the HTLV-1-TAX. The TGF-ß1 promoter is also activated by various other factors, including proto-oncogene products. The detailed analysis of transcriptional regulation of TGF-ß2 and 3 is in progress; expression of both TGF-ß2 and TGF-ß3 is induced by cAMP. Recently, we demonstrated that the retinoblastoma gene product, RB, induces TGF-ß1 gene expression through the Rb control element (RCE) identified from c-fos promoter. The TGF-ß2 promoter is also activated by Rb through the distinct promoter element other than RCE.

CA 007 ANALYSIS OF VESSEL FUNCTION BY DIRECT GENE TRANSFER IN VIVO, Gary J. Nabel[1], Zhiyong Yang[1], Rik Derynck[2], Christian Haudenschild[3], Thomas Maciag[4], and Elizabeth G. Nabel[1], [1]Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI, [2]U.C.S.F., San Francisco, CA, [3]Boston University, Boston, MA, [4]American Red Cross, Rockville, MD.

A variety of growth factors stimulate vascular cell proliferation and vessel formation in vivo. Although the genes encoding many factors have been cloned and their mechanism of action defined in vitro, definition of their role in vivo has been more difficult to analyze. To address this problem, we have developed methods to deliver recombinant genes directly into cells of the arterial wall in vivo. With this approach, recombinant genes are delivered by direct gene transfer (with DNA/liposome conjugates or retroviral vectors) or by cell-mediated gene transfer (with endothelial or vascular muscle cells). Our initial studies demonstrated the feasibility of this direct gene transfer approach using reporter genes. More recently, we have begun to analyze the effects of different recombinant growth and angiogenic factors in vivo. At least three recombinant genes have been expressed by direct gene transfer in porcine iliofemoral arteries where they induce neointimal hyperplasia within the vessel wall. These genes include PDGF B, a secreted form of acidic fibroblast growth factor, and transforming growth factor-β. Although these recombinant genes each stimulate vascular cell proliferation in vivo, they exert otherwise distinct effects on matrix and vessel formation. Analysis of vessels transduced with these recombinant genes will be described. The generation of intimal hyperplasia may represent an early common response to injury which can exert different effects on vessel repair.

Adhesion-Pathobiology (Joint)

CA 008 MONONUCLEAR LEUKOCYTE ADHESION TO ENDOTHELIUM, John Harlan[1], Timothy Carlos[2], Nicholas Kovach[1], Theresa Deisher[1], Terri Haddix[1], Kevin Montogomery[1], Timothy Pohlman[1], and Robert Winn[1], [1]University of Washington, Seattle and [2]Montefiore-University Hospital, Pittsburgh.

Adherence to endothelium is a pivotal event in the emigration of mononuclear leukocytes from bloodstream to extravascular sites of inflammation and immune reaction. This adhesive interaction is mediated by cell surface molecules expressed on leukocyte and the endothelial cell. For peripheral blood monocytes multiple receptor-ligand interactions are involved. The adherence of monocytes to resting cultured human umbilical vein endothelial cell (HUVEC) is mediated primarily by interaction of CD11/CD18 with ICAM-1, whereas adherence to cytokine-activated HUVE involves CD11/CD18/ICAM-1 and VLA-4/VCAM-1 and to a lesser extent SLe^X/ELAM-1. Studies in vivo support these in vitro observations as marked inhibition of monocyte emigration is observed with the combination of blocking monoclonal antibodies to CD18 and CD49d (VLA-4α). Mononuclear leukocyte adhesion to endothelium is regulated primarily at the level of avidity for leukocytes and cell surface expression for endothelium. In marked contrast to peripheral blood lymphocytes, treatment of monocytes with phorbol ester significantly reduces monocyte adherence to resting and cytokine-treated endothelium. Reduction in binding is associated with a decrease in binding of VLA-4 to VCAM-1. Induction of VCAM-1, but not ELAM-1, protein and mRNA by cytokines is inhibited by the protein kinase C (PKC) inhibitor staurosporine and by down-regulation of PKC by pretreatment with phorbol ester, demonstrating that regulation of these adhesive ligands for mononuclear leukocytes can be dissociated.

CA 009 THE PATHOPHYSIOLOGY OF THE VCAM1-VLA4 PATHWAY, Roy R. Lobb,Biogen Inc., 14 Cambridge Center, Cambridge, MA 02142

Vascular cell adhesion molecule-1 (VCAM1) is an adhesion molecule expressed *in vitro* on cytokine-activated endothelium, and *in vivo* on inflamed vascular endothelium, as well as on macrophage-like and dendritic cell types in both normal and inflamed tissues. VCAM1 is a member of the immunoglobulin (Ig) superfamily. Alternative splicing in human endothelium *in vitro* generates at least two forms of VCAM1, with either six or seven extracellular Ig-like domains. VCAM1 interacts with the beta-1 integrin VLA4 on mononuclear leukocytes, basophils, and eosinophils. VCAM1 can activate human T cells in conjunction with coimmobilized monoclonal antibody (mAb) OKT3, indicating that the VCAM1/VLA4 pathway mediates signal transduction as well as cell-cell adhesion. Thus, the VCAM1/VLA4 interaction may play a pathophysiologic role in immune responses as well as in leukocyte recruitment into sites of inflammation. Recent studies on VCAM1-dependent signal transduction, the relationship between VCAM1 structure and function, the functional role of the seventh inserted Ig-like domain, and the *in vivo* effects of blocking mAbs to VLA4 will be presented.

Adhesion-LEC-CAMS

CA 010 P-SELECTIN: A LEUKOCYTE-BINDING ADHESION MOLECULE ON PLATELETS AND ENDOTHELIAL CELLS: *ROLE IN* INFLAMMATION AND THROMBOSIS. Barbara C. Furie and Bruce Furie. Center for Hemostasis and Thrombosis Research, New England Center and Tufts University School of Medicine, Boston MA 02111.

P-selectin on platelets and endothelial cells and E-selectin on endothelial cells are leukocyte receptors that recognize lineage-specific sugars on neutrophils and monocytes. The ligands for these receptors contain the Lex core and sialic acid. Since others have shown that both E-selectin and P-selectin bind to sialylated Lex, we evaluated whether E-selectin and P-selectin recognize the same counter-receptor on leukocytes. Theinteraction of HL60 cells with Chinese hamster ovary cells expressing P-selectin or E-selectin was studied. To determine whether a protein component is required in addition to sialyl Lex for either P-selectin or E-selectin recognition, HL60 cells were digested with multiple proteases. Cells treated with these proteases bound E-selectin but not P-selectin. Fucosidase or neuraminidase treatment of HL60 cells markedly decreased binding to both E-selectin- and P-selectin-expressing CHO cells. Purified P-selectin inhibited CHO-P-selectin binding to HL60 cells, but incompletely inhibited CHO-E-selectin binding to HL60 cells. However, purified soluble E-selectin inhibited CHO-P-selectin and CHO-E-selectin binding to HL60 cells equivalently and completely. COS cells, unable to bind to E-selectin or P-selectin, bound ELAM but not P-selectin upon transfection with α1,3 fucosyltransferase or α1,3/1,4 fucosyltransferase. Similarly, LEC11 cells expressing sialyl Lex bound E-selectin- but not P-selectin-expressing CHO cells. *Sambucus nigra* lectin, specific for the sialyl-2,6βGal linkage, inhibited P-selectin but not E-selectin binding to HL60 cells. Although sialic acid and Lex are components of the P-selectin ligand and the E-selectin ligand, these results indicate that the ligands are related, having overlapping specificities, but are structurally distinct. A protein component and a sialyl-2,6βGal structure on the P-selectin ligand may contribute to its specificity for P-selectin. Based on its cell adhesion function in *in vitro* experiments, P-selectin is thought to play a role in inflammation and thrombosis by mediating the interaction of leukocytes with platelets bound in the region of tissue injury and with stimulated endothelium. To evaluate the role of P-selectin in platelet-leukocyte adhesion *in vivo*, we have employed an arteriovenous shunt model in baboons. We determined the effect of antibodies that inhibit P-selectin-mediated platelet-leukocyte interaction on the uptake of leukocytes in thrombus within the shunt. An *ex vivo* Dacron graft implanted within the arteriovenous shunt is thrombogenic, accumulating platelets and fibrin within its lumen. These bound platelets express P-selectin, as demonstrated by nuclear imaging of a Dacron graft using ^{123}I-labeled anti-P-selectin antibodies. The interaction of leukocytes with P-selectin expressed on platelets immobilized on the graft blocks leukocyte accumulation into the thrombus. These results indicate that leukocyte accumulation within the thrombus is mediated by P-selectin.

CA 011 SELECTIN-CARBOHYDRATE MEDIATED RECRUITMENT OF LEUKOCYTES IN INFLAMMATION, James C. Paulson*, Karl-E. Arfors#, Elissa Keogh*, Laurie Phillips*, Margaret Polley*, Ulrich H. von Andrian#, Bill Way*, Jeff Winkelhake*, *Cytel Corporation, San Diego, CA 92121, #La Jolla Institute for Experimental Medicine, La Jolla, CA 92037.

Two members of the selectin family of adhesion molecules, the E-selectin and P-selectin, mediate recruitment of leukocytes to sites of inflammation and tissue injury following their expression on endothelial cells of post-capillary venules. These selectins recognize endogenous carbohydrate ligands on neutrophils, monocytes, and certain other leukocytes which are members of a sialylated and fucosylated class of structures typified by sialyl-Lex, NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc-. Although the two selectins recognize similar carbohydrate ligands, the E-selectin is expressed in response to inflammatory cytokines and bacterial endotoxin while the P-selectin is expressed in response to thrombin, histamine and oxygen radicals, predicting that they play different and complimentary roles in recruitment of leukocytes. Results from several laboratories suggest that the E-selectin and P-selectin mediate the initial interaction or rolling of leukocytes on activated endothelium. Recent results on the mechanism of selectin mediated recruitment of leukocytes, their detailed carbohydrate specificities, and their role in leukocyte mediated disease will be presented.

CA 012 GRANULAR TARGETING OF VON WILLEBRAND FACTOR AND P-SELECTIN, Denisa D.Wagner,Joost A. Koedam, Tanya N. Mayadas, Agnes M. Journet and Elisabeth M. Cramer, New England Medical Center and Tufts University, Boston and Hopital Lariboisier, Paris, France.

Endothelial cells contain specific storage granules called Weibel-Palade bodies that are rapidly released during vascular injury or in areas of inflamation. We have shown that these elongated granules contain two adhesive proteins: von Willebrand factor (vWf) and P-selectin (PADGEM, GMP-140, CD 62). vWf, a major component of the granule, is a soluble multimeric protein important for platelet adhesion. In contrast, P-selectin is a component of the granular membrane which functions as a receptor for monocytes and neutrophils after it is exocytosed. P-selectin and vWf have the same tissue specificity of expression and are found in & granules of platelets in addition to Weibel-Palade bodies. We are interested in the function and formation of these storage granules.To study the signals on vWf and P-selectin that direct them to the storage granules, we express the proteins in a pituitary cell line (AtT-20) that can store proteins in a regulated manner. We have found that vWf was stored in these cells in granules similar in size and shape to Weibel-Palade bodies and distinct from the endogenous ACTH granules. The large prosequence of vWf was actively involved in the proteins storage. It is likely that a linear targeting sequence does not exist in the propeptide since deletion of either the C-terminal or N-terminal half of the propeptide abolished storage. The function of the propeptide in vWf storage may rather be in directing non covalent aggregation of the protein that may be necessary for granule formation. Covalent multimerization of vWf was not a prerequisite for storage. To investigate whether the mechanism of targeting of the transmembrane receptor P-selectin to granules is specific for megakaryocytes and endothelial cells/and or dependent on vWf,we have expressed P-selectin cDNA in AtT-20 cells. In contrast to vWf which formed new Weibel-Palade body-like organelles in these cells, the immunofluorescence staining pattern for P-selectin closely resembled that of ACTH. Fractionation of the P-selectin transfected cells resulted in the codistribution of P-selectin and ACTH in cellular compartments of the same density. Immunoelectron microscopy using a polyclonal anti-P-selectin antibody confirmed its presence in the membranes of electron dense endogenous ACTH granules. Binding experiments with radiolabeled monoclonal antibody to P-selectin indicated that there was also surface expression of P-selectin on the AtT-20 cells. After stimulation with the secretagogue 8-Br-cAMP the surface expression increased 2-fold, concomitant with the release of ACTH. Our results indicate that in the AtT-20 cells P-selectin follows the regulated pathway of secretion and that it contains an independent sorting signal which directs it to storage granules.

Vascular Cell Phenotype (Joint)

CA 013 ENDOTHELIAL CELL-MURAL CELL INTERACTIONS, Patricia A. D'Amore, Children's Hospital and Harvard Medical School, Boston, MA 02115

Endothelial cells (EC) and mural cells (smooth muscle cells [SMC] in large vessels and pericytes in the microvasculature) make frequent contact throughout the vascular system. In addition to the observations of physical contact between the two cell types, a number of tissue culture studies have provided evidence that mural cells influence EC growth and behavior and vice a versa. We are interested in this interaction from both developmental and functional viewpoints. Embryonic studies suggest that EC are able to recruit mural cells and subsequently influence their behavior. In EC-mural cell cocultures, we have observed morphologic changes in pericytes and SMC that are consistent with this concept. In order to investigate the factor or factors that might be responsible for EC recruitment of mural cells, we have applied an under agarose migration assay in which we examined the effect of EC on SMC migration. Coculture of EC and SMC in wells separated by 1-2 mm resulted in a dramatic increase in SMC migration toward the EC when compared to their migration in the absence of EC. Using neutralizing antibodies against platelet derived growth factor BB and basic fibroblast growth factor (bFGF) (that we have produced and characterized) we demonstrated that these two factors are responsible for nearly all of the increased SMC migration. In addition, we observed that neutralizing antibodies against bFGF were capable of blocking virtually all of the baseline (unstimulated) SMC migration. Thus, we speculate that the elaboration of these two factors by EC in developing vessels, leads to the attraction of the mural cells to the newly forming vessels. Once at the vessel, we suspect that the mural cells and EC make contact through specific cell adhesion molecules. To identify the molecules involved in this interaction we have developed an *in vitro* binding assay in which EC adhesion to a mural cell monolayer is quantified. Using this system, we have determined that EC-mural cell adhesion is mediated by at least two classes of molecules. Addition of blocking antibodies against the beta-1 and alpha-5 integrin subunits or the synthetic peptide GRGDSP revealed that 30-50% of the binding is the result of integrin-matrix interactions. The remainder of the adhesion is due to as yet unidentified molecules. We suspect that this component of the heterotypic interaction is mediated by specific cell adhesion molecules that are important in the subsequent formation of gap junctions between EC and mural cells and that the interaction is critical for the expression of a differentiated endothelial cell phenotype and in the modulation of mural cell growth and function.

CA 014 TRANSCRIPTIONAL REGULATION OF THE SMOOTH MUSCLE ALPHA ACTIN PROMOTER: EVIDENCE FOR INVOLVEMENT OF HELIX-LOOP-HELIX FACTORS. Gary K. Owens, Richard Shimizu, Maria Thompson, Colleen McNamara, and Randal S. Blank. Department of Physiology, University of Virginia, School of Medicine, Charlottesville, VA 22908.

In previous studies, we utilized deletion mapping of the chicken smooth muscle (SM) alpha actin promoter to define sequences required in cis for SM-specific transcriptional activation of a heterologous reporter gene. In these studies, we examined the ability of promoter deletion mutants to direct expression of a linked chloramphenicol acetyltransferase (CAT) fusion gene following transient transfection into rat aortic SMC. In contrast to observed activities in chicken embryonic myoblasts and fibroblasts, addition of sequence between -151 and -257 (p257CAT) resulted in large increases in CAT reporter activity. Additional deletion analysis localized this activity to a region between -209 and -257. Sequence analysis of this region of the rat and chicken promoters revealed a conserved consensus E box at -214 in the chicken and -213 in the rat gene that closely matches the preferred DNA binding site for MyoD. However, no MyoD mRNA was detected in SMC by Northern analyses. Gel shift analyses using SMC nuclear extracts provided evidence for specific protein interactions with a 276 bp DNA SM alpha-actin promoter fragment (-257 through +19). To further test whether the SM alpha-actin promoter could be regulated by helix-loop-helix (HLH) transcriptional regulatory factors, rat aortic SMC were co-transfected with SM alpha-actin promoter/reporter plasmids as well as with expression vectors containing cDNA's encoding the HLH factors, ITF-1, MyoD, MRF4, Myf5, and myogenin. Co-transfection of the promoter/reporter with the skeletal factors MyoD, MRF4, Myf5, or myogenin failed to significantly trans-activate the E-box- containing promoter (p257CAT). However, co-transfection with ITF-1, a ubiquitous regulatory factor which heterodimerizes with muscle-specific factors, resulted in a marked stimulation of CAT reporter activity. Taken together, these data support a role for HLH factors in the trans-activation of the SM alpha actin promoter and suggest a complex interplay of positive and negative factors in the coordinate regulation of SM-specific genes. Supported by PHS Grants R01-HL38854, P01-HL19242, and T32-HL07355.

CA 015 ON THE ORIGIN OF JUXTAGLOMERULAR SMOOTH MUSCLE CELLS, Curt D. Sigmund[1], Craig A. Jones[2], Edward Novak[2], Richard T. Swank[2] and Kenneth W. Gross[2], [1]Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52241, [2]Department of Molecular & Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263.

The classical site of renin expression in mammals is the juxtaglomerular (JG) cells of the kidney. These cells have the ability to regulate expression and secretion of renin in response to a number of neural, humoral and physiological cues. Renin expressing JG cells lie in the most distal region of the afferent arteriole near the glomerular hilus in a region bridging the circulatory and excretory systems. Although JG cells are epithelioid in appearance, containing a well organized secretory apparatus, they are generally thought to be derived from smooth muscle. Nevertheless, the distinction between the renin expressing smooth muscle cells, commonly termed JG cells, and the non-renin expressing cells which lie only a few cells further upstream is not altogether clear. In fact, these non-renin expressing cells can be induced to express renin under physiological stress or by pharmacological treatment. In addition, experiments performed by a number of laboratories has clearly established the developmental profile of renin expression, that is the shift in cell-specific expression from smooth muscle cells of the earliest detectable intra-renal arteries ($\approx$ 15 days of gestation in the mouse and rat) through the developing renal vascular tree and finally to JG cells ($\approx$ 18.5 days of gestation through neonatal life). Despite many years of experimentation, our knowledge of the biology of the JG cell remains restricted to its expression of renin. Certainly, a major limiting factor in studying these cells is their relative paucity in the kidney; JG cells make up substantially less than 0.1% of the total cellular mass of the kidney. In an effort to better understand the cell biology of the JG cell we sought to isolate a permanent immortalized JG cell line that maintains its differentiated characteristics. To this end, we employed oncogene mediated tumorigenesis in transgenic mice. Transgenic mice were constructed with a fusion gene consisting of a tissue- and cell-specific renin regulatory element fused to the potent oncogene SV40 T antigen with the overall goal of specifically targeting production of the oncoprotein in renin expressing cells. Kidney was among the sites exhibiting tumorigenesis. The tumors expressed high levels of renin mRNA and contained renin and were used to isolated a tumoral cell line. We have thus far isolated several non-clonal cell lines and a single clonal cell line derived from kidney tumors. The clonal cell line (As4.1) maintains high level expression of renin mRNA over long periods of continuous culture ($>$ 35 passages). They have the capacity to regulate expression of renin over a 10 fold range dependent on the culture conditions. Renin activity measurements demonstrate the cells contain and secrete active renin and preliminary pulse-chase experiments suggest the constitutive secretion of prorenin and retention of intra-cellular active renin. The contributions of classical constitutive and regulated pathways for secretion of renin are now being pursued. It is our hope that the construction of cDNA and subtraction libraries from these cells will help to identify novel JG cell specific genes, genes involved in the expression and realization of renin and help establish their relationship to renal vascular smooth muscle cells.

Growth Regulatory Molecules (Joint)

CA 016 HEPARIN-BINDING EGF-LIKE GROWTH FACTOR, STRUCTURAL AND BIOLOGICAL PROPERTIES. Michael Klagsbrun, Moshe Marikovsky, Judith Abraham, Stewart Thompson, Deborah Damm, and Shigeki Higashiyama, Children's Hosptial and Harvard Medical School, Boston, MA 02115; and California Biotechnology Inc., Mountain View, CA 94043

Heparin-binding EGF-like growth factor (HB-EGF), an O-glycosylated polypeptide of about 86 amino acids, is a newly-identified member of the EGF family (Higashiyama et al., Science 251: 936-939, 1991). Like EGF and TGFα, HB-EGF binds to and phosphorylates the EGF receptor. Unlike the non-heparin-binding EGF and TGFα, however, HB-EGF is eluted from heparin affinity columns with about 1-1.2 M NaCl. Structurally, HB-EGF appears to consist of two major domains: a C-terminal, EGF-like domain that is about 40% homologous to EGF and TGFα; and a hydrophilic N-terminal extension of 35-45 amino acids that has no counterpart in EGF and TGFα. This two domain structure is reminiscent of another recently-identified EGF family member, amphiregulin. Results of experiments employing synthetic peptides and site-directed mutagenesis have indicated that the EGF-like C-terminal domain is involved in EGF receptor binding, while heparin binding involves sites mostly in the N-terminal extension and in the first several amino acids of the C-terminal EGF-like domain. The heparin-binding sites in HB-EGF appear to be responsible not only for the interaction of this growth factor with immobilized heparin, but also for binding of HB-EGF to cell surface heparan sulfate proteoglycans, which have been identified as low affinity receptors.

The biological properties of HB-EGF suggest possible physiological and pathological roles for this growth factor. Physiologically, HB-EGF may play a role in the macrophage-mediated response in injury, in that (i) HB-EGF mRNA and protein are produced by macrophage-like cells; (ii) HB-EGF is mitogenic for fibroblasts and keratinocytes; and (iii) HB-EGF is found in pig wound fluid created by medium partial thickness excisional wounds made on the backs of pigs. Pathologically, HB-EGF could be involved in the smooth muscle cell (SMC) hyperplasia associated with atherosclerosis, restenosis, and/or hypertension, in that HB-EGF is a potent mitogen for SMC (ED_{50} on bovine aortic SMC of 100 pg/ml), and SMC in culture express HB-EGF. We have found that fetal human vascular SMC and human newborn thoracic SMC both express the HB-EGF mRNA; in the case of the newborn thoracic SMC, the level of the HB-EGF transcript can be dramatically increased by treating the cells with the HB-EGF protein or with serum. In addition, the conditioned medium of the fetal human vascular SMC has been shown to contain growth factor activity that elutes from heparin columns with 0.8-1.2 M NaCl, that competes with ^{125}I-EGF for binding to A-431 cells, and that cross-reacts with anti-HB-EGF antibodies on Western blots. We conclude that HB-EGF is both produced by, and is a mitogen for SMC and that smooth muscle cell hyperplasia may involve an HB-EGF autocrine loop.

CA 017 IL-1α VERSUS FGF-1: ANTAGONIST HUMAN ENDOTHELIAL CELL FUNCTIONS FROM SIMILAR STRUCTURAL FEATURES, T. Maciag, Laboratory of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.

The regulation of human endothelial cell proliferation is regulated by members of the FGF family while the process of non-terminal human endothelial cell differentiation as well as the terminal process of human endothelial cell senescence is regulated by IL-1α, a member of the IL-1 family. The FGF and IL-1 families contain similar features including (1) weak structural similarity, (2) similar crystallographic features, and (3) the evolution of two prototype members lacking a signal sequence for secretion, as well as additional family members containing a signal sequence. Further, nuclear localization of the FGF and IL-1 prototypes have been reported. While these similarities are interesting, the human endothelial cell responds to FGF-1 and IL-1 differently. Because the FGF and IL-1 prototype family members exert their action by the occupancy of high affinity cell surface receptors, the mechanism utilized for the secretion of these prototypes is an important yet poorly understood pathway. Recent data from this laboratory suggest that FGF-1 may be transported into the extracellular environment by a heat-inducible polypeptide suggesting that stress may be involved in the regulation of secretion. This pathway may be of interest because the IL-1 prototypes are well known for their roles as mediators of inflammation whereas the FGF prototypes are potent inducers of angiogenesis.

CA 018 GROWTH INHIBITION BY TGFß, Harold L. Moses, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

TGFß1 is the prototype of a large family of genes involved in growth control, extracellular matrix production, and development. TGFß1 has marked stimulatory effects on connective tissue formation. It is chemotactic for fibroblasts, an indirect mitogen for certain mesenchymal cells and a stimulator of extracellular matrix deposition. The TGFßs are also potent inhibitors of proliferation of most cell types in culture. *In vivo* studies have indicated that the predominant effect of TGFß1 on cell proliferation is inhibition. We have investigated the mechanism of TGFß1 inhibition of skin keratinocyte proliferation. Earlier studies demonstrated that TGF-ß1 inhibition of keratinocyte proliferation involves suppression of c-*myc* transcription and indirect evidence suggested that pRB may be involved in this process. Skin keratinocytes transformed by SV40 and human papilloma virus-16 (HPV-16) or HPV-18 were demonstrated to be resistant to the growth inhibitory effect of TGFß. TGFß was unable to suppress c-*myc* mRNA levels in these DNA tumor virus transformed cells. Transient expression of HPV-16 E7 gene, adenovirus E1A, and SV40 large T antigen (TAg) blocked the TGFß1 suppression of c-*myc* transcription in a transient assay using human *myc*/CAT constructs. These DNA tumor virus oncoproteins have been demonstrated to bind the protein product of the retinoblastoma gene (pRB). Mutants of E1A and TAg that do not bind pRB are transformation defective and expression of these proteins in the keratinocytes failed to block the TGFß1 suppression of c-*myc*. These studies suggested that a cellular protein(s) that interacts with a conserved domain of the DNA tumor virus oncoproteins blocks TGFß1 suppression of c-myc transcription and keratinocyte growth. Several cellular proteins, in addition to pRB, bind to the same conserved domain in the viral oncoproteins, and any of these proteins could be necessary for suppression of c-*myc* transcription in response to TGFß1 treatment. More recently, transient expression of pRB in skin keratinocytes was shown to repress human c-*myc* promoter/CAT transcription as effectively as TGF-ß1. The same c-*myc* promoter region, termed the TGFß Control Element (TCE), was required for regulation by both TGFß1 and pRB. Oligonucleotides containing the TCE bound to several nuclear factors in mobility shift assays and a cellular protein of approximately 106 kD in Southwestern assays. Binding of these factors could be demonstrated in cells with or without normal pRB, and the binding of some factors was rapidly inhibited by TGFß1 treatment of TGFß-sensitive but not TGF-ß-insensitive cells. These data indicate that pRB can function to inhibit c-*myc* transcription and suggest the involvement of cellular factor(s) in addition to pRB in the TGFß1 pathway for suppression of c-*myc* transcription and growth inhibition. The possible involvement of pRB in the TGFß1 pathway for suppression of c-*myc* transcription has a number of implications. Tumor suppressor genes may function in the response pathway for diffusible growth inhibitors analogous to nuclear protooncogene involvement in the growth factor pathway. This predicts that one mechanism for loss of the growth inhibitory response to TGFß would be inactivation of the retinoblastoma gene.

Regulators of Endothelial Function

CA 019 STRUCTURE-FUNCTION STUDIES OF ACIDIC FIBROBLAST GROWTH FACTOR (FGF-1), Wilson H. Burgess, Anne M. Shaheen, Brian Hampton, Robert Friesel and Jeffrey A. Winkles, Laboratory of Molecular Biology, American Red Cross, Rockville, MD 20855.

The fibroblast growth factor (FGF) family presently consists of seven structurally related polypeptides. Two of the proteins, FGF-1 and FGF-2, have been characterized under many different names, most often as acidic and basic fibroblast growth factor, respectively. Functions associated with FGF-1 and FGF-2 include stimulation of mitogenesis, chemotaxis, neurite extension, plasminogen activator activity and mesoderm induction. They are also capable of inducing angiogenesis and wound repair *in vivo*. The precise mechanisms by which the two FGFs are able to elicit these pleiotropic responses remain largely unknown. Binding of these proteins to high-affinity cell surface receptors leads to stimulation of protein tyrosine kinase activity, phosphorylation of phospholipase C-γ, and activation of immediate-early gene transcription. However, the relationship of these events to FGF-stimulated responses is unclear. A better understanding of the structural basis for the different activities of these proteins should lead to the development of agonists and antagonists of specific FGF functions.

We demonstrated that site-directed mutagenesis of lysine 132 of human FGF-1 to a glutamic acid residue results in a mutant FGF-1 that has normal receptor-binding activity and is capable of stimulating tyrosine kinase activity and proto-oncogene expression but is not able to elicit a mitogenic response in target cells (1). A similar dissociation of early events such as proto-oncogene expression from the mitogenic response is observed when human wild-type FGF-1 is used in the absence of added heparin. In contrast, this mutant FGF-1 is equipotent to wild-type protein in its ability to promote mesoderm induction in *Xenopus* animal caps. These results demonstrate that proliferation associated and differentiation associated activities of FGF-1 can be dissociated at the structural level.

Studies of the intracellular fate of the mutant FGF-1 following addition to cultured cells indicate significant differences exist from the fate of the wild-type protein. These differences in sorting or degradation may be related to the mitogenic deficiencies of the mutant. Additional studies related to differences in the status of the three cysteine residues in the wild-type and mutant FGF-1 are in progress. These studies were prompted following elucidation of the complete primary structure of chicken FGF-1 which revealed the presence of only two cysteine residues (positions 30 and 97). The isolated chicken FGF-1 is highly active in mitogenic assays despite the fact that the two cysteine residues are engaged in an intrachain disulfide bond. Chicken FGF-1 also exhibits a significantly reduced heparin dependence for mitogenic activity when compared to the human protein. The relationship between heparin-dependence and cysteine status of these proteins will be described.

1. Burgess, W.H., Shaheen, A.M., Ravera, M., Jaye, M., Donohue, P.J. and Winkles, J.A., *J. Cell Biol.*, **111**, 2129 (1990).

CA 020 THE PRODUCTS OF THE INT-2/FGF3 GENE, Clive Dickson, Paul Kiefer, Piers Acland, Marc Mathieu, Vera Fantl, Irene Marics, David MacAllan, James Close and Gordon Peters, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

Int-2/Fgf3 was discovered as a proto-oncogene transcriptionally activated by mouse mammary tumor virus in murine breast tumors. In adult mice expression of int-2 is normally restricted to brain and testis but shows a complex spatio-temporal pattern from day 6 till birth in the developing mouse embryo. Structural analysis of the gene reveals three promoter regions that generate multiple RNA species all of which appear to encode the same open reading frame. Initiation of protein synthesis occurs at alternative codons; a CUG and a downstream AUG. Use of the AUG codon results in a primary product of 28.5kD which contains an amino-terminal signal sequence for entry into the constitutive secretory pathway. In contrast, initiation of protein synthesis at the CUG codon generates a protein extended by 29 residues which compromises the function of the signal peptide and results in a proportion of this product gaining access to the cell nucleus. To determine the structural basis for nuclear localization, the protein has been mutated at regions with candidate targeting signals. This has led to the identification of three regions containing basic amino acid residues that appear to markedly influence nuclear transport. However, int-2 dependent transformation of NIH3T3 cells only requires the secreted product initiated from the AUG codon. In a focus assay using different int-2 cDNAs, the presence of sequences upstream of the AUG initiation codon, including the CUG and an AUG in the +1 reading frame reduce the efficiency of transformation. This effect is probably mediated by competition for ribosomes which results in a reduced level of translational initiation of the secreted product. Pulse labeled int-2 accumulates in the culture medium with relatively slow kinetics due to the efficient sequestration of the protein by the cell surface and extracellular matrix proteoglycans. Competition studies using soluble glycosaminoglycans, specifically heparin, heparan sulfate and dermatan sulfate displace int-2 binding in a dose dependent manner. At concentrations that remove most of the int-2 from the cell surface and extracellular matrix, the transformed cells revert to a near normal phenotype and loose the ability to grow in semi-solid medium. These observations suggest that int-2 bound to low affinity receptors can facilitate the morphological transformation of cells.

CA 021 THE ENDOTHELINS — MOLECULAR ANALYSIS OF BIOSYNTHESIS AND ACTIONS, Masashi Yanagisawa, Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto, Japan; and Howard Hughes Medical Institute and Department of Molecular Genetics, The University of Texas Southwestern Medical Center, Dallas.

The first member of the endothelin (ET) family, ET-1, was initially identified as a 21-amino-acid potent vasoconstrictor peptide produced by vascular endothelial cells in culture. Subsequent studies demonstrated three separate ET-related genes in the human and other mammalian genomes, which encode for the three distinct isopeptides, ET-1, ET-2 and ET-3. cDNA cloning of ET isopeptides in human revealed that they are expressed in multiple tissues with distinct distribution patterns: while only ET-1 is detected in the vascular endothelium, ET-2 and/or ET-3, in addition to ET-1, are expressed in other tissues such as the brain, lung and kidney. Active ETs are produced via the proteolytic cleavage of ~40-residue inactive intermediate called 'big ETs', catalyzed by a membrane-bound neutral metalloprotease(s) called 'endothelin converting enzyme.' This unique enzyme is sensitive to the protease inhibitor phosphoramidon, and apparently distinct from any other metalloprotease known.

Apart from their potent and long-lasting vasoconstrictor/pressor activities, ETs possess a wide spectrum of non-vascular actions in various tissues. Furthermore, the existence of at least two subtypes of ET receptors with different selectivities to the three isopeptides has been demonstrated. We have recently cloned two distinct subtypes of ET receptor; in vascular tissues for example, the smooth muscle cells express the 'ET_A receptor' which shows the rank order of affinity ET-1 ≈ ET-2 » ET-3, whereas the endothelial cells express the 'ET_B receptor' with the affinity rank order of ET-1 ≈ ET-2 ≈ ET-3, which may mediate the ET-induced EDRF release. These ET receptor mRNAs are expressed also in other ET-producing tissues listed above, suggesting the importance of ET family peptides as *locally* acting mediators.

In cultured endothelial cells, the production of ET-1 is augmented by various chemical and mechanical stimuli, including thrombin, TGF-β, IL-1 angiotensin II, vasopressin, and increased shear rate. In contrast, EDRF attenuates ET-1 release from isolated porcine aorta. Our on-going studies in isolated perfused rat mesenteric arteries have shown that both immunoreactive ET-1 and ET-1 mRNA is significantly up-regulated in response to a low concentration (10^{-10} *M*) of vasopressin. Although this vascular bed shows no acute vasoconstrictive response to this dose of vasopressin, an extremely slow vasoconstriction is observed 3-6 hours after the initiation of the vasopressin challenge. Furthermore, the induction of ET-1 mRNA and the slow constrictor response are both completely abolished by concomitant administration of the mRNA synthesis inhibitor actinomycin D. These results suggest the possibility that endogenous *de novo* production of ET-1 may mediate the slow constrictor response of the mesenteric vascular bed to circulating vasopressin.

Angiogenesis

CA 022 TUMOR MICROCIRCULATION—BOTH A BARRIER AND A TARGET FOR CANCER THERAPY, R. K. Jain, Steele Laboratory for Tumor Biology, Dept. of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

The advent of hybridoma technology and genetic engineering has led to the development of various novel therapeutic agents potentially useful for cancer treatment. These agents include monoclonal antibodies (MAbs) conjugated with radionuclides, toxins, drugs, cytokines, enzymes, effector cells, and so forth; growth factors; biological response modifiers (BRMs); extracellular matrix peptide products; immunotoxins; lymphokine activated killer (LAK) cells, and tumor-infiltrating lymphocytes (TILs). Because of their potent toxicity to cancer cells *in vitro*, and in some *in vivo* model systems, some of these agents have been heralded as "breakthrough drugs" or "magic bullets." Although the possibility of using these agents for cancer therapy remains attractive, clinical results have not yet lived up to the earlier promises of their perceived potential. Similarly, many of the 40 FDA-approved low-molecular-weight conventional drugs, although effective for the treatment of lymphomas, have had minimal impact on solid tumors (e.g., breast, lung, colon, brain) which account for the majority of cancer related deaths in the United States. A key factor limiting their effectiveness in treatment has been the inadequate and nonuniform localization of these molecules and cells in tumors. Cellular factors alone such as heterogeneity of tumor-associated antigen and inherent or acquired resistance (e.g., MDR) have failed to explain the poor penetration of tumors by these agents.

Only in recent years have the peculiarities of tumor microcirculation been recognized as determinants of drug distribution. Three physiological barriers responsible for the poor localization of macromolecules in tumors have been identified: (i) heterogeneous blood supply, (ii) elevated interstitial pressure, and (iii) large transport distances in the interstitium. The first barrier limits the delivery of blood-borne molecules to well-perfused regions of a tumor; the second barrier reduces extravasation of fluid and macromolecules in the high interstitial pressure regions and also leads to an experimentally verifiable, radially outward fluid motion in the tumor periphery which opposes the inward diffusion; and the third barrier increases the time required for slowly moving macromolecules to reach distal regions of a tumor. Binding of antibody to an antigen further lowers the effective diffusion rate of the antibody by reducing the amount of mobile antibody. Because of micro- and macroscopic heterogeneities in tumors, the relative magnitude of each of these barriers would vary from one location to another and from one day to the next in the same tumor, and from one tumor to another.

In this presentation I will summarize our recent findings of the physiological barriers to transport of genetically engineered molecules and cells in a tumor and discuss some strategies to overcome them for therapeutic benefit. I will also point out some therapies where the tumor vasculature or the interstitium may be the targets for cancer treatment [R.K. Jain, "Vascular and Interstitial Barriers to the Delivery of Therapeutic Agents in Tumors," *Cancer and Metabolism Reviews*, **9**: 253-266 (1990)].

CA 023 DRUG DELIVERY SYSTEMS FOR ANGIOGENESIS STIMULATORS & INHIBITORS
Robert Langer, Massachusetts Institute of Technology, Cambridge, MA 02139

One of the important issues in the testing and eventual use of angiogenesis stimulators and inhibitors (and for all informational proteins for that matter) is their delivery. This is particularly important when such molecules are unstable or rapidly destroyed in the body, as are most peptides and proteins. We have developed and report here on the development of the first polymer systems for the controlled release of large molecules (M.W. >1000) such as polypeptide hormones (e.g., insulin, growth factors, vaccines). Pellets or microspheres made of hydrophobic polymers such as ethylene-vinyl acetate copolymer or polylactic acid can release many different macromolecules in bioactive form for over 100 days in vitro and in vivo. Methods of enhancing release on-demand of substances may be accomplished using approaches involving magnetism and ultrasound. We have also synthesized bioerodible polymers, in particlar polyanhydrides, that show surface erosion and lead to near constant release rates of incorporated drugs. By altering the hydrophobicity of the polymer backbone, release times from 1 week to 6 years can be achieved. Studies examinig these novel systems for the delivery of tumor angiogenesis factors, a cartilage-derived inhibitor of angiogenesis , and heparin-cortisone will be described.

Late Abstracts

THE STRUCTURAL BASIS OF INTEGRIN FUNCTION, Mark H. Ginsberg, Joseph Loftus, Timothy E. O'Toole, Ron Bowditch, MaryLynn Bajt, Jari Ylanne, Xiaoping Du and Edward F. Plow, The Scripps Research Institute, La Jolla, CA 92037.

Progress is being made in understanding the basis of integrin function, and generalities are emerging. It was thought that integrins recognize a common tripeptide Arg-Gly-Asp (RGD sequence), however, many integrin ligands lack this tripeptide sequence. Nevertheless, for certain integrins, this sequence in linear and constrained peptides, and in small snake venom proteins, binds with high affinity. Nevertheless, it is possible that multiple interactive sites are utilized by integrins to recognize their ligands. To evaluate this possibility, monoclonal anti-fibronectin (Fn) antibodies which inhibit its binding to purified integrin $\alpha_{IIb}\beta_3$ were developed. The epitopes for three of these antibodies were mapped to a region which lacks RGD, recombinant fragments containing these epitopes bound to purified $\alpha_{IIb}\beta_3$ and inhibited the binding of fibronectin. Thus, this integrin uses RGD and additional sites in Fn to recognize its ligand. Within integrins, multiple regions in both the α and β subunits have been implicated as ligand binding sites. Chemical crosslinking studies have identified a conserved 63 residue sequence in the β_3 subunit and a 20 residue sequence in the α_{IIb} subunit as being involved in the binding of small peptide ligands. Peptide and antibody approaches have confirmed the functionality of the α chain site and the site in β_3. Further, an additional site in β_3 has been identified by this approach by use of integrin peptides and antibodies. Functionality of both β chain sites is strongly supported by identification of natural ligand binding mutants in $\alpha_{IIb}\beta_3$, which result in a bleeding disorder. A critical feature of integrin function is the modulation of integrin affinity by cells. This modulation is due to a change in the structure of the integrin itself, as it can be brought in certain purified integrins in solution by use of conformation-specific monoclonal antibodies.

REGULATION OF PAI-1 GENE EXPRESSION IN THE VASCULAR WALL. David J. Loskutoff, Mark Keeton, Michael Sawdey, Yutaka Eguchi and Jacob Schneiderman, Committee for Vascular Biology, The Scripps Research Institute, La Jolla, CA 92037

Plasminogen activation provides an important source of localized proteolytic activity during fibrinolysis, ovulation, angiogenesis, 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 type 1 PA inhibitor (PAI-1), an efficient, endothelial cell-derived inhibitor of both urokinase and tissue-type PA. Cell culture studies have shown that PAI-1 synthesis can be stimulated by a variety of agents including endotoxin (LPS), cytokines, growth factors, and hormones. PAI-1 levels in plasma also increase in response to some of these molecules, and these increases frequently correlate with thrombotic disease. Although these changes in plasma PAI-1 levels must reflect earlier, tissue-specific biosynthetic and/or metabolic events, little is known about the tissues, or cells within them, that actually produce this important inhibitor under various conditions. We have begun to employ the mouse as an in vivo model to address these questions. In my presentation, I will initially review the biochemistry, cell and molecular biology of PAI-1. I will then summarize recent observations employing nuclease protection assays, in situ hybridization, and immunochemical analysis to determine the tissue distribution and cellular localization of PAI-1 mRNA and antigen in normal mice and in mice treated with LPS and TNF. These studies indicate that PAI-1 is synthesized by endothelial cells in a variety of tissues. Evidence will be presented to suggest that elevated PAI-1 levels in the aorta and kidney may contribute to the thrombotic problems associated with atherosclerosis and glomerular nephritis, respectively.

CHARACTERIZATION OF THE ACTIVATION OF LATENT TGF-ß, Daniel B. Rifkin, Department of Cell Biology, New York University Medical Center, New York, NY 10016

Transforming growth factor-ß (TGF-ß) is a potent 25 kDa homodimer cytokine which has a ubiquitous distribution and a multiplicity of effects depending upon the target cell. Although the production of TGF-ß has been observed in many cells and tissues, the cytokine is released in a latent form (LTGF-ß) in which the propeptide of the immature precursor remains associated with the mature cytokine via non-covalent interactions rendering the complex inactive. It has been obscure how the mature growth factor is generated. We have found that cocultures of several different cell types including endothelial cells, smooth muscle cells, pericytes and fibroblasts activate LTGF-ß, whereas the homotypic cultures do not. This activation requires cell-cell contact, cells of two different types, plasminogen activator and plasmin, and the binding of LTGF-ß to the cell or matrix surface. The surface binding is controled via the interaction of mannose 6-phosphate residues in the propeptide with the M6P/IGF-II cell surface receptor as well as an additional protein covalently bound to the propeptide. The activation reaction is self-limiting since in many cells TGF-ß is a potent inducer of plasminogen activator inhibitor I (PAI-1). Therefore, the formation of TGF-ß results in the production of an inhibitor of an enzyme necessary for the activation of LTGF-ß. Agents which increase the production of plasminogen activators or the M6P/IGF II receptor, such as retinoids, basic FGF, and insulin, stimulate LTGF-ß conversion to TGF-ß. This unique mechanism for the generation of a potent cytokine based upon cell-cell interaction affords an interesting mechansims to control developmental and/or physiological phenomena.

Vascular Pathogenetic Mechanisms; Endothelial Cell Migration and Differentiation

CA 100 STIMULATION OF ICAM-1 mRNA IN ISCHEMIC AND REPERFUSED CANINE MYOCARDIUM, Donald C. Anderson, Gilbert L. Kukielka, Lloyd H. Michael, Keith A. Youker, Caryl L. Lane, C. Wayne Smith, Anthony M. Manning, Hal K. Hawkins, and Mark L. Entman, Department of Pediatrics & Medicine, Baylor College of Medicine, Houston, TX 77030.

Previous studies have demonstrated an important role for CD11/CD18-ICAM-1 interactions in adhesion-dependent cytotoxic reactions of canine neutrophils with isolated myocytes or endothelial cells (EC) *in vitro*. To define a role for ICAM-1 in inflammatory injury of ischemic and/or reperfused myocardium *in vivo*, the regulation of canine ICAM-1 mRNA and expression of ICAM-1 was assessed in myocardial segments of: 1) canine animals subjected to coronary artery occlusion (1h) followed by reperfusion (1-24 h), 2) animals administered LPS (0.4 mg/kg/3 h I.V.) (positive control) and 3) untreated (negative control) animals. Northern blots of RNA isolated from ischemic myocardium (blood flow = 0.04-0.28 ml/min) or normally perfused segments ($\bar{x}$ = 1.12 ml/min) were performed using a canine ICAM-1 cDNA prepared by homology cloning. Low or undetectable levels of ICAM-1 [immunoperoxidase stain with anti ICAM-1 MAb (CL-18)] or ICAM-1 mRNA (2.9 kb) were present in myocardium of untreated animals & in normally perfused segments for up to 3 h of reperfusion. Increased levels of ICAM-1 mRNA were consistently identified in ischemic/reperfused segments as well as in myocardial tissue of LPS treated animals; mean fold increases of ICAM-1 mRNA were 2, 10, or > 20-fold greater than baseline after 1, 3, or 24 h of reperfusion, respectively. After 24 h of reperfusion, normally perfused segments also demonstrated increased levels of ICAM-1 mRNA (>10 fold increase). In further protocols, cardiac lymph was collected prior to & for 24 h after ischemia-reperfusion. Incubation of canine EC with reperfusion lymph (collected at 1,3,5 & 24 h) elicited: 1) a >10 fold increase of ICAM-1 mRNA, 2) a 2-5 fold increase of surface ICAM-1 expression (CL18 binding shown by EIA), & 3) markedly increased ICAM-1-dependent adhesion for neutrophils. These studies demonstrate that ICAM-1 is elicited in ischemic myocardium at a transcriptional level by factors present in cardiac lymph within 1 h of reperfusion. They support a pathologic role for ICAM-1 in the process of myocardial ischemia-reperfusion injury & the development of therapeutic strategies targeting this adhesion receptor or its ligands.

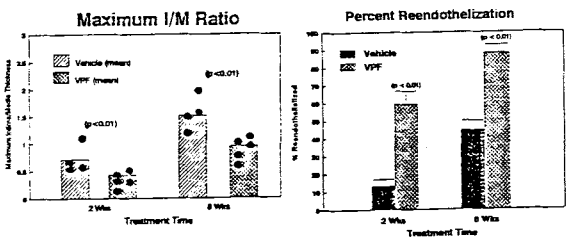
CA 101 ALTERATIONS OF TYPE IV COLLAGEN PRODUCTION BY HUMAN ENDOTHELIAL CELLS IN CULTURE AT HIGH GLUCOSE CONCENTRATION; EFFECT OF TREATMENT WITH AN ALDOSE REDUCTASE INHIBITOR, Anne-Marie Borsos, Ahmed Bakillah, Raouf Salami, Raymonde Guillot and Michel Sternberg, Equipe de Recherches sur la Biochimie et la Pharmacologie des Vaisseaux et du Rein, Faculté de Médecine, 45 rue des Saints-Pères, 75006 Paris, France.

In order to study the mechanisms of type IV collagen (CIV) accumulation in diabetic basement membrane (BM) and of the effect of aldose reductase inhibitors on BM thickening, we have measured CIV production and ^{14}CPro incorporation by human umbilical vein endothelial cells in presence of various concentrations of glucose (Glc 5 and 16 mM) and sorbinil (0, 0.1 and 0.2 mM) during 6 days after beginning of confluence (day 7 to day 13). Glc 16 mM reduced the number of cells to 61%* of 5 mM control. It increased CIV amount secreted in the medium (223%***) or associated with the matrix and cells (235%*) and Pro incorporation in collagenous (255%***) or noncollagenous (197%***) proteins secreted in the medium and in total proteins associated with matrix and cells (191%*), when compared with the 5 mM control, expressed by living cell. Sorbinil 0.1 mM normalized the number of cells to 80%++. It reduced CIV amount to 191% in the medium and 202% in the matrix and cells and Pro incorporation to 99%+++ in the collagenous and 116%+++ in the non-collagenous proteins of the medium and to 27%++ in the total proteins of matrix and cells. Thus Glc is cytotoxic at high concentration but stimulates protein synthesis. Sorbinil appears to correct these effects without specifity for CIV.

*,p<.05;***,p<.001 vs 5 mM Glc;++,p<.01;+++,p<.001 vs 16mM Glc

CA 102 VASCULAR PERMEABILITY FACTOR (VPF) AND POSTANGIOPLASTY REENDOTHELIALIZATION, Allan D. Callow, Scott Stevens, Dan Connolly, Charles Rodi, Ellen McMahon, Eric T. Choi, Jeffrey Trachtenberg, Richard Ornberg and Una S. Ryan, Washington Univ 63110, Monsanto Co 63167, St. Louis, MO

Hypothesis: 1) Restoration of an intact confluent endothelium induces endothelial cells and smooth muscle cells, damaged by balloon angioplasty to resume a quiescent, nonproliferative status and recover normal functional response; 2) rapid restoration of a structurally and functionally competent endothelial layer may suppress the process of restenosis. VPF, an extremely potent polypeptide mitogen for endothelial cells but not for vascular smooth muscle cells and fibroblasts was administered to New Zealand White rabbits undergoing balloon denudation of carotid arteries. Specimens were harvested at 2 wks and 8 wks and examined for percent reendothelialization, intima/media ratio and response of arterial rings to an agonist KCl. Animals were divided into two groups, those receiving VPF and those receiving vehicle only. Percent reendothelialization was markedly greater in VPF animals than in those receiving vehicle only ($p<0.01$). Maximum I/M ratio (cellular proliferation) in vehicle administered (control) animals was significantly greater than that of the animals receiving VPF ($p<0.01$) (Wilcoxon rank sum). Measuring isometric force of arterial rings suspended in a muscle bath revealed that the VPF treated animals recovered greater functional response than those receiving vehicle alone ($n=10$). Conclusions: VPF administration showed: 1) more rapid and complete reendothelialization than in control animals; 2) an ameliorating effect on intimal hypertrophy; 3) partial restoration of vasomotor response to potassium chloride.



CA 103 VITRONECTIN-THROMBIN-ANTITHROMBIN III COMPLEXES ARE BOUND AND INTERNALIZED BY HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS.

Hetty C. de Boer[1], Klaus T. Preissner[2], Bonno N. Bouma[1], Philip G. de Groot[1]. Department of Haematology[1], University Hospital Utrecht, The Netherlands. Haemostasis Research Unit[2], Kerckhoff-Klinik, Max-Planck Institute, Bad Nauheim, Germany.

When thrombin, the key enzyme of the blood clotting system, is activated, it will be inhibited by its main plasma inhibitor antithrombin III (ATIII) by the formation of a binary complex (TAT). In human serum this complex exists associated to a third plasma protein, vitronectin (VN). We have investigated whether VN-TAT complexes can be cleared by the endothelium. Therefore serum derived, purified VN-TAT was radiolabeled and bindingassays were performed on cultured human umbilical vein endothelial cells (HUVEC). At 4°C, VN-TAT complex is bound to HUVEC in a time- and concentration dependent manner. Kinetic analysis revealed an apparent K_d of 16 nM and 170.000 binding sites per endothelial cell. Competition experiments showed that binding is mediated by the VN-moiety of the complex (the other components did not influence the binding). Using synthetic peptides, direct proof was obtained that the primary binding site was located between amino acids 348 and 361 of VN, which comprises two consensus sequences for glycosaminoglycan recognition. Purified heparan sulfate inhibited VN-TAT binding to HUVEC by 75%, indicating that these proteoglycans present at the cell-surface are the binding sites for VN-TAT. At 37°C VN-TAT complex was internalized and transported to the subendothelial compartment. Cytochalasin B and colchicine (inhibitors of transcellular transport), inhibited the deposition of the complex in the matrix, indicating that transcytotic processes are involved. Here we describe a new mechanism by which VN-associated TAT is cleared by endothelial cells. The deposition of VN-TAT in the matrix may be important in cell-binding and cell-migrating processes.

CA 104 SELECTIVE SUSCEPTIBILITY OF HEPATIC ENDOTHELIAL CELLS TO DACARBAZINE TOXICITY, A MODEL FOR HEPATIC VENO-OCCLUSIVE DISEASE. Laurie D. DeLeve and Neil Kaplowitz. Div. of Gastrointestinal and Liver Disease, University of Southern California School of Medicine, Los Angeles, CA 90033 Little is known about the mechanisms leading to hepatic veno-occlusive disease (HVOD). HVOD due to dacarbazine (DTIC), a chemotherapeutic agent, reportedly causes significant mortality. DTIC is activated by cytochrome P450, but nothing is known about the target cell or detoxification. The present studies were designed to test the hypothesis that hepatic endothelial cells (ENDOS) are the target of DTIC toxicity and to examine the role of GSH detoxification. Methods: DTIC (1.5, 3 and 6mM) toxicity was studied in vitro in murine hepatocytes and ENDOS after a 16 hour incubation by MTT viability testing. Actual time of onset of toxicity was studied in ENDOS both by MTT and by ethidium homodimer dye exclusion on a fluorimetric microplate reader. GSH detoxification was studied by manipulating GSH and assessing the effect with MTT. Points along the dose-response curves were compared by paired t-test. Results: DTIC is not toxic to hepatocytes at up to 6mM (n=4). The table shows the loss of ENDO viability at DTIC 3 and 6mM (n=4: ENDOS vs hepatocytes p=0.08 at 3mM and 0.004 at 6mM ; p<0.01 for t-test of slopes of the two dose-response curves). There is a decline in GSH after 8 hours of DTIC followed by the onset of toxicity at 11 hours (8 hrs drug plus 3 hours MTT incubation or after 11 hours of drug by dye exclusion). Depletion of endogenous GSH by 4mM buthionine sulfoximine (BSO) exacerbates toxicity (n=5: DTIC alone vs DTIC/BSO p=0.015 at 3mM and 0.002 at 6mM), whereas exogenous GSH (3mM) protects against DTIC (n=10: DTIC vs DTIC/GSH p=0.012 at 3mM and 0.00006 at 6mM). Blocking utilization of exogenous GSH with 4mM BSO or with 0.5mM acivicin, a g-glutamyl transpeptidase inhibitor, abolishes the protective effect against DTIC (DTIC/GSH plus BSO or acivicin N.S. vs DTIC alone).

Table: Means +/- s.e. normalized to the solvent control (100%).

DTIC	DTIC	+BSO	+GSH	+GSH/BSO	+GSH/acivicin
1.5mM	96+/-8	76+/-8	106+/-10	94+/-11	80+/-15
3mM	62+/-6	24+/-9	90+/-10	62+/-10	46+/-8
6mM	10+/-1	11+/-1	66+/-1	36+/-1	23+/-7

Conclusions: 1.ENDOS exhibit selective susceptibility to the toxicity of DTIC which suggests that activation in ENDOS causes HVOD. 2. Exogenous GSH protects ENDOS by providing cysteine for GSH synthesis; thus, enhancing GSH synthesis in ENDOS offers a promising approach to prevention of HVOD from DTIC.

CA 105 ENHANCED HISTAMINE-INDUCED PROSTACYCLIN SECRETION FROM HUMAN AORTIC ENDOTHELIAL CELLS DERIVED FROM THE HIGH ATHEROSCLEROTIC PROBABILITY ZONE, Carl A. Hansen, Yuri Romanov and Alexander Antonov, Weis Center for Research, Geisinger Clinic, Danville, PA 17822 and the Institute of Experimental Cardiology, Cardiology Research Center of the USSR, Moscow.

Recent studies on the morphology of endothelial cells from human aorta have revealed zones of high and low probability (HPZ, LPZ) for atherosclerotic development. Endothelial cells from these zones were isolated from 6 donors, maintained in culture through 3 passages and the histamine-induced prostacyclin secretory response determined. Media from HPZ histamine-stimulated cells contained 1.8 times more 6-keto-prostaglandin-$F_{1\alpha}$ (403 ± 55 % of control) than media from the paired LPZ cells (223 ± 20 % of control). Removal of external Ca^{2+} reduced the histamine-induced accumulation of 6-K-PG-$F_{1\alpha}$ in LPZ cell cultures by 30 % (158 ± 17 % of control), but over 2 fold in HPZ cells to a rate similar to that of the LPZ cells (189 ± 20 % of control). Thapsigargin, which mobilizes internal Ca^{2+} stores and activates Ca^{2+} entry, resulted in a similar trend of enhanced 6-K-PG-$F_{1\alpha}$ secretion from HPZ cells (377 ± 68 vs 299 ± 36 % of control for HPZ and LPZ respectively). Removal of external Ca^{2+} attenuated this response in both zones to rates similar to that induced by histamine under Ca^{2+} free conditions (157 ± 30 and 135 ± 15 % of control for HPZ and LPZ respectively). These data suggest that the enhanced prostacyclin secretory response from HPZ cells is related to a difference in the Ca^{2+} entry process. Current research is focused on hormone-induced Ca^{2+} signaling differences between HPZ and LPZ cell populations. *Supported by a AHA Grant-in-aid, Pennsylvania Affiliate.*

CA 106 PLATELET DERIVED IL-1 MODULATES ENDOTHELIAL CELL FUNCTION, Catherine M. Hawrylowicz*, Gareth Howells, and Marc Feldmann, *Dept. of Immunology, St. Mary's Hospital Medical School, Paddington, London W2 1PG & Charing Cross Sunley Research Centre, Hammersmith, London W6 8LW.

Interleukin-1 (IL-1) plays a central role in the inflammatory response. We recently demonstrated rapid, cell associated expression of IL-1 by human platelets following activation (1). We have now addressed whether platelet derived IL-1 is in a functionally relevant form that can alter cytokine production and adhesion molecule expression by cultured endothelial cells in a manner comparable to recombinant IL-1.

Thrombin activated human platelets stimulated increased expression of the ICAM-1 adhesion molecule and secretion of the cytokines IL-6 and GM-CSF by cultured umbilical vein and saphenous vein endothelial cells. Unstimulated platelets were much weaker inducers, whilst thrombin and supernatant from thrombin activated platelets had no effect. Induction of cytokine production and ICAM-1 expression by the endothelial cells was abrogated by prior treatment of the platelets with antibody specific for IL-1. Since one of the earliest events following vascular injury is believed to be the rapid adhesion and activation of platelets, platelet delivered IL-1 may provide an important early stimulus for changes to the vascular endothelium following insult.

(1) C.M. Hawrylowicz, S.A. Santoro, F.M. Platts, & E.R. Unanue. 1989. Activated platelets express IL-1 activity. J. Immunol. 143: 4015.

CA 107 THE ROLE OF ADHESION MOLECULES IN LEUKOCYTE MEDIATED GLOMERULAR INJURY. Kent J. Johnson, James Varani, Michael Mulligan, C. Wayne Smith+, Donald C. Anderson+ and Peter A. Ward. Univ. of Michigan Med. Sch., Ann Arbor, MI and +Baylor College of Medicine, Houston, TX.

Most types of glomerulonephritis are associated with leukocyte (neutrophil, monocyte) infiltration which is responsible for much of the glomerular injury. The purpose of these studies was to determine the role of adhesive interactions between leukocytes and resident glomerular cells (endothelial and mesangial) in leukocyte localization and glomerular injury. In *in vivo* studies acute neutrophil-dependent anti-GBM nephritis in rats was associated with the upregulation of the endothelial cell adhesion molecules ELAM-1 and VCAM-1 in the glomeruli. The expression of these molecules peaked at 6 hours which correlates with the peak of the neutrophil influx. Similiar upregulation of these adhesion molecules could also be induced by TNFα infusion into the renal artery of normal rats. Specific antibodies against TNFα and the leukocyte integrin CD-18 markedly inhibited the glomerular injury and proteinuria. This inhibition was associated with suppression of ELAM-1 and VCAM-1 expression in the glomeruli. In correlative *in vitro* studies we found that TNFα stimulated rat endothelial and mesangial cell expression of ICAM-1 and to a lessor extent VCAM-1 and ELAM-1. Furthermore, neutrophil adherence to and killing of mesangial cells in culture appears dependent on the upregulation of adhesion molecules by both cells. Anti-ICAM-1 antibody partially inhibited the adherence of neutrophils to the mesangial cells and neutrophil-mediated killing of the mesangial cells was inhibited by anti CD11/CD18 antibodies. Thus these *in vivo* and *in vitro* studies support the concept that adhesion molecules are important in the pathogenesis of glomerulonephritis not only for leukocyte localization but also in leukocyte mediated injury to resident glomerular cells. Furthermore, a primary effect of TNFα in this system appears to be that of upregulation of these adhesive interactions.

CA 108 IGA IMMUNE COMPLEX INDUCED LUNG INJURY: MOLECULAR DETERMINANTS AND ROLE OF MACROPHAGE PRODUCTS. Michael S. Mulligan, Jeffrey S. Warren, Peter A. Ward, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

Acute lung injury that develops after intrapulmonary deposition of IgA immune complex is mediated by oxygen radicals and is neutrophil independent. The source of tissue-damaging oxyradicals (including $HO^{\bullet}$) appears to be the activated alveolar macrophage. Complement depletion with cobra venom factor or complement blockade by treatment with human recombinant soluble complement receptor-1 was also highly protective in this model. Systemic administration of antibody to CD18 was highly protective against IgA immune complex-induced injury. A modest amount of protection was demonstrated with intratracheal instillation of anti-CD11b. Treatment with anti-TNFα had no protective effects in this mode. Correspondingly, very little TNFα is recoverable in the BAL from injured animals. Although immunohistochemical analysis revealed limited upregulation of ELAM-1 in the pulmonary microvasculature, treatment with anti-ELAM-1 had no protective effects. Airway instillation of the L-arginine analogue N^{G}-monomethyl-L-arginine acetate (NMA) was highly protective against the development of IgA immune complex induced injury. These protective effects were reversible with L-arginine but not with D-arginine. All protective interventions were associated with reduced numbers of retrievable macrophages in the BAL. The requirements for L-arginine complement and the β_2 integrins for the development of IgA immune complex alveolitis are also found in the acute lung injury following deposition of IgG immune complexes. However, the lack of a role for neutrophils, TNFα and ELAM-1 in the IgA model stand in sharp contrast to the mediator requirements in IgG immune complex lung injury.

CA 109 SHIGA TOXIN-ASSOCIATED HEMOLYTIC UREMIC SYNDROME (HUS): COMBINED CYTOTOXIC EFFECTS OF SHIGA TOXIN, IL-1β AND TNF-α ON HUMAN VASCULAR ENDOTHELIAL CELLS, IN VITRO, Tom G. Obrig and Chandra B. Louise, Department of Microbiology and Immunology, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642

There appears to be a relationship between Shiga toxin-producing Shigella or E. coli and the development of vascular complications in humans following bacillary dysentery. Recently we established that human renal vascular endothelial cells are extremely sensitive to and may be the primary target of Shiga toxin, a 70kDa protein complex. Our working hypothesis is that endotoxin-elicited interleukin-1 (IL-1) or tumor necrosis factor (TNF) may combine with Shiga toxin to facilitate vascular damage characteristic of hemolytic uremic syndrome (HUS). The cytotoxic effects of Shiga toxin, IL-1 and TNF on cultured human umbilical vein endothelial cells (HUVEC) have been examined. Individually, Shiga toxin and TNF were directly cytotoxic to HUVEC, and combinations resulted in a synergistic cytotoxic effect. In contrast, IL-1 was neither cytotoxic to HUVEC nor did it enhance cell death in combination with Shiga toxin. The synergistic response of HUVEC to Shiga toxin and TNF were both dose- and time-dependent and could be neutralized by monoclonal antibodies directed against Shiga toxin or TNF. Individual cell lines displayed considerable variability in sensitivity to Shiga toxin and TNF. These results are consistent with a role for Shiga toxin in the development of HUS and support the idea that immune mediators could facilitate the disease process. (Supported by USPHS grant AI24431 from the NIH).

CA 110 LOW LEVELS OF LIPOPOLYSACCHARIDE ENHANCE TNF-α INDUCED ENDOTHELIAL INJURY James A. Royall, Department of Pediatrics, University of Alabama at Birmingham, 35233

With sepsis, initial circulation of endotoxin (LPS) is followed by endogenous release of tumor necrosis factor-α (TNF). Levels of LPS (μg/ml range) much greater than found in septic shock (up to 1 ng/ml) are required to injure endothelial cells (EC). The affect of clinically relevant LPS exposure on subsequent TNF-induced EC injury was evaluated.

The permeability coefficients (P_{ec}) to albumin and sucrose of EC monolayers from bovine aorta (BA), pulmonary artery (BPA), and "lung" (BL) were measured. Exposure was to 1 ng/ml LPS for 2 hr, then to 100 or 500 U/ml TNF for 12 hr. LPS at 1 ng/ml does not increase EC permeability. Change in permeability above control (Δ Perm) was calculated as (P_{ec}Exposed - P_{ec}Control)/P_{ec}Control.

Δ Perm ($\bar{x} \pm$ SE, n=6) 1 ng/ml LPS then 100 U/ml TNF

EC Type	Albumin		Sucrose	
	TNF	LPS/TNF	TNF	LPS/TNF
BA	1.5 ± .4	3.3 ± .2	.63 ± .1	1.2 ± .1
BPA	1.5 ± .7	3.4 ± .9	.67 ± .2	1.5 ± .2
BL	2.0 ± .2	4.37 ± 1.3	.69 ± .1	1.4 ± .3

Analysis by two-way ANOVA indicated LPS pre-exposure resulted in a significant increase in subsequent TNF induced permeability for both alb and suc at 100 U/ml. At 500 U/ml TNF, which induces near maximal increased permeability, LPS pre-exposure had no effect.

These results suggest that clinically relevant levels of LPS enhance subsequent TNF induced EC injury which may be an important pathogenic mechanism with sepsis.

(Supported by an American Lung Association Research Grant)

CA 111 NOVEL ENDOTHELIAL SCAVENGER RECEPTORS MEDIATE INTERNALIZATION AND DEGRADATION OF VARIOUS CONFORMATIONALLY-ALTERED PROTEINS. Jan E. Schnitzer, Dept. of Med. & Path., Cellular & Molecular Med., Univ. Calif. -San Diego, La Jolla, CA 92093-0651

In the past decade, several different scavenger receptors have been identified and studied extensively with regards to their potential role in protein catabolism and possible link to atherosclerosis and diabetes. In this study, we show using ligand blotting that two endothelial proteins (gp30 and gp18), previously identified as albumin binding proteins, interact more avidly with various modified albumins than with native albumin. Albumin was modified by chemical means using formaldehyde or maleic anhydride and by surface adsorption to microscopic gold particles. The detection of gp30 and gp18 was not dependent on disulfide bond reduction. Several ligands which bind to other known scavenger receptors, inhibit binding of modified albumin to gp30 and gp18. These modified albumins bind to the surface of cultured endothelial cells at 4°C, are subsequently internalized with warming to 37°C, and ultimately are degraded by the cell. Both the total cell-associated ^{125}I-protein and its degradation were inhibited by the same ligands that interferred with blotting of gp30 and gp18. Albumin-gold particles appear to be internalized via both coated pits and noncoated plasmalemmal vesicles, both of which seem to deliver their ligands to endosomes/lysosomes. In summary, it appears that two novel scavenger receptors have been identified and partially characterized. These proteins appear to mediate internalization of various modified proteins by classic endocytotic mechanisms and by a new pathway involving noncoated plasmalemmal vesicles. (Supported in part by a AHA Grant-In-Aid and by dNIH HL43278 and HL17080.)

CA 112 GROWTH FACTOR AND GROWTH FACTOR-RECEPTOR EXPRESSION IN AIDS-RELATED KAPOSI'S SARCOMA *IN VIVO* SUGGESTS PARACRINE AND AUTOCRINE MECHANISMS OF TUMOR MAINTAINANCE

M. Stürzl*, H. Brandstetter*, C. Zietz+, N.H. Brockmeyer++, S. Anders*, B. Speiser+, W.K. Roth* and P.H. Hofschneider*. *Max-Planck-Institut für Biochemie, Abteilung Virusforschung, D-8033 Martinsried, FRG; *Pathologisches Institut der LMU München, D-8000 München 2, FRG; *Universitätsklinik Essen, Hautklinik, D-4300 Essen 1, FRG.

Recently, it has been shown that proliferation of Kaposi's sarcoma (KS) derived cells *in vitro* is dependent on the presence of PDGF [Roth et al. (1989) *Oncogene* **4**, 483-487]. In order to strengthen the hypothesis that PDGF is also a major growth factor for KS cells *in vivo*, we performed *in situ* hybridization and immunohistochemical staining for PDGF and PDGF receptors in tissue sections of AIDS-related KS. The data suggest that Kaposi's sarcoma is made up of two different types of tumor cells. (i) The main population are spindle shaped cells with elongated nuclei (KS-s cells). They reveal a strong expression of PDGF ß-receptors and lack PDGF-A and PDGF-B expression. (ii) A minor population of KS cells express PDGF ß-receptor as well as PDGF-A and PDGF-B (KS-p cells). These cells are often grouped in whirls and surrounding vascular slits. In comparison to KS spindle cells, they reveal spherical nuclei with evenly distributed chromatin and inconspicous nucleoli. PDGF α-receptor is not expressed in either form of KS cells. Additionally, we examined the expression of EGF, TGFα, EGF-receptor and several interleukins (1α, 1ß, 6) in Kaposi's sarcoma by immunohistochemistry and by *in situ* hybridization. The data suggest that paracrine and autocrine mechanisms of growth factor action are crucial for the molecular pathogenesis of Kaposi's sarcoma. The interruption of these mechanisms might be an opportunity for therapeutic treatment of Kaposi's sarcoma.

CA 113 REGULATION OF GMP-140 EXPRESSION IN ENDOTHELIAL CELLS, Mathew A. Vadas, Jennifer R. Gamble and Yeesim Khew-Goodall, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Adelaide, South Australia 5000

Granule membrane protein-140 (GMP-140), a member of the LEC-CAM family of adhesion molecules, is a membrane-associated glycoprotein stored in the α-granules of platelets and Weibel-Palade bodies of endothelial cells. Upon activation of endothelial cells with various agents (e.g. thrombin, histamine, phorbol ester (PMA) and complement proteins C56-9), the granules translocate to and fuse with the plasma membrane resulting in cell surface expression of GMP-140. GMP-140 expressed on the surface of endothelial cells mediates the binding of neutrophils and monocytes to the endothelium and therefore plays an important role in inflammation. Soluble GMP-140 has recently been purified from normal human plasma. An alternatively spliced form of GMP-140 mRNA lacking the transmembrane domain, which is potentially the source of plasma GMP-140, has also been isolated. Binding of GMP-140, in both solid and fluid phase, to neutrophils and monocytes results in inhibition of superoxide release. We speculate that in fluid phase, GMP-140 provides a tonic inhibition of neutrophil activation in the circulation, and exhibited on endothelial cells it may prevent inappropriate neutrophil activation during events such as transmigration.

We have examined the expression of both the transmembrane-containing (TM+) and transmembrane-lacking (TM-) mRNAs of GMP-140 during endothelial cell activation by agents such as PMA. Our results show that PMA treatment results in a biphasic decrease in TM+ GMP-140 mRNA expression at 2h and 24h after addition. The TM- mRNA, in contrast, is unaltered in its level of expression at 2h after PMA addition but is similarly decreased at 24h. The mechanism(s) by which these mRNA levels are regulated is the subject of current investigation.

CA 114 RESISTANCE OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS TO KILLING BY NEUTROPHILS RESULTS FROM LOSS OF INTRACELLULAR IRON AND IS RESTORED WITH EXOGENOUS IRON, James Varani, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

First passage human umbilical vein endothelial cells (HUVECs) were sensitive to killing by activated neutrophils and reagent hydrogen peroxide (H_2O_2). Catalase and deferoxamine prevented killing while soybean trypsin inhibitor and superoxide dismutase did not. In these regards, HUVECs are similar to previously characterized endothelial cells from bovine and rat. Although first passage HUVECs were killed by activated neutrophils, sensitivity fell off rapidly as the cells were maintained in culture. At passage 2 and beyond, HUVECs were highly-resistant. The cells also became resistant to killing by reagent H_2O_2. The acquisition of resistance to killing was not accompanied by a failure to up-regulate neutrophil adhesion molecules or to support neutrophil adhesion. Levels of intracellular anti-oxidants (glutathione and total thiols though not catalase activity) increased as a function of passage in culture. However, levels of glutathione and total thiols in late-passage (resistant) HUVECs were similar to levels in late-passage rat pulmonary artery endothelial cells, which were sensitive to killing by activated neutrophils. Cell-associated iron in HUVECs fell as a function of time in culture. By passage 2, the amount of total iron measurable with the Ferrozine reagent was only about 30% of the amount recovered from first passage HUVECs. In contrast, intracellular iron content in rat pulmonary artery endothelial cells remained high through 20 or more in vitro passages. The loss of iron from HUVECs may underlie much of their resistance to killing because when the cells were pre-treated with iron under conditions in which it could be taken up, sensitivity to killing by activated neutrophils and by H_2O_2 was restored.

Early Effector Changes - Vascular Cell Gene Regulation; Adhesion-Pathobiology

CA 200 FLAVONE ACETIC ACID AUGMENTS CYTOKINE-STIMULATED ENDOTHELIAL CELL TISSUE FACTOR PRODUCTION BY A RECEPTOR-INDEPENDENT MECHANISM, Judith C. Andersen, Candace I. Shelly and Sami Diab, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201

Flavone acetic acid (FAA), an unusual chemotherapeutic agent, although cytotoxic to cultured mouse and human solid tumor cell lines, is tumoricidal in vivo only in transplanted tumors with well-developed vascular supplies in which tumor necrosis results from vascular thrombosis with ischemic infarction. FAA alone, in sub-toxic but tumoricidal doses (50 - 200 ug/ml), affected neither morphology nor function of multiply passed human umbilical vein endothelial cells (EC). FAA augmented strikingly, in dose-dependent fashion, tissue factor (TF) synthesis and display induced in EC by either recombinant human tumor necrosis factor-alpha (TNF) or natural human interleukin one (IL-1) without alteration in response time or duration. FAA dose-dependence persisted (i.e. TF production/cell at fixed cytokine concentration increased) well into the range in which direct FAA cytotoxicity was observed (500-1000ug/ml); FAA-cytokine mixtures were more toxic than either component alone in comparable concentration. Preincubation of FAA with EC produced augmentation comparable to that seen with simultaneous FAA-cytokine addition; addition of FAA after tissue factor expression had begun was without effect. Simultaneous EC exposure to FAA, TNF, and IL-1 produced additive augmentation. Basal and augmented TF production were abolished by rabbit antibodies to the stimulating cytokine but not by irrelevant rabbit immunoglobulin. FAA affected neither short-term nor equilibrium cytokine binding to EC; receptor number and affinity remained constant . Although FAA's mode of action has not been determined, preliminary data suggest a nuclear target and may implicate EC sensitization to induction of the "procoagulant phenotype" in FAA's in vivo anti-tumor mechanism. FAA may prove a useful reagent in exploring and modulating in vitro EC reactivity to inflammatory mediators; delineation of its mechanism of sensitization may reveal a critical control point in in vivo EC homeostasis.

CA 201 Retroviral-Mediated Transduction of Human Microvascular Endothelial Cells: Expression of FVIII In Vitro and In Vivo. P.N. Belloni, L. Couto, L. Ross, P. Ho-Faix, and M. Hancock. Department of Cell Biology, Somatix Therapy Corp., Alameda, CA. 94501.

Human microvascular endothelial cells (MVEC) have been investigated as a potential target for genetic augmentation to treat various haemostatic disorders such as hemophilia. MVEC were isolated from human adipose tissue and characterized for expression of Ac-LDL and UEA-I receptors and cytoplasmic vWF. A retroviral vector (MFG-FVIII) containing a deleted form of the human FVIII gene was constructed based on MMLV, that uses the LTR promotor. HMVEC were transduced at a high efficiency with a single exposure of MFG-FVIII particles. Transduced HMVEC were subjected to southern-analysis to determine the average copy/cell. FVIII expression was determined qualitatively by immunohistochemical staining and quantitated using the KABI-Coatest that measures the conversion of factor X to Xa. FVIII was found in the cytoplasm of the transduced HMVEC in a perinuclear pattern. Transduction had no apparent effects on expression of vWF. Factor VIII was efficiently produced and secreted into the culture media at a rate of 2.3 ug/5 x 10^6 cells/24 hr. In vivo expression of hFVIII was achieved in subcutaneous matrigel implants containing transduced HMVEC and detected by immunohistochemical analysis. These results suggest that human microvascular endothelial cells may serve as a cell-based delivery system after genetic augmentation.

CA 202 INHIBITION OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION WITH ANTISENSE OLIGONUCLEOTIDES, C. Frank Bennett, Ming-Yi Chiang, and Hedy Chan, Department of Molecular and Cellular Biology, ISIS Pharmaceuticals, Carlsbad, CA 92008

Increased expression of the endothelial-leukocyte adhesion molecules ICAM-1, VCAM-1 and ELAM-1 have been demonstrated in a variety of inflammatory disorders. We have designed a series of oligonucleotides which hybridize to the human ICAM-1 mRNA to determine the feasibility of using antisense oligonucleotides to inhibit expression of endothelial cell adhesion molecules and to identify the optimum target site on ICAM-1 mRNA. Oligonucleotides were synthesized using phosphorothioate chemistry and screened for activity in human umbilical vein endothelial cells and A549 cells using an ICAM-1 ELISA. Oligonucleotides which hybridized to the AUG translation initiation codon, 5'- cap site, and specific 3'-untranslated regions inhibited ICAM-1 expression in a sequence specific manner. Oligonucleotides which hybridized to specific 3'-untranslated region sequences promoted a loss of the ICAM-1 mRNA, while oligonucleotides which hybridize to the AUG translation initiation codon did not. None of the oligonucleotides had an effect on ICAM-1 transcription, thus they were inhibiting ICAM-1 expression at a post-transcriptional step. Active oligonucleotides inhibited adherence of HL-60 cells to TNF-α treated human umbilical cells in a sequence specific manner. These data demonstrate that antisense oligonucleotides specifically inhibit ICAM-1 expression by at least two mechanisms and are a viable approach for inhibition of the expression of endothelial cell adhesion molecules.

CA 203 SYNERGIC INDUCTION OF METALLOPROTEINASES BY PMA AND TNFα IN HUMAN ENDOTHELIAL CELLS.

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The remodeling of connective tissue extracellular matrix occurs in a number of fundamental biological processes such as cell migration during development, wound healing, angiogenesis... This remodeling implies the coordinated synthesis and degradation of the proteic components of the matrix. Among the enzymes implicated in this degradation, metalloproteinases play the key role. Therefore, investigation of accepted angiogenic factors on the regulation of metalloproteinases should be informative. We report here that TNFα and PMA have important effects on the amount of metalloproteinases secreted by human umbilical endothelial cells as shown by zymography analysis. While none of these agents alone has significant activity on the 92 kd collagenase (type IV collagenase), together, they greatly increase the production of this enzyme. Maximal induction was observed 48 h after treatment. The optimal concentrations of TNF α and PMA were respectively 5ng/ml and 10nM. This optimal concentration of PMA indicates that protein kinase C is most probably involved in this synergy.The 92kd collagenase has been implicated in the degradation of the basement membrane. Our results are therefore consistent with an involvment of this enzyme in migratory process of endothelial cells.

CA 204 CLONING OF IL1-INDUCIBLE GENES IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS: PRELIMINARY CHARACTERISATION, Ferruccio Breviario, Elisabetta d'Aniello, Josée Golay, Elisabetta Dejana, Alberto Mantovani and Martino Introna. Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milano, Italy.

In an attempt to understand more directly the molecular mechanisms involved in the cellular response to interleukin-1 (IL1), we have made several cDNA libraries from mRNA isolated from human umbilical vein endothelial cells (HUVEC), after 2 to 6 passages, and stimulated for 1 hour with 20 ng/ml human recombinant IL1-ß, in the presence of 10 µg/ml cycloheximide. The cDNA libraries were differentially screened with radiolabelled cDNA derived from mRNA isolated from either untreated or IL1- treated HUVEC. 8 different cDNA clones induced by IL1 were identified and partially sequenced. 5 out of 8 corresponded to genes which had previously been identified and characterised as IL1- inducible genes in human endothelial cells (PAi-1, N-CAF, GRO-α, GRO-ß and ELAM-1). The other 3 cDNA clones on the contrary represent novel genes. In all cases, Northern analysis confirmed that these novel genes are induced upon exposure of HUVEC cells to IL1-ß for 1 hour in the presence of cycloheximide. The 3 new genes are identified as clones 100, 133 and 170 and their mRNA size has been estimated to be of 3.5, 1.6 and 2.0 kb respectively. The characteristics of the cDNA clones will be discussed.

CA 205 IDENTIFICATION OF CELL-TYPE- AND CYTOKINE-SPECIFIC TRANSCRIPTIONAL REGULATORY REGIONS OF THE HUMAN ICAM-1 GENE. S.W. Caughman, L. Cornelius, K. Degitz, N. Shibagaki, L.-J. Li, Department of Dermatology, Emory University, Atlanta, GA, 30322.

The regulated expression of the leukocyte adhesion molecule ICAM-1 by endothelial cells (EC) and other tissues is critical to the evolution of inflammatory responses, and the inducibility of ICAM-1 expression appears to be sensitive to a variety of cell-type- and cytokine-specific signals. Human dermal microvascular EC (MEC) constitutively express ICAM-1; human keratinocytes (HK) do not. IL-1 induces ICAM-1 in MEC, but fails to do so in either HK or A431, a human squamous carcinoma cell line, even though both have IL-1 receptors and express ICAM-1 on exposure to IFN-γ. We have characterized a human ICAM-1 genomic clone containing a potent basal promoter region and 4 kb of 5' flanking sequence (JBC 266:14024, 1991). Various upstream portions of the ICAM-1 gene were subcloned into heterologous reporter gene (CAT) plasmids, and their effect upon CAT expression assessed after transient transfection into MEC and A431. An 883 bp region upstream of the basal promoter was identified which conferred enhanced constitutive expression of CAT in MEC and suppressed expression of CAT in A431. IL-1 treatment further enhanced CAT expression in transfected MEC but had no effect in identically transfected A431. However, A431 transfected with this construct did display a reversal of repressed expression and a multi-fold, dose-dependent enhanced expression of CAT in response to IFN-γ. Further characterization of this human IFN-γ-sensitive response region revealed it to be functional across species lines if transfected cells were treated with the species-appropriate IFN-γ, demonstrating that the final molecular signaling mechanism within the cell is evolutionarily conserved. PCR-generated nested deletions of the 883 bp region identified a 90 bp fragment which conferred marked IFN-γ-dependent enhancement of transcription. Sequence analysis of this fragment failed to reveal homologies to elements previously associated with either IFN-γ or IFN-α/β responsiveness. These data implicate the existence of both cell-type- and cytokine-specific responsive elements in the 5' regulatory region of the human ICAM-1 gene, and demonstrate that regulatory effects directed by such elements are dependent upon their cellular context. They also identify a specific IFN-γ-responsive subregion within this gene, and provide the basis for identification of the precise nucleotide composition of this *cis*-element(s), and for the potential isolation and characterization of the *trans*-acting protein whose activation and functional domain is presumptively common to both murine and human cells.

CA 206 ACCESSORY FUNCTION OF FcRII TRANSFECTED ENDOTHELIAL CELLS, Rob M.W. de Waal, Hans R.Westphal, Wil J.M. Tax, Robert A.P.Koene and Dirk J. Ruiter, Department of Pathology and Department of Internal Medicine, Division of Nephrology, University Hospital Nijmegen, The Netherlands

Efficient T cell activation requires the presence of antigen-presenting or accessory cells. Accessory cell function consists of two distinct mechanisms: presentation of antigen in the context of MHC leading to cross-linking of the T cell receptor, and, delivery of one or more secondary signals. In anti CD3-induced T cell proliferation *in vitro*, TCR crosslinking is mediated via FcRs present on the accessory cell. Endothelial cells are completely inactive in this respect since they lack FcRs. They are, however, potent producers of second signal activity. We were able to show this in experiments in which TCR crosslinking is artificially provided for by an anti-CD3 coated solid phase. Inhibition studies indicated that the second signal did not consist of endothelial IL-1 and IL-6 production. To overcome the lack of cross-linking capacity, endothelial cells were transfected with FcRII DNA, ligated into a pCDX vector. Expression of FcRII ranged between 0.1 and 1.0%, as could be demonstrated by staining with a specific moab. Accessory cell activity of the transfected cells was subsequently shown in anti CD3-induced T cell proliferation. Only IgG1 moabs were mitogenic which is in accordance with FcRII specificity.

CA 207 CHARACTERISATION OF A HUMAN UMBILICAL VEIN ENDOTHELIAL CELL LINE TRANSFECTED WITH THE EARLY REGION OF SV40. Fickling, SA., Vallance, P., Tooze, J., Easparathasan, V., Reader, J., Johnstone AP., and Whitley, GStJ. Department of Cellular and Molecular Sciences, St George's Hospital Medical School, London SW17 ORE. U.K.

Human umbilical vein endothelial cells were transfected by electroporation (1) with the early region of simian virus 40 (SV40). Although not immortal the resultant 'cell lines' divide rapidly (population doubling time of 35h) in culture for up to 24 passages in medium supplemented with 5% (v/v) FCS/NCS and 2.5µg/ml endothelial cell growth supplement. Several of these lines express basal levels of ICAM-1 and MHC class 1 but not MHC class II, both ICAM-1 and MHC class 1 could be further induced by tumour necrosis factor and gamma interferon. One particular cell line designated SGHEC-7 retained a number of differentiated endothelial cell functions throughout this period. These functions include a dose dependent production of tissue plasminogen activator in response to histamine, thrombin and phorbol myristate acetate, elevated intracellular calcium in response to histamine, thrombin and ATP and also cytokine induced expression of nitric oxide synthase. Rapid deterioration in cell viability is observed after passage 25, accompanied by an increase in chromosomal abnormalities. These cell lines will facilitate long term large scale studies into the physiology and pathology of the human vascular endothelium.

(1) Whitley GStJ., Nussey SS., Johnstone AP. (1987). Mol. Cell.Endocrinol. **52**, 279-284.

CA 208 HYPOXIA ASSOCIATED PROTEINS: A SET OF ENDOTHELIAL CELL-SPECIFIC, STRESS-SPECIFIC PROTEINS. K.K. Graven, E.W. Dickson, G.L. Weinhouse, H.W. Farber. Pulmonary Center, Boston University School of Medicine, Boston, MA 02118

Exposure to hypoxia (0% oxygen for 4-24 hours) induces a specific set of stress proteins (HAPs: MW 56,47,39,36,34kD) in cultured bovine aortic and pulmonary arterial endothelial cells (EC). These proteins are cell-associated, upregulated in a time- and oxygen concentration-dependent manner, and are distinct from heat shock proteins (HSPs). In vitro translation experiments demonstrate elevated levels of several mRNA transcripts in acutely hypoxic cells. These mRNAs encode for proteins of similar MW to HAPs (48,40,37,35,32kD) while mRNAs from heat-shocked EC encode for a separate set of proteins. Thus, upregulation of HAPs appears due to increased transcription or decreased mRNA degradation. Induction of HAPs is EC specific; HAPs are not upregulated in human lung fibroblasts (FB), bovine pulmonary arterial smooth muscle cells (SMC), or mouse renal tubular epithelial cells exposed to hypoxia. On the contrary, FB exposed to hypoxia upregulate proteins corresponding to conventional HSPs (MW 90,70,68kD) and glucose regulated proteins (GRPs: MW 104,78kD). In SMC, in addition to conventional HSPs, a 32kD protein (heme oxygenase) is upregulated. In human aortic and pulmonary arterial EC exposed to hypoxia, as in bovine EC, the induced proteins correspond to HAPs (MW 57,46,39kD). Investigation of proteins induced in EC by other forms of stress, such as "chemical hypoxia" using sodium cyanide (NaCN), inhibition of glycolysis using 2-deoxyglucose (2-DOG), glucose deprivation using glucose-free media, glucose excess using 5X usual glucose concentration, a change in the redox potential using dithiothreitol (DTT), or oxidant stress using hydrogen peroxide (H_2O_2) demonstrates that HAPs are upregulated only by exposure to hypoxia. EC exposed to NaCN, H_2O_2, and 5X glucose do not upregulate detectable proteins; EC exposed to 2-DOG, glucose deprivation, or DTT upregulate GRPs (104,78kD). Finally, crude EC subfractionation localizes HAPs primarily in the cytosolic fraction following 4-24h of hypoxia. The induction of these unique stress proteins may be important to EC survival during exposure to acute hypoxia.

CA 209 ENDOTHELIAL CELL NUCLEAR PROTEINS WHICH INTERACT WITH ACIDIC AND BASIC FIBROBLAST GROWTH FACTOR. Victor B. Hatcher[1], Christina A. Samathanan[1], Stanley A. Friedman[2], Xi Zhan[2], Vicken Yaghajian[1], Portia B. Gordon[1] and Thomas Maciag[2], Department of Biochemistry[1], Albert Einstein College of Medicine, Bronx, NY 10461. Laboratory of Molecular Biology[2], American Red Cross, Rockville, MD 20855.

Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) are translocated into the nucleus. (Imamura et al. Sci. 249:5460-5465, 1990; Bouche et al. Proc. Natl. Acad. Sci. 84:6770-6774, 1987). Endothelial cell nuclei and an enriched fraction containing nuclear envelopes from human umbilical vein endothelial cells were prepared utilizing homogenization, centrifugation through 2 M sucrose followed by centrifugation through a discontinuous sucrose gradient. The nuclear envelopes were solubilized, biotinylated and reacted with either aFGF-Affi-Gel 102 or bFGF-Affi-Gel 102. The nuclear proteins were eluted, separated by SDS-PAGE and blotted to PVDF. The results from the blots indicated that a 63 kDa and a 52 kDa nuclear protein interacted with aFGF. Only the 52 kDa nuclear protein interacted with bFGF. aFGF and bFGF expressed different specificity with regard to their interaction with human endothelial cell nuclear proteins. In the binding and transport of the FGFs into the nucleus, aFGF and bFGF may interact with different nuclear proteins. The 63 kDa endothelial cell nuclear protein (p63) which interacts with aFGF and heparan sulfate proteoglycan was purified. The p63 nuclear protein interacted with a synthesized peptide containing the aFGF-NLS (CGGGNYKKPKL) and to a lesser degree to a synthesized NLS mutant (CGGGNYTKPKL). In an in vitro nuclear binding assay in which saturable binding of ^{125}I-HSA-NLS was demonstrated, significantly less binding of ^{125}I-HSA-NLS mutant to the isolated endothelial cell nuclei was observed. The p63 endothelial cell nuclear protein significantly inhibited nuclear binding of both the ^{125}I-HSA-NLS and the ^{125}I-HSA-NLS mutant conjugates. Thus, the endothelial cell nuclear protein (p63) has been purified which interacts with aFGF-NLS and appears to be involved in human endothelial cell nuclear binding.

CA 210 AUTOREGULATION OF bFGF GENE EXPRESSION IN BOVINE AORTIC ENDOTHELIAL CELLS. Po-Tsan Ku and Patricia A. D'Amore, Children's Hospital and Harvard Medical School, Boston, MA

Basic fibroblast growth factor (bFGF), unlike most other growth factors, lacks a N-terminal signal sequence, thereby generating a lot of controversy regarding its potential mode of release. In addressing this problem, it was demonstrated that transient breaks in plasma membrane of endothelial cells (EC) induced by scraping, release bFGF into the culture medium (McNeil et al., J Cell Biol 109: 811, 1989). Since little is known about the regulation of bFGF expression, we were interested to learn whether EC might have a "feedback" system to influence bFGF expression following sublethal injury. We have used Northern analysis to examine the expression of bFGF mRNA in bovine aortic EC following scraping. Enhanced expression of bFGF mRNA, especially the 3.7 Kb message, was detected in EC following scraping: maximal increases were observed at 6 hr and gradually returned to control levels. We hypothesized that bFGF released into the culture medium following scraping might lead to the autoinduction of bFGF expression. This concept was supported by the observation that EC treated with 5 $^{ng}/_{ml}$ of recombinant bFGF expressed increased levels of bFGF mRNA. Further insight into the molecular signals regulating bFGF gene expression was obtained by performing the scraping experiment in the presence or absence of suramin, a polyanionic compound which has been demonstrated to inhibit the interaction between growth factors and their receptors on target cells. Preliminary results indicated that presence of suramin blocked the induction of the 3.7 Kb message at 6 hr following scraping, while having little effect on the expression of the 7.0 Kb message. On the other hand, when suramin was present in the culture medium for 10 hr following scraping, expression of the 7.0 Kb message was enhanced, while that of the 3.7 Kb was unchanged. These observations imply the differential regulation of the 7.0 Kb and 3.7 Kb bFGF mRNA. Further, they suggest that cells making bFGF may be able to "replenish" their bFGF levels following loss due to sublethal cell injury.

CA 211 SPARC REGULATES PROTEINS OF THE EXTRACELLULAR MATRIX DURING ANGIOGENESIS *IN VITRO*. Timothy F. Lane, M. Luisa Iruela-Arispe, Helene Sage, Dept. Biological Structure, University of Washington, Seattle, WA 98195.

Angiogenesis *in vitro*, the formation of capillary-like structures by cultured endothelial cells, is associated with changes in the expression of extracellular matrix proteins. Sparc is a secreted collagen-binding glycoprotein that diminishes the adhesion of endothelial cells *in vitro*. Sparc also forms stable complexes with several proteins known to be relevant to the formation of blood vessels and to endothelial cell growth. Previously, we demonstrated that Sparc expression is specifically increased in cells that spontaneously organize into endothelial cords and tubes (Iruela-Arispe, M. L., Hasselaar, P. and Sage, H. (1991) *Lab. Invest.* **64**, 174-186). We now show that addition of purified Sparc protein, or an N-terminal synthetic peptide (Sp_{5-23}), to cultures of bovine aortic endothelial cells containing cords and tubes resulted in a dose-dependent decrease in the synthesis of fibronectin mRNA and protein. Sparc decreased the level of fibronectin mRNA by 75% over 48 h; this effect was blocked by anti-Sparc immunoglobulins. In addition, we observed that levels of thrombospondin mRNA were diminished 80% by Sparc in a dose-dependent manner. Over a similar time course, both mRNA and protein levels of type 1 plasminogen activator inhibitor (PAI-1) were enhanced by Sparc and the Sp_{5-23} peptide. In contrast, no changes were observed in the levels of either type I collagen mRNA or secreted gelatinase. The effects seen with Sparc were distinct from those obtained with endotoxin. Endotoxin had no influence on the production of fibronectin or thrombospondin and required serum to induce PAI-1. These results demonstrate that Sparc modulates the synthesis of a number of secreted proteins and identify an N-terminal acidic sequence as the region of the protein responsible for this activity. The regulation of specific protein synthesis by Sparc may be important to achieve an optimal ratio among different components of the extracellular matrix that affect cellular morphology and proliferation during capillary formation *in vitro*.

CA 212 THE ADHESION OF NEISSERIA MENINGITIDIS TO HUMAN ENDOTHELIAL CELLS IS SUBJECT TO PHASE VARIATION. X. Nassif and M. So. Dept. of Microbiology & Immunology, Oregon Health Sciences University, Portland, OR 97201.

Neisseria meningitidis is an extracellular human pathogen which has the ability to cross the blood-brain barrier (BBB) and produce meningitis. The infant rat is the only known animal in which N.meningitidis replicates and produces infection. After intraperitoneal injection of 10^6 CFU, bacteria are found in the blood with subsequent invasion of the cerebrospinal fluid (CSF). Histopathological examination of the meninges during the first hours of the infection showed bacteria adhering to the endothelial cells of the meningeal capillaries, suggesting that this is the first step in translocation from the circulation to the CSF. In vitro, we found that N.meningitidis adheres to human umbilical vein endothelial cells (HUVEC) and human brain endothelial cells (HBEC). This phenotype was not observed with Neisseria lactamica, a closely related nonpathogenic Neisseria species.

Strains of N.meningitidis vary in their adhesiveness to endothelial cells, some being 200 times more adhesive than others. Interestingly, the endothelial cell-associated bacteria from a poorly adhesive give rise subsequently to a highly adhesive population. Two hypotheses could explain an increase in adhesion after passing bacteria onto cells; (i) induction of the expression of a bacterial gene by contact with a eucaryotic cell component (ii) selection of a subpopulation of bacteria which spontaneously produce an adhesin. This second hypothesis could be explained by the phenomenon of phase variation. Among 15 isolated colonies of a poorly adhesive strain one was found to be highly adhesive, without having been passed onto cells. This result strongly suggests that N.meningitidis adhesion to endothelial cells is subject to phase variation. Comparison of low and high adhesive isolates of the same strain showed the same profile of major outer membrane proteins and no difference in piliation as demonstrated by electron microscopic analysis of negatively stained cells. Work is currently in progress to characterize this adhesin, its endothelial cells receptor and assess its role in the invasion of the CSF by N.meningitidis.

CA 213 TRANSCRIPTIONAL REGULATION OF THE HUMAN CYCLOOXYGENASE GENES IN ENDOTHELIAL CELLS, Karen Neilson, Susan Appleby and Timothy Hla, Laboratory of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rockville, MD 20855

Cyclooxygenase catalyzes the rate limiting step in the synthesis of inflammatory protaglandins. Recently, we cloned the second form of the human cyclooxygenase gene (hcox2). Hcox2 shares 60% amino acid identity with hcox1. Currently, we are examining the enzymatic properties of the hcox2 polypeptide in a cos cell expression system. Both hcox1 and hcox2 mRNAs are expressed in human umbilical vein endothelial cells (HUVEC). However, the level of hcox2 mRNA is greatly increased in the presence of phorbol myristic acetate (PMA), an inducer of HUVEC differentiation. Hcox2 mRNA also appears to be induced in the presence of FGF. To understand the transcriptional regulation of the hcox2 gene by angiogenic factors, we have isolated the putative promoter region. Using PCR primer extension techniques, a 300 bp fragment which is expected to define the transcriptional start site of the hcox2 gene was obtained. This fragment was used to probe a human genomic Southern blot. A 4 kb EcoRI band and a 6 kb SacI band hybridized with the 300 bp hcox2 probe. Next, a human genomic library was screened with the radiolabeled 300 bp 5' hcox2 probe. Five positive phage clones were plaque purified. Four out of the five positives contained the 4 kb EcoRI fragment and the 6 kb SacI fragment. Sac1 and EcoRI digestion of these four clones yielded a 3.5 kb band which hybridized with the 300 bp hcox2 probe on a Southern blot. These fragments are being characterized by DNA sequencing. Deletion analysis in conjunction with reporter constructs of the promoter sequences are anticipated to determine the cis-acting elements responsible for the transcriptional regulation of the hcox2 gene by angiogenic factors. Since cyclooxygenase plays a key role in the synthesis of prostaglandins, understanding the transcriptional regulation of the cyclooxygenase genes will lead to further insight into the inflammatory processes including angiogenesis.

CA 214 LIPOXYGENASE METABOLITES ARE INVOLVED IN RED BLOOD CELL - ENDOTHELIAL CELL ADHESION. Yamaja B.N. Setty, Carlton D. Dampier, and Marie J. Stuart, Div. of Hematology-Oncology, St. Christopher's Hospital for Children, Temple University School of Medicine, Philadelphia, PA.

As an initial investigation into the possible role of lipoxygenase (LO) metabolites in red blood cell (RBC) - endothelial cell (EC) adhesion, the effect on adhesion of the LO inhibitor NDGA (10 uM) was evaluated using a static incubation system (in the absence of plasma) and using CR-51 labeled RBCs. Since baseline RBC-EC adhesion was inhibited by approximately 40% in the presence of NDGA, the role of the LO metabolites 12-HETE, 15-HETE and 13-HODE on the adhesive process has been further assessed. Both the mono-HETEs derived from arachidonic acid (12- and 15-HETE) significantly increased RBC-EC adhesion. In dose response experiments, mean adhesion was 152 ± 10, 166 ± 19, 177 ± 22 and 118 ± 15% (mean ± SE, n=10) in the presence of 1, 10, 100 and 1000 nM 12-HETE respectively, with statistically significant increases ($P<0.05$ to $P<0.01$) in adhesion noted at 1, 10 and 100 nM 12-HETE. 15-HETE had a similar effect with a statistically significant increase ($P < 0.01$) in basal adhesion to 161 ± 21% (mean ± SE, n=9) at a 15-HETE conc. of 1 nM. In contrast, the linoleic acid derivative 13-HODE (100 pM to 1 uM; n=10) had an inhibitory effect on basal adhesion at 1 uM (72 ± 5% of control; $P < 0.01$). Final studies were aimed at evaluating whether EC arachidonate metabolism was activated during the adhesion process. Following overnight labeling of ECs with 14C-AA, monolayers were incubated with washed RBCs for 30 min. Cells and supernatant were analyzed for AA release and eicosanoid production. RBCs stimulated EC arachidonate release by 89% compared to control monolayers (17,507 ± 2689 in controls vs 30,348 ± 2,609 cpm per well in RBC treated ECs, n=9, $P < 0.001$). An increase in both cyclooxygenase (CO) and LO metabolites was also noted. Total CO and LO metabolites in control ECs was 3,952 ± 297 and 1,072 ± 204 vs 4,733 ± 227 ($P < 0.005$) and 1,309 ± 247 ($P < 0.05$) in RBC treated ECs. Our studies have documented a role for LO metabolites in modulating basal RBC-EC adhesion.

CA 215 THROMBOMODULIN GENE REGULATION IN F9 EMBRYONAL CARCINOMA CELLS: INDUCTION BY cAMP AND RETINOIC ACID. Hartmut Weiler-Guettler, Ker Yu and Robert D. Rosenberg. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Thrombomodulin (TM), an endothelial surface glycoprotein involved in antithrombin-mediated anticoagulative pathway regulation, is also expressed in embryonic yolk sac parietal endoderm. In response to retinoic acid (RA) and cAMP, F9 embryonal carcinoma (EC) cells mimic early embryo inner cell mass differentiation into visceral and parietal endoderm. To study developmental regulation of the mouse TM gene, its expression during F9 EC cell differentiation was analysed at the mRNA- and transcriptional level. No TM mRNA was detectable in EC stem cells or in visceral endoderm cells. In response to 1 µM RA, TM mRNA is induced to low levels after 48 h and increases 2-3 fold during the next 48 h. Treatment of EC stem cells with 0.5 mM dibutyryl cAMP and 0.5 mM theophylline (CT) induces TM mRNA within 12 h. This effect is due to elevated cAMP levels, since 0.5 mM butyrate and 0.5 mM theophylline had no effect on TM expression. Induction of F9 EC differentiation into parietal endoderm by exposure to a combination of RA and CT results in 10-12- (50-60-) fold higher mRNA levels than in CT- (RA-) treated cells. Synergism of both hormones was evident only after 12-24 h. In cells treated for 72 h with RA before adding CT, synergistic enhancement of TM mRNA induction occured within less than 6 h and did not require the continued presence of RA. Nuclear run-on experiments showed, that hormone-dependent TM mRNA induction is regulated transcriptionally. Gene expression experiments were performed to characterize regions of the human TM-gene promotor involved in developmental regulation. Transient coexpression of TM-reporter gene constructs and the human retinoic acid receptor β results in activation of the TM-promotor in differentiated F9 cells. Thus, it may be concluded that [1] TM is coregulated with a battery of genes transcriptionally activated during RA and cAMP induced F9 EC cell differentiation into parietal endoderm, [2] establishment of an enhanced cAMP-responsivness is RA-dependent and [3] that a retinoic acid responsive element is present in the human TM gene promotor.

Adhesion-LEC-CAMS; Vascular Cell Phenotype

CA 300 ICAM-2, A SECOND LIGAND FOR LFA-1, PROVIDES A COSTIMULATORY SIGNAL T-CELL RECEPTOR-INITIATED ACTIVATION OF HUMAN T CELLS, Jan Chalupny, Nitin K. Damle, Kerry Klussman, and Alejandro Aruffo, Department of Cellular Interactions, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Activation of T cells requires both signals delivered by the T-cell receptor (TCR) and those derived from costimulatory interactions between certain T-cell surface accessory molecules and their respective ligands on antigen presenting cells (APC). The costimulatory role of ICAM-1 has been demonstrated. Here using a soluble form of ICAM-2 we show that like its homologue ICAM-1, ICAM-2 can provide costimulatory signals during the activation of both resting and antigen-primed CD4+ T cells. The costimulatory effects of ICAM-2 are dependent on its co-immobilization with mAb directed at the CD3/TCR complex. ICAM-2 mediated costimulatory signals result in the induction of IL-2 receptor and IL-2 expression. The costimulatory effects of ICAM-2 is blocked by mAb directed at either the CD11a or the CD18 molecules. The interactions between LFA-1 and ICAM-2 may provide important co-stimulatory signals during the early stages of CD4+ T-cell activation by ICAM-2^{high}, ICAM-1^{low} cells such as endothelial cells at the early stages of inflammation.

CA 301 CHARACTERIZATION OF A GAMMA-GLUTAMYL TRANSPEPTIDASE POSITIVE, SUBPOPULATION OF ENDOTHELIAL CELLS IN A TUBE-FORMING CLONE OF RAT CEREBRAL RESISTANCE-VESSEL ENDOTHELIUM, Lawrence De Bault, Bao-Le Wang and Paula Grammas, Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

There is evidence to suggest that growth factors can modulate the phenotype of endothelial cells (ECs) *in vitro*, ie., "Cobblestone-Like" (CL) vs. "Spindle-Shaped" (SS). The objective of this study was to characterize the EC phenotypes expressed in a clone of cerebral resistance-vessel ECs, RV150-ECT, that spontaneously form tube-like structures. Of interest were ECs positive for γ-glutamyl transpeptidase (γ-GTP), an enzyme important to the biochemistry of the blood-brain-barrier (BBB). RV150-ECT cells (passages >37) were seeded onto glass coverslips or in 12-well cluster dishes and sampled on days 5, 7, 14, 21, and 28. Coverslips were fixed in acetone for γ-GTP light microscopy (LM) enzyme cytochemistry, and wells were fixed in 3% paraformaldehyde plus 0.5% glutaraldehyde for electron microscopy (EM). In the case of immunocytochemistry, fixation was followed by pre-embedding goat-anti-γ-GTP "Immunogold-Silver Staining". Although most of the ECs in the culture were negative at the LM level, a subpopulation of strongly γ-GTP positive cells were found randomly dispersed in early stages of the culture, which later became associated with the apical surfaces of multicellular cell aggregates and with the tube-like structures. LM and EM examination of ECs demonstrated that three morphological phenotypes were present, CL-ECs, SS-ECs, and a "Oval-Shaped" (OS) cells. The OS-ECs were γ-GTP positive, large, with loose cytoplasm and large nucleus, and contained numerous microfilaments and few microvilli. In contrast, SS-ECs were compact, had a dense cytoplasm, few microfilaments, and smaller nuclei, while CL-ECs had an intermediate morphology. These data demonstrate that in long term serial cultures of ECs from cerebral resistance-vessels, ECs will spontaneously express 2 or more distinct phenotypes. These results suggest that the phenotypically distinct EC populations reflect inherent differences in EC biology and that the OS phenotype preferentially expresses high γ-GTP levels, a biochemical property of BBB endothelium. We propose, the OS-γ-GTP positive cells on the apical surface of the cultures represent the terminal differentiation stage of a phenotype that arises as part of the tube-forming process. This may be similar to other processes observed in lumen development where cells within multicellular aggregates naturally degenerate creating the luminal space.

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CA 302 CYTOKINE REGULATION OF PROLIFERATION, ICAM-1 EXPRESSION, AND IL 6 PRODUCTION OF HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS (HDMEC) IN VITRO, Michael Detmar, Susanne Tenorio, Uwe Hettmannsperger, Martin Owsianowski, Constantin E. Orfanos, Dept. of Dermatology, Steglitz Medical Center, Free University of Berlin, Berlin, Germany.

HDMEC play a major role in the mediation of inflammatory skin diseases as psoriasis. To characterize cytokines that influence the proliferation, expression of cell adhesion molecules, and cytokine production of dermal endothelium, we established a model for the isolated cultivation of HDMEC in vitro.

HDMEC were released from children foreskins by enzymatic and mechanical treatment and were purified by continuous Percoll density gradient centrifugation. 2nd passage HDMEC were treated with the recombinant cytokines IL 1a, IL 1b, IL 6, GM-CSF, IFNa, IFNg, TNFa, EGF, PDGF, or NGF for up to 4 days. Proliferation was determined by cell counts, thymidine incorporation, and MUH fluorescence, and the expression of ICAM-1 was assessed by APAAP immunocytochemistry and immunoelectron microscopy. IL 6 production was investigated by ELISA and by Northern Blot hybridization.

Staining with Ab against factor VIII related antigen and detection of Weibel Palade bodies confirmed the pure EC origin of monolayer cultures. IL 1a, IL 1b, GM-CSF, EGF, and PDGF dose-dependently stimulated the proliferation of HDMEC, while IL 6, TNFa, and NGF were not effective; IFNa and IFNg inhibited the proliferation. In contrast to HDMEC, HUVEC growth was not stimulated by IL 1. ICAM-1 expression was induced by IL 1a, IL 1b, IFNg, and TNFa, mainly located on microvillous cell protrusions at the culture surface. Both IL 6 protein and mRNA were induced by LPS, IL 1, and TNFa, but not by IFNg or other cytokines.

These findings confirm the distinct character of HDMEC compared to other EC with differential regulatory effects of each of the tested cytokines. While HDMEC themselves seem not to be primary targets for IL 6, they are potent producers of this inflammatory cytokine.

CA 303 ENDOTHELIAL CELLS MODULATE MURAL CELL PROLIFERATION AND MIGRATION IN VITRO.

A. B. Dodge, J. E. Gabriels and P. A. D'Amore. Children's Hospital and Harvard Medical School, Boston, MA 02115

Endothelial cells (EC) are in contact with subjacent macrovascular smooth muscle cells (SMC) or microvascular pericytes and may communicate via soluble mediators or direct cell-cell contact. Myoendothelial junctions in large vessels occur through fenestrae in the internal elastic lamina, whereas basement membrane discontinuities permit direct contact between pericytes and EC. Evidence suggests that control of mural cell growth and migration may involve such heterotypic interactions. Using cocultures that either permit or prevent physical contact between EC and mural cells, we have investigated the effects of EC on SMC and pericyte proliferation. Mitomycin C growth-arrested EC were cultured either in direct contact with mural cells or plated into millicell inserts positioned 1-2 mm above SMC or pericyte cultures to allow continual exchange of soluble mediators. In both coculture models, EC stimulated an approximate 3-4 fold increase in SMC or pericyte numbers over a nine day time course. Approximately 50% of this stimulatory activity was blocked with neutralizing antibodies against bFGF. The anti-human bFGF completely blocked the mitogenic response of SMC and pericytes to recombinant human bFGF, but had no effect on control mural cell growth in media supplemented with 10% calf serum. Conditioned media derived from growth-arrested EC stimulated an approximate 1.5 fold increase in mural cell proliferation; 50% of this stimulatory activity was lost by boiling the conditioned media. Using an under agarose migration assay we demonstrate that EC induce SMC migration. Nearly all of this activity was inhibited with neutralizing antibodies against bFGF and PDGF-BB. In contrast to the lack of effects of anti-human bFGF on SMC proliferation neutralizing anti bFGF blocked baseline migration. Collectively, these results suggest that macro/microvessel EC regulation of mural cell proliferation and migration, events associated with vascular development and remodeling, may be partly mediated by factors such as bFGF and PDGF-BB.

CA 304 EXTENDED LIFE SPAN OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS TRANSFECTED WITH V-FMS AND ADENOVIRUS E1A ONCOGENES. T.Venkat Gopal, Norma Graber, and Michael Seidman. Otsuka America Pharmaceutical, Inc., 9900, Medical Center drive, Rockville, MD 20850.

Early passage (P2 or P3) human umbilical vein endothelial cells (HUVEC) were transfected with the oncogenes v-fms and adenovirus E1A using a slightly modified DEAE-dextran method. Actively growing growth centers were seen in the oncogene transfected cells after the 7th or 8th passage but not in the control culture.

The uncloned population of the actively growing cells from the transfected culture was tested for various endothelial cell specific markers at different passages. These cells, referred to as HUEF-1, displayed the adhesion molecules, ELAM-1 and VCAM-1, on the surface upon stimulation with IL-1, similar to primary HUVEC. The IL-1 activated HUEF-1 bound neutrophils, and this binding was blocked by a ELAM-1 specific monoclonal antibody. These cells also produced ICAM-1 constitutively which was further elevated by IL-1 treatment. These cells also displayed other endothelial cell specific markers, such as the synthesis of von Willebrand factor and factor VIII, as well as the transfected oncogene products. These cells have been grown successfully up to passage 40 now. The oncogene transfected cells which resembled the primary HUVEC in regard to growth properties and morphology maintained many of the endothelial cell properties even in the late passages. These results show that the oncogenes v-fms and E1A can extend the life span of HUVEC, at the same time maintaining many of the endothelial cell specific properties, without transformation.

CA 305 DISTINCT MOLECULAR PATHWAYS OF SHEDDING OF LAM-1 (L-SELECTIN) Geoffrey S. Kansas and Thomas F. Tedder, Dana Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115

LAM-1, a member of the selectin family of cell adhesion molecules, mediates the initial attachment of leukocytes to endothelium at sites of inflammation and to the high endothelial venules of peripheral lymph nodes. LAM-1 is unique among leukocyte adhesion receptors in that it is rapidly *shed from the cell surface of all leukocyte classes in response* to most stimuli. LAM-1 expressed by transfected U937, K562, 300.19 and COS-7 cells is also shed, indicating that the proteolytic machinery responsible for shedding of LAM-1 is expressed on cells which do not normally express LAM-1. The size of the shed LAM-1 molecule suggests proteolytic cleavage at a membrane proximal site. The highest degree of homology of LAM-1 with its murine homologue MEL-14 is in the transmembrane (TM) and cytoplasmic regions; these regions have no significant homology with the corresponding regions *of the other selectins, ELAM-1 and PADGEM.* LAM-1 expressed by transfected K562 cells is shed in response to both TNF and PMA. Replacement of the TM and cytoplasmic regions of LAM-1 with the corresponding regions of PADGEM prevents shedding in response to both TNF and PMA. Interestingly, cell surface expression of this construct is increased in response to PMA, paralleling the normal expression pattern of PADGEM. Deletion of the cytoplasmic tail of LAM-1 also prevents shedding in response to TNF, but shedding in response to PMA is unaffected. These data *indicate that cytokines and phorbol esters induce LAM-1* shedding via different pathways, and suggest that PMA directly activates the exoprotease(s) responsible for shedding. In addition, these data suggest that cell surface expression of the selectins is regulated by the TM and cytoplasmic regions of these adhesion receptors.

CA 306 THE β_4 SUBUNIT OF INTEGRIN IS EXPRESSED ON SUBSETS OF ENDOTHELIA IN MICE, Stephen J. Kennel, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 3783l

More than 15 subunits of the integrin family of cell surface adhesion molecules have been identified. The MoAb 346-11A binds to the β_4 subunit in mice. Sequence analysis of a cDNA clone coding for this epitope localizes reaction of the MoAb to a portion about half way through the extracellular domain at the beginning of the cysteine rich region. Sites of β_4 expression in mice were detected by autoradiographic analysis of tissues collected from mice 24 or 48 h after iv injection of ^{125}I MoAb 346-11A. This non-quantitative technique emphasizes detection of antigen exposed in the vascular space. These data show that in addition to expression in epithelia of several organs, the endothelia of intermediate vessels throughout the body are sites of β_4 expression. In particular endothelium of larger vessels in lung, but not capillaries, vessels in thymus, spleen, and Peyers patches, and portal vessels of the liver, but not the central veins are positive. Recent evidence indicates that the $\alpha_6\beta_4$ integrin is involved in hemidesmisome mediated attachment of stratified squamous epithelia to basement membrane. The fact that the β_4 subunit is expressed on certain endothelia which do not have detectible hemidesmisones may indicate a different specialized function at these sites. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.

CA 307 INCREASED LEVELS OF CELLULAR GLUTATHIONE IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS, Laura E. Lantry, Susan R. Mallery, Ralph E. Stephens, Departments of Pathology and Oral Pathology, The Ohio State University College of Medicine, Columbus, OH 43210.

Cellular glutathione (GSH) is the primary intracellular free thiol, and plays an important role in cytoprotection and the maintenance of protein thiol status. We have shown that GSH is an integral intracellular component for cell cycle progression from G1 to S, which coincides with a marked increase in activity of two cellular thiol dependent enzymes, ribonucleotide reductase and DNA polymerase α. The MVE's of the chorio-capillaris of the eye were chosen due to their unique position in the vascular system. Cellular levels of GSH were determined by the method of Eyer and Podhransky. This method is a dual beam, kinetic spectrophotometric assay conducted at 412 nm vs. 550 nm. This system was selected because it is not affected by any endogenous intra-sample glutathione reductase inhibitors. Sample levels of GSH were determined versus a ten point standard curve, at the range of 0.2 nM - 2.0 nM of glutathione per ml, which was conducted concurrently. The amount of GSH was determined by the Lowry method, reported as nmol/mg protein. Our results show that log phase MVE's possess nearly 2 fold greater levels of GSH (60.8 ± 3.6 nmol/mg protein, $n=6$), than log phase fibroblasts or keratinocyte cultures (32.1 ± 15.1, $n=25$; 33.0 ± 6.2, $n=10$, respectively). The microvasculature is the primary site for cellular adhesions and vascular tissue exchange, potentially exposing the MVE's to many cytotoxic species, e.g. reactive oxygen species (ROS). Therefore, an increase in GSH levels may reflect an adaptation of the MVE's to their unique location and function, allowing rapid response to circulating stimuli.

CA 308 PLASMINOGEN ACTIVATION AND ASTROGLIAL-INDUCED NEURAL MICROVESSEL MORPHOGENESIS; Laterra J., Indurti R.R. and Goldstein, G.W.; The Kennedy Research Institute and The Johns Hopkins Medical School, Balt. MD 21205.

Astrocytes ensheathe central nervous system capillaries and regulate endothelial differentiation. In addition, neonatal rat astrocytes and astroglial cells of the C6 line induce CNS microvessel endothelial cells to organize into capillary-like structures in vitro. These structures resemble microvessels in size, shape, associated extracellular matrix and presence of central lumens. We examined the functional relationship between plasminogen activator (PA) activity and astroglial-induced microvessel morphogenesis in vitro. Microvessel morphogenesis was quantitated by computer-assisted image analysis and plasminogen activation determined by chromogenic assay. Culture supernatants of endothelial cells grown alone in a cobblestone monolayer contained 2.9 units/ml of net PA activity. PA activity was undetectable in C6 astroglial culture supernatants or in supernatants of endothelial-astroglial cocultures following capillary-like structure formation. In contrast, supernatants from C6 cultures contained 1.6 U/ml net PA inhibitory activity and endothelial-C6 cocultures undergoing capillary-like structure formation contained 5.0 U/ml net PA inhibitory activity. The serine protease inhibitor, aprotinin (1000 U/ml), increased astroglial-induced microvessel morphogenesis by 98 $\pm$ 40 percent. These findings demonstrate that inhibition of plasminogen activation occurs with astroglial-endothelial interactions and may modulate astroglial-induced endothelial cell differentiation.

CA 309 HMEC-1: ESTABLISHMENT OF AN IMMORTALIZED HUMAN MICROVASCULAR ENDOTHELIAL CELL LINE, Thomas J. Lawley, Robert A. Swerlick, Francisco J. Candal, and Edwin W. Ades, Department of Dermatology, Emory University School of Medicine, and the Centers for Disease Control, Atlanta, GA 30322.

The study of human microvascular endothelial cells has been limited, because these cells are difficult to isolate in pure culture, are fastidious in their in vitro growth requirements, and have a very limited lifespan. In order to overcome these difficulties, we have transfected human dermal microvascular endothelial cells (HDMEC) with a PBR-322-based plasmid containing the coding region for the simian virus 40 A gene product, large T antigen, and immortalized them. These cells, termed CDC/EU.HMEC-1 (HMEC-1), have been passaged 56 times to date and show no signs of senescence, whereas normal HDMEC undergo senescence at passages 8-10. HMEC-1 exhibit typical cobblestone morphology, express and secrete von Willebrand's Factor, take up acetylated low-density lipoprotein, and rapidly form tubes when cultured on matrigel. HMEC-1 grow to densities three to seven times higher than HDMEC and require much less stringent growth medium. HMEC-1 will grow in the absence of human serum, while HDMEC require culture medium supplemented with 30% human serum. These cells express cell-surface molecules typically associated with microvascular endothelial cells, including CD36, CD31, and epitopes identified by EN4, PAL-E, and H3/5-47 mAb. As with HDMEC, HMEC-1 have high baseline expression of ICAM-1, which is upregulated by stimulation with TNF-α. They do not constitutively express ELAM-1 or VCAM-1 but can be induced to express both by TNF-α. Furthermore, stimulation with IFN-γ induces expression of MHC class II and increased expression of MHC class I. HMEC-1 specifically bind lymphocytes in cell adhesion assays. Thus HMEC-1 is the first immortalized human microvascular endothelial cell line that retains the morphologic, phenotypic, and functional characteristics of normal human microvascular endothelial cells.

CA 310 VASCULOGENESIS IN THE DEVELOPING RAT ADRENAL MEDULLA: POSSIBLE INDUCTION BY HETEROTYPIC CELL-CELL CONTACTS BETWEEN PARENCHYMAL CELLS AND ENDOTHELIAL CELL PRECURSORS, Peter I. Lelkes and Brian R. Unsworth#, University of Wisconsin Medical School, Milwaukee Clinical Campus, # Marquette University, Milwaukee, WI.

The organspecific differentiation of endothelial cells (ECs) is poorly understood. It has been suggested that EC heterogeneity is caused by both genetic and epigenetic factors, such as cues derived from the parenchymal cells. Based on our in vitro findings of organspecific, inductive interactions between adrenal medullary endothelial and parenchymal cells, we have proposed that similar differentiative signals occur during organogenesis in vivo. To test this hypothesis, we are reexamining the ultrastructure of the embryonic and neonatal adrenal medulla from the vantage point of vascular development. While the main blood vessels are well developed at E15, up to postnatal D5 nests of chromaffin cells (CCs) were found in avascular spaces, lacking (mature) fenestrated capillaries. Concomitantly, heterotypic cell-contacts, including gap junctions, were observed between CCs and "non-distinct" mesenchymal cells. The latter cells were found to contain some EC-specific markers, suggesting that they may represent local mesenchymal EC precursors. Upon neonatal maturation, some of these "mesenchymal cells", still in close contact with CCs, were found to extend EC-like processes and to differentiate into fenestrated capillaries. Until D10, individual CCs were found in close contact with fenestrated ECs, without an intervening basement membrane, which was established only during later stages of neonatal development. Our results suggest, that in the adrenal medulla heterotypic cell contacts with parenchymal cells might induce vasculogenesis, i.e. the organspecific, in situ differentiation of ECs from precursor cells. We hypothesize that inductive, heterotypic interactions between parenchymal and endothelial cells might be a common mechanism of vascular development in other endocrine organs as well.

CA 311 THE ROLE OF ENDOGLIN IN ENDOTHELIAL CELLS, Michelle Letarte and Anne Gougos, Division of Immunology and Cancer Research, Hospital for Sick Children and Dept of Immunology, University of Toronto, Toronto, Canada, M5G 1X8

Endoglin is a major glycoprotein of human endothelial cells, a dimer of Mr=170,000. The sequence deduced from that of a cDNA revealed a novel type I integral membrane protein with an accessible RGD tripeptide. We postulated that endoglin might be implicated in RGD mediated adhesion events. Adhesion of U-937 cells, labelled with the fluorescent dye BCECF, to monolayers of endothelial cells derived from human umbilical vein (EC) was monitored using the FCA multiwell fluorimeter. Pretreatment of EC with antibodies to endoglin stimulated by 5-20-fold the adhesion of U-937 cells in a dose-dependent fashion and with maximum IgG concentrations of 20 ug/ml. The increase in adhesion was not observed with F(ab')2-fragments and was inhibited by antibodies to the appropriate Fc receptor I or II, both present on U-937 cells. The increased adhesion was inhibited in the presence of RGD but not RGE peptides and by cytochalasin B. The effects of antibodies directed at other adhesion molecules of EC cells were tested in the same system. Although VLA-1 to VLA-6, LFA-3 and I-CAM are expressed on EC cells, only anti-VLA-5 and anti-β1 integrin were capable of stimulating adhesion. Since integrins such as VLA-2 and VLA-3 are structurally related to VLA-5 and are present at similarly high levels on EC cells, cross-linking via IgG is not sufficient to explain the increased adhesion observed and the inhibition by RGD. The binding of anti-endoglin antibodies might alter the conformation of the molecule and facilitate the binding to an endoglin ligand via RGD. Future studies should address these questions and establish if endoglin is involved in the regulation of adhesion. The role of endoglin in the proliferation of EC, in response to growth factors is also being investigated; several extracellular matrix components are known to interact with growth factors which in turn can regulate the production of matrix proteins by endothelial cells.

CA 312 ELAM-1 IS CONTINUOUSLY SYNTHESIZED IN HUMAN ENDOTHELIAL CELLS (HUVEC) AT LATE -PHASE (24-HOURS) AFTER IL-1 STIMULATION, J.J. Li, D. Ayer, C. Power, C. Siegfried, J-Y. Bonnefoy and J-J. Mermod, Glaxo Institute for Molecular Biology, Chemin des Aulx, 1228 Plan-Les-Ouates, Geneva, Switzerland

Although in vitro experiments suggest that endothelial leukocyte adhesion molecule (ELAM-1) disappears quickly from cytokine-stimulated HUVECs, this finding can not explain why ELAM-1 is detected at 72-hours during an antigen-specific delayed type hyper-sensitivity in human skin. To resolve this discrepancy, we used immunoperoxidase staining on cultured HUVECs and observed that ELAM-1 was present strongly on cultured HUVECs up to 36-hours after IL-1 stimulation. In order to confirm this observation, pulse-chase labelling and autoradiography were used to reexamine ELAM-1 synthesis in activated-HUVECs. Our results confirms that ELAM-1 induced within 4 hours of IL-1 exposure indeed disappeared by 21 hours. However, new ELAM-1 was continuously being resynthesized and the intensity of the 35SMet/Cys labelling suggests that a significant amount of ELAM-1 was synthesized at 24 hours after IL-1 exposure. To further correlate the expression of ELAM-1 to its functional status, an assay of HL-60 cell adhesion to HUVECs was used. We consistently showed that the number of HL-60 bound to HUVECs 24-hours after IL-1 exposure was 55-65% of that of the 4-hours IL-1 stimulated HUVECs. Futhermore, a mAb which recognizes the lectin/EGF domain (13A) blocks HL-60 binding to HUVECs by 58% suggesting that HUVECs retain a significant capacity to bind specifically to HL-60 cells at 24-hours after IL-1 stimulation. Most interestingly, we show that the complement regulatory domain of ELAM-1 seems to play some role in HL-60 adhesion, since a mAb recognizing the CR domain (13P) can block HL-60 binding to HUVECs but with different kinetics from that of 13A. In summary, our results suggest that ELAM-1 may continuously serve as an important adhesion site for inflammatory cells even at the late-phase after IL-1 stimulation.

CA 313 DOWNREGULATION OF THE LIGAND FOR THE PERIPHERAL HOMING RECEPTOR ON HIGH ENDOTHELIAL CELLS, Reina E. Mebius*, Philip R. Streeter#, Susan R. Watson@, Irving L. Weissman*, and G. Kraal¥. *Howard Hughes Medical Institute, Stanford University Medical Center, Stanford, CA 94305, #SyStemix, Palo Alto, CA 94303, @Genentech, South San Francisco, CA 94080, ¥Dept. of Cell Biology, Vrije Universiteit, Amsterdam, the Netherlands.

Recirculating lymphocytes in the bloodstream can migrate into lymphoid organs by adhering to specialized high endothelial venules (HEV). The interaction of lymphocytes with the wall of HEV is governed by specific lymphocyte surface receptors, called homing receptors (HR), and adhesion molecules on the HEV, called vascular addressins. In mouse the endothelial ligand for the peripheral HR is a ~50-kD sulfated, fucosylated, and sialylated glycoprotein and can be recognized by a soluble homing receptor-IgG chimaera (LEC-IgG). Additionally, the peripheral vascular addressin, involved in directing lymphocyte homing to peripheral lymph nodes, is defined by mAb MECA-79. It has been suggested that mAb MECA-79 recognizes the ligand for the peripheral lymph node HR

It is not known which factors are regulating the expression of specialized adhesion molecules on HEV, although interruption of afferent lymphatic vessels results in a dramatic decrease of the capacity of HEV to bind lymphocytes with a concomitant flattening of the HEV. We studied the expression of the peripheral HR ligand on high endothelial cells and the expression of the peripheral addressin on these cells after interruption of afferent lymphatics. One week after occlusion the HEV became flat walled and the ligand for the HR disappeared completely by both immunohistochemistry and the inability to immunoprecipitate a radioactive sulphated ligand with LEC-IgG. Staining with mAb MECA-79 showed expression of the vascular addressin only on the ablumenal side of the venules, suggesting that MECA-79 recognizes more than only the ligand for the HR. Functional in vivo homing studies revealed that these vessels support minimal lymphocyte traffic from the blood. These data show that functioning afferent lymphatics are centrally involved in maintaining the continuous expression of the endothelial ligand for the peripheral lymph node homing receptor.

CA 314 REGULATION OF ENDOTHELIAL CELL SURFACE ADHESION MOLECULE GENE EXPRESSION BY SYNTHETIC AND VIRAL DOUBLE STRANDED RNA Margaret K. Offermann*#, M. Kelly Hagan# and Russell M. Medford+ *Winship Cancer Center, +Division of Cardiology, # Division of Hematology and Oncology, Department of Medicine, Emory University, Atlanta, GA. 30322

Dysfunction of vascular endothelial cells has been implicated in a number of diseases. One of the earliest features of atherogenesis is the increased adhesion of inflammatory cells, such as monocytes, to vascular endothelial cells. Cell surface adhesion molecules, such as ELAM-1, ICAM-1 and VCAM-1, have been shown to play a role in inflammatory cell-endothelial cell adhesion and interaction. We have tested the hypothesis that an exogenous agent, such as a virus, may be an etiologic factor in atherogenesis leading to abnormal expression of endothelial cell surface adhesion molecules. Poly(I:C), a synthetic double stranded RNA, was used to induce endogenous α/β-interferon in human vascular endothelial cells (HUVEC). As expected, the expressions of interferon-sensitive major histocompatibility class I and 2'5' oligoadenylate synthetase genes were significantly induced. Surprisingly, poly(I:C) at either 1 or 100ug/ml elicited a dramatic induction of the expression of mRNAs encoding the interferon-insensitive cell surface adhesion molecules ICAM-1, VCAM-1 and ELAM-1. A similar pattern was observed using Sendai virus. Both in magnitude and duration, the poly(I:C) induction was at least several fold greater than that observed with interleukin-1 (IL-1, 100 U/ml). Analogous to IL-1, poly(I:C) induction of ELAM-1 was not blocked by the protein synthesis inhibitor, cycloheximide. In contrast, VCAM-1 mRNA induction was partially blocked. Although poly(I:C) and Sendai virus are known potent inducers of α/β-interferon, it is clear from these studies that their effects on endothelial cells are not limited to α/β-interferon regulated functions. Indeed, poly(I:C) and Sendai virus are much more effective inducers of HUVEC cell surface adhesion molecule gene expression than saturating amounts of IL-1. The magnitude and duration of induction of these cell surface adhesion molecules by either poly(I:C) or Sendai virus suggest that viral infection could play an important role in the initiation and maintenance of the vascular endothelial cell dysfunction observed in the earliest stages of atherogenesis.

CA 315 ENDOTHELIAL ADHESION MOLECULES INVOLVED IN MONOCYTE EXTRAVASATION ARE REGULATED VIA SCAVENGER RECEPTOR. Tessa Palkama, Pirkko Mattila, Marja-Leena Majuri, Mikko Hurme and Risto Renkonen. Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland.

Monocyte adherence to the endothelium, their penetration to the subendothelial space and excessive lipid accumulation (foam cell formation) are the initial events in atherogenesis. Scavenger receptors have been reported to play an important role in foam cell formation, since modified LDL can be taken up via scavenger receptors in a non downregulated fashion. In this study we demonstrate that stimulation of scavenger receptors in endotelial cells induces the expression of endothelial adhesion molecules involved in monocyte extravasation. Polyinosinic acid (poly I), a known scavenger receptor ligand, induced the mRNA and protein expression of intercellular adhesion molecule-1 (ICAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule (VCAM) on human endothelial cell line EA.hy 926 and human umbilical vein endothelium. Polycytidylic acid (poly C), a structurally related compound to poly I, which does not bind to the scavenger receptor, was used as a negative control, and had no effect on adhesion molecule expression. Scavenger receptor mediated adhesion molecule expression could be inhibited by H7, a protein kinase C (PKC) inhibitor, while HA 1004, a preferential protein kinase A inhibitor had no effect on adhesion molecule expression. The role of PKC in scavenger receptor mediated adhesion molecule upregulation was further confirmed by the ability of poly I to activate PKC, when measured with [^{3}H]phorbol dibutyrate binding, while poly C again was ineffective.

CA 316 TNFalpha DOWNREGULATES THE EXPRESSION OF THE INTEGRIN ALPHA6/BETA1 ON HUMAN ENDOTHELIAL CELLS. Paola Defilippi, Paola Rossino, Lorenzo Silengo and Guido Tarone, Department of Genetics, Biology and Medical Chemistry, University of Torino, 10126 Torino, Italy.

Cell surface receptors for the extracellular matrix (integrins) mediate the interaction between the endothelial cells and the basal membrane. Endothelial cells explanted from human umbilical vein (HEC) express a large panel of integrins on their surface, including receptors for fibronectin, laminin, collagens and vitronectin. In this paper we show that the treatment with the tumor necrosis factor alpha, TNFalpha, downregulates the expression of the laminin receptor alpha6/beta1 integrin in cultured HEC. The treatment with TNFalpha for more than 15 hours decreases the level of expression of the alpha6/beta1 complex at the cell surface at the 20% of the control value. The downregulation of the alpha6/beta1 integrin is due to a decreased expression of the alpha6 subunit, while the beta1 integrin subunit remains constant. The rate of synthesis of the alpha6 subunit is specifically decreased in treated cells. Northern blot analysis shows that the decreased level of synthesis of alpha6 subunit is due to the downregulation of alpha6 mRNA in TNFalpha treated HEC. We also found that in addition to TNFalpha, interleukin 1 beta (IL-1beta) is able to decrease the expression of the alpha6/beta1 integrin on HEC. We have previously shown that treatment of umbilical vein endothelial cells with TNFalpha induces the expression of the integrin alpha1/beta1 (Defilippi et al., J.Cell Biol., 114, 855-863, 1991), and that a combined treatment with TNFalpha and IFNgamma downregulates the expression of the vitronectin receptor alphav/beta3 (Defilippi et al., J.Biol.Chem., 266, 7683-7645, 1991) These data indicate that TNFalpha regulates the expression of specific integrin complexes on the surface of endothelial cells. This event may play an important role during inflammatory processes.

CA 317 ENDOTHELIAL CELL ADHESION MOLECULES INVOLVED IN THE INCREASED NK CELL ADHESION TO HUMAN ENDOTHELIAL CELLS STIMULATED WITH SALMONELLA BACTERIA, Mari Pinola and Eero Saksela, Dept. of Pathology, Univ. of Helsinki, Finland

Previously we have shown that pretreatment of human umbilical vein endothelial cells (EC) with Salmonella bacteria leads to augmented adhesion of human NK cells to the endothelium. In this study we analysed the roles of three known EC adhesion molecules VCAM-1, ICAM-1 and ELAM-1, in the Salmonella Minnesota mR595 stimulated increase of the NK adhesion to EC using monoclonal antibodies against these structures.

On Salmonella -stimulated EC, VCAM-1 and ICAM-1 were the major binding sites involved in the adhesion, and their effect was additive in mAb inhibition experiments. We could demonstrate the induction of both structures on cultured EC after 24 hr Salmonella-stimulation by fluorescence microscopy. Also in Facscan analysis the intensity of ICAM-1 expression increased by Salmonella pretreatment together with the appearance of VCAM-1. The transient peak of ELAM-1 expression was already over after 24 hrs, at the time of our adhesion experiments, and thus anti-ELAM-1 antibody had no effect.

Leukocyte - endothelial cell adhesion and its regulation are essential and complex initial aspects of lymphocyte migration. Stimulation of endothelium with whole Salmonella bacteria may thus be important in providing anchorage sites for the increased migration of NK cells towards the site of inflammation.

CA 318 GROWTH REGULATION OF NORMAL AND TRANSFORMED ENDOTHELIAL CELLS. A. RayChaudhury and P.A. D'Amore, Laboratory for Surgical Research, Children's Hospital & Harvard Medical School, Boston, MA 02115

We are investigating the mechanisms of endothelial growth control by focusing on the effects of various growth effectors on normal cultured capillary endothelial cells (EC) and transformed EC expressing the polyoma middle T oncogene (BENDO cell line; from W. Risau, Munich, Germany). BENDO cells have an indefinite life span, a much higher proliferation rate than normal capillary EC and, in contrast to the latter, are not appreciably growth-stimulated by fibroblast growth factor. We examined the levels of mRNA for the extracellular matrix protein thrombospondin (TSP), which is known to be an inhibitor of neovascularization and has also been reported to inhibit EC growth. (Our preliminary studies confirm the ability of TSP at concentrations > 200 ng/ml to partially inhibit EC growth). Both rapidly growing and slow-growing (i.e., serum-deprived) normal capillary EC produce significant levels of TSP mRNA. In contrast, BENDO cells express virtually no TSP mRNA. Furthermore, normal capillary EC but not BENDO cells are strongly growth-inhibited by transforming growth factor-beta (TGF-ß), and TGF-ß increases total extracellular matrix proteins about two-folds in normal cultured capillary EC. However both normal and transformed EC show an increase in TSP mRNA levels on treatment (3-6 hr) with TGF-ß, indicating that at least some TGF-ß receptors and intracellular pathways that mediate TGF-ß effects are functional in BENDO cells. These results point to the involvement of TSP in EC growth regulation and also offer evidence to support the concept that TGF-ß acts, at least in part, by altering the extracellular matrix proteins qualitatively and/or quantitatively.

CA 319 **Lymphocyte traffic into rejecting cardiac allograft is CD11a- and CD49d- dependent,** Risto Renkonen, Juha Pekka Turunen and Pirkko Mattila, Department of Bacteriology and Immunology and Transplantation Laboratory, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland.

Acute cardiac allograft rejection is characterized by infiltration of leukocytes into tissue parenchyma. Here we demonstrate the site of entry of inflammatory white cells into a rejecting heart allograft and define the adhesion ligands involved. Lymphocyte binding to frozen sections prepared from day 3 rejecting cardiac allografts was significantly increased compared to sections made from normal hearts (number of bound lymphocytes, 914 ± 483 per mm^2 vs. 309 ± 144, respectively, $p<0.05$) or syngeneic grafts. The bound lymphocytes were located exclusively only on the top of the capillary structures, and not on any other sites on the heart vasculature. We further wanted to analyse, which of the cloned endothelial adhesion molecules and their counter-receptors would be involved in the increased lymphocyte binding. Lymphocyte pretreatment with mAb anti- CD11a or anti-CD49d inhibited this binding more than 50 %. This inhibition on lymphocyte binding could not be increased by combining these two antibodies. Lymphocyte binding to endothelium has been shown to been atleast partly organ-specific. Therefore we asked whether increased lymphocytes adhere to cardiac allografts is organ-specific. To answer to this question we carried out monosaccharide inhibition studies and stained the cardiac allografts with a monoclonal anti-rat peripheral lymph node antibody. Lymphocyte binding to lymph node high endothelial venules (HEV) has been shown to be inhibited by mannose-6-phosphate (M6P) and to kidney peritubular capillaries by mannose-1-phosphate (M1P). In the present study neither of these carbohydrates had any effect. This suggests an organ-specific homing into heart allografts. Taken together we demonstrate here an significant increase in the number of lymphocytes adhering to cardiac allografts compared to syngeneic grafts or normal heart controls. The bound lymphocytes are on the capillary endothelium and not on any other locations. This increased binding can be partly increased by anti-CD11a and anti-CD49d antibodies suggesting that ICAM-molecules and VCAM-1 are essential in lymphcoyte entry to allograft. Monosaccharide inhibition studies demostrate that the mechanism of lymphocyte adhesion to cardiac capillary endothelium differs from tadhesion to kidney allografts or peripheral lymph node high endothelium.

CA 320 REGULATION OF VCAM-1 ON HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS. Robert A. Swerlick, Kwang H. Lee, Thomas J. Lawley, Department of Dermatology, Emory University, Atlanta, GA 30322

Immunopathologic studies have suggested significant variability in VCAM-1 induction on endothelial cells (EC) in different vascular beds in vivo. We therefore examined the regulation of expression of VCAM-1 on microvascular EC derived from human skin (HDMEC) and compared it to large vessel umbilical vein EC (HUVEC) cultured under identical conditions. VCAM-1 was not found on unstimulated cells, but was induced on both HDMEC and HUVEC by TNF alpha in a dose and time dependent manner. TNF induced maximal expression on HUVEC after 6 hours which persisted up to 72 hours while maximal VCAM-1 expression on HDMEC was seen after 16 hours and fell to less than half maximal levels by 72 hours. Interestingly, stimulation of HDMEC with IL-1 alpha at doses as high as 1000 u/ml failed to induce significant VCAM-1 on HDMEC. However, IL-1 stimulation of HUVEC also resulted in a dose and time dependent induction of VCAM-1. Responsiveness of HDMEC to IL-1 was shown by induction of ELAM-1 expression and increases of ICAM-1 after stimulation with IL-1. In addition, while TNF stimulation of HDMEC resulted in maximal expression of VCAM-1 mRNA after 16 hours and loss of mRNA after 48 hours, TNF stimulation of HUVEC resulted in substantially greater amounts of VCAM-1 mRNA which clearly persisted up to 48 hours. Functional experiments correlated with cell surface expression and mRNA expression in that VCAM-1 dependent binding of Ramos cells was detected only to TNF stimulated HDMEC and not to IL-1 stimulated HDMEC. This data demonstrates that VCAM-1 is inducible on skin microvascular endothelial cells in vitro, but the regulation of induction is distinct from large vessel endothelial cells.

CA 321 CARBOHYDRATE SPECIFICITY OF THE SELECTINS, R.Aaron Warnock, Ellen L. Berg and Eugene C. Butcher, Department of Pathology, L-235, Stanford University, Stanford, CA 94305, and the Center for Molecular Medicine, Veterans Administration Medical Center, Palo Alto, CA 94304.

ELAM-1(E-selectin), GMP140(P-selectin) and LECAM-1(L-selectin) are structurally related calcium dependent mammalian lectins that comprise the selectin or LEC-CAM gene family. Each member contains three extracellular domains consisting of an N-terminal domain homologous to other mammalian C-type lectins, an epidermal growth factor-like domain, and a variable number of repeat sequences homologous to complement regulatory or complement binding proteins, followed by a transmembrane domain and a short cytoplasmic tail. We have compared the specificity of the selectin family members for binding to carbohydrate structures by measuring the ability of mouse L1-2 pre-B cells stably transfected with cDNAs for E-selectin, P-selectin, and L-selectin to bind to a variety of sugar substrates. These substrates include neoglycoconjugates containing oligosaccharides covalently bound to human serum albumin, as well as isolated native ligands. Included among the native ligands tested is the peripheral lymph node addressin, a high endothelial venule ligand for L-selectin, as well as the cutaneous lymphocyte antigen, CLA, defined by the HECA 452 antibody. The results suggest that the selectins can display greater specificity for their natural ligands than for proposed oligosaccharides.(Supported by grants from the N.I.H. and the Veterans Association).

CA 322 IN VITRO CHARACTERISATION OF LEC-CAM-IgG CHIMAERAS, Susan R.Watson, Christopher Fennie, Donald J. Dowbenko, *Brian Brandley and Laurence A. Lasky, Department of Immunobiology, Genentech, Inc., S. San Francisco, CA 94080 and *Glycomed, Department of Cell Biology, Alameda, CA 94501

The LEC-CAM family of molecules, ELAM, GMP140 and the peripheral lymph node homing receptor(PLN-HR), have been shown to have a similiar structure. The N terminal lectin domain is linked to an EGF domain that is followed by a variable number of complement-like binding domains and the ligand(s) for these molecules has been shown to be carbohydrate in nature. In order to study these molecules we have made chimaeras of each of the receptors by linking them to the Fc portion of human IgG1 and have expressed them at high levels in a eukaryotic system. These reagents were initially characterised in an ELISA assay using antibodies specific for each family member. Each chimaera was able to block in specific assays. Thus the PNL-HR construct was able to block the binding of lymphocytes to high endothelial venules(HEV) in a frozen section assay, GMP140-IgG chimaera was able to block thrombin-activated platelets binding to HL60 cells and the ELAM-IgG chimaera was able to block neutrophil binding to cytokine activated HUVEC. These chimaeras are being used to investigate the interactions of these receptors with their carbohydrate ligand(s) both in ELISA and cell-based assays.

Angiogenesis; Wound Repair and Disease

CA 400 b-FGF in-vivo ANGIOGENIC POTENTIAL AND THE EFFECT OF THROMBOSPONDIN, David BenEzra, Brenda Griffin and Genia Maftzir, Department of Ophthalmology, Hadassah University Hospital, Jerusalem, Israel

In 1978, we demonstrated that bovine FGF induced a mild angiogenic stimulus in the rabbit cornea and postulated that the induction of this process was associated with the proliferation of the corneal epithelium (Am J Ophthalmol 86:455, 1978). More recently, using human recombinant b-FGF and performing the experiments in a well-controlled double-masked system, we observed the following:

1) Positive in vivo angiogenesis is recorded in all experiments with stimulating concentrations of 250 ng of b-FGF or more.

2) The earliest signs of angiogenesis are clinically observed 48 to 72 hours after initiation of the stimulus. The new vessels reach a maximal length 4 to 6 days later, involuting rapidly thereafter.

3) The intensity and extent of angiogenesis is closely associated with increased concentrations of b-FGF. However, the kinetics of the new blood vessel growth remain constant at all concentrations.

4) Histologically, corneal epithelial proliferation and an increase in the number of "active" keratocytes are observed before the formation of new blood vessels.

5) Corneal cell infiltrates are not observed even at high concentrations of b-FGF. Various degrees of leukocytes infiltration are, however, evident histologically.

6) Human TSP, although by itself not angiogenic, increased the angiogenic potential of low concentrations of b-FGF. Furthermore, when both TSP and FGF are present, clinical leukocyte infiltrates are observed within the stimulated corneas. These results are somewhat contradictory to data reported by others. Possible explanations for these discrepancies will be offered.

CA 401 LINING OF VIABLE AND DEVITALIZED HUMAN AND PORCINE VASCULAR WALL WITH CULTURED HUMAN ADULT VENOUS ENDOTHELIUM. L. Bengtsson, B. Ragnarsson, A. Haegerstrand. Depts. of Anatomy and Cardiovascular Surgery, Karolinska Institute, Stockholm Sweden.

With the aim to create a confluent endothelial lining using cultured adult human venous endothelial cells (AHVEC) on viable and nonviable human and porcine vessels, we have performed high density (10^5 cells/cm^2) seeding on mechanically deendothelialized pieces of viable or devitalized human saphenous vein (HSV) and porcine aorta (PA). The purpose was to investigate the possibility to use autologous endothelial cells to improwe the performance of cardiovascular bioprosthesis

Cell culture was performed in MEM with addition of pooled human serum, cholera toxin, isobutylmethylxantine and antibiotics and cells were used for seeding after 6-7 passages.

After deendothelialization by scraping, one piece of HSV and PA respectively were kept in Ham's F12 with addition of 10% newborn calf serum at 4°C for 3 days and for devitalization, another two specimens kept in deionized water for 3 days. After trypsination, cell seeding was performed and the seeded specimens were kept for seven days in cell culture medium to allow adhesion and spreading.

Morphological evaluation was performed by scanning and transmission electron microscopy, by immuno-histochemistry of von Willebrand factor, laminin and collagen IV as well as by hematoxylin-eosin staining. The origin of endothelial cells as derived from cultures was verified by vital staining during culture by growth in carbocyanine dye-containing medium, according to a recently described method.

The results showed that after complete deendothelialization, a confluent lining of cultured AHVSCs can be obtained on both viable and nonviable human and porcine vascular tissue. Both SEM and TEM revealed an endothelium with similar appearance as their native counterparts. The AHVECs retained positive staining for von Willebrand factor. Laminin and collagen IV immunoreactivity was also closely related to the endothelium, corresponding to a basal membrane.

We suggest that cultured autologous endothelium may be seeded on donor tissue to create a less thrombogenic and possibly also less antigenic luminal surface on vascular bioprosthesis.

CA 402 SPARC INHIBITS DNA SYNTHESIS IN CULTURED ENDOTHELIAL CELLS. Sarah E. Funk, Timothy F. Lane, and E. Helene Sage, Dept. of Biological Structure, University of Washington, Seattle, WA 98195.

Sparc (secreted protein, acidic and rich in cysteine) is a Ca^{++}-binding glycoprotein which is produced by subconfluent cells in culture and by proliferating, migrating, and/or differentiating cells *in vivo*. Both Sparc and a 20-amino acid synthetic peptide (peptide 2.1) from a non-Ca^{++}-binding, cysteine-rich region of Sparc have been shown to inhibit the incorporation of [^{3}H]thymidine and delay the onset of S-phase in synchronized cultures of bovine aortic endothelial cells (BAEC) after release from quiescence. In this study we investigate the conditions under which this modulation occurs in different cell types. The degree of suppression of DNA synthesis is more pronounced in the absence of fetal calf serum and has been shown to be unrelated to contamination by endotoxin. Human umbilical vein endothelial (HUVE) cells, transformed fetal bovine aortic endothelial cells (AGO-7373), and bovine capillary endothelial cells all exhibit a sensitivity to Sparc and peptide 2.1 similar to that seen with BAEC. The growth of human foreskin fibroblasts and fetal bovine nuchal ligament fibroblasts, however, was not suppressed by Sparc and exhibited increased incorporation of [^{3}H]thymidine in the presence of 0.4 mM peptide 2.1. A synthetic peptide from another region of Sparc (peptide 2.3) contains a sequence, Gly-His-Lys, which has been shown to stimulate growth in some cell types. This peptide increased [^{3}H]thymidine uptake in BAEC in a dose-dependent manner at concentrations between 1-80 µg/ml. Higher doses inhibited cell attachment. That certain domains of the Sparc molecule have growth-stimulatory or inhibitory properties with respect to specific cell types may be critical to understanding the role of Sparc in the modulation of cell proliferation during wound repair and remodeling.

CA 403 A SMALL SEGMENT OF THE GREAT SAPHENOUS VEIN AS A SOURCE OF 20 MILLION ENDOTHELIAL CELLS. C.Gillis, L. Bengtsson, A. Haegerstrand. Depts. of Anatomy and Cardiovascular Surgery, Karolinska Institute, Stockholm,Sweden.

Growth properties of endothelial cells derived from adult human great sapheneous vein (HSVECs) was investigated for studies on adult human ECs and endothelialization of cardio-vascular prosthetic graft materials. HSVECs were isolated by collagenase treament of residual segments (3-5 cm) of the great saphenous vein from patients undergoing coronary by-pass-surgery. HSVECs were cultured in gelatin-coated wells and stimulatory effects was assayed in 4-7th passage cells by increase in cell number after 4 days of culture in various media. Effects of fetal calf-serum (FCS) and human serum (HS) were compared and effects of compounds that increase intracellular cAMP levels, i.e. cholera toxin (CT) and isobutylmethylxanthine (IBMX) were also examined. HSVECs were inspected daily for morphology and micrographs were taken. HSVECs cultured on gelatin-coated glass cover slips were used for immunohistochemical analysis of presence of vWF-factor.Supernatants from thrombin-stimulated HSVECs at 4th and 7th passage was assayed for presence of 6-keto PGF_{1a} by means of ELISA-technique.

The addition of serum induced a concentration dependent increase in cell number. It was shown that HS was more efficient than FCS in stimulating growth of ECs. Addition of CT increased the cell number when added to medium containing 40 and 50% HS and this effect was further potentiated by IBMX. Maximal increase (2 to 3 times) in cell number was observed in MEM containing 40% HS with the addition of CT and IBMX.

Using this medium, HSVECs could be passaged 6 times (occasionally up to 10 times) without appearant loss of either proliferation rate or cobblestone morphology and 20 million cells could repeatedly be harvested from one segment.The HSVECs also retained expression for vWF. Unstimulated HSVECs at passage 4 and 7 liberated prostacyklin ranging from 4,0-15,8 and 3,0-7,9 ng/10^5 cells, respectively. Cells at both passages responded to stimulation with thrombin (0.5 U/ml) with a mean increase of 298 and 254% compared to unstimulated cells, respectively.

We conclude that by using a small segment of HSV, ECs may be isolated and easily propagated in autologous (if preferred) human serum with the addition of cAMP elevating compounds and that this appears as simple and reliable alternative to previously described techniques for studies on adult human ECs and *in vitro* endothelialization of vascular prosthetic materials.

CA 404 CULTURED ADULT HUMAN ENDOTHELIAL CELLS LINING CIRCULAR ePTFE GRAFTS-METHOD AND IMPLICATIONS. A. Haegerstrand, L. Bengtsson, C.Gillis. Depts. of Anatomy and Cardiovascular Surgery, Karolinska Institute, Stockholm,Sweden.

Endothelialization of prosthetic vascular materials has been shown to increase patency of vascular grafts. Both peroperative seeding/sodding of ECs and *in vitro* preendothelialization have been implied to reduce graft failure. Peroperative techniques have appeared to cause moderate increases in patency in clinical trials. Recently, expanded polytetrafluoroethylene (ePTFE) -grafts treated with a commercially avaliable fibrin glue and cultured endothelial cells have been reported to reduce thrombogenicity in patients. Different approaches have been undertaken to increase adherence of endothelial cells to synthetic materials. In this study we investigated the adherence and short term resistance to *in vitro* flow conditions of cultured adult human saphenous vein endothelial cells (HSVECs) lined on ePTFE grafts.
HSVECs were initially allowed to adhere for 10 + 20 min in a device specially designed for graft-clamping and rotation. Untreated, serumprotein- (sp) or collagen-coated 8 mm ePTFE grafts, with adhered cells, were then placed in culture medium for 2 days and the relative cell number was examined by presence of radioactively labeled cells and by morphological evaluation using scanning electron microscopy (SEM). By SEM, it was shown that HSVECs formed complete monolayers on both sp- and collagen-coated ePTFE grafts, whereas untreated grafts failed to allow significant cell attachment. The loss of adhered cells subjected to plasma flow (500 ml/min, 5 min, BP 180/50), as measured by loss of radioactivity, was 23 and 11% on sp- and collagen-coated grafts respectively. It was shown that intact monolayers were present on both sp- and collagen-coated grafts also after *in vitro* flow conditions.

This technique was designed to enable endothelialization using the patients blood-derived serum as a major source for both adherence of cells to ePTFE-grafts, as well as for growth during the culture period. Provided the cells remain attached for longer periods when exposed to *in vivo* blood flow, sp-coated grafts may be a clinically useful alternative to previously described methods for peroperative or *in vitro* endothelialization of vascular grafts. The use of endothelialized grafts may also be practical in studies on interactions between endothelial cells and other blood cells/elements in *in vitro*-circulation models as well as for function of ECs transfected with genetic material of theoretical and/or clinical interest.

CA 405 DIFFERENTIAL GROWTH INHIBITION OF HUMAN MELANOMA CELL LINES FROM DIFFERENT STAGES OF TUMOUR PROGRESSION BY HUMAN ENDOTHELIAL CELLS, Erik J. Hegmann, Janusz W. Rak and Robert S. Kerbel, Cancer Program, Reichmann Research Building, Sunnybrook Health Science Centre, Toronto, Ontario, Canada M4N 3M5.

Human melanoma cell lines established from primary or metastatic lesions at different stages of tumour progression have been co-cultured with human dermal microvascular endothelial cells or human umbilical vein endothelial cells. Both endothelial cell types exerted an inhibitory effect on melanoma cell proliferation. However, melanoma cell lines representative of early-stage curable disease, e.g. radial growth phase lesions, were more sensitive to growth inhibition than cell lines isolated from more advanced (metastatic) lesions. Endothelial cell conditioned medium was able to reproduce the co-culture effect. The growth inhibitory activity was partially abrogated by treatment with neutralizing monoclonal antibodies against human IL-6 but not by monoclonal antibodies neutralizing human TGF-β_1 and TGF-β_2, IFN-γ, IL-1α or TNF-α. In addition, the proliferation of IL-6 resistant melanoma cell lines was inhibited by 50% at high concentrations of endothelial cell conditioned medium. These findings indicate that IL-6, and other as yet unidentified growth factors may be important mediators of endothelial cell - tumour cell interactions. It is postulated that factors released by endothelial cells can promote selection of more malignant melanoma variants *in vivo*, thus participating in clonal dominance (tumour progression).

CA 406 EFFECTS OF OXYGEN FREE RADICALS ON NEOVASCULARIZATION IN SKIN FLAPS, Michael J. Im, Richard J. Edwards, Lesley Wong, and Paul N. Manson, Division of Plastic Surgery, The Johns Hopkins University School of Medicine, Baltimore, MD 21205
Superoxide radicals are produced by NADPH oxidase in the plasma membrane of activated neutrophils and macrophages during the inflammatory phase of wound healing. It is reported that vascular endothelium in vitro is damaged by toxic oxygen species. The ultimate lethal effect of superoxide is mediated via the hydroxyl radicals (OH·). In this study, the effects of oxygen free radicals on neovascularization in vivo have been evaluated indirectly by utilizing deferoxamine, an iron chelating agent to prevent the production of OH·. An island skin flap, with its sole blood supply via the inferior epigastric vessels, was utilized as a model for the investigation of neovascularization. Island skin flaps, 3 x 3 cm, were elevated in the abdominal/groin area of rats (Sprague-Dawley) and immediately sutured in their donor sites. The vascular pedicle with or without accompanying nerve was ligated on day 3 postoperatively. Flap viability was assessed by planimetry on day 7 following pedicle ligation. Ligation of the vascular pedicle produced 37 ± 5.3% (n=15) and 69 ± 5.8% (7) of flap area survival in the denervated and innervated flaps, respectively. Flap survival reflects the establishment of neovascularization in wounds. Denervation of the flaps was detrimental to neovascularization. Treatment with a single dose (50mg/kg) of deferoxamine increased the survival rate from 37% to 69 ± 6.7% (9) in the denervated flaps. The results suggest that neovascularization (angiogenesis) is inhibited by toxic effect of free radicals on vascular endothelium.

CA 407 TRANSFORMING GROWTH FACTOR-ß PROMOTES PROLIFERATION OF ENDOTHELIAL CELLS THAT EXHIBIT ANGIOGENESIS IN VITRO. M.Luisa Iruela-Arispe and E.Helene Sage, Department of Biological Structure, University of Washington, Seattle, WA, 98195.

Transforming growth factor ß (TGF-ß) has been implicated in the regulation of angiogenesis *in vivo*, although the molecular mechanisms by which this growth factor stimulates the formation of new blood vessels have remained unclear. Several studies have demonstrated that TGF-ß inhibits the proliferation of subconfluent endothelial cells in culture, but there is little information on the proliferative response of endothelial cells during the formation of nascent cords and capillaries. We have examined the effect of TGF-ß1 on endothelial cell cultures exhibiting spontaneous organization of cords and tube-like structures. Under serum-free conditions, treatment of bovine aortic endothelial cells (BAEC) with various doses of TGF-ß promoted a 25-40% increase in the uptake of [^{3}H]-thymidine. Similar results were obtained with rat brain capillary endothelial cells (REC) that were forming cords *in vitro*. However, sparse or confluent cultures of BAEC or REC showed a significant decrease in thymidine uptake after TGF-ß treatment, as previously described. To examine potential effects of subendothelial matrix produced by tube-containing cultures, we grew endothelial cells on substrates of type I collagen. Under these conditions, the reduction in proliferation by TGF-ß was still observed. The expression of type I collagen and fibronectin protein and mRNA was unaffected by TGF-ß in cord-containing cultures. Thus, TGF-ß stimulated endothelial proliferation in cultures that were forming cords and/or tubes and exhibited an inhibitory effect with respect to endothelial cell monolayers. We speculate that TGF-ß can act as a positive stimulator of endothelial cell growth during angiogenesis. However, this stimulatory effect is transient and would not be apparent on endothelial cells in mature blood vessels.

CA 408 ACIDIC FGF-*PSEUDOMONUS* EXOTOXIN (aFGF-PE) CHIMERIC PROTEIN ELICITS ANTI-ANGIOGENIC EFFECTS ON ENDOTHELIAL CELLS. June Merwin, Mark Lynch, Ira Pastan,* Joseph Madri,+ and Clay Siegall, Bristol-Myers Squibb, CT; *NCI, NIH, Bethesda, MD, +Yale, New Haven, CT

It has recently been shown that the aFGF-PE chimeric proteins are cytotoxic to a variety of tumor cell lines and are aFGF-receptor specific. While aFGF-PE might be considered as a possible chemotherapeutic toxin, limited knowledge is available concerning its effect on the endothelium. This study investigated whether the aFGF-PE fusion protein could function as a possible anti-angiogenic agent. Both rat epididymal fat pad microvascular endothelial cells (RFCs) and Human umbilical vein endothelial cells (Huvecs) were grown in 24 well tissue culture plates to examine dose-dependent cytotoxicity by aFGF-PE. ^{3}[H] leucine incorporation studies indicated that after 24 hours in culture, aFGF-PE had a significant effect on protein synthesis when concentrations >100 ng/ml were used. In cultures incubated with 1000 ng/ml aFGF-PE, protein synthesis was inhibited 77% (Huvecs) and 92% (RFCs). Since the RFCs were more responsive to aFGF-PE in cytotoxicity assays, these cells were grown in a three-dimensional (3-D) collagen type I gel and incubated with either TGF-β_1, aFGF-PE or a combination of both. TGF-β_1 elicits optimal *in vitro* angiogenesis in 3-D cultures; namely, rapid formation of complex tubular structures. RFCs treated with aFGF-PE were unable to mount an angiogenic response suggesting that aFGF-PE chimeric proteins may be useful as anti-angiogenic agents. Further investigations on cell viability, time course and dose dependence in the 3-D system are presently underway.

CA 409 MONOCROTALINE (MCT) ALTERS ENDOTHELIAL CELL (EC) REGULATION OF POLYAMINES, COLLAGEN AND TRANSFORMING GROWTH FACTOR-ß1 (TGF-ß1). Jack W. Olson, Santosh Arcot and Mark N. Gillespie. University of Kentucky, College of Pharmacy, Lexington, KY 40536. We have demonstrated that the development of MCT-induced pulmonary hypertension in rats is a polyamine dependent process in which TGF-ß1 probably has a role. ECs are believed to contribute to the inappropriate cellular growth, differentiation and collagen production underlying the MCT-caused vascular remodeling process. Therefore, we tested whether *in vitro* MCT treatment could alter EC regulation of TGF-ß1, collagen and the polyamines, spermidine and spermine. Porcine pulmonary artery ECs were treated for 24 hours with MCT (2.5, 25, 250 or 2500 µM) and harvested 7 days after MCT removal. EC spermidine content was increased 9 fold while spermine was decreased 40% by MCT. ^{14}C-spermidine uptake was increased (2.1 vs 11.2 pmol/min/10^6 cells; vehicle vs MCT). Northern analyses demonstrated both ornithine decarboxylase, the first and generally rate-limiting enzyme in polyamine biosynthesis, and TGF-ß1 mRNA were significantly elevated following MCT. Incorporation of ^{3}H-proline into collagen was decreased by MCT. The above responses were observed only at 2500 µM MCT. These *in vitro* results suggest that ECs have the potential to contribute to the altered regulation of polyamines, collagen and TGF-ß1 observed *in vivo* during MCT-induced hypertensive pulmonary vascular disease. (Supported by NIH HL-44084 & 36404).

CA 410 EXPRESSION OF COAGULATION FACTOR XIII, A AND S SUBUNITS, BY HUMAN ENDOTHELIAL CELLS IN CULTURE, M. Lia Palomba, Giulio Nalli, Sergio Siragusa, Sandra Carbone, Anna Samaden and Franco Piovella, Istituto di Clinica Medica 2°, IRCCS Policlinico S. Matteo, University of Pavia, 27100 Pavia, Italy

Factor XIII (FXIII) circulates in plasma as a tetramer of two non-identical polypeptides, namely A and S chains. The A subunit dimer has been found in many cell types, while the S subunit has to date only been localized in platelets. FXIII catalyzes the crosslinking of Fibronectin (Fn) molecules as an important step in the incorporation of Fn fibrillar components in the extracellular matrix (EM). A role for FXIII has also been suggested in the crosslinking of von Willebrand factor (vWf) to other subendothelial EM proteins. We applied immunofluorescence technique using polyclonal antibodies to localize FXIII A and S subunits within cultured human endothelial cells (ECs) and in the subendothelial EM. Their relationship with Fn and vWf were also studied. ECs incubated with anti FXIII A showed little intracellular antigen, and a weak positivity outside the cells, as filamentous matrix. FXIII S was found both intracellularly, as fine perinuclear granules or larger formations scattered in the cytoplasm, and extracellularly, in fibers connecting subconfluent cells. The presence of both antigens in ECs cultures has also been confirmed by Western blot analysis. Immunofluorescence double-staining for FXIII S and vWf showed different localization of the antigens, with partial co-distribution in Weibel-Palade bodies. Various degrees of co-localization have been observed in the EM, between both FXIII subunits, vWf and Fn, according to the functional state of ECs. In a particular set of experiments ECs were grown in the presence of 0.1 uM Dexamethasone, a substance which modifies the expression of Fn and vWf, and induces the deposition of a thicker EM. In these preparations the amount of FXIIIA-positive extracellular fibers was increased, with no intracellular expression of the peptide. In the same samples the amount of FXIII S intracellular granules was greatly reduced. Our data agree with the possibility that FXIII plays a role in the process of adhesive proteins co-polymerization and incorporation in the subendothelial EM.

CA 411 DEVELOPMENT OF MONOCLONAL ANTIBODY FGF RECEPTOR STRUCTURE / FUNCTION PROBES, Margaret E. Schelling, Subramaniam Venkateswaran and Vincent D. Blanckaert, Department of Genetics and Cell Biology, Washington State University, Pullman, WA 99164-4234

Monoclonal antibodies against the FGF receptor may help define receptor epitopes involved in FGF binding and signal transduction, processes which mediate coronary and tumor angiogenesis. Monoclonal antibodies against the Coronary Venular Endothelial cell (CVEC) FGF receptor were made as previously reported (J. Cell Biol.109, 247a; FASEB J. 4(3),A487). Screening for FGF receptor positive clones was done using membrane preparations from CVECs expressing the FGF receptor (J. Tissue Culture Methods, submitted) both by Enzyme Linked Immunosorbent Assay (ELISA) and Western blot. Rabbit anti human FGF receptor polyclonal antibody was used as the internal positive control for the assays. Culture supernatants of five clones (4H1, F5P1B6, F5P2F10, F5P2E7 and F5P3B5) tested positive for the FGF receptor. Preliminary results suggest that these clones are secreting IgM antibodies. Ascites production of the above clones in Balb/c mice primed with Incomplete Freund's adjuvant (IFA) and further characterization of these monoclonal antibodies, as well as the production of additional antibodies, is underway. (Supported by AHA-WA 515 and NIH HL41378 grants awarded to MS).

Late Abstracts

GERM LINE TRANSMISSION OF A TARGETED MUTATION IN THE MURINE CD18 GENE. R.W. Wilson, A. Bradley, W.E. O'Brien, C.W. Smith, D.C. Anderson, and A.L. Beaudet. Institute for Molecular Genetics, Department of Pediatrics, and the Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

Leukocyte adhesion deficiency is an inherited immunodeficiency resulting from mutations in the leukocyte integrin CD18. To facilitate studies on this immunodeficiency we sought to obtain a murine line with a mutation at the CD18 locus using homologous recombination in embryonic stem cells. An 8.9 kb portion of the murine CD18 gene containing two exons was used to make the targeting construct. The neomycin resistance gene was inserted into a unique *Pvu*II site within the downstream exon. The construct was linearized within the CD18 gene to create an insertion-type vector. Following electroporation and selection in G418, homologous recombinants were screened by Southern blotting. We observed targeted events at a remarkably high frequency of approximately 1 in 10. Two independent targeted clones were used to generate chimeras and two of the chimeras passed the targeted gene to their offspring. Southern blot analysis of the heterozygous and homozygous animals revealed that the insertion resulted in the expected duplication of the 8.9 kb genomic sequence including the vector sequence. Preliminary northern blot analysis and reverse transcriptase polymerase chain reaction amplification of mRNA from various tissues of homozygous animals demonstrated an absence of the CD18 mRNA. Immunostaining showed substantial reduction of the CD18 protein on the cell surface of leukocytes from homozygous animals. Homozygous animals are maintained in a pathogen-free environment and show no signs of illness. Further study of these animals is in progress.

DISRUPTING THE *ICAM-1* GENE IN THE MOUSE. James E. Sligh, Jr., Christie M. Ballantyne, Allan Bradley,and Arthur L. Beaudet. Institute for Molecular Genetics and Department of Medicine, Baylor College of Medicine and Howard Hughes Medical Institute, Houston, Texas 77030.

Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin (Ig) superfamily, and exhibits heterophilic binding to members of the β_2 integrin family of proteins. In order to test the role of ICAM-1 *in vivo*, we are attempting to produce a mutation in the *Icam-1* gene by targeted recombination in murine embryonic stem (ES) cells. Screening of genomic libraries revealed the *Icam-1* gene is composed of seven exons. Exon 1 contains the 5' untranslated and the peptide leader sequences. Exons 2 through 6 encode Ig domains 1 through 5, with each exon encoding a single Ig domain. Exon 7 contains the transmembrane domain, cytoplasmic domain, and the 3' untranslated sequence through the polyadenylation site.

Several plasmids were prepared for homologous recombination in ES cells. A 5.5 kb segment of the murine *Icam-1* gene was subcloned into pBluescript, and a neomycin resistance gene under the control of the RNA polymerase II promoter was inserted within exon 5. These constructs were cut with restriction endonucleases to generate either replacement or insertion vectors. After electroporation and 9 days of G418 selection, individual ES cell colonies were picked and screened for homologous recombination by PCR, using a primer within the neo cassette paired with a primer lying in the *Icam-1* gene, but outside of the region contained within the electroporated plasmids. PCR screening produced 11 positive clones from 326 G418 resistant colonies using an insertion vector. All of these clones were confirmed to be homologous recombinants by Southern blotting. Numerous chimeric animals were generated by injecting these clones into blastocysts, however the contribution of the ES cells to the animals has been less than 30 percent. These mice are being bred in attempts to obtain germline transmission, and additional chimeras are being sought using various ES cell clones. Animals which are heterozygous and homozygous for the gene defect will be examined for phenotype, when obtained.

ROLE OF LECAM-1 (L-SELECTIN) FOR LEUKOCYTE ADHESION IN THE PRESENCE OF SHEAR STRESS *IN VIVO* (ROLLING), Klaus Ley, Gerhard Linnemann, Ulf Wintzer, Peter Gaehtgens, Dept. Physiology, Freie Universität Berlin, D-1000 Berlin 33, Germany.

L-selectin is an adhesion molecule on lymphocytes, granulocytes and monocytes involved in mediating lymphocyte adhesion to high endothelial venules of peripheral lymph nodes (PLN-HEV) and leukocyte rolling in venules of exposed rat and rabbit mesentery. We investigated whether both adhesion events also share other functional similarities. The mannose-6-phosphate polymer PPME and the sulfated fucose polymer fucoidin are known to bind and functionally block the lectin domain of L-selectin. When micro-infused into rat mesenteric venules, fucoidin inhibited leukocyte rolling by 93% at 300 μg/ml, and by 50% at 3 μg/ml. By contrast, PPME (up to 300 μg/ml) from two different sources consistently had no effect. Similar findings were obtained after intravenous injection of PPME and fucoidin. The carbohydrate-containing ligand for L-selectin on PLN-HEV is known to be sialidase-sensitive. To test whether this is also true for a putative venular endothelial ligand, we micro-infused three different preparations of bacterial neuraminidase (from v. cholerae, s. typhimurium and c. perfringens) at activities of up to 1 U/ml for up to 30 min (buffer pH 6.1, 5 mM Ca^{++}). This treatment is sufficient to block lymphocyte binding to PLN-HEV, but leukocyte rolling remained unchanged after local neuraminidase perfusion of venules. By contrast, micro-infusion of free sialic acid (1.6 mM), but not glucuronic acid reduced leukocyte rolling by 84%. We speculate that either the ligand for L-selectin in venules may be very different from the PLN-HEV ligand, or that L-selectin may not function as a lectin in leukocyte rolling.
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