INFLAMMATION, GROWTH REGULATORY MOLECULES AND ATHEROSCLEROSIS

Organizers: Russell Ross and Michael A. Gimbrone

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Vascular Pathogenetic Mechanisms (Joint)

A 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 (·NO) reacting with O_2^- generates the reactive product, peroxynitrite anion ($ONNO^-$) and, ultimately, the highly reactive and toxic hydroxyl radical (HO·). 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.

Early Pathogenetic "Footprints" - Endothelial Cell Dysfunction

A 002 **EXPRESSION OF VASCULAR CELL ADHESION MOLECULE-1 BY ENDOTHELIAL CELLS DURING ATHEROGENESIS.** Myron I. Cybulsky, Noriaki Kume, Tucker Collins, and Michael A. Gimbrone, Jr. Vascular Research Division, Departments of Pathology, Brigham and Women's Hospital and Harvrd Medical School, Boston.

Endothelial-leukocyte adhesion molecules expressed at sites of atherogenesis (ATHERO-ELAMs) may participate in the recruitment of circulating monocytes to the arterial intima. We generated a monoclonal antibody, designated Rb1/9, and identified a candidate ATHERO-ELAM which: 1) supports mononuclear leukocyte adhesion, 2) is inducibly expressed on the endothelial surface, and 3) is detectable in early atherosclerotic lesions. Aortas from rabbits fed a 1% cholesterol diet for 8-12 weeks and from 4-14 month old Watanabe heritable hyperlipidemic rabbits showed specific Rb1/9 immunohistochemical staining localized to endothelium covering early and advanced (foam cell-rich) atherosclerotic lesions. Aortic endothelium in uninvolved areas of these hypercholesterolemic animals, as well as endothelium lining the aorta and vena cava in normal rabbits, did not stain. The NH_2-terminal sequence of the protein purified by Rb1/9 immunoaffinity chromatography (NH_2-FKIETFPES-RSLAQIGDSVSLT) was highly homologous to human vascular cell adhesion molecule-1 (VCAM-1), an inducible member of the immunoglobulin (Ig) gene superfamily, initially described as a protein with 6 extracellular Ig-like domains. Molecular cloning of rabbit VCAM-I identified an additional 276 base pair Ig-like domain located between domains 3 and 4. This domain, designated domain 4 (the remaining domains were renumbered) or AS-1, was present in cDNA cloned by PCR from IL-1-activated human umbilical endothelial cells (HUVEC). The 7 Ig-like domain form was the predominant VCAM-1 mRNA species and polypeptide expressed on the surface of activated HUVEC. Domain 4 (AS-1 domain) is most homologous to the existing NH_2-terminal domain (domain 1). It is encoded by a single exon of the human VCAM-1 gene, indicating that the 2 forms of mRNA arise by alternative splicing. Rabbit VCAM-1 is expressed by endothelium in two forms, with either 7 or 8 Ig-like extracellular domains. Both forms were identified in a rabbit endothelial cell cDNA library, and are expressed on the endothelial surface. The eighth Ig-like domain in the rabbit is located between the seventh and transmembrane domains. It is homologous to domains 3, 6, and 7, and arises by alternative mRNA splicing. Alternative splicing of the VCAM-I gene by arterial endothelium may generate structures that have an important role in the pathogenesis of atherosclerosis. In addition to its potential role in mononuclear leukocyte recruitment, the expression of VCAM-1 may be a correlate of endothelial cell dysfunction in atherogenesis and thus provide a potential diagnostic marker for lesion detection.

A 003 Possible Roles for Cytokines in Ahterogenesis, Peter Libby and Steven K. Clinton, Department of Medicine, Brigham & Women's Hospital, Boston, Massachusetts, 02115.

Cytokines may contribute to deranged functions of vessel wall cells during atherogenesis. A broad definition of this category of mediators can include many proteins that regulate inflammation, immunity, and host responses to infectious agents. The immunostimulatory interleukins and related leukocyte activating and chemoattractant proteins are one important class of cytokines. Other cytokines include tumor necrosis factor (TNF), colony stimulating factors (CSFs), the macrophage activating and chemoattractant protein MCP-1, and the interferons. Cytokines can modulate the properties of endothelial Cells (EC) that render their surface able to maintain blood in a liquid state. Interleukin 1 (IL1) or TNF can alter the balance of anticoagulant, antithrombotic, and fibrinolytic mechanisms of EC in a fashion that reduces hemocompatibility. Adhesive interactions between leukocytes and endothelium contribute importantly to normal leukocyte trafficking, the mobilization of host defenses, and in the pathogenesis of certain diseases. Early in the response to atherogenic diets in many species, blood monocytes adhere to the endothelium of lesion-prone areas of arteries, enter the intima, and accumulate lipids. IL1, TNF, and interferon gamma can modulate the expression of leukocyte adhesion molecules (LAMs) on the endothelium and other cells that mediate such adhesive interactions. Cytokines can also influence the growth of EC and smooth muscle cells (SMC) by either direct or indirect mechanisms. TNF and IL-1 tend to inhibit EC proliferation in vitro. IL1 treatment stimulates proliferation of SMC, at least in part by inducing autocrine growth factor production. Interferon gamma limits the proliferation of both EC and SMC. IL1 or (to a lesser extent) modified lipoproteins can modulate the expression of the hematopoietic growth factors granulocyte-monocyte CSF (GM-CSF) and monocyte-CSF (M-CSF) in cultured human vascular cells. This is a potentially important point as mononuclear phagocyte recruitment, infiltration, and even multiplication are characteristic of human atheroma. In addition to effects on cell growth, cytokines can regulate the production of lipid mediators by vascular wall cells. For example, IL1 and TNF potently stimulate elaboration of prostaglandins and platelet activating factor by vascular cells. Cytokines such as IL1 and TNF can also stimulate expression of genes for other cytokines by human vascular wall cells (e.g. IL1, IL6, IL8, MCP-1, GM- and M-CSF as well as peptide growth factors). Cytokine gene expression might link "risk" factors implicated in the initiation of atherosclerosis to altered cell function since macrophage-derived foam cells and/or other cells types in rabbit atheroma may produce cytokines at various times during the initiation, evolution, and complication of the lesions. A variety of experimental studies do indicate that inducible expression of cytokine genes in vascular tissue might contribute to locally altered function of EC and SMC during atherogenesis in vivo.

Early Effector Changes - Vascular Cell Gene Regulation (Joint)

A 004 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 β-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.

A 005 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)

A 006 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.

A 007 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.

Cytokines and Immune Mechanisms

A 008 MONOCYTE TRANSMIGRATION INDUCED BY MODIFICATION OF LOW DENSITY LIPOPROTEIN IN COCULTURES OF HUMAN AORTIC WALL CELLS IS DUE TO INDUCTION OF MONOCYTE CHEMOTACTIC PROTEIN 1 SYNTHESIS AND IS ABOLISHED BY HIGH DENSITY LIPOPROTEIN, Mahamad Navab[1], Susan S. Imes[1], Gregory P. Hough[1], Susan Y. Hama[1], Lori A. Ross[1], Richard W. Bork[1], Anthony J. Valente[2], Judith A. Berliner[1], Davis C. Drinkwater[3], Hillel Laks[3], and Alan M. Fogelman[1], [1]Department of Medicine, UCLA, Los Angeles, [2]Department of Pathology, University of Texas Health Sciences Center, San Antonio, [3]Department of Surgery, UCLA, Los Angeles

Incubation of cocultures of human aortic endothelial (HAEC) and smooth muscle cells (HASMC) with low density lipoprotein (LDL) in the presence of 5-10% human serum resulted in a 7.2-fold induction of mRNA for monocyte chemotactic protein 1 (MCP-1), a 2.5-fold increase in the levels of MCP-1 protein in the coculture supernatants, and a 7.1-fold increase in the transmigration of monocytes into the subendothelial space of the cocultures. Monocyte migration was inhibited by 91% by antibody to MCP-1. Media collected from the cocultures that had been incubated with LDL induced target EC to bind monocyte but not neutrophil-like cells. Media collected from cocultures that had been incubated with LDL induced monocyte migration into the subendothelial space of other cocultures that had not been exposed to LDL. In contrast, media from separate cultures of EC or SMC containing equal number of EC or SMC compared to coculture and incubated with the same LDL did not induce monocyte migration when incubated with the target cocultures. High density lipoprotein (HDL), when presented to cocultures together with LDL, reduced the increased monocyte transmigration by 91%. Virtually all of the HDL mediated inhibition was accounted for by the HDL_2 subfraction. HDL_3 was essentially without effect. Apolipoprotein AI was also ineffective in preventing monocyte transmigration while phosphatidylcholine liposomes were as effective as HDL_2 suggesting that lipid components of HDL_2 may have been responsible for its action. Preincubating LDL with β-carotene or with alpha-tocopherol did not reduce monocyte migration. However, pretreatment of LDL with probucol or pretreatment of the cocultures with probucol, β-carotene or alpha-tocopherol prior to the addition of LDL prevented the LDL induced monocyte transmigration. Addtion of HDL or probucol to LDL *after* the exposure to cocultures did not prevent the modified LDL from inducing monocyte transmigration in fresh cocultures. We conclude that cocultures of human aortic cells can modify LDL even in the presence of serum, resulting in the induction of MCP-1, and that HDL and antioxidants prevent the LDL induced monocyte transmigration.

A 009 MECHANISMS THAT REGULATE THE EXPRESSION OF CHEMOTACTIC CYTOKINES: POTENTIAL ROLES IN ATHEROSCLEROSIS AND DTH REACTIONS, Steven L. Kunkel and Robert M. Strieter, Departments of Pathology and Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109-0602

A number of chronic inflammatory reactions are characterized by the elicitation and accumulation of specific leukocyte populations. The majority of these cells are derived predominantly from the circulating pool of peripheral white blood cells. Once recruited to an inflammatory site, these leukocytes play key roles in the amplification and perpetuation of the immune response. Recent evidence suggests that the elicitation of mononuclear cells is dependent in part upon a family of small molecular weight polypeptides that appear to possess specificity with regard to the recruitment of certain leukocyte populations. Two members of this chemotactic cytokine family are interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP). While IL-8 has been identified as a chemoattractant for neutrophils and lymphocytes, MCP possesses absolute specificity for monocytes. A variety of cells have been identified as a source of IL-8 and MCP, including monocytes, human endothelial cells, connective tissue cells (smooth muscle cells and fibroblasts) and parenchymal cells (epithelial cells and hepatocytes). Interestingly, monocytes and endothelial cells can synthesize chemotactic cytokines in response to a number of stimuli, while fibroblasts and epithelial cells demonstrate stimulus specificity. For example, endothelial cells can respond to LPS, interleukin-1 (IL-1), and tumor necrosis factor (TNF) and produce significant quantities of MCP while fibroblasts and epithelial cells respond only to IL-1 and TNF. This data suggests that the in vivo synthesis of IL-8 and MCP from the latter cell types is dependent upon host-derived mediators (IL-1/TNF) resulting in chemotactic factor generation via cytokine networking. Recent experimental evidence from our laboratory has demonstrated the endothelium and perivascular stromal cells are likely sources of MCP for the initiation and maintenance of either atherosclerotic or granulomatous lesions. Endothelial cells treated with low density lipoprotein (LDL, 5 ug/ml) or acetyl-LDL demonstrate a time dependent increase in both de novo MCP mRNA and antigen expression. The latter was assessed by a sensitive ELISA for MCP. Maximum expression of MCP occurred 8 hours post challenge and persisted for the next 24 hours. The production of antigenic MCP by LDL and acetyl-LDL treated endothelial cells was approximately 40 and 60%, respectively, of the response generated by 2 ng/ml of TNF. We next determined whether ex vivo organ cultures of human abdominal aortas, ranging from normal to inflammatory abdominal aortic aneurysms could express antigenic MCP. These studies were aimed at testing the hypothesis that the production of chemotactic cytokines by the cellular constituents of arterial vessel walls are likely associated with the immunopathology of developing atherosclerosis. Culture supernatants from infrarenal normal aortas, occlusive, abdominal aortic aneurysm, and inflammatory aneurysm were found to synthesize 16.5, 36, 31, and 68 ng/ml, respectively, of MCP. Interestingly, the cellular infiltrates of inflammatory aneurysms are histologically similar to that found in delayed type-hypersensitivity (DTH) granulomas. Both of these inflammatory lesions are characterized by the accumulation of lymphocytes and mononuclear phagocytic cells. Using an experimental model of synchronously developing pulmonary granulomas, we have identified the expression of both steady state levels of MCP mRNA and polypeptide that is coincident with the elicitation of monocytes into the developing granulomatous lesion. In addition, the presence of MCP in the granuloma was associated with elevated levels of interleukin-4 and gamma interferon, known inducers of MCP expression by non-immune cells. Localization studies using immunohistochemistry demonstrated that MCP antigen was associated with cells found around the vessel wall (perivascular stromal cells). These studies demonstrate that chemotactic cytokines are important mediators in the initiation and maintenance of inflammatory lesions that are characterized by an abundance of mononuclear phagocytic cells.

Vascular Cell Phenotype (Joint)

A 010 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.

A 011 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.

A 012 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)

A 013 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.

A 014 IL-1α VERSUS FGF-1: ANTAGONIST HUMAN ENDOTHELIAL CELL FUNCTIONS FROM SIMILAR STRUCTURAL FEATURES,
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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.

A 015 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.

Receptor Molecules and Transduction Mechanisms

A 016 G-PROTEIN-COUPLED RECEPTORS AS PROTO-ONCOGENES: Role in Mitogenesis and Tumorigenicity. Lee F. Allen, Susanna Cotecchia, Marc G. Caron, and Robert J. Lefkowitz, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC.

The biological effects of hormones and many drugs are initiated through their interaction with specific receptor macromolecules. The guanine nucleotide binding protein (G-protein) -coupled receptors encompass a broad superfamily of integral transmembrane proteins which transduce extracellular stimuli through G-protein intermediates. These, in turn, impact on specific intracellular signaling pathways, and generate second messengers which mediate the ultimate physiologic response to agonist-induced receptor activation. The adrenergic receptors comprise a subfamily of these receptors, which are activated by catecholamines. They share the characteristic structural features of a G-protein-coupled receptor, consisting of 7 hydrophobic transmembrane spanning domains joined by three intra- and extra-cellular loops. The three major groups of adrenergic receptors (ADRs), α_1, α_2, and ß, each couple to different G-proteins, and activate distinct second messenger systems. The α_1-ADRs activate phospholipase C (PLC) via a pertussis toxin-insensitive G-protein, whereas the ß and α_2-ADRs increase or decrease adenylyl cyclase activity via stimulatory (Gs) or inhibitory (Gi) G-proteins respectively. The ability of each receptor group to interact with its specific G-protein effector system determines the pattern of cellular events mediated by the specific G-protein-coupled receptor.

Elucidation of the primary structure of many G-protein-coupled receptors has enabled investigations of the structural basis for receptor function, and for the specificity of coupling to second messenger systems. The membrane spanning domains appear to play a key role in the formation of the ligand binding pocket. The third intracellular loop, one of the most divergent regions of the receptor, appears to contain important determinants of the specificity of receptor/G-protein coupling. Chimeric receptors involving substitution of selected regions of the third intracellular loop of the β_2-ADR into the α_2-ADR or the α_1B-ADR loop into the β_2-ADR support a key role for this structural domain in mediating the specificity of receptor/G-protein coupling. Through selective mutagenesis, a sequence of 27 amino acid residues in the N-terminal portion of the third intracellular of the α_1B-ADR has been identified, that appears to contain the minimal structural determinants required for coupling the receptor to the activation of phosphoinositide (PI) hydrolysis. The critical importance of these structural domains in the third intracellular loop for determining the specificity of G-protein-coupled receptor interaction with its effector system is confirmed by the generality of this phenomenon to other families of G-protein-coupled receptors, eg the muscarinic cholinergic (MAch) receptors (M1, M2). The carboxyl terminus of the third intracellular loop also appears to have important effects on G-protein coupling and agonist binding. Mutational alteration of the α_1B-ADR by substitution of three amino acid residues ($Arg^{288} \rightarrow$ Lys, $Lys^{290} \rightarrow$ His, and $Ala^{293} \rightarrow$ Leu) results in both an increase in the binding affinity of norepinephrine (NE), and its potency in stimulating PI hydrolysis. In addition, this activating mutation appears to render the α_1B-ADR constitutively active, resulting in the stimulation of PI hydrolysis in the absence of agonist-induced receptor activation. Further, point mutations at residue 293 appear sufficient to increase the affinity and potency of NE, and induce constitutive activity.

Several hormones, neurotransmitters and growth factors, involved with the regulation of cell proliferation, activate signal transduction pathways that, like the α_1-ADR, result in PI hydrolysis. Some act through receptors that couple to G-proteins, while other growth factors can stimulate PLC activity directly through receptor-mediated activation of tyrosine kinase. This stimulation of the inositol phospholipid pathway appears to play a crucial role in regulating the mitogenic response, activating nuclear transcription and DNA synthetic activity, and ultimately results in cell division. Several subfamilies of G-protein-coupled receptors, which activate PI hydrolysis (the serotonin (1C) and MAch (M1, M3, and M5) receptors), have been shown to result in agonist-dependent alterations in cell growth and lead to transformation. Recent studies on the α_1B-ADR have also demonstrated the potential of this receptor to induce transition from quiescence to active cellular proliferation, and abrogate normal growth control mechanisms. Introduction of functional receptors induces neoplastic transformation of rat and mouse fibroblasts, and identifies the α_1B-ADR as a proto-oncogene. The prominent functional role of the α_1B-ADR in several body systems, e.g. hepatic regeneration and vascular smooth muscle proliferation, suggests a potential role for this G-protein-coupled receptor proto-oncogene in hepatic tumorigenesis and in atherogenesis. Mutational alteration of the α_1B-ADR, resulting from the replacement of three amino acids in the third cytoplasmic loop (288, 290, 293) induces constitutive activity, and appears to result in the activation of this proto-oncogene. "Oncomutant" expressing cell lines demonstrate a more aggressive transformed phenotype, and appear constitutively active with agonist-independent focus formation and maximal growth rates. Mutational alteration of this receptor, which renders it constitutively active, thus appears to activate the transforming potential of this proto-oncogene, and plays an important role in triggering uncontrolled cell proliferation in vitro.

G-protein-coupled receptors are emerging as an important class of growth factor receptors which can modulate signaling pathways that control cell proliferation. Mutational alteration of key amino acids in the carboxyl terminus of the third intracellular loop, important for receptor/G-protein coupling, may prove to be a generalized mechanism for inducing constitutive activity, and activating this new class of G-protein-coupled receptor proto-oncogenes. Subversion of these or other structurally related receptor genes in vivo by spontaneously occurring mutations of this type may, therefore, lead to uncontrolled cell growth and human disease, eg neoplasia and atherosclerosis. Identification of such mutant receptors may provide specific disease markers and provide targets for therapeutic intervention.

A 017 SIGNAL TRANSDUCITON BY THE PDGF RECEPTORS, FGF RECEPTORS, AND BY A RECENTLY IDENTIFIED RECEPTOR FOR VASCULAR ENDOTHELIAL GROWTH FACTOR/PERMEABILITY FACTOR, L.T. Williams[1,2,3], J.A. Escobedo[1,2,3], W.J. Fantl[2,3], A. Klippel[1,2,3], C. de Vries[2,3], and N. Ferrara[4] Howard Hughes Medical Institute[1], Cardiovascular Research Institute[2], University of California, San Francisco[3], and Genentech Inc., South San Francisco, California[4].

The first step in signal transduction by tyrosine kinase growth factor receptors is ligand-induced formation of receptor oligomers, a process that facilitates receptor autophosphorylation. When phosphorylated on tyrosines, the receptors form non-covalent complexes with several cytoplasmic proteins that have been implicated in the regulation of cell proliferation. We identified the sites on the platelet-derived growth factor (PDGF) receptor and fibroblast growth factor (FGF) receptor that mediate binding of the receptors to signaling molecules. Each of these sites consists of a tyrosine in the context of a short sequence that determines which signaling molecule binds to the site. The interactions of each signaling molecule with the receptor can be selectively disrupted by either mutating the sites on the receptor or by adding short tyrosine-phosphorylated peptides that mimick the sites and compete for binding to the signaling molecules. In this way, we have been able to selectively block binding of PDGF receptors to phosphatidylinositol 3-kinase and GTPase activating protein, and binding of the FGF receptor to phospholipase C-γ. Selective disruption of each of these pathways has distinct functional consequences on the ability of the receptor to stimulate cellular response associated with growth factor action. We have also studied the sites on the signaling molecules that bind the phosphotyrosine-containing sequences on the receptors. These sites lie within SH2 domains of each signaling molecule. The interaction of each SH2 domain with its corresponding phosphotyrosine sequence is of high affinity (K_D equals 0.1 to 5 nM) and is extremely specific.

In a different set of experiments, we have identified a cDNA that encodes a receptor for vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). This factor is a highly specific endothelial cell mitogen that induces an increase in capillary permeability. VEGF/VPF has some sequence similarity (20-25% identity) to PDGF. We cloned the cDNA that encodes a receptor for VEGF/VPF. This receptor has multiple immunoglobulin domains and a kinase insert region, establishing it as a member of the PDGF receptor family. The receptor binds VEGF/VPF with a K_D of approximately 20 pM. Some of the signal transduction properties of the VEGF/VPF receptor differ from those of the PDGF receptor.

Vascular Disease Progression and Regression

A 018 REGRESSION OF ATHEROSCLEROSIS IN MONKEYS. Donald D. Heistad, Mark L. Armstrong, Frank M. Faraci, and Richard Padgett, University of Iowa College of Medicine and VA Medical Center, Iowa City, IA 52242.

There is clear morphological evidence of regression of atherosclerosis in monkeys, with striking reabsorption of vascular lipids. We have measured vasodilator capacity, which is the best functional index of structural changes in resistance vessels. Regression of atherosclerosis, with reabsorption of vascular lipids, is not accompanied by consistent improvement in maximal vasodilator capacity. Fibrosis of arteries during regression may restrict improvement in vasodilator capacity. Vascular fibrosis, together with "remodeling" of arteries, probably account in large part for the finding that the magnitude of regression that has been demonstrated by angiographic studies also is disappointingly small.

Vasospasm is a common clinical complication in atherosclerosis. Major hypotheses concerning the pathophysiology of vasospasm focus on the role of platelets and leukocytes. The hypotheses are that platelets or leukocytes, which release several potent vasoconstrictor agonists, may produce arterial spasm, especially if endothelium over atherosclerotic lesions is dysfunctional.

In relation to the platelet hypothesis, serotonin (which is released by platelets) is a possible mediator of vasospasm. Atherosclerosis greatly potentiates constrictor responses to serotonin in retinal, cerebral, coronary, and limb blood vessels in monkeys in vivo. Retinal vasoconstriction may contribute to amaurosis fugax, with transient ischemia and blindness. We have observed that a regression diet for 18 months abolishes augmented constrictor responses of the retinal, cerebral, and limb circulation to serotonin.

In relation to the leukocyte hypothesis, activation of leukocytes with the chemotactic peptide f-met-leu-phe (fMLP) produces marked constriction of large arteries in atherosclerotic, but not normal, monkeys. After regression of atherosclerosis, vasoconstrictor responses to serotonin return to normal, and abnormal constrictor responses to fMLP are improved but not abolished.

Thus, these studies suggest that hyperreactivity to vasoactive products that are released by platelets and leukocytes returns towards normal with regression of atherosclerosis. The findings may have important implications for reduction in susceptibility to vasospasm after regression, and for evaluation of therapeutic approaches to atherosclerosis.

Genetic Models of Vascular Disease

A 019 EXPRESSION OF THE RENIN/ANGIOTENSIN SYSTEM IN TRANSGENIC RATS: J.J. Mullins[1], J. Peters[2], S. Bachmann[3], M. Bader[2], M. Lee[2], J. Wagner[2], K. Zeh[2], U Hilgenfeld[2], M. Kaling[2], D. Ganten[2] and G.Barrett[1] and K. Murakami[4]. [1]AFRC-Centre for Genome Research, University of Edinburgh, [2]Dept.Pharmacology and [3]Dept. Anatomy, University of Heidelberg, [4]Institute of Biochemistry, University of Tsukuba.

The renin-angiotensin system, which is one of the major machanisms of blood pressure homeostasis, has been intensively studied physiologically and pharmacologically, but the role it plays in the development of hypertension is still largely unknown. To investigate their individual roles in the physiology of blood pressure control, we are introducing RAS gene constructs into the germline of the mouse and the rat. Though widely used, transgenic technology is almost exclusively limited to the mouse since this species is genetically well characterised and its short generation time and ease of handling facilitate experimentation. To overcome size limitations of the mouse in certain areas of cardiovascular research, and to complement mouse studies already underway, we have established transgenic techniques in the rat.

We have generated transgenic rats harboring the mouse Ren-2 gene in their genome which exhibited extreme hypertension (systolic pressure 180-260 mm Hg). Since the hypertensive phenotype segregates with presence of the transgene, this suggests that expression of the mouse renin gene is responsible for the hypertension. The transgene is expressed in a tissue-specific manner, the highest expression being found in the outer adrenal cortex. We have further characterised the morphology of these animals, and now demonstrate that they exhibit symptoms typical of the vascular damage due to extreme hypertension. Evidence suggests that alterations of adrenal steroidogenesis may, at least in part, be responsible for the phenotype

Transgenic rat strains have been constructed which express the human angiotensinogen gene. Human angiotensinogen was expressed primarily in the liver and kidney and was present at high concentration in the circulation. When administered recombinant human renin, these animals exhibited a pronounced rise in blood pressure which reponded to specific human renin inhibitors. This demonstrates the high degree of species specificity of the renin/angiotensinogen reaction and the possibility of using specifically tailored transgenic strains for developing and testing new pharmacological agents.

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.

MICROVASCULAR CELL MODULATION BY EXTRACELLULAR MATRIX AND SOLUBLE FACTORS, Joseph A. Madri, Ph.D., M.D. and Martin Marx, M.D., Department of Pathology, Yale University School of Medicine, New Haven, CT 06510

Blood vessels are comprised of a variety of cell types including vascular endothelial and smooth muscle cells. In large vessels these two cell types appear to exhibit stable phenotypes. In contrast, in the microvasculature, endothelial cells and pericytes or mesangial cells (analogues to smooth muscle cells in large vessels) appear to exhibit "plastic" phenotypes in vivo and in vitro.
In vivo, during inflammatory responses microvascular endothelial cells undergo angiogenesis and have been shown to express PDGF receptors. In vitro, under certain conditions, microvascular endothelial cells have been shown to express α smooth muscle actin as well as PDGF receptor α and β chains while retaining their vonWillebrand Factor expression. In addition, α smooth muscle actin isoform expression as well as proliferative rate and multicellular organization appear to be modulated in vitro by extracellular matrix composition and organization and soluble factors such as TGF-β1. Microvascular endothelial cells plated and grown in two-dimensional cultures express α smooth muscle actin and PDGF receptor α and β chains. Under such culture conditions these cells exhibit a substantial proliferative response to PDGF BB, a modest proliferative response to PDGF AB and no response to PDGF AA. In contrast, in three-dimensional culture, as these cells form complex capillary-like structures in response to TGFβ1, they lose their expression of α smooth muscle actin protein and mRNA as well as their responsiveness to PDGF isoforms and subsequently their expression of PDGF receptor α and β chains. These changes are consistent with the notion that microvascular endothelial cells can modulate their phenotype and responsiveness to soluble factors, depending upon extracellular matrix composition and organization.
In parallel studies using mesangial cells isolated from rat glomeruli similar modulation of PDGF receptor α and β chains and PDGF isoform responsiveness was observed in two- and three-dimensional cultures. Specifically, culture on several matrix components resulted in expression of PDGF receptor β chain and smaller amounts of PDGF receptor α chain. PDGF BB, and to a lesser extent PDGF AB, elicited proliferative responses, while PDGF AA had no appreciable effect on proliferation. In contrast, in three-dimensional cultures, these cells formed multicellular aggregates which lead to gel contraction and progressively lost their PDGF receptor expression and responsiveness with time in culture. These findings are consistent with the notion that, like microvascular endothelial cells, the mesangial cell also exhibits a "plastic" phenotype which is responsive to matrix composition and organization.

EXPRESSION OF RECOMBINANT PDGF BB IN THE VESSEL WALL FOLLOWING DIRECT GENE TRANSFER, Elizabeth G. Nabel[1], Zhiyong Yang[1], Suzanne Liptay[1], Christian C. Haudenschild[2], Gary J. Nabel[1], [1]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI, [2]Boston University, Boston, MA.
Multiple cell types in atherosclerotic and restenosis lesions elaborate growth regulatory proteins, including the dimeric forms of PDGF. PDGF plays a role in cellular proliferation following vascular injury. To determine the biological effects of PDGF BB in vivo, a plasmid expression vector, pSVL, containing the cDNA for the coding region of the PDGF B gene (v-sis) was used and a second plasmid in the pMEX eukaryotic expression vector was constructed. Primary cultures of endothelial and vascular smooth muscle cells were transfected in vitro to test for transfection efficiency. Transfected cells were analyzed for the ability to grow in low fetal calf serum and were assayed for secretion of recombinant PDGF BB protein into culture supernatant. The recombinant PDGF B gene was introduced into porcine iliofemoral arteries by liposomal transfection using a catheter delivery method, previously developed in our laboratory. Transfer of PDGF B DNA in vivo was confirmed by PCR. Expression of recombinant PDGF BB protein was demonstrated by immunohistochemistry using a monoclonal antihuman PDGF B antibody. The expression of recombinant PDGF BB protein in porcine arteries was associated with marked intimal proliferation three weeks following transfer, compared with control porcine vessels transfected with a reporter gene (ß-galactosidase). These findings suggest that the overexpression of recombinant PDGF BB stimulates intimal cell hyperplasia in the vessel wall in vivo.

THE DISTINCTIVE FUNCTIONS OF SELECTINS, INTEGRINS, AND IG FAMILY MOLECULES IN REGULATION OF LEUKOCYTE INTERACTION WITH ENDOTHELIUM, Timothy A. Springer, The Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.
Adhesion molecules together with cytokines and chemoattractants regulate leukocyte interaction with endothelium and subsequent events such as migration through the endothelium and within tissues, and cell-cell interactions that are important in inflammatory responses including those of granulocytes and monocytes with host cells and foreign pathogens, and antigen-specific responses of lymphocytes. Adhesion receptors are also used by cells to sense information about the environment that can stimulate signalling pathways. Three families of adhesion receptors have distinct functions in cell interactions. Ig family molecule density on the surface regulates cell interactions; some molecules are inducible by cytokines such as ICAM-1 and VCAM-1 and others are constitutively expressed such as ICAM-2. Integrin adhesive activity can be regulated independently of surface expression by intracellular signals acting on integrin cytoplasmic domains that appear to affect the conformation of the extracellular ligand binding domain. Selectins mediate attachment of leukocytes ot the vessel wall under flow conditions at which integrin and Ig family members are ineffective. The initial steps in leukocyte accumulation have been reconstituted in on vitro system. Neutrophils in a parallel plate flow chamber roll on artificial phospholipid bilayers containing the selectins CD62 or ELAM-1. The results are the same on bilayers containing CD62 and ICAM-1, expect when a chemoattractant is infused, activation of integrins causes the rolling neutrophils to arrest and then spread. The accompanying adhesion developed through the integrin-ICAM-1 interaction is more than 100-fold stronger than through selectins.

Vascular Pathogenetic Mechanisms; Early Pathogenetic "Footprints" - Endothelial Cell Dysfunction

A 100 DOWNREGULATION OF ENDOTHELIAL CELL EXPRESSION OF HLA-I IN RESPONSE TO CYTOMEGALOVIRUS INFECTION.
NC Alampi, ME Wohlford, DD Sedmak; Dept of Pathol, Ohio State Univ, Columbus, OH 43210.
CMV infects endothelial cells (EC) *in vivo* and is capable of establishing a state of persistent infection; however, mechanisms of CMV persistence are poorly understood. The endothelium may be of primary importance in mediating immune responses to CMV because it serves as the interface between the immune system and underlying tissues. Because HLA Class I (HLA-I) antigens serve as major determinants of the immune response to virally infected cells, we have examined the expression of HLA-I by CMV-infected endothelial cells *in vitro*, on a population level by flow cytometry and on an individual cell level by dual immunoperoxidase staining. Population changes in HLA-I expression, as measured by flow cytometry, showed that infected endothelial cell cultures separated into a subpopulation of HLA-I positive cells and a subpopulation of HLA-I negative cells. Dual immunoperoxidase staining of such cultures revealed that CMV infection of an EC was associated with loss of HLA-I by that cell. Non-infected cells remained HLA-I positive. These results suggest that the HLA-I negative subpopulation detected via flow cytometry consisted of CMV infected cells. Immunoperoxidase staining also demonstrated that HLA-I downregulation occurred in endothelial cells as early as six hours post infection. Neither IFNγ nor TNF treatment of infected cells reversed CMV-induced HLA-I downregulation. Flow cytometric analysis showed that cultures still separated into a subpopulation of HLA-I negative cells and dual immunoperoxidase staining showed that CMV infected cells remained HLA-I negative. Interestingly, there appeared to be a marked upregulation of HLA-I in those cells immediately adjacent to the CMV-positive plaques, as compared to those cells distant from infected cells. This study demonstrated that CMV infection of endothelial cells resulted in surface and cytoplasmic downregulation of HLA-I. These findings suggest a mechanism by which CMV-infected cells may evade immune recognition, a phenomenon which may serve to promote viral persistence.

A 101 INDUCTION OF ENDOTHELIAL FERRITIN: A CYTOPROTECTIVE STRATEGEM OF THE VESSEL WALL.
G Balla, HS Jacob, J Balla, M Rosenberg, K Nath, F Apple, JW Eaton and GM Vercellotti, Dept. of Medicine, Lab. Med. & Pathology, University of Minnesota, Minneapolis, MN 55455
Heme, a hydrophobic iron chelate, rapidly enters porcine aortic endothelial cells (PAEC) and within 30 min, enhances H_2O_2 or activated PMN-mediated injury *in vitro* (Lab Invest 64:648, 1991). To our surprise, when PAEC are treated for prolonged periods (16 h) with hemin (10 μM), the cells, rather than being hypersusceptible to oxidant injury, become resistant. We hypothesize that this adaptation to oxidant stress may reflect synthesis of a natural iron chelator, such as ferritin, to limit the reactivity of endogenous or heme-derived intracellular iron. PAEC exposed to hemin (10 μM) rapidly synthesize ferritin as measured by ELISA; within 4 h, PAEC ferritin increases 4-fold and by 20 h, 10 fold. Heme is metabolized by heme oxygenase (HO) a heat shock protein, which can open the porphyrin ring and release heme iron. Hemin (10 μM) induces HO mRNA 100-fold and HO activity 50-fold within 4 hours. To determine whether HO or ferritin is the critical cell protectant, we differentially induced each protein. Pre-incubations with cell-permeant iron:pyridoxal isonicotinoyl hydrazone chelate (but not with impermeable, free inorganic iron) or with a combination of hemin and tin protoporphyrin IX (an inhibitor of HO) causes substantial increases in intracellular ferritin without any increment in HO. In both cases, there is marked protection against hemin-catalyzed, H_2O_2-mediated PAEC injury. Conversely, during incubations with both hemin and desferrioxamine or with sodium arsenite, high levels of HO, but not ferritin, accrue, yet without associated protection against later challenge by hemin and H_2O_2. This suggests that HO serves mainly to provide intracellular iron from added hemin; iron, in turn drives the synthesis of ferritin, the proximate defense against oxidant damage. Furthermore PAEC incubated with exogenous apoferritin accumulate substantial ferritin (presumably by pinocytosis) and become strikingly resistant to oxidant stress. This beneficial effect is specific for apoferritin, as PAEC pre-incubated with apo-transferrin, apo-lactoferrin, and albumin remain sensitive to iron-driven oxidation. Under diverse experimental conditions an inverse correlation between intracellular ferritin content and the susceptibility of PAEC to oxidant challenge can be appreciated. Ferritin heavy chain ferroxidase activity is essential to sequester ferric iron and is required for endothelial cytoprotection by exogenous ferritin. Mutated recombinant human heavy chain ferritin, devoid of ferroxidase activity (Broxmeyer et al. PNAS 88:770, 1991), when added to PAEC for 14 h, fails to prevent oxidant damage. In conclusion, we suspect that ferritin may be a crucial intracellular buffer for the control of reactive iron and suppression of intracellular iron-synergized oxidant events.

A 102 CELL SURFACE EXPRESSION OF A MONOCYTE ADHESION PROTEIN ON CYTOKINE ACTIVATED HUMAN ENDOTHELIAL CELLS AND ATHEROSCLEROTIC HUMAN AND WATANABE RABBIT CORONARY VESSELS,
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The adhesion of circulating monocytes to the endothelial lining of blood vessels is an early, critical event in the pathogenesis of atherosclerotic plaque formation. To assess the role of cytokine induced endothelial cell adhesion molecules in this process, monoclonal antibodies (mAb) were generated that recognized cell surface molecules present only on human umbilical vein endothelial cells (HUVE) treated with tumor necrosis factor alpha (TNF) or interleukin 1 (IL-1). One such mAb, IG9, inhibited the adhesion of the promyelomonocytic U937 cell line and the promyelocytic HL60 cell line to TNF or IL-1 treated HUVE by 35% and 25% respectively. The adhesion of lymphocytes and granulocytes to cytokine activated HUVE was unaffected. Immunoprecipitation of 125-I labelled, TNF treated HUVE cell surface proteins with the IG9 mAb identified a 130 kD protein under non-reducing conditions and two proteins of 145 kD and 55 kD under reducing conditions in SDS-PAGE gels. Cell surface expression of the IG9 protein was detected after a 3 hr incubation with cytokine and persisted for up to 48 hr, with maximal expression at 4-9 hr. Tunicamycin, an inhibitor of N-linked glycosylation, reduced the reactivity of the IG9 mAb to TNF treated HUVE by 50% while transforming growth factor beta eliminated IG9 protein induction. To determine whether the IG9 protein is expressed in human vessels, sections of inflamed lung tissue and of atherosclerotic coronary arteries representing a range of lesion involvement were analyzed immunohistochemically. The IG9 mAb specifically identified lung vessels with extensive mononuclear inflammation within and around the vessel. Coronary artery sections with any type of pathology were also reactive with the IG9 mAb. Uninvolved vessels in both tissues were unreactive. Preliminary data indicated that the IG9 protein was detected in atherosclerotic vessels of the hypercholesterolemic Watanabe rabbit.

A 103 T CELLS FROM RT6-DEPLETED DIABETES RESISTANT (DR) BB RATS INDUCE ENDOTHELIAL MHC ANTIGENS,
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RT6 is a rat maturational alloantigen expressed on ≈50% of CD4⁺ and ≈70% of CD8⁺ T cells. *In vivo* depletion of RT6⁺ T cells induces autoimmune diabetes in DR-BB rats, <1% of which normally become hyperglycemic. Anti-RT6 antibody (Ab) treated DR rats develop pancreatic insulitis within 10 and diabetes within 30 days. Endothelial MHC class I hyperexpression and *de novo* MHC class II expression may be early events in inflammatory autoimmune insulitis. These endothelial changes are probably induced by lymphocyte-derived cytokines. We undertook to determine if the induction of autoimmunity in DR rats by depletion of RT6⁺ T cells concomitantly produces populations of RT6⁻ cells capable of inducing endothelial cell (EC) MHC antigen expression. Purified DR T cells were added to *in vitro* cultures of MHC histocompatible rat EC, cocultured for 4 days, and endothelial MHC antigen expression determined by flow cytometry. We studied: 1) total lymph node T cells from untreated DR rats; 2) *in vitro* purified RT6⁻ T cells from untreated DR rats; and 3) total T cells from DR rats depleted of RT6⁺ T cells *in vivo* by treatment with anti-RT6 Ab for 4-21 d. EC cultured alone and with IFN-γ were used as negative and positive controls, respectively. Results: Total T cells from intact DR rats and *in vitro* purified RT6⁻ T cells induced minimal EC class I hyperexpression and no class II expression. When stimulated by lectin (con A), these populations induced maximal EC MHC expression. In contrast, isolated RT6⁻ T cells obtained after 4 days of *in vivo* depletion induced substantial EC class I and II MHC expression without mitogen stimulation. Con A activation further augmented class II expression. Isolated RT6⁻ T cells obtained after 21 days of *in vivo* depletion (*i.e.* at the onset of diabetes) stimulated maximal EC MHC antigen expression in the absence of con A. Addition of con A did not augment expression. We conclude that depletion of RT6⁺ cells *in vivo* activates a population of RT6⁻ T cells that can induce EC MHC expression. Unsorted T cells and RT6⁻ T cells obtained by *in vitro* depletion are not activated. Altered T cell activity may lead to endothelial activation *in vivo* and contribute to an inflammatory process leading to diabetes.

A 104 NEOINTIMAL PROLIFERATION AND ENDOGENOUS GROWTH FACTOR RELEASE IN AN ORGAN CULTURE OF HUMAN CORONARY ARTERY,
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Intimal smooth muscle cell proliferation characterizes the onset of arterial atherosclerosis. Its progression may be influenced by the release of growth factors from the vessel wall in response to different stimuli. Investigations in humans have been hampered by the lack of readily available viable tissue. To overcome this we attempted to develop an organ culture model of human coronary artery intimal proliferation.
Segments of artery obtained from heart transplant recipients were cultured for 14 days in the presence of 30% foetal calf serum. Tissue viability assessed by ATP concentration (nmol/g wet weight) remained high during culture (240 ± 34 [SEM], day 0, n=18 and 272 ± 52, day 14, n=10). A new intimal layer was observed in histological sections of cultured artery and cell proliferation was demonstrated by ^{3}H thymidine incorporation (505 ± 80 dpm/μg DNA, n=13). Autoradiography showed that the majority of proliferating cells were in the neointimal layer with few dividing cells in the media. These cells were identified as smooth muscle cells with α-actin antibody.
In a separate set of experiments segments of artery were cultured in the absence of serum for a 24 hour period. Tissue viability was retained (221 ± 52, n=5, ns, vs day 0) and ^{3}H thymidine incorporation occurred (638 ±122, n=7). Conditioned medium taken from these experiments caused proliferation in a swiss 3T3 fibroblast bioassay (88 ± 15%, n=5, stimulation over basal medium).
In conclusion we have shown that intimal proliferation occurs in organ culture of human coronary artery. In addition endogenous growth factors are released from arteries cultured in the absence of serum. The characterization of these factors may help in the development of new anti-atherosclerotic therapies.

A 105 HEPARIN REMOVES ENDOGENOUS bFGF RELEASED INTO THE VESSEL WALL AFTER ARTERIAL INJURY,
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Heparin is known to inhibit smooth muscle cell (SMC) proliferation after balloon catheter denudation of the rat carotid artery and we have recently shown that initiation of this proliferation is mediated by bFGF released from damaged SMC. Although the mechanism for inhibition of SMC proliferation by heparin is unclear, we present evidence that this might be due to removal of bFGF from the injured vessel wall. Injection of [^{125}I]-bFGF into rats after arterial balloon catheter injury showed an approximate 100 fold increase in uptake of radiolabel when compared to endothelialized arteries. A single i.v. injection of heparin (300U/kg) caused an approx. 40% reduction of radiolabel in the denuded vessel wall with a concomitant increase of [^{125}I]-bFGF in the plasma. The half life of [^{125}I]-bFGF in the plasma was significantly prolonged after injection of heparin. Determination of endogenous bFGF in the vessel wall was also measured semiquantitatively by Western blot analysis on homogenates of rat carotid arteries. Injection of heparin after balloon catheterization caused a substantial reduction in extractable endogenous bFGF when compared to controls receiving saline. Since bFGF is known to induce SMC proliferation after injury these data suggest that inhibition of SMC proliferation by heparin is mediated in part by removal of released bFGF from the vessel wall. (Supported by NIH grants HL 400103 and HL 03174).

A 106 ANGIOTENSIN II STIMULATION OF RAT AORTIC SMOOTH MUSCLE CELLS LEADS TO RAPID TYROSINE PHOSPHORYLATION OF CELLULAR PROTEINS,
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Angiotensin II (AII) has important effects on vascular contraction and cell growth, yet the intracellular biochemical mechanisms mediating these events are not fully characterized. Protein tyrosine phosphorylation has been shown to be a critical component in the signalling pathways activated by many mitogenic growth factors, including platelet-derived growth factor (PDGF). To investigate whether AII stimulates protein tyrosine phosphorylation, serum-starved rat aortic smooth muscle cells were stimulated with saturating concentrations of either PDGF-BB homodimer (50 ng/ml), or AII (1 μM) for various times. Cell lysates were then subjected to immunoblot analysis with monoclonal antiphosphotyrosine antibodies. PDGF-BB treatment induced rapid tyrosine phosphorylation of several proteins, including the 185 kD PDGF-receptors, phospholipase C-γ (145 kD), and GAP (125 kD). AII induced tyrosine phosphorylation of at least two cellular proteins of apparent molecular weights 75 kD and 125 kD respectively. Tyrosine phosphorylation of these bands was observed within 1 min of AII stimulation and was maximal at 30 min. Tyrosine phosphorylation of these two proteins was also observed following treatment of the cells with the protein kinase C (PKC) agonist phorbol 12-myristate 13-acetate (PMA). Furthermore, when cells were pretreated with PMA for 24 hr to down-regulate endogenous PKC, AII stimulation failed to induce protein tyrosine phosphorylation. Thus, the AII-mediated protein tyrosine phosphorylation involves activation of PKC. These results indicate that AII signalling in vascular smooth muscle cells may be mediated in part by tyrosine phosphorylation of cellular proteins.

A 107 HEPARIN INHIBITION OF PDGF-INDUCED MITOGENESIS IN A-10 AND BOVINE AORTIC SMOOTH MUSCLE CELLS,
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Abnormal proliferation of vascular smooth muscle cells is believed to play a major role in the development of atherosclerosis, pulmonary arterial hypertension and in the failure of vascular surgical procedures. Physiologically, vascular smooth muscle cell growth is thought to be closely regulated by the actions of plasma and tissue mitogens and by the antiproliferative activities of heparin and heparan sulfate that are produced in the blood vessel wall. Although the antiproliferative effects of heparin are well documented the specific mechanisms of activity are still under investigation. The A-10 cell line (ATCC CRL 1476), derived from rat thoracic aorta provides a good model for heparin inhibition of vascular smooth muscle cell proliferation in response to serum and PDGF. These cells respond in a like manner to mitogens and heparin when compared to either vascular smooth muscle cells derived from bovine calf aorta or bovine calf pulmonary artery. We have found that heparin inhibits phosphotidylinositol 4-phosphate (PIP) kinase activity. In A-10 cells, heparin inhibits mitogenesis and reduces cellular levels of PIP_2 in a dose dependent manner. PDGF induced calcium mobilization, monitored using the intracellular dye fura-2, was attenuated by heparin pretreatment. Additional effects of heparin on PDGF induced signal transduction will be discussed. This work was supported by the American Heart Association-Hawaii Affiliate Grant #HF-02-91.

A 108 INHIBITION BY RETINOIDS OF PDGF-STIMULATED CALCIUM INFLUX AND CELL PROLIFERATION IN AORTIC SMOOTH MUSCLE CELLS, Lawrence J. Mordan and Mark Estacion, Molecular Oncology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI 96813
Retinoids have been shown to be effective antiproliferative agents in studies using a variety of murine fibroblasts cell lines and thus may be of use in controling the proliferation of vascular smooth muscle cells forming atherosclerotic plaques. We have evaluated the ability of various natural and synthetic retinoids to inhibit PDGF-stimulated mitogenesis and increases in the intracellular calcium concentration in the A10 rat aortic smooth muscle cell line. Confluent cultures stimulated with human recombinant PDGF (10 ng/ml) in the presence of plasma-derived serum (5%) were inhibited in a dose dependent manner from progressing to DNA synthesis by submicromolar concentrations of retinoids. Like fibroblasts, aortic smooth muscle cells were dependent upon physiological extracellular calcium concentrations (1.8 mM) for efficient cell cycle progression. The PDGF-induced intracellular calcium increase was composed of a transient peak in the intracellular calcium concentration followed by a new steady state level 2 to 3 fold higher than prestimulated concentrations. Various retinoids were found to inhibit the sustained calcium increase, which was shown to be due to the influx of extracellular calcium. The sustained calcium increase was insensitive to verapamil (10uM) and, from preliminary patch-clamp electrophysiological measurements, was dependent upon the functional expression of T-type, low voltage-activated plasma membrane calcium channels. These data suggest that retinoids, both natural and synthetic, may be effective prophylactic agents in the prevention of atherosclerotic plaques resulting from the proliferation of vascular smooth muscle cells. Supported by UPSHS Grant CA 51498.

A 109 ASSOCIATION OF TGFβ'S (1-3) WITH ARTERIAL STRUCTURAL REMODELLING IN A SHEEP MODEL OF PULMONARY HYPERTENSION, Elizabeth A. Perkett, Ron W. Pelton, Barbara Meyrick, Leslie G Gold and Harold L. Moses. Departments of Pediatrics, Medicine, Pathology, and Cell Biology. Vanderbilt University, Nashville, TN 37232-2986 and Department of Pathology, New York University Medical Center, New York, NY 10016

Chronic pulmonary hypertension is associated with arterial structural changes including increased medial thickness of muscular arteries and muscularization of peripheral arteries. We have previously found that TGFβ is present lung lymph from normal sheep and that increased levels are associated with the development of chronic pulmonary hypertension induced by chronic vascular injury[1]. In the present study we looked for the presence of TGFβ mRNA's and proteins in lung biopsy tissue from control sheep and from sheep during the development of pulmonary hypertension (secondary to 12 days of air embolization). In situ hybridization was performed using ^{35}S-labelled riboprobes made to murine constructs for TGFβ1, TGFβ2 and TGFβ3. TGFβ proteins were localized by immunohistochemistry using specific antibodies for TGFβ1, TGFβ2 and TGFβ3. The localization patterns in control sheep lung tissue were similar to findings in murine lungs[2]. Hybridization of all three TGFβ mRNA's was seen in the cell layer (smooth muscle cells and fibroblasts) underlying the respiratory epithelial of large preacinar airways. Hybridization was also seen in the medial layer of muscular arteries. Protein localized in the same areas, but also was found in respiratory epithelium. Following one day of injury, TGFβ1 and TGFβ3, but not TGFβ2, showed marked immunoreactivity in the edema fluid. This apparent increase in protein was not associated with a change in mRNA localization. After 12 days of injury, marked vascular remodelling was present in the peripheral arteries of the lung and immunoreactivity and mRNA hybridization for all three β's was seen in the medial and adventitial layer of these remodelled arteries. These findings suggest that TGFβ's may be associated with vascular remodelling of pulmonary hypertension. We speculate that the initial appearance of TGFβ1 and β3 in edema fluid may reflect injury and the source of the TGFβ may be both from inflammatory cells, as well as cells within the lung. The initial release of TGFβ may trigger a repair response leading to the expression of TGFβ's in remodelled arteries.

[1]Perkett et al. J. Clin. Invest. 86:1459-1464, 1990.
[2]Pelton et al. Am. J. Respir. Cell Biol. in press, 1991.

A 110 LIPOPROTEIN LIPASE (LPL) INCREASES LIPOPROTEIN RETENTION BY SUBENDOTHELIAL CELL MATRIX, Uday Saxena*, Michael G. Klein and Ira J. Goldberg, Department of Medicine, Columbia University, New York, NY, 10032
LPL is a normal constituent of the arterial wall. To explore the role of LPL in lipoprotein transport and retention, bovine milk LPL was added to cultured bovine endothelial cells grown on semipermeable filters prior to addition of VLDL or LDL. LPL increased ^{125}I-LDL (1-100 ug) retained by the subendothelial cell matrix by 5-24 fold; VLDL (200ug) retention increased 8 fold in the presence of LPL. This retention was decreased by monoclonal antibodies to LPL. The presence of LPL did not affect the binding of HDL to the matrix. ^{125}I-LDL binding to extracellular matrix increased when LPL was directly added to the matrix or was added to the basolateral side of the endothelial cell monolayers. LPL treatment did not aggregate LDL. Dissociation of LPL from LDL decreased LDL binding to glycosaminoglycans. In the presence of LPL, VLDL transport across the monolayer increased by 18%. High molar ratios of oleic acid to bovine serum albumin (3:1) in the medium increased VLDL transport approximately 30%. LDL transport increased 37% in the presence of VLDL and LPL and increased 42% when oleic acid was added to the media. We hypothesize that LPL on or within the arterial wall may promote atherogenesis by two mechanisms, 1) LPL mediated subendothelial cell matrix retention of VLDL and LDL may lead to conversion to more atherogenic forms and 2) lipolysis of triglyceride-rich lipoproteins on endothelial surfaces may increase VLDL and LDL transport across the arterial intima.
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A 111 ENDOTHELIAL CELLS INFECTED WITH CYTOMEGALOVIRUS FAIL TO EXPRESS HLA CLASS II ANTIGENS AND mRNA IN RESPONSE TO IFN-γ. DD Sedmak, DA Knight, ME Wohlford, WJ Waldman; Dept of Pathology, Ohio State Univ., Cols., OH 43210
The endothelial cells (EC) in Transplant-associated arteriosclerosis (TxAA) lesions have been shown to express HLA class II antigens, in contrast to the lack of expression in normal, nontransplanted vessels. Cytomegalovirus (CMV), which has been shown to infect ECs, has been epidemiologically associated with TxAA. In this study we tested the hypothesis that CMV infection of EC upregulates interferon-γ (IFN-γ) induction of HLA class II antigens and mRNA. EC infected in vitro for 0 to 72 hrs with CMV strain AD169 (MOI: 5 PFU/cell) or an EC-propagated clinical isolate were incubated with 50 to 400 units/ml of IFN-γ for 48 to 96 hours. Flow cytometry of these cultures revealed significantly fewer cells with surface staining for HLA DR as compared to induced but sham-infected cultures. The percent of cells failing to express HLA DR positively correlated with the number of infected EC, the latter as assessed by immunohistochemistry. Dual-labelling immunohistochemistry of infected cultures, utilizing monoclonal antibodies (mAb) to HLA class II monomorphic determinants and a mAb to a CMV early antigen, demonstrated that greater than 80% of infected cells expressed neither surface nor cytoplasmic HLA DR alpha, HLA DR beta, or HLA DP antigens, in contrast to expression of these antigens in noninfected cells within the cultures. Northern blot analysis, utilizing a full length cDNA probe to HLA DR, revealed that infected non-treated ECs did not contain HLA DR mRNA. Infected, IFN-γ treated EC cultures contained less than 10% of the HLA class II mRNA present in non-infected IFN-γ treated cultures. In conclusion, CMV infection of EC does not result in expression of HLA class II antigens and inhibits their induction by IFN-γ, a phenomenon which appears secondary to inhibition of HLA class II transcription. These findings, rather than provide a direct link between CMV-infected EC and TxAA, suggests a here-to-fore undescribed mechanism of CMV persistence.

A 112 Antisense Approach To Smooth Muscle Proliferation.

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Exuberant smooth muscle proliferation is the primary process responsible for restenosis after angioplasty as well as the long-term failure of coronary bypass grafts. The proliferating smooth muscle cells (SMCs) undergo a phenotypic change and express a variety of new genes not present in the quiescent state. We targeted two such genes, an oncogene c-myb and a nonmuscle isoform of a myosin heavy chain (NMMHC) with antisense sulfurthioate oligonucleotides. Both oligonucleotides suppressed SMC proliferation equally well (70%) *in vitro* in a rat aortic SMC cell line (SV40LT-SMC) while antisense c-myb was far superior (90%) in a mouse SMC cell line (BC3H1). The effect of oligonucleotides was concentration- and growth state-dependent. Treatment with antisense but not sense oligonucleotides resulted in a marked decrease in the corresponding mRNA and protein levels. Antisense NMMHC but not antisense c-myb, had no effect on proliferation of rat fat pad endothelial cells in culture and neither oligonucleotide affected quiescent SMCs.

In conclusion: 1) Antisense oligonucleotides could be useful for prevention of cellular proliferation and can be selectively targeted to proliferating rather than quiescent SMCs or endothelial cells; 2) Both c-myb and nonmuscle myosin play a key role in SMC proliferation.

A 113 INHIBITION OF PHOSPHATIDYLINOSITOL 4-PHOSPHATE KINASE BY HEPARIN. A POSSIBLE MECHANISM FOR THE ANTIPROLIFERATIVE EFFECTS OF HEPARIN.

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Heparin and related glycosaminoglycans are important modulators of vascular smooth muscle cell growth, and may be involved in pathological processes such as atherosclerosis. Since polyphosphoinositide metabolism is a major mechanism for regulating cellular activities, including proliferation, the effects glycosaminoglycans and polyanionic compounds on the activities of phosphoinositide kinases were characterized. Heparin and heparan sulfate caused dose-dependent inhibitions of rat brain cytosolic phosphatidylinositol 4-phosphate (PIP) kinase activity, with half-maximal inhibitory concentrations of approximately 0.5 and 5 µM, respectively. PIP kinase was also inhibited by several dextran sulfates, but was not sensitive to inhibition by keratin sulfate, chondroitin sulfate or hyaluronic acid. Polynucleotides and acidic polypeptides were only weakly inhibitory. Heparin did not alter either the PIP-or Mg^{2+}-dependence of PIP kinase. Addition of heparin to brain membranes suppressed PIP kinase activity without affecting phosphatidylinositol (PI) kinase activity. Heparin interfered with the ability of a GTP analogue to stimulate PIP kinase activity in these membranes, suggesting that it uncouples the kinase from an activating guanine nucleotide regulatory protein. In cultured A-10 vascular smooth muscle cells, heparin caused dose- and time-dependent inhibitions of [^{3}H]thymidine incorporation into DNA. Similar treatments with heparin reduced cellular levels of phosphatidylinositol 4,5-bisphosphate (PIP_2) without changing PI and PIP levels. Therefore, heparin-mediated inhibition of PIP kinase appears to lead to reductions of PIP_2 levels which may attenuate cellular proliferation.

A 114 ROLE OF IMMEDIATE EARLY GENES (IEG) IN AUTOCRINE GROWTH OF RAT AORTIC SMOOTH MUSCLE CELLS (SMC) IN-VIVO FOLLOWING VASCULAR INJURY,

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Vascular SMC proliferation is a major pathologic feature of arterial occlusive disease. Autocrine activation of growth-related IEG's and growth factor genes (GFG) in vascular SMC may play a key role in this process. Demonstrated is the sequential in-vivo induction of several IEG and GFG mRNA's in rat aortic SMC after aortic balloon de-endothelialization (BDE). BDE leads to rapid induction of IEG's (c-fos, c-jun, c-myc, egr-1, JE, thrombospondin-TSP). This is followed by later activation of GFG's (PDGF-A, TGF-beta 1, bFGF). Evidence for an IEG mediated autocrine growth process is further supported by the spontaneous re-activation of SMC IEG's (c-myc, JE, TSP) at 7 days after BDE which is followed less than 1 day later by a 2 fold increase in SMC thymidine incorporation. Blood-borne factors are necessary for full IEG expression since BDE in the absence of blood results in only partial induction. Further, the dependence of de-novo protein synthesis for GFG activation is demonstrated by attenuation of GFG mRNA induction after BDE in rats treated with cyclohexamide (7.5mg/kg i.p.). These data support the hypothesis of SMC autocrine growth in-vivo and suggest that blood-borne products are necessary for full IEG expression and IEG proteins may be necessary for GFG activation and DNA synthesis.

A 115 ACTIVATION OF T CELL SUBSETS BY CYTOMEGALOVIRUS-INFECTED ENDOTHELIAL CELLS.

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Cytomegalovirus (CMV), a source of serious complications among immunosuppressed individuals, infects endothelial cells *in vivo*, and has been epidemiologically associated with atherogenesis, allograft rejection, and transplantation-associated accelerated arteriosclerosis (TxAA). As the interface between the immune system and underlying tissues, the endothelium may be of primary importance in mediating pathogenic immune responses to CMV. In an effort to model such immune interactions *in vitro*, and to evaluate the antigen presenting potential of the endothelium, we have investigated responses of highly purified (monocyte-depleted) T lymphocytes to CMV-infected allogeneic human umbilical vein endothelial cells (HUVE). Proliferation assays demonstrated dramatically enhanced responses by CMV-seropositive donor-derived T cells cocultured with CMV-infected HUVE, as compared to those elicited by non-infected cells. Similarly, as determined by limiting dilution analysis of IL-2-producing cells, the frequency of T cells responding to infected HUVE was generally found to exceed by an order of magnitude those responding to uninfected cells. Responses of CMV-seronegative donor-derived T cells were minimal in all assays regardless of the CMV status of the stimulator population. Analyses of T cell subsets revealed a substantial contribution by $CD4^+$ cells to the observed responses, as demonstrated by their consistently enhanced activation (proliferation and IL-2 production) by CMV-infected HUVE. Responses of $CD8^+$ populations, however, have varied among donors. The marked activation of $CD4^+$ cells is particularly intriguing since we have shown that CMV-infected HUVE do not express HLA Class II antigens, and, indeed are refractory to their induction by IFN_γ. These studies show that purified T-cells can respond to CMV in the exclusive context of endothelial cells. As TxAA is characterized by sub-endothelial T cell infiltration, these findings support a role for CMV/endothelial interaction in TxAA pathogenesis, and perhaps in classical allograft rejection.

A 116 NON-GLYCOSYLATED MURINE RECOMBINANT SPARC EXHIBITS BIOLOGICAL ACTIVITY ON ENDOTHELIAL CELLS.

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The Ca^{+2} binding glycoprotein Sparc alters endothelial cell morphology and is associated with angiogenesis in vitro. To determine whether glycosylation is critical for biological activity, murine recombinant Sparc (rSparc) was expressed as a secreted protein in Saccharomyces cerevisiae. cDNA comprising the 5'-triosephosphate isomerase (TPI) promoter, the barrier signal sequence and third domain, the α-factor KEX2 site, Sparc, and TPI terminator were sequentially ligated in a 5'-3' orientation. The single glycosylation site was mutated in one construction to prevent synthesis of a hyperglycosylated product. Immunoblot analysis of wild-type rSparc expressed in an mnn9 (glycosylation defective) mutant yeast strain demonstrated secretion of a product of reduced M_r 40,000-41,000. Non-glycosylated, mutated rSparc expressed in a wild-type yeast strain exhibited a reduced apparent M_r of 40,000 on SDS-PAGE. Both wild-type and mutated rSparc, as well as Sparc from murine parietal yolk sac cells, induced a rounded morphology in spread bovine aortic endothelial cells (BAEC). BAEC stained with rhodamine-phalloidin showed a diminution of stress fibers, and a relocalization of actin filaments primarily at the cell margins, prior to overt cell rounding. Both wild-type, and mutated rSparc could compete for the binding of ^{125}I-Sparc to BAEC. These data indicate that glycosylation of SPARC might not be required for biological activity. The availability of rSparc, in conjunction with site-directed mutagenesis, facilitates investigation of Sparc sequences that are critical for biological activity.

Early Effector Changes - Vascular Cell Gene Regulation; Adhesion Pathobiology

A 200 GMP140 (PADGEM/CD62) BINDS TO CHRONICALLY-STIMULATED BUT NOT RESTING CD4+ T LYMPHOCYTES VIA SIALYLATED AND SULFATED PROTEOGLYCAN DETERMINANTS,

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CD62 is a 140 kDa glycoprotein expressed by activated platelets and endothelial cells. CD62 has been shown to play an important role in adhesive interactions between granulocytes, platelets and vascular endothelial cells during inflammation. Here using a soluble form of CD62 we show that CD62 binds to antigen-stimulated CD4+ T cells. Freshly isolated CD4+ T cells do not bind GMP140, but upon priming and subsequent stimulation with alloantigen these cells express CD62-reactive molecules on their cell surface. The binding of CD62 to stimulated T-cells is almost completely abolished by pretreatment of T cells will neuraminidase or trypsin and is Ca^{2+} dependent. Binding of CD62 to chronically stimulated T cells can be blocked with soluble sulfated glycans such as dextran sulfate, fucoidan, and heparin. The ability of activated T cells to bind CD62 may facilitate the adhesion of primed T cells at sites of platelet and endothelial cell activation in acute or chronic vascular injury.

A 201 BIMODAL PROLIFERATION OF FIBROBLASTS IN RESPONSE TO IL-1 CORRELATES WITH PDGF SECRETION AND RECEPTOR EXPRESSION, AND WITH MAP2-KINASE ACTIVITY,

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Interleukin-1 (IL-1) stimulates proliferation of fibroblasts as well as smooth muscle cells indirectly via autocrine induction of PDGF-AA. Our current studies demonstrate a bimodal proliferative response in fibroblasts to IL-1. Low concentrations of IL-1 (0.25 ng/ml) induce more extensive PDGF A-chain gene expression than higher concentrations (10 ng/ml). Additionally, higher concentrations of IL-1 also decrease PDGF receptor α-subunit mRNA and protein expression, whereas PDGF receptor β-subunit expression is not altered. Hence, IL-1 induces greater proliferation of fibroblasts at low concentrations than at high concentrations. As a consequence, the required elements for the autocrine PDGF-AA loop induced by low concentrations of IL-1 are less available at higher concentrations of IL-1. We further evaluated the possible mechanisms by which the bimodal response of fibroblasts to low versus high concentrations might be manifest. Neither low nor high concentrations of IL-1 (receptor occupancy ca. 4-15% versus 50%, respectively) modulate the affinity nor the number of IL-1 receptors within the first 30 minutes after addition of IL-1. However, within the first hour after addition of IL-1, we identified a difference in the timecourse of transiently activated MAP2-kinase between low versus high concentrations of IL-1. Low concentrations of IL-1 induced a later, but more sustained increase in MAP2-kinase activity than high concentrations. This temporal shift in induction of MAP2 kinase may be involved in the differential response to low versus high concentrations of IL-1. This work was supported in part by NIH grants HL-18645 and HL-03174 and a grant from Bristol-Myers Squibb Co.

A 202 ENDOTHELIAL CELL ADHESION MOLECULE INTRODUCTION BY α-THROMBIN.

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Thrombin is a serine protease which cleaves fibrinogen to form fibrin clots. It has recently been demonstrated to induce calcium fluxes in cells which express its specific receptor. The role of thrombin in leukocyte-endothelial cell (EC) adhesion was assessed by quantitating mononuclear leukocyte binding to resting or thrombin-treated (4 hr) human umbilical vein EC monolayers. Leukocyte binding increased by 40%, and was a consequence of augmented surface expression of ICAM-1, and induced expression of ELAM, as determined by flow cytometry. Northern blot analysis demonstrated an induction of mRNA for these adhesion molecules in a dose response fashion, and with a time course, paralleling the surface expression. Secreted IL-1 was not responsible for the noted induction, as determined by failure of neutralizing anti-IL 1α or β to inhibit the response. The protein kinase C inhibitor staurosporine completely abrogated adhesion molecule expression, as did the protein synthesis inhibitor cycloheximide. We conclude that the elaboration of thrombin in micro- and macrothrombi may lead to an endothelial proadhesive state for circulating mononuclear leukocytes. This may be a trigger for early events in inflammatory forms of vascular injury, including atherosclerosis.

A 203 USE OF AORTIC RINGS AS AN IN VITRO MODEL FOR STUDYING CHANGES IN GENE EXPRESSION DURING VASCULAR INJURY.

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Vascular injury leads to many changes in gene expression, often resulting in hypertrophy or hyperplasia. In vitro models for cell injury frequently use cell culture techniques to study the cellular changes and molecular events. We have found that incubation of intact rat or rabbit aortic rings in a serum-free medium, using conditions where the rings are metabolically intact for up to 96h, leads to changes in protooncogene expression, extracellular matrix components and DNA synthesis that reflect the changes reported to occur during vascular cell injury in other experimental models. During the initial 4h incubation of rat aortic rings, c-myc, c-fos and HSP-70 were induced, and in the presence of cycloheximide, superinduction was observed. During the incubation time period between 6-24h steady state mRNA levels for fibronectin increased several-fold, and both collagen and elastin mRNA levels decreased markedly. A selective induction of the alternatively spliced form of fibronectin designated EIIIA was apparent. These changes in extracellular matrix components did not occur during the initial 4h incubation. Changes in actin isoforms also were observed with a decrease in α-actin between 6-24h. The changes occurring within a 24h incubation period were not affected by addition of polymyxin B, suggesting endotoxin was not a factor. Autoradiography showed that between 24-72h incubation of rabbit aortic rings, the number of cells incorporating labeled thymidine into DNA increased several-fold, primarily in medial smooth muscle cells. Addition of dibutyryl cAMP selectively reduced the induction of fibronectin expression within the initial 24h incubation, and reduced thymidine incorporation at the later times. Thus, in vitro incubation of aortic rings offers a convenient experimental model to study phenotypic changes in vascular cell types under experimental conditions where the cells are differentiated, in contact with an extracellular matrix similar to that found in vivo, and where functional interrelationships between endothelium and smooth muscle have been clearly documented.

A 204 ENHANCEMENT OF FIBRONECTIN BINDING TO ADHERENT CELLS BY LIPOPROTEINS.

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Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) rapidly enhanced fibronectin (Fn) binding to normal and osteosarcoma fibroblastic cells. Enhancement was 2-4 fold over control levels. LDL was active below 5 ug/ml. Binding studies indicate that LDL increased the number of Fn binding sites but this increase was not due to an increase in receptor synthesis since the binding experiments were done in the presence of cycloheximide. LDL and VLDL were able to enhance Fn binding to Familial Hypercholesterolemic (FH) fibroblasts. In addition, acetylation of LDL did not diminish Fn binding in non-FH cells. Together, these results suggest the LDL receptor and positively charged amino acids on apo-B do not play a role in this effect. LDL had to be present with Fn to evoke an enhancement response. Cells preincubated with LDL and then washed extensively bound normal levels of iodinated Fn. The region of fibronectin responsible for cell surface and extracellular matrix binding has been localized to the 70-kDa amino-terminal fragment of Fn. LDL enhanced the binding of the 70-kDa fragment of Fn but did not enhance binding of the carboxyl-terminal fragment of Fn that includes the RGD cell adhesion site. Our results lead us to hypothesize that an apolipoprotein, or other lipid binding protein, interacts with Fn and a cell surface receptor and enhances Fn binding.

A 205 MACROPHAGE-COLONY STIMULATING FACTOR (M-CSF) GENE EXPRESSION IN VASCULAR CELLS AND IN EXPERIMENTAL AND HUMAN ATHEROSCLEROSIS.

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The infiltration of monocytes into the vascular wall and their transformation into lipid-laden foam cells characterizes early atherogenesis. We examined the expression of macrophage-colony stimulating factor (M-CSF), a protein which regulates monocyte growth and differentiation, by cultured human vascular endothelial (EC) and smooth muscle cells (SMC). Bacterial endotoxin (LPS), interleukin-1 (IL-1α), or tumor necrosis factor (TNFα) induced M-CSF mRNA accumulation in a time- and concentration-dependent fashion in both EC and SMC. Vascular cells stimulated with these agents also showed increased fluorescent antibody staining for M-CSF protein and released immunoreactive M-CSF in a time-dependent manner. In contrast, iron-oxidized low density lipoproteins failed to consistantly increase M-CSF mRNA or the synthesis and secretion of immnuoreactive protein. Northern analysis of mRNA isolated from the atheromatous aorta of rabbits fed a 1% cholesterol diet showed elevated M-CSF mRNA compared to controls. Furthermore, polymerase chain reaction analysis of M-CSF mRNA in human atheromata showed higher levels than found in non-atherosclerotic arteries and veins. As we found no mRNA for the M-CSF receptor, c-fms, in cultured EC or SMC by Northern analysis, macrophages are likely the primary target for M-CSF within atheromatous vessels. We therefore investigated the effects of M-CSF on monocyte functions related to foam cell development. Treatment of cultured human monocytes with recombinant human M-CSF (10^3 U/ml) led to the accumulation of mRNA for the acetyl-LDL (scavenger) receptor and apolipoprotein E (apo E) over a 72 hr period. These studies establish that vascular EC and SMC produce substantial M-CSF in response to a variety of stimuli. The local production of M-CSF during atherogenesis may contribute to macrophage survival and proliferation or activate specific macrophage functions such as expression of the scavenger receptor and secretion of apo E.

A 206 THE EXTRACELLULAR MATRIX SURROUNDING VASCULAR SMOOTH MUSCLE CELLS CONTROLS THEIR PROLIFERATION AND GENE EXPRESSION.

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The dedifferentiation of vascular smooth muscle cells (VSMCs) is likely to be a key event in the development of many vascular diseases. In the intact vessel, VSMCs are embedded in an interstitial matrix composed predominantly of collagen type I and fibronectin and surrounded by a basement membrane consisting of collagen type IV, laminin, and other components. When removed from the vessel and plated onto tissue culture plastic, VSMCs dedifferentiate, proliferate, and are highly invasive. Since the extracellular matrix (ECM) can play an important role in controlling the proliferation and differentiation of cells in many other tissues, we examined the effect of a reconstituted basement membrane (Matrigel) on the behavior of isolated VSMCs. In contrast to their behavior on plastic, VSMCs plated onto Matrigel did not proliferate or initiate new DNA synthesis, even when cell density was low and serum growth factors were present. Instead, the cells migrated to form multicellular networks exhibiting a cellular organization with features similar to those seen in the intact aorta. Electron microscopy revealed the presence of contractile elements within the cells, while Northern and Western analyses indicated that smooth-muscle-specific mRNAs and proteins were re-expressed by these cells. "Differentiated" VSMCs exhibited a reduced invasive potential in which net matrix metalloproteinase (MMP) activity was decreased through a reduction in 72kD gelatinase mRNA expression, the major MMP expressed by these cells. In addition, the "differentiated" VSMCs no longer expressed a proliferation-dependent gene with homology to the ID (inhibitor of differentiation) gene, which was expressed at high levels in proliferating VSMCs. These results suggest that the ECM surrounding VSMCs in vivo plays an important role in controlling their proliferative, differentiative, and invasive potential.

A 207 SEX STEROIDS INCREASE MONOCYTE BINDING TO ENDOTHELIAL CELLS.

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A significant event in the atherosclerotic process is believed to be the adherence of monocytes to the endothelial cell surface and their subsequent migration into the intima. We studied the effect of sex steroids on human monocyte binding to a monolayer of human umbilical vein endothelial cells (HUVEC) using ^{3}H-adenine labelled monocytes. Preincubation of monocytes and HUVEC with 17β-estradiol at pharmacological (10^{-5}M) and physiological (10^{-10}M) concentrations increased monocyte binding to HUVEC by 52% and 49% respectively ($p<0.05$). Progesterone in pharmacological (10^{-5}M) and physiological (10^{-9}M) concentrations and the combination of 17β-estradiol (10^{-10}M) and progesterone (10^{-9}M) also increased monocyte binding by 66%, 64% and 66% respectively ($p<0.05$) but pharmacological concentrations (10^{-5}M) of the combination of estradiol and progesterone had no effect. Further experiments demonstrated that preincubation of monocytes but not HUVEC with sex steroids increased binding to HUVEC. Sex steroids did not influence non-specific binding of monocytes to gelatin plates.

These results suggest a possible regulatory role for sex steroids in the binding of monocytes to endothelial cells through an effect on the monocytes. This may be due to altered expression of adhesion molecules on monocytes or alteratively an indirect effect due to release from monocytes of factors that affect endothelial cells. These observations may have important implications in understanding the link between oral contraceptives and arterial disease.

A 208 FETAL SPECIFIC GENES IN RABBIT AORTIC SMOOTH MUSCLE CELLS, David K.M. Han and Gene Liau, Laboratory of Molecular Biology, American Red Cross, Rockville, MD 20855

We have employed two approaches to study the changes that vascular smooth muscle cells (SMC) undergo during blood vessel development. 1) Identification of genes that are expressed in fetal but not in adult SMC. 2) Analysis of the expression of smooth muscle contractile genes. Here, we report the isolation of a fetal specific gene (F-31) and identification of insulin-like growth factor-II (IGF-II) as genes expressed in fetal but not in adult SMC. F-31 recognized a 2.3 kb mRNA which was high in fetal aortic smooth muscle (20-25 days gestation), low but detectable in newborn smooth muscle, and completely shut off in the adult. IGF-II expression was detectable in fetal smooth muscle but not in newborn or adult smooth muscle. By contrast, mRNA levels of α-smooth muscle actin, and α-tropomyosin were unchanged, whereas smooth muscle myosin heavy chain mRNA expression increased during this period. Analysis of cultured SMC obtained from the respective tissues revealed *in vitro* F-31 and IGF-II expression mimicked *in vivo* expression. Next, we examined the influence of culture conditions on the expression of these genes. We found F-31 and IGF-II were both expressed in proliferating, preconfluent fetal cells but were down regulated in postconfluent fetal SMC. However, we found that postconfluent cells treated with 0.5% serum, insulin and transferrin expressed significant levels of F-31 but not IGF-II. Morphological comparison of SMC in culture revealed that proliferating fetal and adult SMC were very similar. However, at high cell density, fetal cell were unable to assume the "hill and valley" morphology, typical of adult SMC. Sequencing of the 1.2 kb F-31 cDNA and subsequent database analysis of F-31 showed that it is similar to the H19 gene. H19 is a developmentally regulated gene in a variety of tissues and is also induced during myoblast differentiation. Our studies on the developmental regulation of F-31 is consistent with the notion that this gene may be involved in SMC development and differentiation.

A 209 INTERACTION AND REGULATION OF AN ENDOTHELIAL-SPECIFIC SURFACE ANTIGEN BY ENDOTHELIAL CELL GROWTH FACTORS.

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An endothelial specific surface antigen (ESSA), detected by the monoclonal antibody H513E3, has been found on vascular endothelial cells, explants of bone marrow endothelium and some marrow stromal cell lines. A somatic cell hybrid line (H5Scl 1.4.4) which also expresses ESSA and Factor VIII-related antigen was used to investigate the modulation of ESSA by a number of regulators including endothelial cell growth factors. Binding of H513E3 was significantly reduced when H5Scl1.4.4 cells were incubated with either endothelial cell growth factor (ECGF) or acidic fibroblast growth factor (aFGF). This was slightly but not significantly influenced by addition of heparin (50ug/ml). Other growth factors and mediators (b-FGF, PDGF-BB, TNFα, TGFβ, GM-CSF, IL-1α, IFN-gamma, LPS, TPA) were tested to determine the specificity of this reduction of antibody binding. Consistently, ECGF, aFGF and bFGF incubation reduced binding by 60-80% and other agents had either a lesser or no effect. TPA however, reduced ESSA sites significantly. The possibility that FGF's bound directly to ESSA was discounted since there was no competition with H513E3 MAb binding at 4°C. Also crosslinking experiments using ^{125}I-aFGF indicated that H513E3 MAb did not immunoprecipitate the FGF receptor complex. Kinetics of ESSA downregulation are relatively slow compared to standard receptor-ligand interactions. RNA synthesis inhibitors appear to partly overcome the degree of aFGF induced downregulation. This suggests an indirect mechanism of ESSA regulation by FGF's which may involve alteration of RNA synthesis.

A 210 LIPOPOLYSACCHARIDE (LPS)-INSTRUCTED, CRYOPRESERVED, HUMAN MONOCYTES (Mo), SEQUENTIALLY CONVERT PLASMA FIBRINOGEN TO FIBRIN, UPON THROMBIN GENERATION, AND SUBSEQUENTLY, PREFERENTIALLY LYSE THE POLYMERIZED FIBRIN FORMED

by Kierulf, P[1], Osnes LT[1], Andersen Å-B Westvik[1], Halvorsen S[2]. The R and D Group, Clinical Chemistry Department[1], Hem. Res.Lab.[2], Ulleval University Hospital, N-0407 Oslo, Norway.

Aim: To examine if cryopreserved, LPS-instructed human Mo may: 1) give rise to fibrin formation in plasma through their thrombin generating (Tissue Factor-linked) potential, 2) lyse the fibrin formed by expressing uPA (i.e. may overrule LPS-induced PAI-2 expression), and 3) preferentially lyse polymerized fibrin (as opposed to soluble fibrin.
Materials and methods: Ampuled (6×10^6 cells/ml), cryopreserved human blood mononuclear (Mo and lymphocytes Ly) were thawed and seeded (2.4×10^4 Mo/well - RPMI-FCS 5%), Mo adhered (2 h 37°C, 5% CO_2), Ly washed off, remaining Mo LPS-stimulated (2µg/well, 4 h) to induce PCA. PCA expression ascertained on duplicate wells, and Mo subsequently incubated with heparinized plasma (15 IU/well). Pro-thrombin activation leading to thrombin generation, fibrin formation and soluble fibrin appearance, were monitored by ELISA F_1+F_2 (Behring Werke) fibrinopeptide A (FPA, Imco, Stockholm) and tPA-chromogenic assay (Kabi, Stockholm) respectively. Fibrinogen and fibrinolysis were monitored by ELISA FgDP and ELISA FbDP (Organin, Technika) respectively.
Results: Fibrin formation (FPA-appearance) was time-related to pro-thrombin activation (F_1+F_2-appearance) indicating Tissue Factor-induced pro-thrombin-cleavage probably to be responsible. Subsequently, polymerized fibrin (as opposed to fibrinogen and soluble fibrin) (FbDP-appearance) was preferentially attacked. Fig. 1.

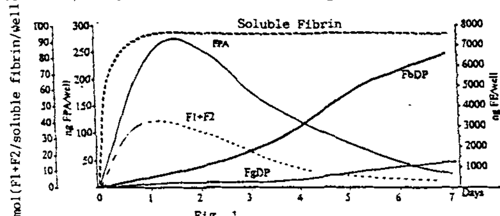

Fig. 1

Conclusions: Repair-delineation, i.e. Tissue Factor-induced conversion of fibrinogen to fibrin upon pro-thrombin cleavage and thrombin generation, whether as part of hemorrhagic arrest, microbial invasion prevention, or tumor encapsulation, is followed by resolution-fibrinolysis by preferential degradation of the inter-cellular polymerized fibrin formed.

A 211 VASCULAR SMOOTH MUSCLE CELL INVASION BUT NOT CHEMOTAXIS IS INHIBITED BY BUFFERING INTRACELLULAR CALCIUM.

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The development and progression of atherosclerosis depends on the migration and proliferation of vascular smooth muscle cells (VSMC) and their interaction with extracellular matrix (ECM). We have found previously that this interaction is due in part to the invasion of reconstituted basement membrane (Matrigel) by VSMC in response to PDGF. We also noted under video micrography that VSMC exhibit spontaneous membrane deformations (SMD). We hypothesized that intracellular calcium dynamics may have an important role in VSMC invasion and SMD. VSMC were exposed to BAPTA-AM (an avid buffer of intracellular calcium after intracellular deesterification) for 45 min. Chemotaxis and invasion in response to PDGF were then examined in a 4 hr modified Boyden assay. BAPTA treated VSMC no longer exhibited SMD and showed a 95% reduction in invasive ability as compared to controls. However, attachment and chemotaxis (migration in response to an attractant) on ECM components (Laminin, Collagen I, or Collagen IV) were unaltered by intracellular calcium buffering. These observations were independent of animal age and cell passage number. Thus, SMD and PDGF mediated invasion of reconstituted basement membrane by VSMC are markedly inhibited by intracellular calcium buffering while attachment and chemotaxis are unaltered. We conclude that the mechanisms governing spontaneous membrane deformations and invasion (but not attachment and chemotaxis) require mobilization of intracellular calcium.

A 212 ANGIOPEPTIN, A SOMATOSTATIN ANALOGUE, DECREASES RAT LEUKOCYTE ADHESIVENESS TO ENDOTHELIAL CELLS,

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It has previously been shown that somatostatin and the stable analogue Angiopeptin (AP) decrease endothelial cell (EC) adhesiveness for normal leukocytes (L). Here we examined whether exposure of L to AP modifies L adhesiveness to unstimulated and IL-1b-activated EC. Monolayers of EC bind 274+12 L/mm2. Exposure of L for 1, 4 and 24 hours to AP (1ug/ml) reduces significantly ($p<0.05$) adhesion of L to 188+10, 185+8 and 172+3 L/mm2, respectively. EC exposed to IL-1b (100U/ml) bind 381+17 L/mm2. Exposure of L for 1, 4 and 24 hours to AP (1ug/ml) reduces significantly ($p<0.05$) binding of L to the IL-1b-activated EC to 237+8, 254+11 and 248+13 L/mm2, respectively. Thus, increased expression of adhesion molecules induced in EC by IL-1b is not sufficient to sustain L adhesion to EC on the similar level to this obtained with L not exposed to AP. AP had no effect on the expression of the adhesion molecules ICAM-1 (CD54) and LFA-1 (CD11a/CD18) by EC and L, as asessed by fluorescence-activated cell sorting. This suggests that AP may modulate either expression of other molecules than ICAM-1 and LFA-1 and/or modulate the affinity of adhesion molecule(s) experssed by L. In summary, these results demonstrate that exposure of L to AP (1ug/ml) reduces within one hour, basal level of L adhesiveness to EC, and abolishes increase in L adhesiveness to IL-1b-stimulated EC.

A 213 REGULATION OF TGFα GENE EXPRESSION BY GLUCOSE AND GLUCOSAMINE IN VASCULAR SMOOTH MUSCLE CELLS,

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Accelerated atherosclerosis and other vascular complications are hallmarks of diabetes mellitus. Abnormal cell proliferation triggered by growth factors could participate in the development of these conditions. We have therefore investigated the effects of glucose and insulin on the transcription of one growth factor found in vascular smooth muscle, TGFα. Glucose at 30mM, but not insulin, stimulated transcription of a TGFα-luciferase construct twofold when transfected in cultured rat aortic smooth muscle cells. The effect was apparent by 30 min and maximal by 12-18 h. Glucosamine was much more potent than glucose in this regard, with a 12-15 fold stimulation seen with 5mM glucosamine. Equivalent effects of sugars on the levels of the native mRNA for TGFα were also seen in these cells. Pharmacologic experiments using azaserine to block activity of the enzyme that synthesizes glucosamine (glutamine:fructose-6-P amidotransferase) are consistent with the involvement of a metabolite of glucose in the hexosamine pathway as being the intracellular mediator of these effects on transcription. Protein kinase C, although a regulator of TGFα transcription in some circumstances, is probably not involved in signalling the effects of glucose. The region of the TGFα promoter that confers glucose sensitivity has been mapped to a proximal 130 bp fragment of that gene.

A 214 INSULIN-LIKE GROWTH FACTOR-1 MODULATES AGGREGANT INDUCED $[Ca^{2+}]_i$ TRANSIENTS IN HUMAN PLATELETS, Alykhan S. Motani, Daniela Salvemini, Erik E. Anggard and Gordon A.A. Ferns, The William Harvey Research Institute, St Bartholomew's Hospital Medical College, London EC1A 6BQ, U.K.
Insulin-like growth factor-1 (IGF-1) potentiated thrombin, collagen and ADP induced aggregation of human platelets ($p<0.0005$). The effect was dose dependent with a threshold of 30 nmol/l and was maximal for IGF-1 concentrations between 0.1 and 0.3 umol/l. Peak potentiation above control values was 258±54% using 10-30 mU/ml thrombin and 369±176% for 0.2-0.5 ug/ml collagen with washed platelets, and 77±22% using ADP (0.5-2.0 ug/ml) with platelet rich plasma. The transient elevation in cytosolic calcium ($[Ca^{2+}]_i$) induced by platelet aggregants was monitored using Fura-2 loaded washed platelets in a Perkin-Elmer LS50 luminescence spectrophotometer (excitation wavelength 340/380 nm, emission wavelength 505 nm) equipped with a constant stirring facility. Although preincubation of platelets with IGF-1 did not cause an elevation in basal $[Ca^{2+}]_i$, it was associated with an enhanced $[Ca^{2+}]_i$ response to subaggregatory doses of both thrombin and collagen. At these agonist concentrations there was an increase in integrated $[Ca^{2+}]_i$ values of 24±12% (n=5, $p<0.05$, t=0-100 sec after addition of thrombin) and 57±22% (n=5, $p<0.05$ t=50-400 sec after addition of collagen). Peak levels of $[Ca^{2+}]_i$ attained after collagen stimulation were increased by 74±27% ($p<0.02$) in IGF-1 treated platelets, but were not significantly augmented when thrombin was the aggregant. IGF-1 did not have a significant effect on platelet degranulation measured as ^{3}H-5HT release from loaded washed platelets.
The William Harvey Research Institute is funded by a grant from ONO Pharmaceutical Co., Japan. GAAF is a recipient of a grant from the University of London Central Research Fund.

A 215 LYSOPHOSPHATIDYLCHOLINE INDUCES MONOCYTE EXPRESSION OF HEPARIN-BINDING EGF-LIKE GROWTH FACTOR mRNA, Toru Nakano[1], Elaine W. Raines[1], Judith A. Abraham[2], Michael Klagsbrun[3] and Russell Ross[1], [1]Department of Pathology, University of Washington, Seattle, WA 98195; [2]California Biotechnology, Mountain View, CA 94043; [3]Children's Hospital and Harvard Medical School, Boston, MA 02115
Heparin-binding EGF-like growth factor (HB-EGF) is a recently described growth factor secreted by activated human macrophages and shown to be a potent stimulant of smooth muscle cell proliferation. However, the physiologic stimuli for its release and its possible role in the stimulation of smooth muscle cell proliferation in atherosclerosis remain undefined. The level of lysophosphatidylcholine (lysoPC), a product of inflammation, is reported to increase in blood of patients with atherosclerosis and is a component of oxidatively modified low-density lipoprotein. Addition of lysoPC to human monocytes induced the expression of HB-EGF mRNA and enhanced the release of heparin-binding mitogenic activity. Bacterial lipopolysaccharide (LPS) also increased HB-EGF mRNA level, and lysoPC further enhanced the LPS-induced response. Glucocorticoid, a potent anti-inflammatory drug, inhibited both lysoPC- and LPS-induced mRNA expression, suggesting that HB-EGF is one of the factors involved in such inflammatory processes. Cycloheximide inhibited LPS-induced HB-EGF mRNA expression, but it did not inhibit lysoPC-induced expression, which indicates that the LPS-stimulated mechanism for HB-EGF mRNA expression involves new protein synthesis while lysoPC-stimulated expression is independent of protein synthesis. The activation of mononuclear cells by lysoPC, which results in production of HB-EGF, may be one of the important events involved in the smooth muscle proliferative response in atherogenesis.
This work was supported in part by NIH grants HL-18645 and HL-03174 and a grant from Bristol-Myers Squibb.

A 216 EFFECTS OF VPF ON LEUKOCYTE ADHESION TO ENDOTHELIAL CELLS IN VITRO,
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We have examined the effects of vascular permeability factor (VPF) on the induction of leukocyte adhesion activity in human endothelial cells in culture. We found that the exposure of human aortic endothelial cells to VPF (5 - 50 ng/ml) resulted in increased adhesion of monocytes, monocytic cell lines (J774, THP-1, U937) and a lymphocytic cell line (Ramos) to the treated cells. The binding of neutrophils and HL-60 cells was not effected by the VPF treatment. Maximal induction of adhesion occurred 4-6 hours following the addition of VPF, and was totally blocked by an antibody to VLA4, a receptor for VCAM-1. Human umbilical vein endothelial cells, which respond to VPF mitogenically, did not exhibit an induction of leukocyte adhesion following VPF treatment. These results suggest that VPF may play a variety of different roles in vascular processes.

A 217 INTERLEUKIN-4 (IL-4) MODULATES THE RESPONSE OF HUMAN VASCULAR ENDOTHELIAL CELLS TO OTHER CYTOKINES, Ewa M. Paleolog, Githa Aluri and Marc Feldmann, Charing Cross Sunley Research Centre, Hammersmith, LONDON W6 8LW, U.K.

Following activation, vascular endothelial cells are able to regulate the response of circulating blood cells, through the expression of adhesion molecules and secretion of soluble mediators such as cytokines. In the present study we demonstrate that the T-lymphocyte product IL-4 modulates these endothelial cell functions both directly, and in response to other cytokines.

IL-4 stimulated human umbilical vein endothelial cells (HUVEC) to secrete IL-6 at levels comparable to those observed in response to IL-1 and TNF. However, production of IL-6 in response to IL-4 was much slower, commencing only 6-24 hours after the addition of stimulus. When IL-4 was added to HUVEC in combination with sub-optimal doses of IL-1ß and TNF, marked synergy was observed, as early as 6 hours after cytokine addition, when IL-4 alone has little or no effect on HUVEC. Similar results were obtained using cultures of human saphenous vein (HSVEC) and human umbilical artery endothelial cells (HUAEC). Although IL-4 failed to induce secretion of the haematopoietic growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF), it significantly enhanced release of this cytokine in response to both TNF and IL-1ß in all three endothelial cell types. Finally, although IL-4 upregulated TNF-induced expression of the adhesion molecule VCAM-1 by HUVEC, this effect was less pronounced in the other cell types.

These results suggest that IL-4, may play an important role in promoting recruitment and activation of leucocytes at sites of inflammatory lesions.

A 218 EXPRESSION OF THE ADHESION MOLECULES ICAM-1 VCAM-1 AND ELAM-1 IN NORMAL AND ATHEROSCLEROTIC HUMAN ARTERIES,

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The expression of adhesion molecules in frozen sections of post- mortem (59) and surgical specimens (5) of coronary arteries, aortas and common carotid arteries was determined by immunohistochemistry, using the avidin- biotin complex method. Five anti-ICAM-1, 3 anti-VCAM-1 and 2 anti-ELAM-1 monoclonal antibodies were employed.

ICAM-1 showed little or no expression in normal arteries: only the endothelium occasionally showed weak or patchy staining. By contrast, strong reactivity was seen in atherosclerotic plaques. Double staining with cell-specific markers showed that macrophages, smooth muscle cells and endothelial cells were all reactive. Endothelial staining was quantitated by colour image analysis, and showed significantly increased expression in all sub-types of atherosclerotic lesions, with the exception of fibrous plaques.

VCAM-1 was expressed in macrophages in atheromas. Endothelial expression was absent in normal areas and usually weak or negative over the lesions, rarely stronger. Little ELAM-1 was seen in the arterial endothelium, even when present in peri- arterial small vessels.

These findings suggest that ICAM-1 could be involved in the recruitment of monocytes to an atherosclerotic lesion. This would allow for self-perpetuating development of a lesion, and might contribute to the characteristic plaque morphology of the disease.

A 219 ACTIVATION-INDUCIBLE ADHESION MOLECULES IN CHRONIC INFLAMMATION ASSOCIATED WITH ADVANCED ATHEROSCLEROSIS,

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An immunohistochemical investigation was carried out to determine the relationship between the degree of chronic inflammation associated with advanced human atherosclerosis and the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1). Monoclonal antibodies specific for these adhesion markers, and their known ligands, were applied to cryostat sections from nine patients undergoing elective repair of atherosclerotic abdominal aortic aneurysms. A spectrum of adventitial/medial chronic inflammation was observed with a predominance of B lymphocytes in the form of lymphoid follicles with germinal centres. The expression of VCAM-1 varied with the degree of inflammation: focal staining was detected in a few adventitial vessels in non-inflamed tissue with an increasing intensity in proportion to the mononuclear cell infiltrate. The central areas of lymphoid aggregates showed VCAM-1 expression in close proximity to B cells. This expression increased with the size of the lymphoid aggregate, the highest intensity being in germinal centres. VLA-4 (VCAM-1 ligand) was also most strongly expressed in germinal centres, indicating that VCAM-1 and VLA-4 may be significant in lymphoid organization. ELAM-1 was detected in approximately 50% of the vessels within the atherosclerotic tissue wall but did not specifically coincide with lymphoid aggregates or correlate with the severity of inflammation. However, in non-inflamed tissue fewer endothelial cells were positive. ICAM-1 was widely distributed with increasing intensity in relation to the degree of inflammation; its ligand, LFA-1, was expressed strongly on many mononuclear cells. These findings suggest that activation-inducible adhesion molecules may play an integral role in the initiation and progression of the chronic inflammation associated with advanced human atherosclerosis. In addition, the pattern and intensity of VCAM-1 expression may underlie the selective presence and distribution of B cells within this tissue.

A 220 SIGNAL TRANSDUCTION AND MEMBRANE FLUIDITY IN MEGAKARYOCYTES AND MEG-01 CELLS.

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Platelets of hyperlipoproteinemic patients show an enhanced aggregation and decreased membrane fluidity. Little is known about the mechanism of the increased susceptability to stimulation. Measurements of platelet membrane fluidity by fluorescence polarization are severly hampered by the high turbidity of the suspensions. We measured the lateral diffusion of lipids in the plasma membrane, by means of Fluorescence Photobleaching Recovery, of single cultured human megakaryocytes (MK) and Meg-01 cells which share many membrane properties with platelets. The composition of the plasma membrane was changed by suppressing cholesterol synthesis with different concentrations mevalonic acid (0 to 10 mM). A short incubation (5 mM, 5 min) did not change the lateral diffusion coefficient (D) of Meg-01 cells ($3.14 \pm 0.87.10^{-9}$ (n=34) versus $3.36 \pm 1.18.10^{-9} cm^2/s$ (n=45), p>0.05). In contrast, after 20 hours incubation with 5mM mevalonic acid, D was increased with 25% to $3.92 \pm 0.66.10^{-9} cm^2/s$ (n=24, p<0.05), indicating that the membrane became more fluid. Under the same conditions Ca^{2+}-mobilization in Meg-01 cells, after stimulation with 5U/ml thrombin, was decreased from 210±24% (n=5) to 165±14% (n=5, p<0.05) of values found in resting cells. Also ^{14}C-serotonin secretion of MK cultured with 0, 2.5, 5.0, and 10.0 mM mevalonic acid was dose-dependently reduced from 68±10% (n=6) to 64±6 (n=5), 56±6% (n=7, p<0.05), and 36±17% (n=7, p<0.001) of total content, respectively. Thus an increase in membrane fluidity leads to impaired Ca^{2+}-mobilization and serotonin secretion. These data indicate that the lateral mobility of lipids in the plasma membrane affects signal transduction through the lipid bilayer.

A 221 SYNTHETIC α-THROMBIN RECEPTOR PEPTIDES ACTIVATE G PROTEIN-COUPLED SIGNALLING PATHWAYS BUT FAIL TO INDUCE MITOGENESIS.

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α-Thrombin stimulates phospholipase C and modulates the activity of adenylate cyclase in a number of cell types *via* G protein-coupled receptors. It is also a potent growth factor, notably for a line of Chinese hamster lung fibroblasts (CCL39 cells). We have recently cloned and expressed a Chinese hamster thrombin receptor cDNA (Rasmussen *et al. FEBS Lett.* 288: 123-128) which displays 79% overall amino acid sequence identity with its human homolog. Both receptors display a putative thrombin cleavage site in the N-terminal extracellular domain and cleavage at this site appears to be required for activation. Synthetic peptides corresponding to 14 residues carboxyl to the presumed thrombin cleavage site of the human receptor have been shown to activate platelets as well as the thrombin receptor expressed in *Xenopus* oocytes. In the present study we have examined the effects of synthetic peptides corresponding to the same region of the hamster receptor (S-42 - L-55), and shorter peptides (2 to 7 residues), on signal transducing systems in CCL39 cells. Our results indicate that hamster receptor peptides of ≥5 residues effectively stimulate phospholipase C in CCL39 cells *via* the thrombin receptor and induce rapid desensitization of the response. The same peptides also inhibit adenylate cyclase in a pertussis toxin-sensitive manner. Although the peptides are potent agonists of serotonin release in platelets, unlike thrombin they by themselves are **not** mitogenic. However, they potentiate DNA synthesis in cooperation with growth factors possessing tyrosine kinase receptors. Hence we conclude that the potent mitogenic action of thrombin cannot be accounted for solely by the activation of the cloned receptor. We postulate the existence of an additional receptor activated by thrombin, which is required for its full mitogenic potential.

Cytokines and Immune Mechanisms; Vascular Cell Phenotype

A 300 IL-1 RECEPTOR EXPRESSION BY HUMAN ENDOTHELIAL CELLS, Ann L. Akeson, Laura B. Mosher, Connie W. Woods, *Shirlee Yonkovich, *Lynn Kochersperger, and *Stephen Yanofsky, Marion Merrell Dow Research Institute, Cincinnati, OH, 45215 and *Affymax Research Institute, Palo Alto, CA 94304
Endothelial cells (EC) respond to small amounts of IL-1. Among the biological effects of IL-1 are induction of EC ligands important for lymphocyte and monocyte binding. We have found that IL-1 increases by 30% to 50% adherence of monocytic cell lines U937 and THP-1 to both human umbilical vein EC and aortic EC. Two distinct receptors for IL-1 have been cloned and sequenced, however the type of IL-1R on EC has not yet been identified. Using solution hybridization we found that both umbilical vein EC and aortic EC have significant levels of mRNA homologous to a cRNA for the type 1 IL-1R. JM-2, a human T cell line, and both U937 and THP-1 cells also had mRNA homologous to the type 1 IL-1R. Raji, a human B cell line that expresses the type 2 IL-1R, had no detectable mRNA for the type 1 receptor. It has previously been shown that THP-1 cells have mRNA homologous to the type 1 receptor but fail to translate the mRNA (Spriggs et al., JBC, 265: 22499-22505, 1990). To determine whether human EC express the type 1 IL-1R, anti-type 1 IL-1R monoclonal antibodies (Mab) were used. Mab 218 specifically blocked binding of IL-1, α or β, to CHO cells transfected with a cDNA for the type 1 receptor but did not block binding to Raji cells. The Mab also blocked binding of ^{125}I-IL-1β to aortic EC. These results indicate that EC do express at least a small number of type 1 IL-1R.

A 301 CONVERSION OF A MONOCYTE CHEMOTACTIC PROTEIN INTO A NEUTROPHIL ATTRACTANT BY SITE-DIRECTED MUTAGENESIS. Clifford J. Beall, Sangeeta Mahajan and Pappachan E. Kolattukudy, Biotechnology Center, The Ohio State University, Columbus, Ohio 43210.
The recruitment of monocytes to artery wall and their differenciation into foam cells has been postulated as an early step leading to atherosclerosis. The small cytokine Monocyte Chemoattractant Protein-1/Monocyte Chemoattractant and Activating Factor (MCP-1/MCAF) may play a significant role in the migration and activation of these monocytes. MCP-1/MCAF has structural similarity to Neutrophil Attractant Protein-1/Interleukin-8 (NAP-1/IL-8), but each protein is specific in attracting its own target cell. We have substituted residues tyrosine 28 to leucine and arginine 30 to valine in MCP-1/MCAF to correspond to those in NAP-1/IL-8. This double substitution causes a drastic decrease in chemotactic activity towards monocytes with the appearance of a novel neutrophil chemotactic activity. We therefore postulate that these two amino acid residues are involved in the binding of these small cytokines to their receptors.

A 302 INFLAMMATORY AND MURAL CELL CYTOKINES COOPERATE IN REGULATING ENDOTHELIAL CELL PROPERTIES, T.J. Brown, *C. Soderland and M. Shoyab, * Cell Systems Corp., Kirkland, WA 98034 and Bristol-Myers Squibb Pharmaceutical Research Instiite, Seattle, WA 98121.
The pathobiology of restenosis, a result of vascular injury following angioplasty, consists, in part, of an inflammatory component (the infiltration of leukocytes) and a mural component (the hyperproliferation of smooth muscle cells) associated with impaired reendothelialization. We questioned whether soluble products from either component might causally contribute to the failure of reendothelialization. Primary human aortic smooth muscle cells (HASMC) were isolated from segments of human aortas. These cultures maintained a SMC-phenotype through multiple passages (>90% positive for α-actin). Serum-free conditioned medium from HASMC contained a potent antiproliferative moiety for cultured bovine aortic endothelial cells (BAEC), which we termed endothelial cell regulatory factor (ECRF). Amino-terminal amino acid sequence analysis identified ECRF as the differentiation factor, leukemia inhibitory factor (LIF). Neutralizing antibodies to LIF completely blocked the biological activity of ECRF. Thus, the cytokine LIF appeared to be a gene product of HASMC. We examined the interaction of LIF with the leukocyte-derived cytokine Oncostatin M (Onco M), a potential gene product of the inflammatory component of restenosis. Combined treatment of BAEC with suboptimal doses of LIF and Onco M resulted in demonstratable cytokine-cytokine synergy for both inhibition of proliferation and stimulation of the thrombolytic phenotype, as measured by increased plasminogen activator activity. Thus, vascular interactions between LIF and Onco M may provide a mechanism for cooperativity between the inflammatory and mural components of restenosis in regulating the reendothelialization process following angioplasty.

A 303 POST-CARDIAC TRANSPLANT CORONARY ARTERIOPATHY IS ASSOCIATED WITH MHC II EXPRESSION AND INCREASED SMOOTH MUSCLE CELL FIBRONECTIN SYNTHESIS REGULATED BY INTERLEUKIN-1 ß. Nadine Clausell, John Coles, and Marlene Rabinovitch, Division of Cardiovascular Research, Hospital for Sick Children, Toronto, Ontario, Canada, M5G 1X8.
Progressive intimal thickening causing coronary artery (CA) occlusion is a major complication of cardiac transplantation. An immune reaction involving infiltration of inflammatory cells in the vessel wall has been implicated in its pathogenesis. Previous studies in our laboratory in the fetal lamb ductus arteriosus have suggested that intimal thickening associated with smooth muscle cell (SMC) migration is related to increased SMC fibronectin (FN) synthesis. Using a piglet heterotopic cardiac transplant model we observed that donor CA SMC produced 2-4 times more FN than host CA SMC associated with increased FN mRNA levels. A monocyte/SMC *in vitro* co-culture system, studied by Navab *et al* .(J. Clin. Invest. 1991 87:1763-1772), showed that increased SMC FN synthesis was regulated by cytokines interleukin-1 (IL-1) and 6 (IL-6). In this study we therefore assessed whether there was evidence of an immune reaction in the transplanted donor CA and whether the increase in FN in donor CA SMC was cytokine-mediated. Expression of MHC II antigens was investigated by immunohistochemistry and SMC FN levels were determined at passage 3 by labelling the cells with [^{35}S]-methionine for 24 h in presence and absence of neutralizing antibodies to TGF ß, IL-1 α and ß and IL-6. There was strong immunostaining for MHC II antigens in donor CA not detected in host CA. Only in the presence of anti-IL-1 ß, was donor CA SMC FN reduced to host levels. Our data suggest that an immune mediated reaction present in the graft coronary arteriopathy is associated with increased SMC FN synthesis which appears to be regulated by IL-1 ß.

A 304 AUTOCRINE GROWTH CHARACTERISTICS OF FETAL RAT VASCULAR SMOOTH MUSCLE CELLS. Colleen L. Cook, Robert Z. Florkiewicz, Kurt R. Stenmark, and Richard A. Majack, Dept. of Pediatrics, University of Colorado, Denver, CO and the Whittier Institute for Diabetes and Endocrinology, LaJolla, CA

Little is known about the factors which drive the replication of medial smooth muscle cells (SMC) during vascular development; even less is known about the factors which supress SMC replication once morphogenesis is complete. Our goal is to achieve an understanding of the phenotypic changes which occur as the developing aorta progresses from a rapidly proliferating to a fully quiescent tissue. SMC were cultured from the truncus arteriosus and abdominal aortas of fetal and neonatal rats. These cells grew in culture in the "hill and valley" morphology typical of adult SMC, express the mRNAs for elastin and α-actin, and stained positively by immunofluorescence for smooth muscle-specific α-actin. The growth characteristics of day 13 truncus arteriosus-derived SMC ($TA_{13}SMC$) were examined in detail. $TA_{13}SMC$ grew autocrinely in serum deprived media for at least 12 days, and expressed bFGF mRNA and protein. Antisense bFGF oligonucleotides, when added to autocrinely growing cultures, specifically inhibited $TA_{13}SMC$ growth. The effect was maximal at an oligonucleotide concentration at 10mM. SMC isolated from later stages of development (i.e. past fetal day 20) did not exhibit autocrine growth in culture. The data collectively suggest that SMC replication early in development is driven by an autocrine mechanism, possibly involving bFGF, and that a stable change in phenotype (to non-autocrine growth) occurs prior to birth.

A 305 MODULATION OF ION CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS BY VASOACTIVE PEPTIDES. Mark Estacion, Molecular Oncology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI 96813.

Vascular smooth muscle cells express functional receptors to vasoactive peptides such as vasopressin and bradykinin. Binding of these peptides leads to transient intracellular calcium signals measured spectrofluorimetrically using the calcium sensitive dye fura-2. Whole-cell patch-clamp recordings were obtained from bovine pulmonary vascular smooth muscle cells and from the rat aortic vascular smooth muscle cell line A10. The A10 cells expressed voltage-gated calcium and potassium currents. A predominant component of the calcium current has properties consistent with type-T calcium channels. The potassium current appears similar to delayed rectifier potassium channels. The bovine pulmonary smooth muscle cells expressed both inward rectifier and delayed rectifier potassim currents. In both cell types an additional component of potassium current was seen in response to the calcium transients caused by vasopressin or bradykinin. A similar modulation was seen in response to ionomycin, a calcium ionophore, suggesting that calcium activated potassium channels are also expressed in these cells. It is hoped that the characterization of the ion channels in vascular smooth muscle cells will lead to better understanding of smooth muscle physiology such as regulation of contractility and of proliferation. This research was supported by a grant from the American Heart Association, Hawaii Affiliate, Inc.

A 306 GM-CSF MODULATES APOLIPOPROTEIN E SYNTHESIS AND ACAT ACTIVITY IN MURINE PERITONEAL MACROPHAGES, Glenn F. Evans, Laura Guthrie, and Steven H. Zuckerman, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285

The macrophage response to LDL and/or modified LDL results in morphologic and biochemical changes which contribute to foam cell formation and atherosclerosis. The relationship between macrophage activation by GM-CSF and changes in both apolipoprotein E (apo E) synthesis and acyl-CoA: cholesterol acyltransferase (ACAT) activity has been investigated. Incubation of macrophages with GM-CSF for a minimum of 16 hours reduces the secretion of immunoprecipitable apo E by 75%. Half maximal inhibition is seen with 0.1-1.0 units/ml and is reversible. Decreased secretion of apo E corresponds with a 60-75% reduction in apo E mRNA levels occurring by 1-3 days. These decreases are not due to decreased metabolism of the cells as both total protein synthesis and fibronectin secretion are increased in GM-CSF treated macrophages. The effect of GM-CSF is heat labile and insensitive to polymixin B, and thus can be distinguished from a similar reduction in apo E secretion by LPS. Although exhibiting reduced apo E secretion, GM-CSF stimulated macrophages had significantly higher ACAT activity following ingestion of Ac-LDL compared to non-GM-CSF treated controls. The inflammatory aspects of GM-CSF and its role in atherosclerosis by regulating macrophage function necessitates a greater understanding of GM-CSF - macrophage interactions within atherosclerotic lesions.

A 307 OSTEOPONTIN EXPRESSION DISTINGUISHES ARTERIAL SMOOTH MUSCLE CELL PHENOTYPES *IN VITRO* AND *IN VIVO*, Cecilia M. Giachelli, Nancy S. Bae, Donna Lombardi, Christopher W. Covin, David Denhardt, Stephen M. Schwartz, Department of Pathology, University of Washington, Seattle, WA 98195

Osteopontin (OPN) is a secreted glycoprotein associated with bone morphogenesis, cell transformation, immune cell activation, and bacterial resistance. We have recently discovered OPN expression in rat vascular smooth muscle cells (SMC). *In vitro*, OPN is a marker for the π phenotype: a phenotype shared by cells derived from the pup rat aortic media and adult carotid neointima but distinct from that seen in cells isolated from adult aortic media. *In vivo*, OPN mRNA and protein are constitutively expressed at low levels in carotid and aortic medial SMC. After balloon angioplasty, OPN mRNA and protein levels increase selectively in proliferating and migrating intimal SMC. To understand factors which regulate OPN in SMC, we have explored the effect of growth state and growth factor treatment on cultured rat vascular SMC. OPN mRNA was expressed during all phases of the SMC cycle, but increased postconfluence. TGFβ (10 ng/ml), bFGF (10 ng/ml), and Angiotensin II (10^{-6} M) stimulated an increase in OPN mRNA expression as well as histone 2b mRNA 24h after treatment compared to vehicle treated SMC. Fetal calf serum (10%) had a minimal effect on the steady-state OPN mRNA levels even though a strong mitogenic response was initiated. The response of OPN to TGFβ may be part of the generalized effect of this growth factor on extracellular matrix molecule production since elastin mRNA expression was similarly increased in TGFβ-treated cells. The effects of bFGF and Angiotensin II, however, appeared to be specific for OPN since elastin mRNA did not vary after treatment of SMC with these agents. These data indicate that several growth regulatory molecules, known to be upregulated after vascular injury, have a common effect of elevating OPN mRNA levels in vascular SMC *in vitro*.

A 308 ORGANIZATION OF ENDOTHELIAL CELLS INTO A CAPILLARY-LIKE STRUCTURE ON BASEMENT MEMBRANE PROTEINS (MATRIGEL): EFFECT OF CELL CYCLE. **James L. Kinsella and Masafumi Kuzuya. LCS, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224**
In vitro models will be important for our understanding of the molecular mechanisms involved in angiogenesis. Confluent bovine aorta endothelial cells (BAEC) (early G_1 or G_0) attached to Matrigel but did not form a network even after several days. When subconfluent BAEC were plated on Matrigel, the cells stop dividing and differentiate within 12 hr into tube-like structures similar to a capillary network. Maximum tube formation was measured after the cells were subconfluent for 12 hr and half maximum stimulation occurred between 6 and 8 hr. Similar results were obtained by refeeding serum-starved subconfluent cells. Subconfluent cells inhibited in late G_1 phase (300 μM L-mimosine for 48 hr) organized into a limited network of cobblestone appearing cells without tube-like structures on Matrigel. Subconfluent cells inhibited in S phase (2 μg/ml aphidicolin for 48 hr) organized into cell aggregates. In cell attachment assays, about 70% of the subconfluent BAEC attached to laminin, the major basement membrane protein in the Matrigel, within 1 hr; while only 30% of the confluent cells were attached. According to Grant et al., Cell 1989, a RGD site on laminin is important for cell attachment and a YIGSR site is important for cell-cell interactions and subsequent tube formation. We found that subconfluent cells prefer the YIGSR site over the RGD site for attachment, while confluent cells had lower attachment to YIGSR but the same for RGD. Using a modified Boyden chamber with 5% serum in the bottom chamber to measure chemotaxis, less than 1% of the confluent cells migrated to the bottom during a 2 hr period. Over 7% of the subconfluent cells migrated to the bottom chamber with the same conditions. We propose that for tube formation to occur, BAEC must be between early and late G_1 phase. The ability to form tubes by subconfluent BAEC may be the result of increased cell migration and greater interaction with the laminin YIGSR sequence.

A 309 INTERFERON-γ SUPPRESSES PDGF PRODUCTION FROM A MONOCYTIC LEUKEMIC CELL LINE, THP-1 CELLS AND BLOOD MONOCYTE-DERIVED MACROPHAGES, Chiya Kosaka, Junichi Masuda, Kentaro Shimokado, Tasuku Yokota, Toshiyuki Sasaguri, and Jun Ogata, Research Institute, National Cardiovascular Center, Osaka 565, Japan
Involvement of the cells and cytokines which mediate immunological response has been recently suggested during atherogenesis by the immunohistological observation of the macrophages and T lymphocytes in atherosclerotic lesions. Regulatory effect of interferon-γ (IFN-γ), a cytokine secreted by activated T lymphocytes, on growth factor production and secretion from macrophages was examined. Platelet-derived growth factor (PDGF) mRNA was induced in THP-1 cells by incubation with phorbol 12-myristate 13-acetate (PMA). IFN-γ treatment following 24-hour preincubation with PMA attenuated PDGF mRNA expression in a dose- and time-dependent fashion. Culture medium was collected 48 hours after addition of IFN-γ, and its mitogenic activity was evaluated in mouse 3T3 fibroblasts by measuring stimulation of [^{3}H]-thymidine incorporation. PDGF-dependent mitogenic activity, estimated by preincubation with anti-PDGF antibody, was decreased from 31500 dpm to 22900 dpm by treatment with 10 I.U./ml of IFN-γ (27.3% reduction), despite the fact that number and viability of the cells did not show significant changes. Radioimmunoassay for PDGF was performed by using ^{125}I-PDGF-AA and ^{125}I-PDGF-BB. The amount of PDGF-AA decreased from 9.4 ng/ml without INF-γ to 7.3 ng/ml with addition of INF-γ (10 I.U./ml). PDGF-BB decreased from 0.063 ng/ml *without INF-γ to 0.031 ng/ml with INF-γ (10 I.U./ml). Blood* monocyte-derived macrophages cultured on plastic dishes also demonstrated the same inhibitory action of INF-γ on PDGF mRNA levels. These results suggest that IFN-γ modulates PDGF production and secretion from macrophages, and that concomitant appearance of T cells and macrophages in atherosclerotic lesions may imply the cellular interaction on the regulatory mechanism of growth factor secretion from macrophages through the action of INF-γ.

A 310 INDUCTION OF MACROPHAGE INTERLEUKIN 1 beta RELEASE BY MINIMALLY MODIFIED LOW DENSITY LIPOPROTEINS, CHOLESTERYL-9-HYDROXYOCTADECADIENOIC ACID AND 9-HYDROXYOCTADECADIENOIC ACID, George Ku, Craig E. Thomas, and Richard L. Jackson, Marion Merrell Dow Research Institute, Cincinnati, OH 45215
Incubation of normal human peripheral blood monocyte-derived macrophages (HPBM) with Cu^{2+}-induced (6 µM $CuSO_4$, 13 min at 37°C) minimally modified low density lipoproteins (MM-LDL, 200 µg/ml) resulted in the release of interleukin 1 beta (IL-1β, 34 pg per 3X10^6 cells) as measured by an ELISA. Native LDL had no effect. As determined by gel electrophoresis, both LDL and MM-LDL contained intact apolipoprotein B. Therefore, the differences between these lipoproteins might be associated with lipid peroxidation products such as cholesteryl-9- and -13-hydroxyoctadecadienoic acid (chol-9-HODE, chol-13-HODE). When HPBM were treated with chol-9- and -13-HODE (33 µM), chol-9-HODE was twice as potent as chol-13-HODE in the induction of IL-1β release, i.e., 56 and 25 pg per 3X10^6 cells, respectively. Since chol-HODE in MM-LDL could presumably be de-esterified by lysosomal esterases, free 9- and 13-HODE were tested for IL-1β inductive activity. Both 9- and 13-HODE (33 µM) induced IL-1β release; however, 9-HODE was more potent than 13-HODE in this regard (128 and 32 pg IL-1β per 3X10^6 cells, respectively). 9-HODE was also capable of inducing IL-1 release from murine peritoneal macrophages (Elisa) and rabbit peripheral blood monocyte-derived macrophages (RIA), suggesting a general pro-inflammatory property of 9-HODE. In the context of atherosclerosis, IL-1β can induce vascular smooth muscle cells (SMC) to release platelet-derived growth factor and increase its receptor levels, which serve to promote SMC proliferation. Thus, 9-HODE-induced IL-1β release may be a mechanism whereby oxidative modification of LDL contributes to atherogenesis.

A 311 PRODUCTION OF METALLOPROTEINASE AND THEIR INHIBITORS BY BOVINE LYMPHATIC ENDOTHELIAL CELLS: MODULATION BY TNFα, Carol Laschinger, Jaro Sodek* and Safia Wasi, Protein Chemistry Laboratory, National Reference Laboratories, The Canadian Red Cross Society, Ottawa, Canada and *Medical Research Council Group in Periodontal Physiology and the Department of Biochemistry, University of Toronto, Toronto, Canada

We have previously shown that bovine lymphatic endothelial cells (BLEC) produce both tissue type plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) (Thrombosis Research, 59, 567-579, 1990). Treatment with the inflammatory cytokine, tissue necrosis factor alpha (TNFα), decreased the net fibrinolytic potential of these cells. PAI-1 activity was stimulated three to seven fold, whereas tPA activity decreased by five fold as compared with untreated cells. The plasminogen activation is one of the mechanisms implicated for the conversion of latent matrix metalloproteinases (MMP) to active enzymes. Therefore to investigate the role of the lymphatic system on the regulation of extravascular fluid movement we have initiated studies to determine whether BLEC produce MMP and their inhibitors and if these activities can be modulated by TNFα. Utilizing enzymography, immunoprecipitation, and immunoblot analyses we have shown that BLEC produces two species of progelatinases (68 kDa major, 92 kDa minor) as well as a tissue inhibitor of MMP (TIMP-1). Treatment with TNFα for up to 72h did not stimulate the production of progelatinases or TIMP-1. These observed effects of TNFα are different from those reported for other cell types such as synovial cells and dermal fibroblast. To elucidate the mechanisms which govern gene expression of these proteins, total cytoplasmic RNA was prepared from BLEC and analyzed by Northern blotting analysis. Hybridization under non-stringent conditions using human PAI-1 cDNA probe revealed marked enhancement of two species (1.6 kb and 3.0 kb) of PAI-1 message after TNFα treatment. TNFα effects on the expression of MMPs and TIMP-1 are under investigation. The production and stimulation of procollagenase is being investigated.

A 312 CONTROL OF CELL GROWTH/DEATH CYCLING - ROLE OF FATTY ACIDS AND CYTOKINES, William S. Lynn, Dorian Coppenhaver and James Wallwork, Departments of Internal Medicine and Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX 77550

Using five models of uncontrolled cell growth, i.e. autoimmune (casein) glomerulitis in NZB mice, obesity in C57Bl6 mice, lymphoid hyperplasia in retrovirus-infested mice, B-16 melanoma growth in mice and cytokine (IL-1, TNF, γ interferon, neurotensin) activated T cells, we have observed that uncontrolled generalized lipolysis with liberation of saturated fatty acids intracellularly is a major modulator of cell growth/death cycling. The type of fatty acid released, notably $\omega3$ fatty acids, is also critical, i.e. saturated fatty acids activate cell death (apoptosis). $\omega3$ unsaturated fatty acids, however, prevent cell death, whether produced by the saturated fatty acids or cytokines. *In vivo*, removal of the saturated fat by calorie restriction also prevents both cell death and uncontrolled cell growth, as produced by retrovirus or by transformed cell transplants or by autoimmune inflammation. With large doses of intraperitoneal tumor cells, both removal of saturated fat, i.e. calorie restriction, and activation of cytotoxic immune cells, e.g. by α interferon, IL-2, are required to control tumor growth/death cycling. These data suggest that cytokines and hormones which control cell growth/death do so to some extent by controlling nutrient supplies, especially fatty acids.

A 313 SELECTIVE INHIBITION OF THE PROLIFERATION OF HUMAN AND RAT VASCULAR SMOOTH MUSCLE CELLS BY ADPRT INHIBITORS, James C. Metcalfe, David G. Grainger, Robin Hesketh and Peter L. Weissberg, Department of Biochemistry, University of Cambridge, CB2 1QW, U.K.

Abnormal proliferation of vascular smooth muscle cells (VSMCs) is a component of several vascular diseases and agents which selectively inhibit VSMC proliferation may therefore prevent or inhibit the progress of these diseases. When arterial VSMCs are dispersed into cell culture, they begin to lose smooth muscle-specific isoforms of myosin heavy chain (SM-MHC) and actin before proliferating and it has been proposed that this de-differentiation is an essential precursor to proliferation. Hexamethylene bisacetamide is the best characterised inhibitor of adenosine diphosphoribosyl transferase (ADPRT) and has been shown to cause morphological and functional differentiation of several transformed tumour cell lines and to inhibit their proliferation. These observations provided the rationale for examining the effects of HMBA on the de-differentiation of VSMCs defined by the loss of SM-MHC and for comparing its effects on the proliferation of VSMCs and other types of cells. HMBA selectively and reversibly inhibited the proliferation of human and rat VSMCs compared with endothelial cells, fibroblasts or lymphocytes, which must remain able to proliferate if an inhibitor of VSMC proliferation is to be of practical use. Half-maximal inhibition of VSMC proliferation occurred at 2.8-5mM HMBA and at >30 mM for other cell types. HMBA also prevented de-differentiation, defined by the loss of SM-MHC, and was shown to act early in the G_1 phase of the cell cycle. More potent inhibitors of ADPRT were also selective inhibitors of VSMC proliferation.

A 314 DIVERGENT SIGNAL TRANSDUCTION PATHWAYS IN ENDOTHELIAL CELLS, Hedwig S. Murphy, James A. Shayman, Peter A. Ward, Department of Pathology and Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109

Rat pulmonary artery endothelial cells respond to human recombinant C5a and TNFα with the ultimate functional outcome of generation of O_2^-, while bradykinin, which is reactive with endothelial cells, induces a very small O_2^- response. In order to investigate intracellular signal-transduction pathways in endothelial cells, the increase in intracellular calcium and inositol(1,4,5)P_3 in response to these peptides was examined. C5a and TNFα-stimulated cells produced a brief elevation in intracellular calcium, the change in which was smaller than the response to bradykinin. In the case of C5a, the calcium response was completely abolished by pertussis toxin pretreatment, while for TNFα and bradykinin, pretreatment with pertussis toxin failed to alter the calcium responses. Inositol(1,4,5)P_3 levels were transiently increased in response to all three agonists. Pertussis toxin pretreatment resulted in inhibition of inositol(1,4,5)P_3 in response to C5a and TNFα and partial inhibition of the response to bradykinin. The O_2^- response to C5a was abolished by pertussis toxin pretreatment, whereas the response to TNFα was insensitive to this pretreatment. These data indicated that endothelial cells are able to produce O_2^- in response to TNFα and C5a, but that they respond via different signal transduction pathways.

A 315 MODIFICATION OF TUMOR NECROSIS FACTOR ALPHA PRODUCTION UPON THE MODULATION OF Na^+/HCO_3^- COTRANSPORT IN LIPOPOLYSACCHARIDE-ACTIVATED HUMAN MONOCYTES, Urszula Orlinska and Robert C. Newton, Inflammatory Diseases Research, The Du Pont Merck Pharmaceutical Co., Glenolden PA 19036.

Tumor Necrosis Factor alpha (TNF alpha) is produced and secreted from monocytes in response to activation with lipopolysaccharide (LPS). Based on our finding that the activation of monocyte is associated with a rise in intracellular pH (pH_i) due to an established role of sodium ion and carbonate in regulating pH_i, we investigated the role of Na^+ and HCO_3^- in TNF production in LPS-activated human monocytes. We observed that the replacement of Na^+ in the culture medium with sucrose, choline chloride, or Li_2CO_3 inhibited TNF production completely. Without HCO_3^- in the culture medium TNF production was inhibited 92%. Phloretin, DIDS, SITS, which are inhibitors of anion transport, inhibited TNF production with IC_{50} of 25 µM, 118 µM and 139 µM, respectively. The amiloride and amiloride analog 5-(N-ethyl-N-isopropyl)amiloride (EIPA), known primarily as inhibitors of the Na^+/H^+ antiporter, inhibited TNF production with an IC_{50} of 3.5 µM and 3.3 µM, respectively. These data suggest that TNF alpha production is dependent primarily on the activation of Na+-dependent HCO_3^- cotransporter with the negligible component of Na^+/H^+ exchanger.

A 316 LIPOPROTEIN LIPASE EXPRESSION AND SECRETION IN INBRED MOUSE STRAINS DIFFERING IN THEIR SUSCEPTIBILITY TO ATHEROSCLEROSIS, Geneviève Renier, Emil Skamene and Danuta Radzioch, Department of Experimental Medicine, McGill Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Canada.
To determine the role of lipoprotein lipase (LPL) in the development of atherosclerosis, we evaluated LPL expression and secretion in mice differing in their susceptibility to develop atherosclerosis. Inflammatory peritoneal macrophages of susceptible C57BL/6J mice showed high LPL mRNA expression. In contrast, A/J mice which are resistant to develop atherosclerosis had about 3- to 4-fold lower LPL mRNA levels as compared to that observed in the C57BL/6J mice. Activation of the macrophage by lipopolyssacharide (LPS) or interferonγ (IFNγ) resulted in all the mice strains in an inhibition of the LPL mRNA expression. Constitutive secretion of LPL was similar in both mouse strains. In contrast, heparin-induced LPL as well as intracellular LPL immunoreactive mass were 2- to 3-fold lower in the A/J mice as compared to those observed in the C57BL/6J mice. These results may suggest a possible role of LPL in the development of atherosclerosis.

A 317 LOW-DENSITY LIPOPROTEIN RECEPTOR-DEPENDENT FORMATION OF EICOSANOIDS IN HUMAN BLOOD-DERIVED MONOCYTES. Peter B. Salbach[§], Eberhard von Hodenberg[§], Uwe Janßen-Timmen*, John A. Glomset[&], Wolfgang Kübler[§], and Andreas J.R. Habenicht*. [§]University of Heidelberg, Medical School, Department of Internal Med., Div. of Cardiology, *Div.. of Endocrinology and Metabolism, 69-Heidelberg, F.R.G.; [&]Howard Hughes Medical Institute Laboratory, University of Washington SL-15, Seattle, Washington 98195, USA

We have shown previously that the low density lipoprotein (LDL) receptor pathway delivers arachidonic acid (AA) for prostaglandin (PG) formation in PDGF-stimulated fibroblasts. Here we studied the possibility that the LDL pathway delivers AA for eicosanoid biosynthesis in cultured human monocytes. When incubated with LDL that was reconstituted with cholesteryl-(^{14}C)-arachidonate (rec-LDL), resting monocytes formed three labeled products of the PG H synthase pathway: 6-keto-PGF$_{1\alpha}$, thromboxane (TX) B$_2$, and PGE$_2$. By contrast, resting monocytes formed only small amounts of products of the 5-lipoxygenase pathway, LTB$_4$ and LTC$_4$ from either rec-LDL or ^{14}C-AA indicating preferential utilization of AA for the PGH synthase reaction. However, when monocytes were first incubated with rec-LDL and subsequently stimulated with the chemotactic peptide, N-formylmethionylleucylphenylalanine or Ca^{++}-Ionophore A 23187, they converted LDL-derived ^{14}C-AA efficiently into LTB$_4$ and LTC$_4$. The production of eicosanoids was mediated by the LDL receptor because chloroquin, an inhibitor of lysosomal activity, and a polyclonal anti-LDL-receptor antibody, prevented formation of eicosanoids from rec-LDL but not from unesterified ^{14}C-AA. When taken together, our results suggest that macrophages utilize LDL-derived AA to produce several distinct eicosanoids that are likely to play important roles in inflammation and possibly cardiovascular disease.

A 318 MYOSIN ISOFORMS AND SMOOTH MUSCLE CELL POPULATIONS IN WATANABE HERITABLE HYPERLIPEMIC AND IN CHOLESTEROL-FED RABBITS, Saverio Sartore[°,§] and Paolo Pauletto*, °Institute of General Pathology, *Institute of Clinical Medicine, and §NRC Unit for Muscle Biology and Physiopathology, University of Padova, Padova, Italy
Monoclonal antibodies specific for smooth muscle (SM) and nonmuscle (NM) myosin isoforms, and immunofluorescence techniques were applied to the study of SM cell composition of the arterial wall lesions in 90-day-old Watanabe heritable hyperlipemic (WHHL), and in cholesterol-fed (CF) New Zealand White (NZW) rabbits. Three SM cell populations were found in the thickened intima (TI) of WHHL rabbits: cells which reacted with anti-SM myosin or anti-NM myosin antibody, exclusively; and cells which showed a double myosin content. This latter SM cell population shared this antigenic pattern with the vascular SM cells from developing and cultured aortic SM tissue. Part of the cells displaying a NM myosin content are also labeled with an anti-monocyte/macrophage antibody. In the media underlying the TI, there was a marked increase of the SM cell population with double myosin content, which is present in trace amount in the media of adult normocholesterolemic NZW rabbits. A similar distribution of myosin isoforms and of SM cell heterogeneity were found in the CF rabbits. Though the cholesterol metabolism and the distribution of cholesterol among the various lipoproteins is different in the two models of atherogenesis, this immunocytochemical study indicates a similar SM cell composition. Moreover, in rabbits in which hypercholesterolemia is of endogenous or exogenous origin the major SM cell population, presents both at the level of the TI and the subjacent media, displays a myosin antigenicity similar to that of "immature" vascular SM cells. These data are compatible with the hypothesis that a process of "phenotypic modulation", "dedifferentiation" or proliferation from a "stem-like cells" may be responsible for SM cell response to the arterial wall injury.

A 319 RECOMBINANT HUMAN MACROPHAGE COLONY STIMULATING FACTOR (rhM-CSF) REDUCES FOAM CELL DEVELOPMENT AND ATHEROSCLEROSIS IN WHHL RABBITS, Robert G. Schaub, Mark Bree, Lori Hayes, Steven Clinton and Joseph Loscalzo, Genetics Institute, Cambridge, MA 02140 and Harvard Medical School, Boston, MA 02115
Previous studies have demonstrated that rhM-CSF, a growth factor for monocytes/macrophages, reduces plasma cholesterol in Watanabe hyperlipidemic rabbits (WHHL) for up to 2 wks by continuous i.v. infusion (CIVI). We extend these findings to an 8 wk CIVI in the WHHL which includes assessment of foam cell development and aortic lesion formation. Ten male WHHL rabbits were surgically implanted with venous catheters. They were also injected with 1% carrageenan (15 ml sc in the mid-abdomen) to promote formation of a macrophage rich granuloma. Five rabbits each were treated with vehicle or rhM-CSF at 100 ug/kg/day (5 wks) followed by 300 ug/kg/day (3 wks). Blood counts were performed daily (4 days) and weekly thereafter. Cholesterol (total [TC], HDL,LDL), triglycerides (TG) and circulating antibodies to rhM-CSF were measured weekly. A transient decrease (50%) in platelet counts (7-14 days) was observed. rhM-CSF (100 ug/kg/day) decreased TC maximally by 14 days (43%). An increase in the dose to 300 ug/kg/day was required to maintain the reduction. At day 21 of the 300 ug/kg/day dose, TC reduction was only 20%. The loss of rhM-CSF response correlated with the appearance of anti-rhM-CSF antibodies. Other lipid parameters were less affected. HDL was unchanged, LDL elevation was reduced and TG was slightly reduced compared to vehicle control. Granuloma weights from treated rabbits (2.8 +/- 1.4 grams) were less than those from vehicle treated animals (7.1 +/- 3.1 grams) ($p<0.05$). Histology revealed the presence of numerous large foam cells in the vehicle treated rabbits. Granulomas from the rhM-CSF treated rabbits were more fibrous and contained fewer foam cells. Aortae were divided into 15 segments and compared for their frequency of lesions. Non-treated rabbits had a higher frequency of lesions in the thoracic and abdominal aorta as compared to the rhM-CSF treated rabbits. The total cholesterol (ug/mg) in granulomas from rhM-CSF treated animals was significantly lower (1.96 +/- 0.72) than vehicle treated rabbits (6.08 +/- 1.46) ($p<0.03$). Aortic TC was also decreased (1.75 +/- 0.44 vs 2.6 +/- 0.29) in treated animals, but liver and spleen TC were unchanged. These results demonstrate that the plasma cholesterol decrease observed with rhM-CSF is accompanied by decreased foam cell development and a moderation of aortic lesion formation.

A 320 MITOGENIC ACTIVITY OF INTERFERON GAMMA FOR HUMAN VASCULAR SMOOTH MUSCLE CELLS IN CULTURE,
Kentaro Shimokado, Tasuku Yokota, Chiya Kosaka, Toshiyuki Sasaguri, Junichi Masuda, Jun Ogata, Stroke and Atherosclerosis Research Laboratory, National Cardiovascular Center Research Institute, Suita, Osaka

Interferon gamma(IFN-γ) is a cytokine secreted by activated T lymphocytes. It has been reported to suppress the multiplication of vascular smooth muscle cells (SMCs), to inhibit migration of cells or to induce expression of MHC-II antigen on the surface of vascular cells. Since some SMCs in the atherosclerotic lesion express MHC-II antigen, it has been proposed that IFN-γ is actually produced in the vascular wall and plays important roles in atherogenesis. In this paper, we present data which show that IFN-γ , under certain circumstances, stimulates proliferation of vascular SMCs by inducing autocrine growth factor(s) and by upregulating PDGF receptor. Recombinant human IFN-γ (1-100 i.u./ml) stimulates ^{3}H-TdR incorporation into quiescent arterial SMCs in culture which are synchronized to G0 by culturing in medium containing 1% plasma-derived serum for 5 days. It also accentuates mitogenic activity of PDGF(BB). When quiescent SMCs are stimulated by PDGF(BB), they enter S phase within 16 hours. On the other hand, SMCs stimulated by IFN-γ start incorporating ^{3}H-TdR with a time lag of 4-6 hours. Synergistic effect with PDGF is also seen after similar time lag. If conditioned medium of IFN-treated SMCs is added to culture instead of IFN-γ itself, the time lag for mitogenicity and synergism with PDGF disappears, suggesting that those effects are mediated partly by substance(s) secreted by IFN-treated SMCs. Mitogenic activity in conditioned medium of IFN-treated SMC can be detected also by mitogenesis assay using swiss 3T3 cells which is originated from mouse and therefore are not responsive to human IFN-γ. Mitogenicity induced by IFN-γ is blocked by anti-IFN-γ antibody but not by anti-PDGF antibody. ^{125}I-PDGF(BB)-binding to SMCs also increases after IFN-treatment. This increase is due to increase in number of PDGF receptor.Based on those observation we conclude that IFN-γ regulates proliferation of vascular SMCs bidirectionally.

A 321 TGF$_β$ HAS A BIPHASIC, CONCENTRATION DEPENDENT EFFECT ON EGF AND PDGF-BB INDUCED SMOOTH MUSCLE CELL PROLIFERATION,
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Abnormal smooth muscle cell (SMC) proliferation is a major pathological feature of atherosclerosis and restenosis following balloon angioplasty. Transforming growth factor-β (TGF$_β$), epidermal growth factor (EGF) and platelet derived growth factor-BB (PDGF-BB) are polypeptide growth factors that are presumably present at the site of vascular injury. The purpose of these studies was to investigate the effects of TGF$_β$ on EGF or PDGF-BB induced SMC proliferation. Briefly, SMC derived from spontaneously hypertensive rats were grown to confluence, growth arrested in a defined serum free media and then treated. ^{3}H-thymidine incorporation was measured at 24 hours. Treatment with EGF (1.7 nM) or PDGF-BB (.7 nM) resulted in 8.4 fold and 73.4 fold increases in 3H-thymidine incorporation respectively (as compared to untreated control). Co-treatment with TGF$_β$ elicited a biphasic response. TGF$_β$ at 10^{-10}M inhibited EGF- (44% decrease) and PDGF-BB- (82% decrease) induced mitogenesis. In contrast, TGF$_β$ at 10^{-11}M increased EGF induced mitogenesis (340%) and TGF$_β$ at 10^{-12}M increased PDGF-BB induced mitogenesis (22%). Treatment with TGF$_β$ alone decreased ^{3}H-thymidine incorporation by 36% at 10^{-10}M and had no effect at lower concentrations. In summary, the concentration of TGF$_β$ is an important determinant of EGF or PDGF-BB induced SMC proliferation *in vitro*.

A 322 THE ROLE OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IN ATHEROSCLEROSIS: IMMUNOHISTOCHEMICAL DETECTION IN HUMAN ATHEROSCLEROTIC LESIONS BY AN ANTI-MCP-1 MONOCLONAL ANTIBODY,
Motohiro Takeya, Teizo Yoshimura, Edward J. Leonard, and Kiyoshi Takahashi, Department of Pathology, Kumamoto University Medical School, Kumamoto 860, Japan, and Immunopathology Section, Laboratory of Immunobiology, NCI-FCRDC, Frederick, MD 21702, USA.

One of the earliest events observed in atherogenesis is the adherence of circulating monocytes to the arterial wall and subsequent migration into subendothelial space. Although various monocyte chemotactic factors (MCF) have been postulated to cause monocyte migration into the arterial wall, there is no evidence for production of MCF in human atherosclerotic lesions in vivo. MCP-1 is a recently characterized 76 amino acid protein that attracts monocytes but not neutrophils. MCP-1 can be produced in vitro by various cells including blood mononuclear leukocytes, endothelial cells, smooth muscle cells, fibroblasts, and tumor cells. MCP-1 may account for monocyte/macrophage accumulation in lesions of delayed-type hypersensitivity, chronic inflammation, certain neoplasms, and atherosclerosis. We previously showed the expression of MCP-1 mRNA in macrophage-rich lesions of atherosclerosis in rabbit (Yla-Herttuala et al., PNAS 88:5252, 1991). In the present study, we investigated immunohistochemically the production of MCP-1 in the atherosclerotic lesions of patients of various ages using highly specific anti-MCP-1 monoclonal antibody. In fatty streak lesions, the earliest visible change of atherosclerosis, endothelial cells and subendothelial macrophages were positively stained for MCP-1. In advanced atherosclerotic plaques, subendothelial macrophages continued to express MCP-1; however, endothelial cells did not. Only scattered macrophages were positive for MCP-1 in old fibrous plaques. These results indicate that MCP-1 plays an important role in the initiation and further accumulation of monocytes in early atherosclerotic lesions.

A 323 ADHESION AND GROWTH OF BOVINE ENDOTHELIAL CELLS ON SPECIFIC EXTRACELLULAR MATRIX MOLECULES

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Endothelial cells grown on uncoated tissue culture plastic *in vitro* adhere to the surface via vitronectin deposited from serum in the culture medium. Vitronectin (Vn) can efficiently coat the surface in the presence of other serum proteins, and can displace previously coated molecules such as serum albumin. Vn may not represent an ideal surface on which to culture endothelial cells, which *in vivo* are in contact with basement membrane. We investigated the effects of different surface coatings of extracellular matrix molecules on the production of extracellular matrix (ECM) components by bovine corneal endothelial (BCE) cells, during short - and long-term cultivation using a culture medium which did not contain Vn. Growth rates were similar on each ECM molecule coated. The production of endogenous ECM by BCE cells was affected by the nature of the molecule coated. The ECM content of laminin, type IV collagen and thrombospondin was high when the cells were cultivated on type IV collagen, but low when the cells were cultured on Vn or type I collagen. Coated laminin and fibronectin induced varying effects, depending on the ECM molecule measured. The production of fibronectin by the BCE cells appeared to be unaffected by the substrate. Cultures passaged and maintained for long periods (up to 10 weeks) on the same substrate displayed similar profiles of matrix production to those cultured for one passage, with one exception. Initial passages on a Vn substratum yielded high production of ECM components, whereas continued passages on this substrate resulted in declining secretion of ECM components with time. It was concluded that Vn and type I collagen are inappropriate substrates for continued production by BCE cells of an ECM which resembles basement membrane in composition.

A 324 DIFFERENTIATION OF MONOCYTES TO MACROPHAGES IS STIMULATED BY SECRETORY PRODUCTS OF HUMAN VASCULAR SMOOTH MUSCLE CELLS, Eberhard von Hodenberg, Eva Pestel, Martina Hautmann, Marion Forster and Wolfgang Kübler, Department of Cardiology, University of Heidelberg, Germany
One of the early steps in atherogenesis is the adhesion of monocytes to the endothelium and migration into the intima. There the cells undergo diffentiation to macrophages, which can become foam cells and/or effect other vascular cells by secretion of cytokines and growth factors. Mechanisms influencing macrophage differentiation in the artery wall are still not clear. We examined the effect of smooth muscle cells (SMC) secretory products on the differentiation of monocytes to macrophages. Human SMC were isolated from the aorta of heart donors and incubated until subconfluency was archieved. Isolation of SMC-secretory products (SMC conditioned medium = SMC-c-medium) was performed after a 48h incubation of SMC in serum free medium. Freshly isolated human monocytes were then incubated in SMC-c-medium, medium supplemented with 10 % serum, or medium without serum. The grade of monocyte/macrophage differentiation was analysed by immunoflueréscent techniques with specific monoclonal antibodies MAX 1,2, and 11. Differentiation of monocytes to macrophages occured after 6 days, when cells were incubated in SMC-c-medium or in medium + 10% serum. Most of the cells incubated in serum free medium died after 3 to 6 days and did not differentiate to macrophages. Known secretory products of SMC such as PDGF, interleucin 1,2 and 6 or TNF alpha, as well as a cocktail of these products all failed to induce a comparable macrophage differentiation. We conclude that SMC can stimulate macrophage differentiation via (a) secretory product(s) which still need(s) to be characterized. By this interaction SMC can effect macrophage foam cell formation and atherogensis.

A 325 GROWTH FACTOR mRNA EXPRESSION IN HUMAN AORTIC AND UMBILICAL VEIN ENDOTHELIAL CELLS, Cynthia R. Wagner, Tony E. Morris, Paul A. Mattox, Gary D. Shipley and Jeffrey D. Hosenpud, Immunology Research, Veterans Affairs Medical Center, and the Department of Cell Biology and Anatomy and the Division of Cardiology, Department of Medicine, Oregon Health Sciences University, Portland, Oregon 97201
Endothelial cells can be potent producers of growth factors for smooth muscle cells which suggests that endothelial cell (EC) growth factor production may be a key factor in the development of atherosclerosis. Chronic rejection in transplant recipients mimics atherosclerosis in many respects. Since transplant-related atherosclerosis is limited to the donor organ, we are examining whether recipient lymphocyte's alloreactivity stimulates growth factor production by donor ECs. For our study ECs are isolated from the aortas of cardiac transplant donors. As this is not a commonly studied EC type we have begun by examining human aortic ECs (HAECs) for basal expression of poly $(A)^+$ RNA (mRNA) coding for several growth factors. Since umbilical vein ECs (HUVECs) are commonly used to study human endothelium, we compared HAEC mRNA expression to that of HUVECs. These two EC types were examined for mRNA coding for acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), transforming growth factor α (TGFα), transforming growth factor β (TGFβ), platelet-derived growth factor A chain (PDGF-A), platelet-derived growth factor B chain (PDGF-B, c-sis), and amphiregulin (AR). We detected mRNA coding for bFGF, TGFα & β, and PDGF-A & B in HAEC; however, we found mRNA coding for TGFα and PDGF-A & B to be variably expressed. We did not detect mRNA coding for aFGF or AR. HUVECs differed in that they consistently expressed mRNA coding for bFGF, TGFβ, and PDGF-A and B chain. We did not find detectable levels of mRNA coding for aFGF, TGFα, or AR in HUVECs. This study suggests that HUVECs have a different pattern of growth factor mRNA expression than HAECs and that the EC type may need to be considered when studying the role of ECs in human vascular diseases.

A 326 CLONING OF RAT MONOCYTE CHEMOATTRACTANT PROTEIN 1 (MCP 1) AND ITS EXPRESSION IN A BACULOVIRUS SYSTEM: ANALYSIS OF MCP 1 IN RAT MODELS OF HUMAN DISEASE, Jeffrey S. Warren, Michael L. Jones, and Peter A. Ward, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109.

Monocyte chemoattractant protein 1 (MCP1), also known as monocyte chemotactic and activating factor, is the human homolog of the murine JE gene product. Purified native human MCP1 possesses potent chemotactic activity for monocytes and can augment monocyte tumoristatic activity. MCP1 is constitutively produced by, or can be induced in, a variety of cell types. While these activities suggest that MCP1 may participate in inflammatory and immunologic processes, the biological role of MCP1 has not been studied in vivo. Because antibodies directed against human MCP1 do not neutralize rat monocyte chemotactic activity or react with rat tissues, we have cloned and expressed rat MCP1 in order to analyze its role in rat models of human disease. A cDNA library was constructed from TNFα-stimulated rat pulmonary artery endothelial cells. The cDNA library was screened with synthetic oligonucleotide probes based on the recently published rat MCP1 cDNA sequence (T. Yoshimura: BBRC 174:504, 1991). Four appropriately sized ($\approx$450 bp) MCP1 cDNA sequences were rescued, amplified by polymerase chain reaction, and ligated in proper orientation into the pJVETLZ baculovirus transfer vector (gift of C. Richardson, Biotech Res. Inst., Montreal, Canada). Spodoptera frugiperda insect cells (Sf-9) infected with baculovirus recombinants (Autographa californica) bearing MCP1 (AcMCP1) directed the expression of monocyte-specific chemotactic activity. Negative controls (noninfected Sf-9 cells, Sf-9 cells infected with wildtype virus, and MCP1-negative recombinant virus (AcpJVELTZ)) contained no chemotactic activity. Preliminary biochemical characterization of material produced in cultures infected with AcMCP1 revealed the presence of at least three 12-22kd variably glycosylated proteins. The production of rat MCP1 has enabled us to begin to investigate its role in the pathogenesis of monocyte-macrophage rich inflammatory processes in which MCP1 is a likely mediator.

A 327 HEPARIN DECREASES THE RATE OF PROLIFERATION OF RAT VASCULAR SMOOTH MUSCLE CELLS BY INCREASING THE DURATION OF THE G_2 TO M PHASE OF THE CELL CYCLE, Peter L. Weissberg, David J. Grainger, Christine M Witchell and James C. Metcalfe, Department of Biochemistry, University of Cambridge, CB2 1QW, U.K.
Seven heparins of varying molecular weight partially inhibited proliferation of primary cultures of rat aortic vascular smooth muscle cells (VSMCs). However, none of the heparins reduced the proportion of cells (>85%) that entered the cell cycle and initiated DNA synthesis and the anti-proliferative mechanism of one heparin (5,000 Da) was analysed in detail:

1. Heparin (100µg/ml) caused a small delay (1.7h ± 0.2h) in the entry into S phase in the first cell cycle of primary VSMCs but did not affect the proportion of cells which completed S phase (>85%) or its duration.
2. Primary VSMCs proliferated more slowly in the presence of heparin so that at 144h after the addition of heparin to the freshly plated cells, the number of cells was reduced by 55% ± 7%. Time lapse photomicroscopy showed, however, that in the presence of heparin, >90% of the cells had divided by 96h and that the duration of mitosis was unaffected. Heparin therefore caused an increase of approx. 2-fold in the cell cycle time of all of the cells by increasing the time taken to traverse from S phase to mitotic metaphase.
3. Heparin blocked the de-differentiation of primary VSMCs, as defined by the progressive loss of smooth muscle-specific myosin heavy chain (SM-MHC). However, heparin had similar effects on the cell cycle of passaged VSMCs, which contain very little SM-MHC, to its effects on primary VSMCs.

We conclude that the increase in cell cycle times occurs independently of inhibition of de-differentiation by heparin.

A 328 SYNERGY BETWEEN IL-4 AND IL-1 IN THE FORMATION OF IL-6 BY ENDOTHELIUM.

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Interleukin-4 (IL-4) is released by activated T cells, and can regulate the behaviour of many cell types including macrophages, fibroblasts and endothelial cells (ECs). In this study, we describe the synthesis of interleukin-6 (IL-6) activity by ECs in response to IL-4, and demonstrate that there is strong synergy in this response between IL-4 and interleukin-1 (IL-1). Human umbilical vein endothelial cells (HUVECs) were cultured in 12 well plates, and stimulated with IL-4 both in the presence and absence of IL-1. Culture supernatants were collected after 24 hours of stimulation, and stored frozen. An IL-6 dependent cell line (7-TD1) was used to quantitate the amount of IL-6 activity of stored culture supernatants. IL-4 was found to stimulate the synthesis of IL-6 activity by HUVECs in a dose dependent manner. This response was not as great as that to IL-1. However, when HUVECs were stimulated with both IL-1 and IL-4, the magnitude of the response was greatly increased as compared to that with either stimulus alone. This enhanced response to IL-4 in the presence of IL-1 was also shown to be dose dependent. Northern blot analysis indicated an increased amount of mRNA for IL-6 in HUVECs stimulated with both IL-4 and IL-1 as compared with cells treated with only one of the two cytokines. We conclude that IL-4 may be an important co-factor in the release of IL-6 by ECs in inflammatory foci, where these cells may be exposed to IL-1. The role of IL-6 in regulating the acute phase response, raises the possibility that IL-4 may indirectly regulate this systemic response, through the activation of IL-1 stimulated ECs.

Vascular Disease Progression and Regression; Genetic Models of Vascular Disease

A 400 LOCALIZATION OF PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1) IN HEALTHY AND ATHEROSCLEROTIC ARTERIES

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The critical event that converts an asymptomatic atherosclerotic plaque into a symptomatic one is the formation of a thrombus on the fissured or ulcerated lesion. A deficiency in fibrinolysis may contribute to occlusive thrombus formation leading to myocardial infarction or stroke.
To study the distribution of PAI-1 protein, we analyzed samples of human healthy and atherosclerotic arteries by immunohistochemistry. Tissue specimens were embedded within 1 h of removal in O.C.T. compound, frozen in cold methyl-butane and cryostat sectioned or fixed in formol, placed in paraffin and sectioned. PAI-1 protein was identified by using a murine anti-PAI-1 MAb IgG1 (MAb 380, American Diagnostica). An isotype-matched murine MAb not reacting with human tissue was used as negative control. Anti-vWF and anti- -actin MAbs were used as endothelial cell and smooth muscle cell (SMC) markers, respectively.
The most prominent specific PAI-1 staining was observed in the media of all arteries assayed. PAI-1 protein colocalized with -actin indicating that SMC contain the majority of the PAI-1 of the arterial wall. Carotid and renal arteries exhibited a stronger PAI-1 signal than aorta. PAI-1 was found in atherosclerotic plaques associated with SMC and foam cells which were also positive for -actin. Northern blot analysis of PAI-1 mRNA isolated from various pig arteries (carotid, pulmonary, coronary and aorta) also showed that PAI-1 is differentially expressed.
SMC proliferation and migration into the intima are early events in lesion formation. The association of PAI-1 protein with SMC may increase the local levels of PAI-1 after plaque rupture which may prevent the fibrinolytic system from being activated and thus favor the persistence of the thrombus.

A 401 CYTOMEGALOVIRUS INDUCED VASCULAR INJURY: STUDY IN THE RAT, Cathrien A. Bruggeman, Selma Herngreen and Angelique H.M. Span, Department of Medical Microbiology, University of Limburg, Academic Hospital Maastricht, 6202 AZ Maastricht, The Netherlands.

There is some evidence that viruses, especially viruses belonging to the herpesvirus family such as cytomegalovirus (CMV) play a role in atherogenesis. Since endothelial alteration is probably associated with the development of atherosclerosis, we looked at the effect of CMV infection on morphological evidence of endothelial injury. For this purpose normo- and hypercholesterolemic rats were infected with rat CMV and the endothelium of the large vessels was observed during the first 4 months post infection by light and electron microscopy. Endothelial cell desquamation was observed starting at 2 weeks post infection. There was a 16 till 20 fold increase in the number of white blood cells adhering to the intima. The adhesion started early post infection and was followed by migration into the subendothelial space, where they progressively were filled with lipid. The degree of the intimal injury increased in the CMV infected hypercholesterolemic animals. After CMV infection the space between the endothelium and the basement membrane was expanded and was filled with basal-like material.
These observations provide support for the concept that CMV infection could be involved in atherogenesis. Although the precise mechanism is unclear the possibility that local inflammation processes due to reactivation of latent virus present in the vessel wall playing a role in this process remains to be further investigated.

A 402 ATHEROGENESIS IN GROWING WATANABE HERITABLE HYPERLIPIDEMIC (WHHL) RABBITS. Clubb, F.J., Jr., Butler, M.M., Willerson, J.T., Buja, L.M. Departments of Pathology and Cardiology at Texas Heart Institute and University of Texas Health Science Center at Houston, Houston, TX 77225. The WHHL rabbit is an established strain that carries a genetic mutation causing a deficiency of low density lipoprotein receptors, which leads to increased serum levels of cholesterol. This in turn results in the development of atherosclerosis. We studied both temporal and topographic distribution of leukocyte (L), monocyte (M), and foam cell (FC) adherence to endothelium and subendothelial lipid accumulation in young WHHL rabbits. Aortas of WHHL (n=32) and control/NZW (n=16) rabbits were perfusion fixed at various intervals (1, 3, 6, and 12 months of age), removed, stained with Sudan IV, and evaluated grossly and microscopically (light, scanning, and transmission). The percent lipid positive surface area progressively increased with age in the WHHL, with the proximal thoracic aorta (PT) consistently more involved than the distal thoracic (DT), proximal abdominal (PA) or distal abdominal (DA) aorta (mean ± SEM: 1mo=3.4±0.8% PT, 0.2±0.1 DT, 1.7±0.6 PA, 0.2±0.1 DA; versus 12 mon=66.1±5.3 PT, 31.8±10.4 DT, 35.1±5.5 PA, 11.4±6.3 DA; versus 0% in NZW). Similarly, the total number of L, M, and FC adherent to the endothelium increased with age (mean ± SEM: 1mo 3.1±1.1 cells/mm^2 PT, 0.7±0.2 DT, 1.7±0.6 PA, 0.2±0.2 DA; versus 12mo= 47.8±7.4 PT, 49.8±7.2 DT, 42.3±5.3 PA, 4.6±1.3 DA; versus 0% in NZW). Thus, the early age groups contained both small lipid positive areas and relatively low numbers of attached L, as well as TEM-identified lipid-laden macrophages and smooth muscle cells in the affected intima. FC and platelets were not observed on the intimal surface in these early age groups. There was a proportional increase in regional lipid and leukocytes at 12 months. These findings suggest that in the early stages of atherogenesis, an association exists between lipid accumulation and leukocyte adherence.

A 403 GROWTH FACTOR ACTIVITY DETECTED IN SERUM-FREE CULTURE OF PIG ARTERIOVENOUS BYPASS GRAFTS, Sheila E Francis, Steven Hunter, Cathy M Holt, Pat A Gadsdon, and Gianni D Angelini, Department of Cardiac Surgery, University of Sheffield, Northern General Hospital, Sheffield S5 7AU, UK

Smooth muscle cell proliferation in the intima is the main cause of late saphenous vein graft failure in man. It has been suggested that the release of growth factors from the vessel wall may provide the initial stimulus for this event.

To test this hypothesis segments of pig arteriovenous bypass graft removed from animals 1 and 4 weeks after implantation were cultured in serum-free media for 24 hours. Tissue viability as assessed by ATP concentration (nmol/g wet weight) was 245 ± 20, (n = 23) in freshly isolated graft and was maintained throughout the culture period, 244 ± 27, (n=13). Cell proliferation, as measured by ^{3}H Thymidine incorporation occurred during culture (920 ± 145 DPM/μgDNA, n=13).

Autoradiography and PC10 immunostaining showed that the proliferating cells were predominantly in the neointimal layer with few dividing cells in the media. In serial histological sections these cells were identified as smooth muscle cells using an antibody against α-actin.

Conditioned media from the cultured grafts were assayed for mitogenic activity using a 3T3 fibroblast proliferation assay. Media from grafts incubated for 15 minutes caused no cell proliferation. However, media from grafts incubated for 24 hours caused significant cell growth (87% ± 15, n=11, above that caused by basal medium).

The results of this study suggest that endogenous factors from the graft may regulate intimal smooth muscle cell proliferation in this model. This finding presents new possibilities toward therapeutic interventions aimed at improving long term vein graft patency rate.

A 404 MACROPHAGE AND REGRESSION OF ATHEROSCLEROSIS, Eugen Koren and Mirna Koscec, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Numerous pathohistological, ultrastructural, biochemical and cell culture studies carried out over the last 30 years provided evidence for both antiatherogenic and pro-atherogenic capabilities of macrophages. Our focus is on antiatherogenic potentials of these pluripotent cells, particularly on degradation of crystalline cholesterol. We have shown that mouse tumor macrophages P388D$_1$ internalize and solubilize radioactively labeled cholesterol crystals in culture (1). These studies were extended to human monocyte derived macrophages in an attempt to elucidate possible mechanism(s) by which these cells degrade cholesterol crystals. Macrophages were cultured with fluorescently labeled cholesterol crystals in a serum-free medium for 96 hours. During this period live cells were systematically analyzed for fluorescence distribution and fluorescence recovery after photobleaching by the use of interactive laser scanning microscope ACAS-570 (Meridian Instruments, Okemos, MI). Electron microscopic and biochemical analyses including cellular lipid and apolipoprotein measurements were carried out as well. Results clearly demonstrated a) ingestion of crystals; b) coating of internalized crystals with multiple phospholipid bilayers; c) mobilization of crystalline cholesterol, its esterification and accumulation in the form of droplets; d) an increase in the production of apolipoprotein E (ApoE), and e) excretion of ApoE and cholesterol into medium. These data confirm antiatherogenic potentials of macrophages and suggest that these cells could contribute to the regression of atherosclerotic lesions in vivo.

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A 405 INHIBITION OF ANGIOTENSIN CONVERTING ENZYME (ACE) DECREASES THE PROGRESSION OF ATHEROSCLEROSIS IN THE HYPERCHOLESTEROLEMIC HAMSTER, Mark C. Kowala, Mary Rosser*, Sean O'Conner*, Ron Recce, Sophie Beyer and Gunnar Aberg. Dept. of Pharmacology and Dept. of Screening and Bio-chemical Research* (Wallingford, CT), Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543

The role of the renin-angiotensin system in atherogenesis was determined by testing the effect of the ACE inhibitor fosinopril on the development of the fatty streak in hyperlipidemic hamsters. Atherosclerosis was induced by feeding chow containing 0.05% cholesterol and 10% coconut oil for 3 weeks. A control group was run along side two groups receiving either fosinopril or captopril at 50 mg/kg/day. Separate studies indicated that only fosinopril significantly decreased mean arterial pressure by 15% at 6 hours after dosing. In the atherosclerosis study, neither fosinopril or captopril reduced blood pressure at 3 weeks. Compared to controls, fosinopril reduced plasma LDL+VLDL cholesterols and total triglycerides by 27% and 40% respectively. Captopril had no effect on plasma lipids. Fatty lesions were analyzed in *en face* specimens of the aortic arch that were stained with oil red O (for neutral lipid). Compared to controls, fosinopril reduced the number of intimal macrophage-foam cells/mm^2, average foam cell size (μm^2) and total foam cell area (μm^2) by 85%, 38% and 90%. Captopril reduced these parameters by 44%, 16% and 53% respectively. In a separate experiment, fosinopril (50 μg/ml) increased the internalization of LDL into cultured Hep G2 cells by 150%, while fosinoprilat and captopril had no effect. To summarize, fosinopril decreased plasma LDL+VLDL cholesterol possibly via an up-regulation of hepatic LDL receptor activity. In addition, fosinopril lowered blood pressure and inhibited atherosclerosis. Captopril reduced the fatty streak without affecting plasma cholesterol and blood pressure. We suggest that the renin-angiotensin system participates in atherogenesis, and inhibiting ACE may modulate the disease through several mechanism(s) including an effect on macrophages.

A 406 ALL ISOFORMS OF PDGF ARE PRESENT IN A BABOON PROSTHETIC GRAFT HEALING MODEL.

Larry W. Kraiss, Elaine W. Raines, Thomas R. Kirkman, Russell Ross and Alexander W. Clowes; Departments of Surgery & Pathology, University of Washington School of Medicine, Seattle, WA 98195.

The factors regulating smooth muscle (SMC) proliferation and intimal thickening in injured arteries and vascular grafts are largely undefined. Previous work in this baboon model utilizing porous expanded polytetrafluoroethylene (ePTFE) vascular grafts has shown that SMC proliferate in regions of neointima covered by endothelium and that PDGF A-chain mRNA co-localizes to these regions. We now report that all three dimeric protein forms of PDGF (AA, AB, and BB) are present in this model of graft healing. Bilateral aorto-iliac ePTFE grafts in place for two months were removed and processed for immunohistochemical studies or snap-frozen for extraction and evaluation of extracted proteins by enzyme-linked immunosorbent assay (ELISA) and Western blotting studies. The graft extracts were also tested for mitogenic activity in a baboon SMC culture system. A monoclonal antibody against PDGF B-chain stained macrophages populating the graft matrix and neointimal SMC. A double affinity-purified polyclonal rabbit antibody to PDGF A-chain similarly stained macrophages in the graft matrix strongly. Neointimal staining was confined to the endothelial layer and first few SMC layers, similar to the localization observed with *in situ* hybridization for PDGF-A mRNA. Dimer-specific ELISAs detected significant amounts of PDGF-AA, AB, and BB in the extract of pooled graft matrix. The AA homodimer was the primary PDGF isoform in the neointimal extract, but some BB may also be present in very small amounts. No PDGF was detected in the perigraft tissue. In Western blots, a 30 kD protein in the intimal extract was recognized by an anti-PDGF-A antibody. The extract of the graft matrix also contained 30-40 kD proteins recognized by both anti-PDGF-A and polyclonal anti-PDGF antibodies. Extracts from both neointima and graft matrix were mitogenic for baboon SMC in culture and a significant portion of the mitogenic activity was blocked by an antibody to PDGF.

Conclusion: In this model of graft-healing, mitogenically active PDGF A- and B-chain are both present. The PDGF-B is primarily localized to the matrix while the neointima appears to express much more PDGF-A than B-chain.

A 407 LASER-INDUCED FLUORESCENCE: A TOOL FOR THE IN SITU STUDY OF ATHEROSCLEROTIC PLAQUE DEVELOPMENT,

Alexandra Lucas, Masis Perk, Yue Wen, Wolfgang Schneider, Bodh Jugdutt, Anees Chagpar, Carolyn Smith, Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G-2B7

We are investigating the in situ use of laser-induced fluorescence spectroscopy for the identification of specific biochemical changes during atherosclerotic plaque development. Fluorescence spectroscopy has been used for laser angioplasty guidance, in vivo. Atherosclerotic fatty, fibrous and calcific plaque content have been identified by selective changes in fluorescence emission spectra. Two avian models of atherosclerosis development were studied: (1) Marek's disease virus (MDV) infected White Leghorn roosters and (2) vascular balloon injury of cholesterol fed roosters. Fluorescence emission spectra (range 360-700nm) were recorded from the endocardial surface of each aorta during excimer laser excitation at 308nm. Specific changes in the fluorescence emission intensity and ratios of intensity in spectra were correlated with histological changes as well as arterial cholesterol and triglyceride content. A decrease in the fluorescence intensity at 440-460nm ($p<0.0005$) was recorded from fibrous and fatty intimal proliferation, as well as a decrease at 425-450nm with intimal thrombus ($p<0.009$). Fuorescence emission from areas of intimal hyperplasia in cholesterol fed roosters was higher intensity than low cholesterol diet roosters at 440-460nm ($p<0.004$). Areas of intimal cellular infiltration in MDV infected roosters had increased fluorescence emission ratios at 415/440nm and 420/460nm ($p<0.029$). The cholesterol and triglyceride content from Folch extractions of aortic segments had a good correlation with multiple regression analysis of 63 fluorescence emission ratios recorded from these segments ($R2= 0.64$ and 0.94 respectively). In order to further delineate the specific changes in fluorescence emission recorded from atherosclerotic lesions, spectra were recorded after intimal injection of ellastin, Types I, III, and IV collagen, cholesterol, triglycerides, LDL, VLDL, retinol, calcium hydroxyapatite, and NADH into the intimal surface of 210 segments of normal mongrel dog aorta. An increase in fluorescence intensity at 415nm was detected with collagen I and LDL-C ($p<0.014$); the ratio 375/455 increased with elastin and collagen III ($p<0.001$). Collagen IV (a normal constituent of the subendothelial layer) and pure cholesterol and triglyceride produced no significant change.

Conclusion: Fluorescence spectroscopy offers a new investigative tool for dissecting the different molecular constituents during atherosclerotic plaque development in a living organism.

A 408 A MONOCLONAL ANTIBODY DIRECTED AGAINST CD62 (GMP-140/PADGEM) INHIBITS PLATELET-PLATELET AND PLATELET-MONOCYTE INTERACTIONS.

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Granule membrane protein (GMP-140), also known as CD62 or PADGEM, is expressed on the platelet surface following degranulation. GMP-140, also expressed by activated endothelial cells, is part of a new family of adhesion molecules (known as Selectins or LEC-CAM) related to the endothelial leukocyte adhesion molecule (ELAM-1) and to the lymphocyte homing receptors in man (Leu-8/TQ1) and in mouse ($gp90^{MEL-14}$). In a an attempt to study the properties of platelet GMP-140 a monoclonal antibody (Mab LYP-20) was raised against GMP-140. LYP20 immunoprecipitated a protein of 128 kD from iodinated platelet lysates, and this protein was demonstrated to be GMP-140 by immunodepletion experiments using a Mab (S12) specific for GMP-140 (S12 is a generous gift from Dr. R. McEver). Western blots experiments indicate that LYP20 is directed against a disulphide bridge-dependent epitope. Using 125I-labelled antibodies, flow cytometry or immunohistochemical techniques it was observed that LYP20 binds specifically to thrombin-stimulated platelets and to human endothelial cells. The epitope recognized by LYP20 may be distinct from that recognized by S12, since S12 does not compete with LYP20, and vice-versa, in binding studies. LYP20 is the first anti-GMP140 Mab to significantly inhibit collagen or thrombin-induced aggregation of platelets. In addition, LYP20 inhibits rosetting of thrombin-activated platelets to U937 cells. These results are the first to demonstrate that the Selectin GMP-140 is involved in platelet-platelet interaction and plays, as previously shown, a key role in mediating platelet-leukocyte interactions.

A 409 IMMUNOCHEMICAL LOCALIZATION OF PDGF AND PDGF RECEPTOR IN ATHEROSCLEROTIC LESIONS OF HYPERCHOLESTEROLEMIC NONHUMAN PRIMATES,

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PDGF is a major mitogen and chemoattractant for smooth muscle cells that are the principal cells responsible for the progressive lesions of atherosclerosis that occlude the affected artery by increasing the thickness of the innermost layer of the artery, the intima. PDGF and its receptors are expressed at low or undetectable levels in normal vessels. However, at all stages of lesion development, PDGF B-chain is detected in macrophages that infiltrate the vessel wall. In this study we examined coordinate localization of PDGF B-chain and its specific cell surface receptor, the PDGF β-receptor subunit, in developing lesions of cholesterol-fed nonhuman primates. We have also investigated proliferation of smooth muscle cells and macrophages in adjacent sections of these arteries to further evaluate the possible role of PDGF in the development of the lesions of atherosclerosis. α-actin positive smooth muscle cells (SMCs) in the intima and superficial medial layers were stained intensely for the PDGF receptor β-subunit in all phases of atherogenesis. Lesions containing numerous PCNA positive cells (proliferating cells) were associated with macrophages that stained more intensely for PDGF B-chain. PCNA positive cells were identified as 44.5% macrophages (HAM-56 positive cells), 25.5% SMCs and 31.0% undefined. PCNA positive cells were distributed predominantly in the intima in all phases of atherogenesis. However, a significant portion of the PCNA positive SMCs (43.4% in fatty streak lesions) were distributed in the first few layers of the media as well as deep in the intima of each lesion. Numerous SMCs, forming perpendicular arrays protruding from the medial layer into the intima, were observed in active lesions. Thus, increased expression of both PDGF B-chain in macrophages and PDGF β-receptor subunit in smooth muscle cells are associated with developing lesions and co-localize with infiltrating and proliferating SMCs.

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Late Abstracts

BASIC FGF IS NECESSARY FOR ARTERIAL SMOOTH MUSCLE CELL MIGRATION AFTER BALLOON CATHETER INJURY.

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The migration of smooth muscle cells from the tunica media to the tunica intima is an obligatory step in the formation of a neointima following injury to the rat common carotid artery. Smooth muscle cell migration was quantified by scanning electron microscopic examination of the intimal surface of this vessel 4 days after injury, the time at which the peak rate of migration occurs. In balloon-injured vessels, smooth muscle cells occupied 10.5±2.0% (mean±SEM) of the intimal surface at this time point. In contrast, in vessels subjected to a gentle denuding injury that does not damage the media, migration was decreased and smooth muscle cells covered only 4.0±1.4% of the intimal surface ($p < 0.05$). Bolus intravenous administration of human recombinant basic fibroblast growth factor (bFGF) to rats at the same time as gentle denuding injury increased the proportion of the intima covered by smooth muscle cells to 24.4±7.1% ($p < 0.01$). These results suggest that medial trauma stimulates smooth muscle cell migration, possibly as a result of the liberation of bFGF from injured smooth muscle cells. In order to test this hypothesis, an affinity-purified rabbit IgG that inhibits the biological activity of human bFGF and cross-reacts with rat bFGF was administered intravenously in phosphate-buffered saline (PBS) to rats at the same time as balloon catheter injury and then every 24 hours. Smooth muscle cell migration was measured after 4 days. The proportion of the intima that was occupied by smooth muscle cells was 20.3±3.2% in normal IgG-treated rats, 11.8±3.8% in animals injected with PBS alone (p = NS), and 4.0±1.1% in animals treated with antibody to bFGF ($p < 0.001$). These results indicate that bFGF is a powerful stimulant of smooth muscle cell migration *in vivo*, and is a necessary component in the migratory response of smooth muscle cells to balloon catheter injury. (Supported by NIH grants HL 18H, HL 30203).

ABERRANT EXPRESSION OF THE IMMEDIATE EARLY GENES *FOS* AND *MYC* IN CULTURED HUMAN ATHEROSCLEROTIC PLAQUE SMOOTH MUSCLE CELLS,

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The proliferation of vascular smooth muscle cells (smc) is a critical event in atherosclerotic plaque formation. We recently reported a collagen gel procedure for developing human plaque smc (p-smc) strains, and showed that non-synchronized p-smc exhibited a 2 to 6 fold enhanced expression of the protooncogene *myc* compared to healthy aorta smc (HA-smc) (Parkes et al, 1991, Am. J. Path. 138: 765). Here we report the cell cycle dependent expression of the immediate early genes *myc* and *fos* which are critical to cell growth and differentiation. The expression of *fos*, which normally precedes that of *myc*, was non-detectable in quiescent cells. Sixty minutes following stimulation with fetal bovine serum, cultures of p-smc displayed a 3 to 7 fold enhanced expression of *fos* compared with HA-smc. Although p-smc expressed greater amounts of *fos* mRNA, the cell cycle dependent expression kinetics of this gene were identical in both plaque derived and healthy aortic smc. In all cases, *fos* message was first detected at 30 min, peaked at 60 min and was undetectable by 120 min following serum stimulation of quiescent cultures. The expression kinetics of *myc* was different in p-smc vs. HA-smc. For HA-smc *myc* mRNA levels first rose at 60 min following stimulation of quiescent cells, and reached a peak at 120 min. For p-smc *myc* started to rise a little earlier, at 30 min, reaching a peak at 90 min. All cell strains displayed a super-induction of both *myc* and *fos* in the presence of the protein synthesis inhibitor, cycloheximide. Moreover in the presence of cycloheximide *fos* continued to be expressed beyond 120 min. These results are in keeping with the idea that de novo synthesis of a repressor molecule(s) is required to turn off expression of *fos*. Results with the transcription inhibitor, actinomycin D, suggest that differences in the amount of *myc* and *fos* mRNA levels observed is not due to differences in messenger stability between p-smc vs. HA-smc. (Supported by NIEHS R01 02143 and Berlex Laboratories Inc.).

NICOTINE EFFECTS ON ENDOTHELIAL CELL GROWTH,

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Several factors, including smoking, predispose to atherosclerosis. Nicotine (N) is a major component of cigarette smoke and has been postulated to play an important role in atherosclerosis. We wished to investigate the role of N on endothelial cell (EC) growth by incubating calf pulmonary artery EC in the quiescent phase of the cell cycle (G_0-G_1) in serum-free conditions with N (free base, 10^{-19}-10^{-4} M), and determining the effects on tritiated thymidine incorporated into DNA and cellular proliferation after 48 hrs. The results showed concentration-dependent effects of N on EC growth. N-stimulated DNA synthesis had a bell-shaped distribution with a peak effect at 10^{-12} M that was 1.8-fold greater than control (serum-free M199 media alone). This peak effect occurred at concentrations that were 10,000-fold lower than N levels present in human plasma (10^{-8} M) after smoking 1-10 cigarettes. Concentrations of N below 10^{-16} M were not significantly greater than control, whereas N was cytotoxic at concentrations greater than 10^{-6} M. Similar effects were observed on EC proliferation with a peak increase in cell number of 2.5-fold greater than control at 10^{-10} M nicotine. Hydroxyurea (1mM), which blocks the transition from G_1 to S-phase of the cell cycle, did not completely abolish DNA synthesis stimulation in response to N suggesting that DNA synthesis was reparative and in response to N-induced DNA injury. This effect was observed at intermediate concentrations of N that were between those which stimulated cellular proliferative and cytotoxicity. In addition, in the presence of fetal bovine serum (0.5%), N-stimulated DNA synthesis was 2-fold greater than in response to N alone. Insulin (10 ug/ml) and platelet-poor plasma (5%) showed no effect on DNA synthesis in the presence of N, however. Our results imply that nicotine plays a role in EC growth control, that the cellular effect is dose dependent, and that platelet factors may play an important role in modulating the proliferative response.

REGULATION OF EICOSANOID PRODUCTION AND CELLULAR SECRETION BY CELL-IMPERMEABLE INHIBITORS OF PHOSPHOLIPASE A_2.

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Cell-membrane phospholipase A_2 (PLA_2) is a key enzyme in eicosanoid production and cellular secretion. Therefore, PLA_2 inhibitors have been considered for regulation of these processes and treatment of related diseases, such as inflammation, allergy and thrombosis. However, inhibitors that penetrate into the cell interfere with the vital phospholipid metabolism and impair the cell viability. We have designed and synthesized cell-impermeable PLA_2 inhibitors (PLIs) by linking known and novel inhibiting molecules (mainly derivatized phospholipids) to macromolecular carriers, such as plasma expanders and the like. The inhibiting moiety interacts with the cell membrane PLA_2 but its internalization is prevented by the carrier. The PLIs were found efficient in 1. Inhibiting cell-membrane PLA_2 activity in various cell types. 2. Inhibiting the production of arachidonic acid, leukotrienes (LTC_4) and prostaglandins (PGE_2) in HL-60 cells and macrophages. 3. Blocking human platelet aggregation and inhibiting thromboxane production. 4. Blocking serotonin secretion from rat basophilic (RBL) cells. 5. Reducing PLA_2-induced edema and adjuvant-induced arthritis in rats. 6. Blocking the secretion of mucoids from IB3 cells derived from the lungs of a cystic fibrosis (CF) patient who underwent heart-lung transplantation. (Excessive secretion and accumulation of mucus in the airways is the main cause for morbidity and mortality in CF patients). These data demonstrate the therapeutic potential of the cell-impermeable PLA_2 inhibitors we have synthesized, and suggest that these preparations may be proposed for treatment of pathological states related to eicosanoid production and excessive cellular secretion.