INFLAMMATION, GROWTH REGULATORY MOLECULES & ATHEROSCLEROSIS

Organizers: Russell Ross and Michael Gimbrone

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Poster Sessions

The Pathogenesis of Atherosclerosis Revisited

E 001 TRANSCRIPTIONAL REGULATION OF THE LDL RECEPTOR BY BASIC FGF: IMPACT of SPECIFIC RECEPTOR TYROSINE AND PROTEIN KINASES, David P. Hajjar, Andrew C. Nicholson, Alison T. Stopeck, and Hsien-Yeh Hsu. Depts. of Biochemistry and Pathology, Cornell University Medical College, New York, NY 10021.

Recently, we showed that TGF-ß enhanced the binding and degradation of LDL in arterial smooth muscle cells (SMC) (Nicholson AC and Hajjar DP, J. Biol. Chem. 267:25982-25987,1992); and, that other cytokines such as TNF and IL-1 upregulate LDL receptor function and gene expression in HEP G2 cells (Stopeck AT et. al., J. Biol Chem 268: 17489-17494, 1993). Since bFGF has been implicated in the regulation of cell proliferation and cholesterol metabolism in the vessel wall, we set out to define the role of bFGF in processes related to cholesterol delivery and trafficking in arterial SMC. In studies reported herein, we show that bFGF increases low density lipoprotein (LDL) binding, uptake, and degradation in SMC in a dose-dependent manner. This increase was paralleled by an increase in LDL receptor mRNA steady state levels. To determine if bFGF activated transcription of the LDL receptor gene, we transiently transfected smooth muscle cells with a gene construct consisting of the 5' upstream promoter region of the DNA from the human LDL receptor gene ligated to a plasmid containing the luciferase gene. We found that bFGF and a protein kinase C (PKC) activator, PMA, significantly induced luciferase activity driven by the LDL receptor promoter, while 25-hydroxy-cholesterol reduced the luciferase activity in bFGF-stimulated cells. These findings show that bFGF and PKC are inducing LDL receptor gene transcription. We also evaluated potential signal transduction pathways induced by bFGF to establish the mechanism(s) leading to the activation of the LDL receptor gene. Activation of FGF receptor tyrosine kinase activity in SMC by ligand binding resulted in tyrosine phosphorylation of one of the FGF receptors and a 90-kD protein as well as increased tyrosine phosphorylation of phospholipase C-gamma. Increased PKC and protein kinase A activities occurred in bFGF treated SMC relative to control cells. Inhibitors of receptor tyrosine kinase and other protein kinases significantly reduced transcription activity and surface expression of LDL-receptor, respectively. Finally, several key enzymes that are central to the regulation of LDL-cholesteryl ester (CE) metabolism were also measured in bFGF-stimulated cells. An increase in ACAT activity and the rate of cholesterol esterification was observed, but there was no effect on the lysosomal or cytoplasmic CE hydrolase activities. Our findings suggest potential signal transduction pathways activated by bFGF which play a role in regulating transcription activity and surface expression of the LDL receptor.

E 002 LIPIDS AND THE PATHOGENESIS OF ATHEROSCLEROSIS, Russell Ross, University of Washington, Seattle

Hyperlipidemia has for some time been associated with increased incidence of atherosclerosis and its clinical sequelae, coronary artery disease, heart attack, and stroke. The means by which hyperlipidemia induces the cellular migratory and proliferative responses characteristic of this increase in disease are being studied and better understood. Lipid insudation and accumulation were initially thought to represent the basis of lesion formation. However, the advanced lesions of atherosclerosis represent the culmination of smooth muscle, macrophage, and T cell proliferation and accumulation. The response-to-injury hypothesis of atherosclerosis proposed that "injury" to the endothelium and to the other cells of the artery wall plays a major role in the initiation and progression of the proliferative lesions of atherosclerosis. Data on oxidized lipoproteins suggest that oxidized LDL and other modifications of lipoproteins may be an important part of this process. Recent studies in hypercholesterolemic nonhuman primates, using the antioxidant, probucol, have demonstrated a statistically significant association between resistance to oxidation of LDL and decreased incidence and size of lesions of atherosclerosis. The lesions of the probucol-treated nonhuman primates contain fewer macrophages and smooth muscle cells, both of which show a marked decrease in PCNA positivity. In addition, newer approaches to studying atherogenesis include the development of transgenic mice. One such model, the apo E-deficient mouse, develops lesions of atherosclerosis similar in type and distribution to those observed in humans and nonhuman primates. This model demonstrates all stages of lesion development, including the entry of monocytes and T lymphocytes into the artery wall and fatty streak formation that precedes the development of intermediate lesions and advanced lesions or fibrous plaques. Other approaches to studying atherogenesis and its sequelae include a restenosis model in rabbits and, potentially, in nonhuman primates, involving the development of an advanced lesion of atherosclerosis, which can be induced to restenose by balloon angioplasty, to study the associated processes of cell migration and replication. Data from these studies are converging to provide further insight into the roles of growth factors, cytokines, and lipid oxidation in the pathogenesis of atherosclerosis.

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Receptors and Signal Transduction (Joint)

E 003 HETEROMERIC KINASE RECEPTORS FOR THE TGF-ß SUPERFAMILY

Jeffrey L. Wrana, Liliana Attisano, Juan Carcamo, Fernando Lopez-Casillas, Franscesc Ventura, Frances Weis and Joan Massague, Cell Biology and Genetics Program and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York City, New York 10021

The TGF-ß superfamily of cytokines represents one of the largest groups of growth and differentiation factors. Studies based on affinity crosslinking of various superfamily members to the cell surface has demonstrated that these ligands generally interact with pairs of membrane receptors that have been designated as type I and type II receptors. In addition, TGF-ß interacts with a cell surface proteoglycan, betaglycan (type III receptor) that functions to control ligand access to the signalling receptors. Type II receptors for activin, TGF-ß and bone morphogenetic proteins (BMPs) have been identified in mammals, Drosophila and C. elegans. These receptors bind their respective ligands with high affinity and share certain structural features. They are composed of a short, cysteine-rich extracellular domain followed by a single transmembrane domain and an intracellular region containing a kinase domain with predicted ser/thr specificity. More recently type I receptors for TGF-ß, activin and BMPs have been identified in mammals and Drosophila. Interestingly, these receptors share common structural features with the type II receptors, including an intracellular kinase domain. Thus the receptors for TGF-ß-related factors are grouped into a superfamily of related transmembrane kinases representing a new class of signalling receptors.

To explore receptor requirements for signalling we have investigated both the activin and TGF-ß systems. Both activin and TGF-ß type II receptors bind their respective ligands with high affinity, while the type I receptors require the presence of their corresponding receptor II in order to interact with ligand. Receptors I and II form stable complexes and while neither type I nor type II receptors can signal when expressed alone, signalling can be restored when both receptor types are coexpressed. Several different type I receptors for TGF-ß and activin have been characterized. Two of these type I receptors, TßR-I and ActR-IB contain kinase domains that are over 90% identical. Interestingly, expression of these receptors in non-responsive cell lines that express activin and TGF-ß type II receptors restored biological responses that were similar for both ligands. In contrast, expression of a second set of type I receptors for activin and TGF-ß (ActR-I and TSR-I, respectively) was unable to restore any of the common responses. These data demonstrate that signalling receptors for the TGF-ß superfamily are composed of heteromeric complexes of related transmembrane receptor kinases. Moreover, the presence of multiple, distinct activin and TGF-ß receptor complexes may represent one way in which the common and distinct biological responses observed for these factors are generated.

Transcriptional Regulation in Vascular Cells (Joint)

E 004 NF-κB AND IκB-α: AN INDUCIBLE REGULATORY SYSTEM IN ENDOTHELIAL ACTIVATION, Tucker Collins, Margaret Read, Maryann Whitley, and Amy Williams, Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, Massachusetts.

Induction of new endothelial cell surface proteins is an important early step in the development of an inflammatory infiltrate. Cytokine-induced expression of endothelial-leukocyte adhesion molecule-1 (E-selectin or ELAM-1) may mediate neutrophil adhesion to endothelium, whereas induced expression of vascular cell adhesion molecule (VCAM-1) may be important in the adhesion of mononuclear cells. Both the corresponding genes are transcriptionally regulated by the inflammatory cytokines. Structural analysis of the promoters of these genes reveals binding sites for the transcription factor nuclear factor κB (NF-κB). These NF-κB sites are necessary but not sufficient for cytokine responsiveness. Endothelial cells express transcripts encoding the p50/p105 (NFKB1) and p65 (RelA) components of NF-κB and the rel related proto-oncogene c-Rel; steady state levels of these transcripts are transiently increased by tumor necrosis factor-α (TNFα). Western blotting revealed that stimulation of endothelial cells with TNFα resulted in nuclear accumulation of the p50 and p65 components of NF-κB. UV crosslinking and immunoprecipitation demonstrated binding of the p50 and p65 components of NF-κB to the E-selectin κB site. Endothelial cells express an inhibitor of NF-κB activation, IκB-α (MAD-3). Protein levels of this inhibitor fall rapidly after TNFα stimulation. In parallel, p50 and p65 accumulate in the nucleus and RNA transcript levels for IκB-α are dramatically upregulated. Recombinant p65 stimulates expression of E-selectin promoter-reporter constructs. IκB-α inhibits p65 or TNFα-stimulated E-selectin promoter-reporter gene expression in transfected endothelial cells. Since multiple genes relevant to the pathobiology of endothelial activation have functional NF-κB sites, activation of this pleiotropic mediator in endothelial cells could coordinate the expression of numerous endothelial products which are important in endothelial activation. Increased expression of IκB-α decreases NF-κB activation and diminishes expression of κB dependent genes. This regulatory mechanism insures that the induction of NF-κB is transient and that the activated endothelial cells returns to a quiescent state. This dynamic balance may be offset during the initial onset of vascular pathobiology.

E 005 TNF INDUCED GENES. Vishva M. Dixit, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109. Programmed cell death (PCD) is of fundamental importance in cancer, development of the immune system, and embryogenesis. In development, it is critical to the establishment of proper neuronal connections and the formation of the immune repertoire. Inhibition of PCD has been associated with the development of a number of malignancies including follicular lymphoma and breast cancer. We have utilized a subtractive hybridization strategy to clone genes capable of conferring resistance to PCD induced by the cytokine tumor necrosis factor(TNF). Significantly, one of the genes capable of inducing resistance to PCD, designated A20, represents a novel class of zinc finger proteins. Interestingly, A20 is induced by a number of viral gene products known to inhibit PCD including the LMP-1 protein of the Epstein-Barr virus and the *tax* gene product of the HTLV-1 virus which causes adult T-cell leukemia. The initial characterization of the regulation of A20 and other TNF-induced genes will be discussed.

Genetically Modified Animals

E 006 HUMAN CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) TRANSGENIC MICE; STUDIES IN GENE REGULATION AND FUNCTION, Alan R. Tall[1], Tsuei Ping Yang[1], Elaine Quinet[1], Helena Oliveira[1], Luis Agellon[1], Tony Hayek[2], Anne-Marie Walsh[2], and Jan Breslow[2], [1]Columbia University and [2]Rockefeller University, New York, NY.

The plasma CETP mediates the transfer of cholesteryl esters (CE) from HDL to apoB containing lipoproteins. Studies in human CETP transgenic mice have helped to elucidate the role of CETP in lipoprotein metabolism and the regulation of CETP gene expression. CETP transgenic mice show small reductions in HDL cholesterol and apoA-I, but much more pronounced decreases in HDL concentration and size when cross-bred with mice expressing human apoA-I or human apoC-III (hypertriglyceridemic mice). Mice expressing higher levels of CETP (as seen in some human dyslipidemias) also show increases in VLDL plus LDL cholesterol and apoB, indicating both CE transfer and LpB accumulation; these mice show downregulation of liver LDL receptors and HMGCoA reductase mRNAs compared to non-Tg littermates. Mice expressing a CETP minigene linked to about 3 Kbp of upstream sequence and 2 Kbp of downstream sequence show expression in appropriate tissues, and marked induction of plasma CETP and liver (hepatocyte) CETP mRNA when placed on a high cholesterol diet. Mice expressing the same minigene linked to the metallothionein promoter do not show induction of mRNA in response to dietary cholesterol, allowing localization of the putative cholesterol response element (ChRE) to a defined region of upstream or downstream sequence. Preliminary results obtained with trangenic mice expressing transgenes with progressive deletions of the flanking sequences suggest that the ChRE resides within the proximal promoter. In addition to altered gene transcription, the CETP gene employs a novel mode of regulation involving alternative splicing of mRNA. Besides the mRNA encoding plasma CETP, all human tissues contain an alternatively spliced variant, in which exon 9 derived sequences have been omitted. Cellular co-transfection of cDNAs encoding full-length and E9-deleted CETP, shows that the E9 deleted form inhibits the cellular secretion of the full-length form, due to retention within the endoplasmic reticulum. The findings suggest a novel use of alternative splicing to generate a poorly secreted protein variant which complexes with the active form and prevents its secretion by cells. This concept is being tested in transgenic mice expressing an E9 deleted transgene. Also, the regulation of splicing is being studied using a transgene with the option to splice out E9.

E 007 *IN VIVO* MAPPING OF CARDIOVASCULAR SIGNALING PATHWAYS IN GENETICALLY MANIPULATED MICE, Kenneth R. Chien, John Ross, Jr., Howard A. Rockman, Lyn Powell-Braxton, John J. Hunter, and Giuseppe Lembo, Department of Medicine, Center for Molecular Genetics and the AHA-Bugher Foundation Center for Molecular Biology, University of California, San Diego, La Jolla, California

Utilizing molecular insights from *in vitro* systems, animal models, human genetics, and epidemiology, a number of genetically-based mouse models of human cardiovascular disease have now been generated. Coupling mouse genetics with recently developed miniaturized technology to quantitatively assess hemodynamics, angiography, and contractility *in vivo*, it is now feasible to genetically dissect complex physiological responses of the cardiovascular system in the intact animal. Toward this end, we have documented that the mouse heart can activate a hypertrophic response that is indistinguishable from that of larger species, including man. Following microsurgical banding of the thoracic aorta, the mouse myocardium acquires the morphological, structural, physiological, and genetic markers of clinical hypertrophy. Studies in *in vitro* cardiac muscle cell systems, have documented that the microinjection of oncogenic Ras protein can activate features of myocardial cell hypertrophy. To determine if Ras can serve a similar role in the intact heart *in vivo*, we fused an oncogenic Ras cDNA to a 250 bp MLC-2 promoter fragment that will confer ventricular-specific expression in transgenic mice. Several independent mouse lines harboring this MLC-Ras transgene were generated, and displayed structural, physiological, and genetic markers of hypertrophy in the left ventricular chamber. We have also monitored the physiological phenotype via microdigitized angiography and miniaturized catheters to assess cardiac function and consequent effects on the mouse aortic tree and branching vessels. While the proximal signals which couple to Ras dependent pathways to initiate this cardiac growth response are not completely clear, recent evidence in both normal and IGF-1 deficient mice suggest that IGF-1 may play a critical role in the activation of this adaptive physiological response. Studies are currently in progress to determine if Ras can rescue the IGF-1 deficient phenotype. These results suggest the utility of using mouse genetics for the *in vivo* mapping of complex physiological responses, such as cardiac hypertrophy. In addition, digitized microangiography should be valuable in monitoring the atherogenic phenotype in the *in vivo* context in a variety of genetically based mouse models of atherosclerosis.

Special Address (Joint)

E 008 MOLECULAR GENETIC ANALYSIS OF CELL ADHESION, Richard Hynes, Howard Hughes Medical Institute, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

A multiplicity of cell surface adhesion receptors and adhesive extracellular matrix proteins contributes to the adhesion of cells during normal development and in physiological and pathological processes in adults. Extensive molecular and *in vitro* cellular studies of these molecules have suggested roles for these adhesive proteins. However, since there are apparently overlapping specificities and functions of the different receptors, counterreceptors and ligands, it is necessary to determine which molecules contribute to which biological processes *in vivo*. Genetic analyses provide one good way to investigate these questions.

Integrin adhesion receptors and some matrix proteins are known to exist in invertebrate species which have been the traditional systems for genetic analyses. A certain amount of genetic dissection of the functions of these molecules during development of fruit flies and nematodes has been conducted and the roles of several adhesive proteins in specific developmental presses have been analyzed. However, in order to analyze the functions of these proteins in developmental processes and diseases characteristic of mammals, it would be desirable to conduct genetic analyses in a mammal; the obvious choice is the mouse.

In recent years techniques have been developed for introducing targeted mutations in genes of interest and establishing mutant strains of mice. In the past two to three years, mutations have been generated in several extracellular matrix proteins (tenascin, fibronectin, thrombospondin, collagens), several integrins, selectins, cadherins and Ig superfamily adhesion receptors. Some of these mutations produce embryonic lethality (eg. fibronectin, integrins, cadherins) and provide insights into the functions of these proteins in early mammalian development. Other mutations (eg. selectins, ICAM-1, β_2 integrin) are viable but have defects in inflammatory responses and yet others (eg. tenascin, thrombospondin) do not exhibit obvious phenotypic defects.

Examples of mutant phenotypes will be discussed to illustrate the types of information which can be gleaned by this approach, some of the complications of interpretation and potential avenues for future research to address these complications.

Cytokines and Their Inhibitors

E 009 CYTOKINES AND THE REGULATION OF CELL ADHESION MOLECULES *IN VIVO*. Steven M. Albelda[1,2], Istvan Juhasz[1], Horng-Chin Yan[1,2], Joseph Pilewski[1], George Murphy[3], Meenhard Herlyn[2], [1]Department of Medicine, Univ. of Penna. Philadelphia, PA 19104, [2]Wistar Institute, Philadelphia, [3]Department of Dermatology, Univ. of Penna., Philadelphia.

Endothelial cell (EC)-leukocyte interactions involve multiple cell adhesion molecules (CAM's) acting in a programmed and sequential manner to form a leukocyte-EC adhesion cascade. In order to fully understand this process, an *in vivo*, rather than *in vitro* model is needed. Two major problems have limited the utility of current animal models. The first is the inability of many of the anti-CAM antibodies developed in human cell culture models to cross-react with animal CAM's. The second is the difficulty in extrapolating animal (particularly rodent) findings to humans.

To circumvent these problems, we have transplanted pieces of normal human skin onto immunodeficient (SCID) mice to create chimeric animals (1). The transplanted skin grafts closely resemble normal skin histologically, retain their human vasculature, show low baseline levels of expression of E-selectin, VCAM-1, and ICAM-1, demonstrate marked upregulation of these CAM's in response to intradermal injection of human cytokines, and have the ability to support an active inflammatory reaction with migration of murine leukocytes into cytokine-injected areas. The skin model has been used to study the role of human endothelial cell CAMs in inflammation. Intravenously injected bioactive anti-human E-selectin mAb completely inhibited leukocyte adhesion and emigration induced by TNF, but had minimal effects on LTB_4 induced inflammation. Anti-PECAM-1 antibodies did not affect leukocyte adhesion, but blocked transmigration.

Human bronchus was also successfully transplanted into SCID mice. Intraluminal instillation of TNF induced similar upregulation of E-selectin, ICAM-1, and VCAM-1 in the submucosal microvascular bed as was seen in the skin, although with slightly different kinetics. Interestingly, upregulation of ICAM-1 and VCAM-1 was observed on the airway smooth muscle tissue.

In conclusion, Human/SCID chimeric mice represent a powerful model system to study the regulation and function of human CAMs on endothelium and smooth muscle in an *in vivo* setting.

1. Yan et al., Human/SCID Mouse Chimeras, J. Clin. Invest., 91:986-996

E 010 INTERLEUKIN-1 AND INTERLEUKIN-1 ANTAGONISM, Charles A. Dinarello, Tufts Univiversity and New England Medical Center, Boston, MA 02111 USA

Interleukin-1 (IL-1) affects nearly every cell type by increasing the expression of a variety of genes associated with the promotion of inflammatory processes. These include upregulation of cyclooxygenase and nitric acid synthases. In animal models of infectious, inflammatory or metastatic disease, the role of IL-1 has been defined by specifically preventing the ability of IL-1 to trigger its type I receptor (IL-1RI). The IL-1RI transmits a signal(s) through the cytoplasmic domain which is structurally related to the fruit fly *Toll* protein. We have recently cloned the promotor most proximal to the 5' UTR of the human IL-1RI gene. The genomic sequences reveal a lack of TATA and CAAT boxes but rather a considerable (75%) GC rich region. The translation of the type I IL-1R appears under a significant suppression and may limit the biological activities of IL-1 by low level of expression. On the other hand, the type II IL-1R, lacking a significant cytoplasmic domain, does not transmit a signal and acts as a decoy receptor preventing the binding to the type I receptor. There are several approaches to reducing IL-1 activity; inhibition of IL-1 converting enzyme which cleaves pro-IL-1β, anti-IL-1RI, the IL-1 receptor antagonist (IL-1Ra) and soluble (extracellular) IL-1RI and IL-1RII. IL-1Ra is structurally related to IL-1 and binds at 4°C to the IL-1RI with near equal affinity as that of *bona fide* IL-1; however, IL-1Ra does not transduce a signal. IL-1Ra has been given to humans in pharmacologic levels (mean plasma levels of 30 μg/ml) without evidence of agonist activity. In addition, there has been no affect on normal homeostatic parameters, suggesting that IL-1 does not play a role in health. In healthy humans given intravenous endotoxin, IL-1Ra reduced endotoxin-associated neutrophilia by 50% and completely reversed endotoxin-induced immunosuppression. IL-1Ra is produced naturally and is elevated in the circulation in several diseases. In a variety of diseases, plasma levels of IL-1Ra are in 100-fold molar excess to those of IL-1β. It is unclear whether these endogenous levels of IL-1Ra are sufficient to block IL-1 activity in disease. A single injection of IL-6 or IFNα does not induce IL-1 but rather high levels of IL-1Ra (10 ng/ml).

E 011 CYTOKINES AS MEDIATORS OF VASCULAR DISEASES, Peter Libby, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115.

The cytokines, mediators of immune and inflammatory responses, orchestrate many systemic defense mechanisms to infection and injury. Recently, much interest has focused on the possibility that cytokines may also modulate local pathological processes that involve blood vessels ranging from atherosclerosis to septic shock. This concept emerges from the observations that each of the principle cell types involved in atherogenesis both produce and respond to a wide gamut of cytokines. These mediators alter key functions of vascular cells and leukocytes associated with the vessel wall that likely contribute to a variety of pathologic processes. For example, the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) regulate the expression of endothelial-leukocyte adhesion molecules (e.g. E-selectin and vascular cell adhesion molecule-1, VCAM-1) that govern leukocyte recruitment in at sites of inflammation or injury. Selective granulocyte and mononuclear phagocyte-tropic chemokines such as monocyte-chemoattractant and activating protein MCP-1 can promote local accumulation and activation of blood-derived leukocytes at such sites. Leukopoietic cytokines such as macrophage-colony stimulating factor (M-CSF) can stimulate terminal differentiation of monocyte-derived macrophages and enhance their replication during chronic states of arterial inflammation such as atherosclerosis. After the initial stage of leukocyte recruitment and activation, cytokines can augment the local production of smooth muscle chemoattractants and mitogens such as platelet-derived growth factor, contributing to the transition from acute inflammatory or vasculitic states to more fibrous lesions. Cytokines can also regulate the synthesis and degradation on components of the extracellular matrix such as collagen that comprise a large proportion of the mass of mature lesions of intimal thickening, the final result of a variety of pathologic and iatrogenic states. Cytokines also regulate the thrombogenic properties of endothelium and macrophages, for example by inducing tissue factor expression and increasing the production of inhibitors of fibrinolysis. Thus, cytokines derived from vascular cells and from recruited leukocytes probably contribute to multiple facets of vascular lesion formation, a concept that provides tools for understanding these processes and potential targets for intervention.

E 012 INTERLEUKIN - 1 (IL-1) AND ENDOTHELIAL CELLS (EC): RECEPTORS AND NEW IL-1-INDUCIBLE GENES. Alberto Mantovani, Francesco Colotta, Fabio Re, Ferruccio Breviario, Victor Vidal Alles, Elisabetta Dejana, Martino Introna, Istituto Ricerche Farmacologiche "Mario Negri" via Eritrea 62, 20157 Milano, Italy.

IL-1 activates EC in a prothrombotic/proinflammatory sense. Two IL-1 receptors (R) have been identified, the type I and the type II (decoy) R Human umbilical vein EC express the type I R and not the type II decoy R, as assessed by northern and PCR analysis and crosslinking, and respond to IL-1 via the type I R. A differential screening approach was used to identify novel IL-1 - inducible genes in EC. PTX3, a novel gene related to pentraxins (C-reactive protein and serum amyloid P component)was selected for further analysis. Sequencing of the full-length cDNA clone and RNase mapping revealed that the PTX3 transcript is 1861 bp long and has a unique transcription start side. The predicted protein sequence of 381 amino-acids is highly similar to pentaxins in its C terminal half where it also contains a typical 8 amino-acid "pentaxin signature" sequence. The N-terminal half of PTX3 shows no similarity to any known protein sequence and initiates with a putative signal peptide indicating that PTX3 is secreted. The genome of PTX3 is organized into 3 exons. Interestingly, the region of homology between PTX3 and pentaxins corresponds to the third PTX3 exon. The PTX3 gene has been localized on human chromosome 3 band q25 by Southern blots of somatic cell hybrids and in situ hybridization. The PTX3 mRNA is induced in endothelial cells by IL-1ß and TNFα but not by IL-6 and Interferon-γ. PTX3, unlike classical pentraxins, is also expressed at the mRNA and protein level by mononuclear phagocytes. The muose gene is highly similar to the human one. PTX3 may represent a novel marker of inflammatory reactions, particularly those involving the vessel wall.

Developmental Processes (Joint)

E 013 EMBRYONIC DEVELOPMENT OF THE ENDOTHELIAL NETWORK STUDIED IN THE AVIAN MODEL, Françoise Dieterlen-Lièvre, Dominique Luton and Luc Pardanaud, Institut d'Embryologie cellulaire et moléculaire du CNRS et du Collège de France, 49bis, av. de la Belle Gabrielle, 94736 Nogent s/Marne cedex, France.

The blood system is a derivative of the mesoderm. We have undertaken to define its early history, i.e. which of the mesoderm substructures are capable of giving rise to endothelial cells and whether a hemangioblastic anlage, endowed with both endothelial and hemopoietic capacities, can be identified at an early step of the segregation process. These questions are best approached in the avian model, because it is possible to perform orthotopic exchanges of selected rudiments between two species, quail and chick, and trace the progeny through a structural cell marker (quail nuclear marker) (1) or monoclonal antibodies (for instance the quail hemangioblastic marker, QH1) (2). We have previously determined that the two sheets of the lateral plate mesoderm have definitely different capacities, only the splanchnopleural or internal layer giving rise to endothelial and hemopoietic cells (3). We now show that the segmental plate, i.e. the paraxial mesoderm that divides into somites, has an important endothelial potential. Segmental plate and somites also appear capable of producing hemopoietic cells, although in our experimental conditions they are less productive than splanchnopleura (4). Whether there is a common progenitor to the two lineages is now studied through an expression analysis of two protooncogenes, c-ets1 and c-myb, respectively linked to the amplification process of endothelial versus hemopoietic progenitors (5, 6).

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2 - Pardanaud, L., Altmann, C., Kitos, P. & Dieterlen-Lièvre F. 1987. Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. Development, 100, 339-349.
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6 - Pardanaud, L. & Dieterlen-Lièvre, F. 1993. Expression of c-ets1 in early chick embryo mesoderm: relationship to the hemangioblastic lineage. Cell Adhesion & Communication (in press).

E 014 ABERRANT INFLAMMATORY RESPONSE IN TGF-BETA-1-DEFICIENT MICE, Tom Doetschman[1], Ron Diebold[1], Mike Eis[1], Ann Kier[2], Greg Boivin[2], Ingrid Grupp[3], Stacy Smith[4], Paul Allen[4], Marcia Shull[1], and Jeff Saffitz[4], [1]Department of Molecular Genetics, [2]Department of Pathology, [3]Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, [4]Department of Internal Medicine, Washington University School of Medicine, St. Louis.

TGF-ß1 knockout mice develop an acute, multifocal, mixed cell inflammatory disease followed by wasting and death usually within the week after weaning. Among the affected organs are stomach, liver, pancreas, heart, lung, diaphragm, salivary, and lacrimal glands. Other disorders such as cardiomyopathy, hyperplasia, and stomach ulcers often accompany the inflammation. To distinguish inflammatory from developmental effects of the TGFß1 deficiency, several approaches have been undertaken: 1) FACS and immunohistochemical techniques are being used to determine whether thymus development and thymocyte activity is abnormal. 2) The time course of inflammation is being monitored so that possible developmental abnormalities can be observed prior to the onset of inflammation. 3) Genetic and therapeutic approaches to inhibit the inflammatory response are being pursued to determine which disorders persist. Our initial results are as follows. Firstly, activated thymocytes appear in the thymus in pre-symptomatic animals and increase to high levels in symptomatic (wasting) animals. Peripheral thymocytes appear activated but exhausted in symptomatic animals. These results suggest abnormal thymic development, improper thymocyte activation, or peripheral activation followed by abnormal trafficking into the thymus. Secondly, inflammation first occurs in many of the affected tissues in general during the second week of life, with some tissues being affected earlier than others. Consequently, abnormalities detected in the first week of life should be independent of inflammation. Thirdly, our genetic approach is to combine the TGFß1 mutation with the *scid*, *lps*, and *beige* mutations to determine the role played by lymphocytes, macrophages, and natural killer cells, respectively, in the inflammatory process. TGFß1/SCID mutant mice have just been born and are being analyzed. Finally, anti-integrin (LFA-1) therapy inhibits inflammation in several tissues revealing disorders that appear to have other origins such as growth control deficiency, impaired ventricular relaxation, and reduced ß-adrenergic response and receptor density. Using these approaches we hope to separate the developmental from the inflammatory disorders and thereby shed light on the function of TGFß1 at the whole animal level.

E 015 REGULATION OF VASCULOGENESIS AND ANGIOGENESIS, Werner Risau, Max-Planck-Institute, D-61231 Bad Nauheim, Germany.

Angioblasts and hemopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac. The development of blood vessels from these early in situ differentiating endothelial cells is called vasculogenesis which can be induced in avian embryonic stem cells by fibroblast growth factors in vitro. Tyrosine kinase receptors, such as the VEGFR-2 (flk-1) and tie-2, specific for the endothelial lineage, are concomitantly induced in angioblasts. Angiogenesis, the formation of blood vessels by sprouting from preexisting vessels, is observed during embryonic development, e.g. organogenesis of the brain. The expression of VEGF and its cognate receptors VEGFR-1 (flt-1) and VEGFR-2 (flk-1) as analyzed by northern blot and in situ hybridization suggests a paracrine control of vasculogenesis, angiogenesis and endothelial cell proliferation during mouse development. Furthermore, the VEGF/VEGFR signaling system is necessary for pathological angiogenesis and tumor growth of human and rat glioblastoma.

E 016 **SMOOTH MUSCLE DIVERSITY AS PART OF THE VASCULAR RESPONSE TO INJURY.** Stephen M. Schwartz, Volkhard Lindner, Edward O'Brien, Rick Johnson, Michael A. Reidy & Cecilia M. Giachelli. University of Washington, Seattle, WA 98195

We have hypothesized that the arterial wall may be comprised of at least two smooth muscle cell (SMC) types. When SMCs from the arteries of two week old pup rats are put into culture and compared with SMC grown from the arteries of adult rats, a number of pup-specific differences appear. Cells with similar properties have been isolated from the artery of the adult rat in 3 ways. First, if the vessel is subjected to balloon angioplasty, a neointima forms and cells from this structure put into culture display the full range of properties seen in pup cells. Cells with similar pup/intimal (π) properties can be derived from the adult vessel wall either by cloning or by selectively isolating SMC in the absence of PDGF.

We have used differential hybridization to identify genes whose expression stably marks the two types of SMC. The growing list of π genes include cytochrome P450 IA1, PDGF-B, type I (alpha 1) collagen, elastin, differential tenascin and osteopontin (OP). Genes which appear unique to the adult SMC phenotype in culture include versican and PDGF-alpha receptor.

The differential libraries made from π cells have proven to be useful in identifying genes showing patterns of overexpression in response to injury or in atherosclerosis Among genes first identified in cultured π cells in vitro, several have shown selective expression in the neointima in vivo as well. These include PDGF-B chain, elastin, cyp1a1, and OP. OP is also a prominent feature of atherosclerosis, especially in areas of calcification. OP, however, is not found in pre-atherosclerotic intima, implying that its expression is somehow chronically controlled within the plaque, either by changes in lineage or by chronic expression of factors that stimulate its expression. OP may play a critical role in formation of atherosclerotic lesions via functions in chemotaxis of leukocytes and smooth muscle cells. Moreover in examining the role of OP in microvascular injury we have found that OP protein is prominent in vessels undergoing malignant arteriosclerosis as well as being a common early component of tubulointerstitial injury in the kidney where it could serve as a chemotactic/adhesive factor for monocytes. In light of its close association with biomineralization in the bone and its striking co-localization with ossified areas of atherosclerotic plaque, it is interesting to speculate that osteopontin may be an important modulator of calcium deposition in a diverse panel of responses to injury.

In summary, constitutive expression of a set of genes by intimal or pup arterial smooth muscle cells in vitro appears to identify markers either of a subset of cells important in vascular response to injury or a common response of vascular smooth muscle cells at injury sites.

Adhesion Pathobiology: Molecular Genetic Approaches (Joint)

E 017 MOLECULAR GENETIC MANIPULATION OF MURINE E-SELECTIN EXPRESSION IN ADULTS AND DURING DEVELOPMENT, David S. Milstone, Peter O'Donnell, and Vannessa Davis, Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115. E-Selectin (endothelial-leukocyte adhesion molecule-1, ELAM-1) belongs to a family of cell surface glycoproteins the members of which share a unique polypeptide domain organization and play key roles in leukocyte adhesion to vascular endothelium. Expression of E-selectin in adult tissues is largely restricted to cytokine-activated postcapillary venular endothelial cells where it is thought to be involved in early leukocyte adhesive events. Although a large body of *in vitro* functional evidence and both *in vitro* and *in vivo* gene expression data support this proposed function, few stringent tests of this hypothesis have been reported. Murine embryonic stem (ES) cells differentiate *in vitro* to form embryoid bodies (EB) containing vascular channels which are lined by morphologically identifiable endothelial-like cells, contain nucleated blood cells and may be analogous to embryonic blood islands. To compare the properties of these endothelial-like cells with those of mature vascular endothelium, we are examining the expression pattern and function of E-selectin, and other adhesion molecules, in ES cells differentiated *in vitro*. E-Selectin mRNA is not induced by LPS in undifferentiated ES cells but is first induced, in a time and concentration-dependent manner, in stages of EB differentiation when vascular channels are not prominent. We are currently determining the relationship of E-Selectin induction to emergence of the endothelial lineage in EB, whether E-Selectin inducible cells are precursors of the endothelial-like cells formed in EB, and whether induction of E-selectin may thus provide a useful marker of the vascular endothelial lineage during development. We have cloned a portion of the murine E-Selectin gene and introduced into ES cells, *via* homologous recombination, a predicted null allele to test the hypothesis that E-Selectin expression is required for differentiation and function of the endothelial lineage in EB. We are also establishing, *via* blastocyst-mediated transgenesis, lines of mice bearing this mutation to test the hypothesis that E-Selectin expression is required for endothelial-neutrophil interactions at sites of acute inflammation and for evolution of a normal acute inflammatory response *in vivo*..

E 018 MOLECULAR BASIS FOR L-SELECTIN FUNCTION IN COMPARISON WITH P- AND E-SELECTIN, Thomas F. Tedder[1,2], Kim B. Saunders[2], Geoffrey S. Kansas[2], Klaus Ley[3], Andreas Zakrzewicz[3], Rosemary M. Gibson[4], Barbara C. Furie[4], and Bruce Furie[4],
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The selectin family of adhesion molecules mediates the initial interactions of leukocytes with endothelium. L-selectin is expressed by most leukocytes, while P- and E-selectin are expressed by activated endothelial cells. The extracellular region of each selectin contains an amino-terminal lectin domain, followed by an epidermal growth factor (EGF)-like domain and multiple short consensus repeat units (SCR). L-selectin mediates lymphocyte attachment to high endothelial venules (HEV) in lymph nodes and also mediates leukocyte attachment to cytokine-activated endothelial cells through an induced endothelial ligand which is independent of both E- and P-selectin. Transfection of L-selectin cDNA into cells which do not normally bind to endothelium confers adhesion and the ability to roll in rat mesenteric venules *in vivo*, demonstrating that expression of L-selectin alone is sufficient to mediate leukocyte rolling. However, removal of the cytoplasmic domain of L-selectin completely abrogates attachment and rolling of transfected cells suggesting that cytoskeletal associations or signals generated through the cytoplasmic domain are involved in regulating L-selectin function. Since the affinity of L-selectin for its ligand is transiently increased following leukocyte activation, this event may also be regulated through the cytoplasmic tail. The increase in affinity can be recapitulated by the binding of a unique mAb to the EGF-like domain of L-selectin, suggesting the involvement of the EGF-like domain in this process. Therefore, the role of the selectin extracellular domains in cell adhesion was examined using a panel of chimeric selectins generated by exchange of domains between L- and P-selectin and between L- and E-selectin. The binding ability of chimeric selectin molecules expressed by cDNA-transfected cells was assessed in two ways. L-selectin function was assessed by the ability of transfected cells to bind HEV and to roll in mesenteric venules. P- and E-selectin-like activities were assessed by the ability of the chimeric receptor to bind the myeloid cell line HL60, that is not bound by L-selectin. Exchange of only the lectin domains between selectins successfully conferred the adhesive and ligand recognition functions of the lectin-derived molecule. However, chimeric selectins which contained both the lectin domain of L-selectin and the EGF-like domain of P-selectin exhibited dual ligand-binding specificity, supporting adhesion both to myeloid cells and to HEV and mesenteric venules *in vivo*. Comparable experiments with L- and E-selectin chimeric molecules provided similar results. In all cases, L-selectin-like function was always correlated with the presence of the L-selectin lectin domain, with no apparent functional contribution provided by the L-selectin EGF-like domain. Exchange of the SCR domains between selectins had no detectable effect on receptor function or specificity. These results suggest a unique role for the EGF-like domains of P- and E-selectin and indicate that the EGF-like domain is in part responsible for ligand recognition and leukocyte adhesion mediated by these selectins, either by binding ligand directly or by contributing to the binding specificity of the lectin domain.

E 019 P-SELECTIN-DEFICIENT MICE, Denisa D. Wagner, New England Medical Center, Division of Hematology-Oncology, and Tufts University School of Medicine, Boston, MA 02111.

Our laboratory studies the response of endothelial cells to vascular injury and inflammatory stimuli. One of our main interests is the expression and function of the adhesion receptors P- and E-selectin. P-selectin is stored in the endothelial storage granules called Weibel-Palade bodies from where it can be rapidly expressed on the cell surface. We have shown that after surface expression P-selectin is rapidly internalized and is directed into newly formed Weibel-Palade bodies. This means that when necessary this molecule can be reexpressed on the plasma membrane without requiring de novo synthesis. E-selectin in contrast is not stored and after expression on the plasma membrane by de novo synthesis is routed into lysosomes where it is degraded. P- and E-selectin mediate adhesion to monocytes and neutrophils and some cancer cells. To study the role of these molecules in normal and pathological processes, we have prepared mice lacking P-selectin by gene targeting in embryonic stem cells (Mayadas et al., 1993), and we are currently preparing animals lacking both P- and E-selectin by the same methodology. The P-selectin-deficient animals are fertile, appear grossly normal and under the conditions of routine mouse husbandry appear generally healthy at one year of age. We have found major changes in leukocyte behavior in the P-selectin-deficient animals. There was a complete absence of spontaneous leukocyte rolling in the mesenteric venules and a delayed recruitment of neutrophils to sites of inflammation. Peripheral neutrophil counts in blood were elevated in P-selectin-deficient animals compared to wild type, likely the result of defective interaction of the neutrophils with the vessel wall. We are now examining the role of P-selectin in later stages of inflammation, in neutrophil demargination, and in wound healing. Since P-selectin is also found in platelet α-granules, the P-selectin-deficient platelets that can be obtained in normal numbers from the mutant animals will be an excellent tool to study the role of this receptor in platelet functions.

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Special Address (Joint)

E 020 AN *IN VIVO* LOOK AT MICROCIRCULATION IN HEALTH AND DISEASE, Rakesh K. Jain, Harvard Medical School and Massachusetts General Hospital, Department of Radiation Oncology, Boston, MA 02114.

The possibility to grow endothelial cells in culture from various organs has increased our understanding of the structure and function of these cells at the molecular level. It has also provided novel hypotheses about the molecular mechanisms of various diseases involving vascular pathophysiology, e.g., cancer, inflammation, and atherosclerosis. A prerequisite for testing these hypotheses *in vivo* is the availability of tissue models which permit non-invasive, continuous and long term monitoring of microvascular events. To this end, we have utilized two chronic preparations: modified Sandison rabbit ear chamber (1) and modified Algire mouse dorsal skin chamber (2) to study microcirculation in normal and neoplastic tissue. The former has the advantage of superior optical qualities and the latter of being able to work with immunodeficient and transgenic species. These preparations have allowed us to test hypotheses generated from *in vitro* studies and to formulate new ones in the following areas: angiogenesis and blood flow in tumors (2-4); metabolic microenvironment in tumors (5,6); transvascular and interstitial transport in normal and tumor tissues (7-11); and leukocyte-endothelial interactions in normal and tumor vasculature (12,13). In this presentation I will highlight our findings in these general areas and focus on parameters which can only be measured *in vivo*. I will also discuss the implications of our findings for cancer detection and treatment (14).

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Adhesion Pathobiology: Anti-Inflammatory Strategies

E 021 REGULATION OF LEUKOCYTE - ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION WITH SYNTHETIC OLIGONUCLEOTIDES, C. Frank Bennett, ISIS Pharmaceuticals, Carlsbad, CA 92008

In response to inflammatory stimuli, expression of a group of proteins (endothelial - leukocyte adhesion molecules) which bind circulating leukocytes are induced on the surface of vascular endothelial cells. Binding of circulating leukocytes to vascular endothelium is an obligatory step in emigration of leukocytes to sites of inflammation. A series of antisense oligonucleotides were designed to hybridize to the mRNA's which encode three such endothelial - leukocyte adhesion molecules; intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule - 1 (VCAM-1) and E-selectin (ELAM-1). Antisense oligonucleotides are short synthetic oligonucleotides (10 to 25 bases in length) designed to hybridize to an RNA (sense strand) encoding the protein of interest. Upon binding to a target RNA, the oligonucleotide may inhibit expression of the target protein by multiple mechanisms, depending in part on the target site of the mRNA and chemical class of oligonucleotide. Several oligonucleotides were identified which selectively inhibited expression of either ICAM-1, VCAM-1 or E-selectin. Oligonucleotides which hybridized to the 3'-untranslated region of either ICAM-1, VCAM-1 or E-selectin mRNA's promoted a selective reduction in mRNA levels, while oligonucleotides which hybridized to 5'-untranslated sequences did not significantly reduce target mRNA levels. ICAM-1, VCAM-1 and E-selectin antisense oligonucleotides each reduced adhesion of HL-60 cells to tumor necrosis factor activated human umbilical vein endothelial cells. These data demonstrate that antisense oligonucleotides are capable of selectively inhibiting the expression of different endothelial - leukocyte adhesion molecules in human umbilical vein endothelial cells An antisense oligonucleotide was also identified which selectively inhibited murine ICAM-1 expression. The murine ICAM-1 antisense oligonucleotide exhibits anti-inflammatory activity in several animal models demonstrating that the oligonucleotides are also active *in vivo*.

E 022 MONOSPECIFIC AND COMMON GLYCOPROTEIN LIGANDS FOR E- AND P-SELECTIN ON MYELOID CELLS, Dietmar Vestweber, Agneta Levinovitz and Martin Lenter, Hans Spemann-Laboratory at the Max-Planck-Institut for Immunology, 79108 Freiburg, Germany .

E- and P-selectin are inducible cell adhesion molecules on endothelial cells, which function as Ca^{2+}-dependent lectins and mediate the binding of neutrophils and monocytes. We have recently identified a 150 kD glycoprotein ligand for E-selectin on mouse myeloid cells, using a recombinant antibody-like form of mouse E-selectin (1). Here, we report that this ligand does not bind to an analogous P-selectin fusion protein. Instead, the chimeric P-selectin-IgG protein recognizes a 160 kD glycoprotein on the mouse neutrophil progenitor 32D cl 3, on mature mouse neutrophils and on human HL60 cells. The binding is Ca^{2+}-dependent and requires the presence of sialic acid on the ligand. On all three cell types, this ligand is co-expressed with the 150 kD E-selectin ligand but not recognized by E-selectin. Removal of N-linked carbohydrate side chains from the 150 kD and the 160 kD monospecific selectin ligands abolishes the binding of both ligands to the respective selectin.

In addition, glycoproteins of 230 and 130 kD were found, which bound both to E- and P-selectin in the presence of Ca^{2+} and could only be isolated from mature mouse neutrophils but not from 32D cl 3 and HL60 cells. The signals detected for these ligands were 15-20 fold weaker than those for the monospecific ligands. Both proteins were heavily sialylated and required sialic acid for selectin-binding. The ratio between the 230 kD and the 130 kD protein varied in different experiments with a tendency to find more of the 130 kD protein after reduction. However, both proteins were detectable in reduced samples and reduction of the 230 kD glycoprotein did not directly generate the 130 kD species. Our data reveal that E- and P-selectin recognize two categories of glycoprotein ligands: one type is monospecific for each of the two selectins and the other is common for both endothelial selectins.

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E 023 IN VIVO RELATIONSHIPS BETWEEN CYTOKINES AND ADHESION MOLECULES, Peter A. Ward, Ara A. Vaporciyan, Michael L. Jones, and Michael S. Mulligan, Department of Pathology, University of Michigan, Ann Arbor, Michigan.

Intrapulmonary deposition of IgG immune complexes results in a series of reactions leading to the influx of neutrophils and damage of alveolar epithelial and vascular endothelial cells. This reaction is CD11a/CD18 (but not CD11b/CD18)-dependent and requires availability of ICAM-1 as well as E-selectin. By the use of either blocking antibodies or soluble TNFα receptor-1, it has been demonstrated that TNFα is required for neutrophil recruitment. Through the use of ^{125}I-antibodies, in vivo upregulation of ICAM-1 occurs in a time-dependent manner following deposition of immune complexes. Under conditions of TNFα blockade, upregulation of ICAM-1 is not only completely inhibited but its vascular expression drops below baseline levels. Furthermore, treatment of rats with recombinant murine IL-4 or IL-10 profoundly suppresses production of TNFα (as measured in bronchoalveolar lavage fluids), resulting in inhibition of neutrophil influx. Again, this treatment results in suppression of ICAM-1 upregulation in vivo. These studies emphasize the role of cytokines in the upregulation in vivo of ICAM-1, increased expression of which is a necessary prerequisite for recruitment of neutrophils. The data also provide evidence for the regulatory functions for IL-4 and IL-10 in the acute inflammatory response.

Pathophysiologic Aspects of Proteases and Their Inhibitors (Joint)

E 024 THE LDL RECEPTOR GENE FAMILY - ROLES IN PROTEASE REGULATION, LIPID METABOLISM AND ATHEROSCLEROSIS, Shun Ishibashi[1], Dennis Burns[2], Thomas Willnow[1], Hideo Otani[1], Robert E. Hammer[3], Michael S. Brown[1], Joseph L. Goldstein[1], and Joachim Herz[1]; [1] Departments of Molecular Genetics and Internal Medicine, [2]Department of Pathology, [3] Howard Hughes Medical Institute and Department of Biochemistry, University of Texas, Southwestern Medical Center, Dallas TX 75235

Four structurally closely related proteins that belong to the low density lipoprotein (LDL) receptor gene family are currently known in mammals. They comprise the LDL receptor, the LDL receptor-related protein (LRP), the Heymann's nephritis antigen gp330 and the very low density lipoprotein (VLDL) receptor. All these constitutively recycling cell surface receptors are composed of the same four structural motifs: complement-type cysteine-rich repeats, epidermal growth factor (EGF) precursor-homologous domains, a single membrane-spanning segment and a short cytoplasmic tail that contains between one and three 'NPxY' endocytosis signals. LRP and gp330 are approximately five times larger than the LDL receptor and the VLDL receptor and a structurally perfectly conserved homologous protein has been found in the evolutionarily distant organism *Caenorhabditis elegans*. LDL receptor and LDL receptor-related proteins function in biologically diverse processes which include the metabolism of lipoproteins, lipoprotein lipase, plasminogen and plasminogen activators and plasminogen activator inhibitor, α_2-macroglobulin, Pseudomonas exotoxin A, lactoferrin and vitellogenin. The activity of LRP and of gp330 can be modulated in vitro by a small vesicular protein, called receptor-associated protein (RAP). A physiological role of RAP as a fast-acting modulator of LRP activity has been proposed. To study the physiological functions of the different members of this gene family we have created strains of mice in which individual genes have been disrupted. Observed phenotypes vary depending on the gene defect and include increased plasma cholesterol levels and increased susceptibility for the formation of atherosclerotic lesions in LDL receptor deficient mice and early embryonic lethality in LRP-deficient mice. Furthermore, we have used somatic cell gene transfer to study the effect of overexpression of the transferred gene in normal and genetically altered mice.

E 025 PAI-1 GENE EXPRESSION IN HEALTH AND DISEASE by David J. Loskutoff, Ph.D., Department of Vascular Biology, The Scripps Research Institute, La Jolla, CA 92037.

Plasminogen activator inhibitor 1 (PAI-1) is a rapid and specific inhibitor of both t-PA and urokinase and may be the primary regulator of plasminogen activation in vivo. A primary hypothesis of our work is that the expression of PAI-1 in vivo is under strict regulation, and that the inappropriate expression of this inhibitor may suppress the normal fibrinolytic system of the tissues and create a local prothrombotic state. This condition may result in pathological fibrin deposition following injury and/or tissue factor expression. In order to test this hypothesis, we have begun to study PAI-1 gene expression in mouse and human tissues, using Northern blotting, in situ hybridization, and immunohistochemistry. PAI-1 mRNA and antigen were detected in most murine tissues from normal animals suggesting a vascular origin. Although no PAI-1 mRNA was detected in endothelial cells (ECs), it was detected in smooth muscle cells (SMCs) throughout the vasculature. Endotoxin (LPS) induced PAI-1 mRNA in most tissues and this increase resulted from specific induction in vascular ECs. The level of PAI-1 mRNA in SMCs was rapidly and dramatically reduced by LPS. PAI-1 was elevated locally in a variety of disorders. For example, it was dramatically increased in the kidneys of mice with glomerulonephritis, in atherosclerotic human vascular tissue, and in the pulmonary arteries of individuals with non-resolving pulmonary emboli. In most instances, the pattern of PAI-1 induction resembled the pattern of fibrin deposition. Thus, the abnormal expression of PAI-1 in tissues may inhibit the clearance of fibrin and contribute to the ongoing pathologies of these disorders.

Strategies to Modify Gene Expression (Joint)

E 026 ADENOVIRUS-MEDIATED GENE TRANSFER INTO VASCULAR CELLS, Robert D. Gerard and Robert S. Meidell, Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235-8573

In an effort to develop gene-based strategies to intervene in the pathogenesis and progression of vascular disease, we have employed recombinant, replication-defective adenoviruses to introduce foreign genes into mammalian cells *in vitro* and *in vivo*. Viruses have been generated which carry genes encoding either reporter enzymes (luciferase, β-galactosidase) or physiologically important proteins (tissue plasminogen activator, plasminogen activator inhibitors, uPA receptor, LDL receptor, apolipoprotein A1, bFGF) expressed from a variety of promoters of viral or cellular origin. Efficient genetic modification has been observed following infection of a wide range of mammalian cell types in culture and several different tissues in living animals, including liver parenchyma, pancreatic islet cells, renal tubular epithelium, skeletal myocytes, and vascular endothelial and smooth muscle cells. Following intravenous administration of recombinant adenovirus to mice or rabbits, foreign genes are preferentially (>99%) expressed in the liver. Histochemical staining demonstrates expression in both hepatocytes (predominantly) and endothelial cells. Mice infected with a virus encoding the human LDL receptor express the protein at a 10-fold higher level than uninfected mice, and show significantly accelerated clearance and reduced circulating levels of cholesterol. Similarly, animals infected with a virus encoding human apolipoprotein A-I accumulate high (> 200 mg/dl) circulating levels of ApoA-I and show increased circulating HDLc levels, suggesting the potential for gene-based therapeutic strategies to reduce cardiovascular risk. Delivery of foreign genetic material to vascular endothelial and smooth muscle cells has been examined using several catheter-based strategies. Intraluminal dwell of high-titer purified stocks of recombinant adenovirus, either by direct instillation into a surgically isolated vessel segment or infusion through a double-balloon catheter, results in efficient and preferential genetic modification of vascular endothelium. In contrast, infusion of recombinant virus stock into the vessel wall under pressure using perforated or Hydrogel-coated balloon catheters targets foreign genes to medial smooth muscle cells, although the efficiency of genetic modification is relatively low. Vascular endothelial cells infected with recombinant adenoviruses encoding human tPA and PAI-1 demonstrate marked overexpression of these proteins. Efficient, directed genetic modification of cells resident in the vessel wall offers the potential to intervene in important pathophysiologic processes, including angiogenesis, vascular thrombosis and restenosis following angioplasty. While adenovirus mediated gene transfer offers the potential of therapeutic application, several important limitations, including cell-type specific targeting, stability of foreign gene expression and the effects of host immune response, remain to be addressed.

The Pathogenesis of Atherosclerosis Revisited; Receptors and Signal Transduction

E 100 TREATMENT OF EXPERIMENTAL CHRONIC VASCULAR REJECTION WITH ANGIOPEPTIN AND HEPARIN DERIVATES IN A RAT MODEL,

Levent M. Akyürek[1], Alkwin Wanders[1], Erik Larsson[1] and Bengt Fellström[2], [1]Departments of Pathology and [2]Medicine, University Hospital, Uppsala, S-751 85 Sweden

Intimal thickening of vessels is a prominent characteristic feature of transplanted organs undergoing chronic rejection. Since accelerated transplant arteriosclerosis is a major cause of late graft failures it is important to investigate and evaluate potential means of prevention and treatment. So far no effective treatment has been established.

As an experimental model we used the orthotopic transplantation of abdominal allogeneic and syngeneic aortic grafts in the rat. The allogeneic grafts were stored in a cold perfusion solution for 1-hour ischemia and syngeneic transplantations for 4-hours before transplantation. Animals were put on a cholesterol diet. After 8 weeks of observation the vessel grafts were explanted and subsequently immunohistochemically monitored for different cell types and mediators. Focal alterations of the intimal and medial layer were measured using an image analysis system. No immunosuppressive drugs were added. The recipient rats were treated with the compounds in question using mini-osmotic pumps. The somatostatin analogue angiopeptin was continously administered in a dose of 100 µg/kg/day and the non-coagulant heparin derivatives OAM 71262 or LA-heparin (no affinity to antithrombin-III) in a a dose of 250 µg/kg/hour.

Angiopeptin treatment led to a reduction of the intimal thickening by over 50% in the syngeneic grafts ($p<0.05$) but not in the allogeneic grafts. No clear effect could be observed on any certain cell type or mediator. In a similar manner, OAM 71262 or LA-heparin reduced the degree of intimal thickening in the syngeneic grafts by over 50% ($p<0.05$) and 30%, respectively. Similarly, no beneficial effect was seen in the allogeneic grafts.

It is concluded that angiopeptin and heparin derivatives may prove to be promising compounds in conjunction with immunosuppressive agents to combat accelerated graft arteriosclerosis.

E 101 Signaling pathways leading to chemotaxis and proliferation of human arterial smooth muscle cells. Are they distinct or common?

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Chemotaxis and proliferation of arterial smooth muscle cells (SMC) both contribute to intimal SMC accumulation, a key event in the development of atherosclerotic lesions and in restenosis following angioplasty. The intracellular signaling pathways leading to chemotaxis and proliferation may be distinct in human SMC. In these cells, factors that act as potent chemoattractants, such as platelet-derived growth factor-BB (PDGF-BB) and insulin-like growth factor I (IGF-I), both stimulate phosphatidylinositol bisphosphate (PIP_2) hydrolysis and diacylglycerol formation within 15-45 seconds, without affecting phosphatidylcholine or phosphatidylethanolamine lipid masses, measured with high-performance liquid chromatography. Hydrolysis of PIP_2 and formation of inositol trisphosphate are consistent with the rapid increase in intracellular calcium levels following stimulation with either PDGF-BB or IGF-I. Factors that act as potent mitogens in SMC, such as PDGF-BB and PDGF-AA, markedly activate the mitogen-activated protein kinase (MAP kinase) cascade, with a maximal activation of MAP kinase kinase and MAP kinase 5 min after stimulation. IGF-I, which is a weak mitogen but has a receptor number equal to PDGF-AA, does not significantly stimulate this signaling pathway.

Taken together, these results imply that phosphatidylinositol turn-over and calcium flux are important for chemotaxis, whereas activation of the MAP kinase cascade is not required for chemotaxis but is important for proliferation. Distinct signaling pathways leading to directed migration and proliferation may be involved in SMC proliferative versus migratory response of the vascular wall to injury.

This study was supported in part by NIH grants HL 18645 and HL 03174, and a grant from Bristol-Myers Squibb Co.

E 102 INTRACELLULAR CATABOLISM OF OXIDIZED LDL,

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We have previously shown that defective intracellular processing of oxidized LDL (OxLDL) by cultured macrophages leads to the intracellular accumulation of large amounts of free cholesterol and of undegraded apolipoprotein (J. Lipid Res. 1992; 33: 819-829). Aim of the present study was to characterize the lipoprotein-derived material which accumulates within macrophages incubated in the presence of OxLDL. J774 murine macrophages were incubated with 125I-OxLDL or acetylated LDL (AcLDL) and cell homogenates fractionated on Percoll density gradients. Upon incubation with OxLDL the bulk of radioactivity was in the lysosome-enriched fraction (48% of cell-associated radioactivity, versus 28% with 125I-AcLDL-incubated cells). Gel filtration chromatography indicated that this fraction contained lipoprotein-derived material with the size of the original particle, in addition some larger, probably aggregated, material eluted at the void volume. Upon salt density gradient fractionation virtually all particles (>90%) floated in the density range of HDL ($d < 1.063$-1.21 g/ml). With the addition of unlabelled OxLDL the peak of radioactivity moved towards a lighter density and some radioactivity appeared to associate to the parent lipoprotein itself. In the case of 125I-AcLDL besides a radioactivity peak in the density range of HDL, a shoulder with higher density indicated the presence of more extensively processed protein. Addition of unlabelled AcLDL moved a relatively high percentage of the total radioactivity to the density of the parent lipoprotein. Upon immunofluorescence microscopy with a monoclonal anti lysosome-associated membrane protein (LAMP) antibody (kindly provided by Dr. August, Johns Hopkins University) and anti apo B the two epitopes colocalized in the same subcellular organelle. Our results suggest that lipoprotein-derived material with the characteristics of a lipoprotein particle accumulates in lysosomes of murine macrophages incubated with OxLDL. This accumulation of OxLDL might be responsible for the poor activation of ACAT by OxLDL in spite of the large accumulation of free cholesterol within macrophages.

E 103 PCNA POSITIVE CELLS ARE DECREASED IN LESIONS OF PROBUCOL-TREATED HYPERCHOLESTEROLEMIC MONKEYS,

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In a recent study of atherosclerosis following diet-induced hypercholesterolemia (HC) in the nonhuman primate, *Macaca nemestrina*, we evaluated the effect of the antioxidant, probucol, on lesion size in relation to the resistance of plasma LDL to oxidative modification. Plasma LDL from probucol-treated HC animals was significantly more resistant to *ex vivo* oxidation than LDL from control HC animals. Intimal lesion area was demonstrated to be inversely correlated with the resistance of LDL to oxidation. To evaluate the effects of probucol on lesion cell proliferation in the present study, intimal lesions of 3 out of 10 animals with normal resistance of LDL to oxidation and 3 out of 6 animals with increased resistance of LDL to oxidation were analyzed by quantitative immunohistochemistry. Using proliferating cell nuclear antigen (PCNA) to estimate cells in cell cycle traverse, the percentage of PCNA-positive [% PCNA(+)] cells was analyzed at 6 sites within the thoracic (T1-T6) and abdominal (A1-A6) aortae and at 4 sites within the iliac artery. A 2-fold decrease in % PCNA(+) cells was found in thoracic aorta sites of animals with increased resistance of their LDL to oxidation; a 3-fold decrease was found in abdominal aorta sites of animals with increased resistance of their LDL to oxidation; and no difference was found in % PCNA(+) cells in the iliac arteries between the two groups. Additional analysis by double immunostaining showed the majority (>70%) of PCNA(+) cells to be macrophages and the remaining PCNA(+) cells to be smooth muscle cells in both groups. To evaluate whether the 3 animals selected from each group were representative of the entire group of 16 animals, %PCNA(+) cells was evaluated at 2 selected aortic sites in all 16 animals. In T2 of animals with normal resistance of their LDL to oxidation, 8.5 ± 1.4% of lesion cells were PCNA(+) compared to 2.5 ± 0.8% ($p< 0.005$) in animals with increased resistance of their LDL to oxidation. In A1, 5.4 ± 1.1% of lesion cells were PCNA(+) in animals with normal resistance to oxidation, compared with 1.4 ± 0.7% ($p< 0.01$) in animals with increased oxidation resistance. These findings suggest that the decrease in lesion size in the probucol-treated animals with increased resistance of plasma LDL to oxidation may be due, in part, to a decrease in cells entering cell cycle traverse.

E 104 CO-ORDINATE REGULATION OF SQUALENE SYNTHASE, HMGCOA REDUCTASE AND LDL RECEPTOR IN HEPG2 CELLS, A D Charles, L K Buckett and F McTaggart, Cardiovascular Dept, ZENECA Pharmaceuticals, Alderley Park, Cheshire, England.
HMGCoA reductase and squalene synthase (SQS) catalyse key steps in cholesterol biosynthesis. The activity of both these enzymes is subject to feedback inhibition by cholesterol, which also down-regulates the level of LDL receptor (LDL-R). For LDL-R and, in part, HMGCoA reductase, this regulation occurs at the level of transcription. We have investigated how SQS is regulated by cholesterol, and the extent to which this correlates with changes in HMGCoA reductase and LDL-R. We have measured the changes in mRNA levels of SQS, HMGCoA reductase and LDL-R in HepG2 cells in response to lovastatin (1μM) or 25-hydroxycholesterol (25μM) over 48h using an RNase protection assay, and correlated these with alterations in enzyme or receptor activity. Incubation with 25-hydroxycholesterol resulted in a significant (40–60%) and co-ordinate decrease in both activity and mRNA levels for SQS, HMGCoA reductase and LDL-R. Most of the inhibition was achieved within 12h, by which time cholesterol synthesis was totally abolished. Lovastatin maximally induced HMGCoA reductase mRNA levels 2-3 fold and SQS mRNA 4-5 fold within 30h. SQS and HMGCoA reductase activities rose steadily over the 48h, increasing 3-4 fold for SQS and 4-5 fold for HMGCoA reductase. After an initial decrease, cholesterol synthesis rose as lovastatin inhibition was overcome. LDL-R mRNA was not maximally induced (2-fold) until 40h after addition of lovastatin. Our data demonstrates that in HepG2 cells, changes in enzyme activity parallel changes in mRNA levels for both SQS and HMGCoA reductase. We observe, however, subtle differences in the regulation of the LDL-R under the same conditions.

E 105 INFECTION OF AORTIC ENDOTHELIAL CELLS BY HUMAN CYTOMEGALOVIRUS
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Injury of the arterial endotheium may be an improtant provocation for the initiation of atherolsclerosis. Human cytomegalovirus (HCMV), a human herpesvirus, is a suspected pathogenic factor in the development of atherosclerosis and a potential cause of endothelial damage. To develop a representative model system to study the pathogenic effects of HCMV on the aortic endothelium, tertiary cultures of human arterial endothelial cells (HAEC) were immortalized by the introduction of the E6 and E7 genes of human papillomavirus type 16 along with the neomycin resistance marker into the genome of the HAEC cells. Neomycin resistant clones that were morphologically similar to the parental cells and positive for expression of von Willibrand factor (vWF) were selected and expanded for further analysis. The resulting HAEC cell line which is thus far immortal in its growth properties, was analyzed for HCMV infectivity with a laboratory and and two clinical isolates of the virus. At 24 hours post-infection (pi), viral antigens were present in 14-22% of cells. Antigen-positive cells increased up to six days pi. There was no significant effect on the abundance or localization of vWF in virus infected cells. Although cytopathology was not very apparent in infected cell monolayers, one step growth curve experiments demonstrated that the HAEC-I cells supported productive infection of the laboratory and clinical strains. Virus infection peaked between five to seven days pi. Enhanced monocyte-endothelial cell adhesion was evident in virus infected cells which correlated with the kinetics of infection and antigen expression. The availability of a permanently-growing human aortic endothelial cell line will be a useful model system to further define the potential role of HCMV in the development of atherosclerosis.

E 106 ACCELERATED GRAFT ARTERIOSCLEROSIS AFTER PROLONGED COLD GRAFT ISCHEMIA TIME IN THE RAT, Bengt Fellström[1], Levent M. Akyürek[2], Erik Larsson,[2] Johannes Waltenberger[3], Keiko Funa[3], Alkwin Wanders[2], Departments of [1]Medicine and [2]Pathology, University Hospital, 751 85 Uppsala, [3]Ludwig Institute for Cancer Research Biomedical Center, Uppsala Sweden
The purpose of this study was to investigate the effect of prolonged cold graft ischemia time on the development of transplant arteriosclerosis (TA). Aorta grafts from DA or PVG rats were stored in a cold perfusion solution *for 1, 4 or 24 hours* before an orthotopical transplantation to PVG recipients. After various observation times (2 until 8 weeks) the grafts were examined for different cell populations. Regional changes of the intima and media layers were measured using an image analysis system. The allogeneic intima layers showed independently of the ischemia time a very prominent increase of the area that occluded approximately 35% of the lumen after 8 weeks. The intima changes seen in the syngeneic grafts that underwent 1 hour ischemia were moderate, whereas the grafts after 24 hour ischemia time showed pronounced TA-like changes that could be as prominent as those seen in the allogeneic grafts. The severity of the intima thickening in the syngeneic group correlated with the ischemia time ($r=0.62$, $p<0.001$, $n=49$) and, in the allogeneic group, with the observation time ($r=0.59$, $p<0.001$, $n=62$). The cell composition found in the intima and media of both groups differed considerably: In the allogeneic group macrophages, T cells, MHC class II expressing cells and smooth muscle cells were observed in the whole vessel wall. In the syngeneic grafts T lymphocytes and cells with a class II expression were absent. Here, almost exclusively smooth muscle cells and macrophages were found. It is concluded that the damage that occurs after prolonged cold ischemia time is sufficient to cause pronounced TA. Furthermore, the pathophysiological mechanism leading to this type of TA is different from that seen in the allogeneic situation.

E 107 MONOCLONAL ANTIBODY TO MURINE MACROPHAGE SCAVENGER RECEPTOR (MSR) INHIBITS MACROPHAGE ADHESION *IN VITRO*.
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Macrophage (Mϕ) adhesion molecules play an important role in the recruitment of these cells to sites of inflammation, including atherosclerotic lesions. Adhesion to culture surfaces *in vitro* is a property of Mϕ that is frequently used to isolate and purify these cells. In addition, Mϕ differ from other cell types in that they are able to adhere to tissue culture plastic in the absence of divalent cations. By exploiting this property in a multiwell adhesion screening assay, we have isolated a rat monoclonal antibody (mAb), 2F8, that inhibits the EDTA-resistant adhesion of murine Mϕ to tissue culture plastic surfaces in the presence of serum. Immunoprecipitation with mAb 2F8 from CHO cells stably transfected with MSR cDNAs, showed that this antibody recognizes both forms of the murine MSR. Analysis of MSR biosynthesis in primary peritoneal Mϕ showed that only the type II form of the receptor was synthesized. Receptor precipitated from Mϕ was able to bind acetylated low density lipoprotein (AcLDL), and uptake of this ligand by Mϕ was partially inhibited by mAb 2F8. Some, but not all MSR ligands inhibited EDTA-resistant Mϕ adhesion.
This receptor, implicated in foam cell formation in atherogenesis, has previously only been shown to mediate endocytosis. We suggest that Mϕ may use the MSR for recruitment to, and/or retention in atherosclerotic lesions, and other anatomical sites where scavenger receptor ligands may be abundant.

E 108 THE INDUCTION OF ENDOTHELIN-RECEPTORS AND THE MODIFICATION OF ENDOTHELIN-INDUCED RESPONSES BY INTERLEUKIN-1ß IN PRIMARY CULTURED SMOOTH MUSCLE CELLS FROM HUMAN AORTA. Yasushi Fujitani, Yukio Sasaki, Toshikazu Okada and Yoshihiro Urade. International Research Laboratories, Ciba-Geigy (Japan) Limited, 10-66 Miyuki-cho, Takarazuka 665, Japan

Endothelin (ET), produced by the vascular endothelium, is the most potent vasoconstrictive peptide and also acts as a mitogen on vascular smooth muscle cells. ET is thought to be involved in vascular remodeling, since the production and release of ET reportedly increase during atherosclerotic process. In this study, to investigate the role of ET under inflammatory conditions in the blood vessel wall, we examined the effects of various cytokines and growth factors on the ET-induced signal transduction pathway in primary cultured smooth muscle cells from human aorta (AOSMC).

AOSMC (5-6 th passages), which possess only ET_A receptors coupled to intracellular Ca^{2+} increase, were treated for various periods (1-24 hr) with IL-1ß (2ng/ml), IL-8 (10 ng/ml), TNFα (5 ng/ml) and TGFß (5 ng/ml). Pretreatment of AOSMC with IL-1ß induced a significant increase in maximal binding capacity (Bmax) of ET_A receptors from 35,000 to 55,000 sites/cell, without affecting the dissociation constant (Kd) of about 40 pM. IL-1ß also induced expression of ET_B receptors (Kd: 20 pM, Bmax: 3,300 sites/cell). Other cytokines or growth factors did not show such effects. The up-regulation of ET_A and ET_B receptors was observed in a transient manner 4-12 hr after treatment with IL-1ß, peaking at 6hr, and was completely inhibited by simultaneous treatment with cycloheximide.

Furthermore, after treatment with IL-1ß, ET-1 evoked an increase in cellular cAMP, which was not observed in the untreated cells. However, ET-induced intracellular Ca^{2+} increase remained unchanged. The ET-induced cAMP increase showed almost identical time courses with the up-regulation of ET receptors and was also abolished by cycloheximide. The induction of the ET-induced cAMP response was specific to IL-1ß. The cAMP increase was completely blocked by BQ-123, an ET_A antagonist, and indomethacin, indicating the involvement of ET_A receptors and prostanoids in ET-induced cAMP increase.

These results suggest that the contractile and mitogenic activities of ET in smooth muscle cells might be altered during vascular remodeling.

E 109 **S100 PROTEINS IN INFLAMMATION**

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S100 proteins are small, acidic Ca^{2+}-binding proteins which are highly conserved and expressed in a cell lineage-specific manner. Although lacking signal peptide sequences for secretion some of these proteins have extracellular activities including glial cell growth activity by S100β. MRP8 and 14 are found in myeloid cells and are present in serum of patients with a number of inflammatory conditions and have been implicated in the pathogenesis of reperfusion injury. MRP8 and 14 coat vascular endothelium adjacent to marginating monocytes and it is proposed that these proteins are involved in the migration of leukocytes into tissues (Hogg N, Allen C, Edgeworth J (1989) Eur J Immunol, 19: 1053). We described a murine S100 protein (CP-10) related to MRP8, which is a potent chemotactic agent for neutrophils and monocytes *in vitro* and *in vivo*. CP-10 is expressed in high levels by bone marrow cells which decrease following differentiation of monocytes and macrophages. The chemotactic domain resides within a "hinge" region peptide, $CP\text{-}10_{42\text{-}55}$, between the two Ca^{2+}-binding EF hand domains. Like TGFβ, CP-10 is more potent (optimal activity at $10^{-12}M$) than classical chemoattractants but does not mobilise intracellular Ca^{2+}, provoke granule enzyme release or induce superoxide. However CP-10 causes profound shape changes and polymerisation and redistribution of F-actin suggesting a specific effect on cell movement. A role for this protein in the pathogenesis of inflammation and atherosclerosis is suggested by its effects on leukocyte migration, on expression of adhesion receptos and on macrophage activation as evidenced by its ability to promote foam cell formation *in vivo*.

E 110 **THE ADHESION MOLECULE E-SELECTIN IS INDUCED BY ANGIOTENSIN II IN HUMAN CARDIAC ENDOTHELIAL CELLS**

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In epidemiological studies increased ACE-activity was associated with higher risk for coronary artery disease. Expression of adhesion molecules on endothelial cells may be one of the initial events in atherosclerosis. We investigated the regulation of adhesion molecules in human cardiac endothelial cells (HCEC) in culture by angiotensins. HCEC were isolated from explanted hearts and grown in culture. Contaminating non-endothelial cells were removed by paramagnetic beads linked to the ulex europaeus lectin. The expression of E-selectin, VCAM, and ICAM was measured on protein level by means of a cell-ELISA and FACS analysis and on mRNA level by RT-PCR. The effect of 4 hour incubation with Angiotensin I (A I), A II, and A III (all 10^{-11}-$10^{-5}M$) was compared with the maximal stimulation by the cytokins TNF (1000 U/ml) and IL-1 (20 U/ml). Low levels of E-selectin and moderate levels of VCAM and ICAM were detected under control conditions. After incubation with AI and AII E-selectin, but not VCAM and ICAM, was enhanced to 20% and 50% of TNF effects, respectively. AI in the presence of the ACE-inhibitor lisinopril ($10^{-6}M$) and AIII were without effect. The data suggest that AII modulates the interaction of leukocytes with endothelial cells by selectively inducing E-selectin. This could be one of the mechanisms leading to a higher risk of coronary artery disease by increased ACE-activity.

E 111 ACTIVE TGF-β IS DEPRESSED FIVE-FOLD IN TRIPLE VESSEL DISEASE PATIENTS COMPARED WITH SYNDROME X PATIENTS, David Grainger, Paul Kemp, James Metcalfe, *Alex Liu, *Richard Lawn, +Andrew Grace, +Peter Schofield and +Anoop Chauhan, Department of Biochemistry, University of Cambridge, CB2 1QW, U.K., *Division of Cardiovascular Medicine, Stanford University, CA 94305 and +Cardiac Unit, Papworth Hospital, Cambs., CB3 8RE, U.K.

Our recent work has led to the working hypothesis that TGF-β is a key regulator of vascular smooth muscle cell proliferation *in vivo*. One approach to testing this hypothesis was to determine whether the development of atherosclerosis is correlated with depressed levels of active TGF-β. We have developed assays for both active and total (active+latent) TGF-β in serum and compared the levels of active and total TGF-β in 32 patients who had at least 50% coronary stenoses of all three major arteries (triple vessel disease; TVD) with 30 syndrome X patients with no indication of coronary artery stenoses by angiography.

All of the TVD group had serum levels of active TGF-β of 1ng/ml or less (below the detection limit of the assay), whereas all the syndrome X group had 2ng/ml or more of active TGF-β (mean 4.07 ± 1.93ng/ml; range 2 to 10ng/ml). Control experiments showed that the differences in active TGF-β between the two groups were not due to the presence of a dominant inhibitor of the assay in the sera of the TVD patients or of an activator of latent TGF-β in the sera of the syndrome X patients. In contrast to the active TGF-β data, the TVD patients had higher levels of total TGF-β (8.84±15.52ng/ml; range <1 to 60 ng/ml) compared with syndrome X subjects (5.57±3.64ng/ml; range 2 to 18ng/ml; $p<0.005$). The data have been analysed to determine the effect of several parameters: a significantly higher proportion ($p< 0.05$) of the total TGF-β was active in women compared with men and in patients with low lipoprotein(a) concentrations. Patients taking aspirin had higher levels of total TGF-β ($p<0.05$); other drugs had no significant effect.

E 112 IDENTIFICATION AND CHARACTERIZATION OF NATURALLY OCCURRING SOLUBLE PDGF-RECEPTORS, Charles E. Hart, William Downey, Debra Gilbertson, Analeen Vermillian, Gayle Yamamoto, and Jay P. Tiesman*, ZymoGenetics Inc., Seattle WA 98105, *Proctor and Gamble, Cincinnati OH 45239.

We have recently identified naturally occurring soluble forms of both PDGF alpha and beta receptors (sPDGF-Ra, sPDGF-Rb). These receptors have been identified in conditioned culture media from a variety of human cell types including vascular smooth muscle, osteosarcoma, glioblastoma, and dermal fibroblasts. Additionally, sPDGF-Ra and sPDGF-Rb polypeptide have been identified in normal human blood plasma and serum. Purification of the sPDGF-Ra has been accomplished via affinity chromatography using both PDGF-BB ligand and anti-PDGF-alpha receptor monoclonal antibody matrices. Purified sPDGF-Ra is approximately 90kD in size as determined by SDS-PAGE, and contains approximately 40kD of carbohydrate, similar to full-length membrane-bound PDGF alpha receptor. We have additionally identified a cell-associated 70kD molecule which corresponds to the truncated cytoplasmic domain of the PDGF alpha receptor. Furthermore, we have found that sPDGF-Ra is able to bind both PDGF-AA and PDGF-BB in solution and compete in a dose dependent manner with membrane-bound PDGF receptor for [125I]-PDGF-AA and [125I]-PDGF-BB binding. The soluble receptors appear to be generated by proteolytic processing of full-length cell-surface receptor. In MG-63 osteosarcoma cells up to 20% of all receptors produced appear to be cleaved liberating soluble sPDGF-Ra to the culture media. If vascular smooth muscle cells produce similar high levels of soluble PDGF receptor *in vivo*, then this could provide for the production of specific PDGF antagonists at high concentrations in a localized environment, adding an additional mechanism for regulating the activity of PDGF *in vivo*. On going studies include determining the source of sPDGF-R found in human plasma, determining the protease(s) involved in cleaving the full-length receptor, and monitoring for changes in circulating plasma levels due to changes in physiology.

E 113 VASCULAR BIOCHEMISTRY OF HOMOCYSTEINE: INTERACTIONS WITH PLASMA PROTEINS, Donald W. Jacobsen, Susan R. Savon, and Ralph Green, Departments of Cell Biology and Clinical Pathology, Cleveland Clinic Foundation, Cleveland, OH 44195

Homocysteine (Hcy), a sulfur-containing amino acid involved in the methionine cycle and transsulfuration pathway, has long been suspected of having both atherogenic and thrombogenic properties. The leading cause of mortality in individuals with cystathionine β-synthase deficiency or inborn errors of cobalamin and folate metabolism, conditions which result in hyperhomocysteinemia, is cardiovascular disease. Recent clinical evidence now suggests that individuals with slight to moderate homocysteinemia are at significantly greater risk for the development of cardiovascular disease. The mechanism of Hcy atherogenicity and thrombogenicity is unknown. Previous studies have focused on the vascular endothelium as the site of attack by Hcy. Indeed, injected Hcy (or its thiolactone) causes desquamation of endothelial cells in animals; *in vitro* it is cytotoxic to cultured endothelial cells. But do these models mimic the real world of homocysteinemia? We have investigated the forms of Hcy in plasma from normal donors and in patients with hyperhomocysteinemia caused by pernicious anemia (PA). We have found that: 1) > 90% of the Hcy in normal donor plasma (total plasma Hcy = 3.4 to 13.7 μM) and in PA plasma (total plasma Hcy = 100 to 400 μM) is protein-bound; 2) < 5% of the Hcy in normal donor plasma exists as free reduced Hcy; and 3) fractionation of PA serum on Sephacryl S200HR reveals that 80% of the protein-bound Hcy is associated with the albumin region of the elution profile. However, ion-exchange chromatography by Mono Q FPLC suggests that proteins other albumin have Hcy associated with them. To model possible interactions between secreted Hcy and plasma proteins, we have conducted *in vitro* homocysteinylation studies between free reduced Hcy and normal donor plasma. We have found that: 1) free reduced Hcy at initial concentrations of 1.0 to 2.0 mM reacts rapidly with plasma protein at 37°C; 2) the protein binding capacity of plasma for Hcy is > 500 μM; 3) free reduced Hcy rapidly displaces protein-bound cysteine (150 μM to 10 μM in < 5 min) suggesting that Hcy, at least initially, interacts with protein by a sulfhydryl/disulfide exchange mechanism; and 4) there is a slow re-association of released cysteine with protein. These studies suggest that, *in vivo*, Hcy entering the circulation is likely to rapidly react with blood proteins by disulfide bond formation. Thus, the suspected atherogenicity and thrombogenicity of Hcy may not be due to Hcy in its free reduced form, but to a plasma protein modified by homocysteinylation.

E 114 TRANSPORT PATHWAYS OF LOW-DENSITY LIPOPROTEINS BY ARTERIAL ENDOTHELIUM OF HYPERCHOLESTEROLEMIC RATS, C.H. Kao[1], V.C. Yang[1], J.K. Chen[2] and J.S. Kuo[3], [1]Department of Biology, Tunghai University, Taichung, Taiwan, [2]Department of Physiology, Chang Gung Medical College, Lin-Kou, Taiwan and [3]Department of Medical Research, Veteran General Hospital, Taipei, Taiwan, Republic of China

The transport pathways of low-density lipoprotiens (LDL) across the endothelium at the branched and unbranched regions of arteries were studies in high cholesterol diet- and normal diet-fed rats. Rat tissues were analyzed by perfusing *in situ* human or rat LDL labeled with colloidal gold or fluorescein 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI). Fatty streaks appeared in the intima of the branched regions of aortic arch after 3 months of high-cholesterol feeding. Atherogenic plaque and denuded endothelium were observed in rats only at 12 months after high-cholesterol feeding. The accumulation of LDL-DiI in the intima was markedly increased in the high cholesterol diet-fed rats as compared to the normal diet-fed rats. In addition, more LDL-DiI accumulated in the branched regions than those in the unbranched regions of both the thoracic aorta and the carotid artery. The intima with lower shear stress (the outer wall of the carotid bifurcation and the inner wall of the aortic arch) contained more LDL-DiI than those in the other areas of the vessel wall. LDL-gold conjugates were observed in the intracytoplasmic vesicles as either free within the cytoplasm or attached to the luminal and the abluminal surfaces and in the subendothelial space. Some of the tracer molecules were found within the open junctions at the branched regions of the aortic arch. These results indicated that the major visible routes for transport of the LDL in both the branched and the unbranched regions of the arteries are intracytoplasmic vesicles. In addition, open junctions play an important role in the LDL transport across the endothelium at the branched regions of the arteries. The normal intercellular spaces, however, were less important in the transendothelial transport of LDL in the branched as well as the unbranched regions of the arteries.

E 115 OXIDIZED LOW DENSITY LIPOPROTEIN SELECTIVELY AUGMENTS CYTOKINE ACTIVATED VCAM-1 GENE EXPRESSION IN HUMAN VASCULAR ENDOTHELIAL CELLS. Bobby V. Khan, R. Wayne Alexander, and Russell M. Medford, Division of Cardiology, Emory University School of Medicine, Atlanta, GA 30322.

Oxidative stress signals in the form of oxidized low density lipoprotein (ox-LDL) and other extracellular as well as intracellular oxidation-reduction (redox) sensitive regulatory signals may play an important role in the pathogenesis of atherosclerosis. Redox sensitive activation of endothelial VCAM-1 gene expression is likely an important target for these signals. To determine whether ox-LDL modulates VCAM-1 gene expression, both human aortic endothelial cells (HAEC) and human umbilical vein endothelial (HUVE) cells were incubated in the presence of 100 μg/ml unmodified, native low density lipoprotein (n-LDL), ox-LDL, or no lipoprotein (control) for 48 hours and then exposed or not to the inflammatory cytokine tumor necrosis factor-α (TNF-α, 100 U/ml) for six hours. Exposure of the HAEC and HUVE cell types to TNF-α markedly induced expression of VCAM-1, ICAM-1, and E-selectin, as determined by ELISA assay. In both HAEC and HUVE cells, ox-LDL augmented VCAM-1 activation by TNF-α by 63% ($p<0.05$) and 45% ($p<0.05$), respectively. Ox-LDL also augmented ICAM-1 expression by TNF-α by 44% ($p<0.05$) but only in HAEC. Ox-LDL had no effect on E-selectin activation by TNF-α in either cell type. No differences from control were observed with HAEC and HUVE cells exposed to n-LDL. These data suggest that oxidative modification of LDL selectively potentiates the expression of cytokine-induced VCAM-1 and ICAM-1 in different endothelial cell types. Furthermore, these studies suggest a functional linkage between extracellular oxidative stress signals such as ox-LDL and intracellular, redox sensitive molecular regulatory mechanisms controlling VCAM-1 gene expression.

E 116 VITAMIN E INHIBITS NEOINTIMAL THICKENING FOLLOWING BALLOON ANGIOPLASTY IN CHOLESTEROL FED RATS.

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In the balloon catheter injury, oxygen free radicals are released and may play a role in initiating smooth muscle cell (SMC) proliferation. It has been reported by Boscoboinik et al *(1991. J. Biol. Chem.266:6188-6194)* that SMC proliferation *in-vitro* may be inhibited by the naturally occurring lipid soluble antioxidant, vitamin E . In this study we have investigated the effects of vitamin E in cholesterol fed rats. Male rats (200-300g) were assigned to one of 3 dietary groups 1) 1 % cholesterol, 2) 0.5% vitamin E + 1% cholesterol, 3) control chow. Balloon catheter de-endothelialisation of the left carotid artery was performed 9 weeks after starting the experimental diets. The rats were sacrificed and perfusion fixed with 4 % paraformaldehyde/PBS ten days after injury. Segments of carotid artery were embedded in paraffin wax and 5μm section were cut and stained with Verhoef-Van Gieson (VVG). Cross sectional measurements of the central carotid portion of injured artery were determined at eight points per section and the intimal/medial ratios derived for each point. A final ratio for each animal was calculated from the means of the replicate sections.

	Control (n=6)	1% Chol (n=5)	1% Chol + 0.5%VitE (n=6)
Intima(μm)	3.70 ± 0.49	4.64 ± 0.53	2.53 ± 0.18*
Media (μm)	5.75 ± 0.30	5.36 ± 0.14	5.49 ± 0.32
Intima/media	0.71 ± 0.07	0.80 ± 0.09	0.51 ± 0.05#

Values are ± SEM *$p<0.01$, #$p<0.05$, compared to cholesterol fed rats with unpaired t-test

We found that both absolute intimal thickness and intima/media ratio of the vitamin E treated rats was significantly lower than those receiving a cholesterol only diet. The data suggest that vitamin E may offer some protection against the development of neointimal thickening following balloon injury. ***This project was supported by Ono Pharmaceutical Co. Ltd, Japan and the British Heart Foundation***

E 117 DIGITAL IMAGING AND FRAP STUDIES OF CHOLESTEROL MOBILIZATION

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Cholesterol crystals are invariably found in advanced, and quite frequently, in early atheromas. Experiments in animals as well as clinical studies in humans have shown that regression of atheromas can be induced by lowering of plasma cholesterol. Morphological changes during the regression comprise significant remodeling of arterial wall including disappearance of crystals. It is commonly believed that so called exchangeable cholesterol can be mobilized and removed from atheroma via HDL. However, the mechanism underlying mobilization of crystalline cholesterol is unknown. We have recently shown that macrophages can phagocytose and solubilize cholesterol crystals during which process multiple bilayers of phospholipids are deposited on the surface of internalized crystals.

The purpose of this study was to explore potential effects of phospholipids on cholesterol crystals. Fluorescently labeled crystals were incubated with three major phospholipids sphingomyelin (S), lecithin (L), and phosphatydilethanolamine (PE) and monitored by fluorescence imaging microscopy, confocal microscopy and fluorescence redistribution after photobleaching (FRAP). Results clearly demonstrated that all three phospholipids were able to mobilize crystalline cholesterol although through different mechanisms. S and L were found to cause a uniform dissolution preserving the original shape of crystals. PE penetrated into crystals causing their fragmentation and considerably faster solubilization. Based on results of this study we conclude that intracellular phospholipids deposited on phagocytosed crystals mobilize cholesterol most probably through the "flip-flop" rotation of phospholipid molecules.

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E 118 The Role of Oxidatively Modified LDL in Rheumatoid Arthritis

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Although atherosclerosis and rheumatoid arthritis are distinct disease states, both are considered chronic inflammatory diseases and may share similar cellular mechanisms in terms of the inflammatory response. Lipid peroxidation products have been detected in rheumatoid synovial fluid and we have previously reported the depletion of vitamin E in rheumatoid synovial fluid when compared to matched serum samples, possibly indicating its consumption in the termination of lipid peroxidation (1). Furthermore, individual proteins of rheumatoid synovial fluid are oxidised as a result of free radical production (2).

The depletion of antioxidants, such as vitamin E and β-carotene, within the LDL molecule renders it susceptible to oxidation, with the consequent formation of oxidatively modified LDL (oLDL). In the atherogenic process, oLDL is taken up via the scavenger receptor expressed on macrophages, giving rise to foam cells and fatty streaks. Recently, we have also identified such foam cells in the rheumatoid synovium (3).

oLDL has several pro-inflammatory properties: it induces the expression of M-CSF, GM-CSF and adhesion molecules on vascular endothelial cells and is cytotoxic to these cells. Since oLDL has several pro-inflammatory properties, we suggest that they are not confined to the arterial intima but that the microenvironment of the rheumatoid joint may also be a site where oLDL plays a role in disease pathogenesis.

Subsequent to the consumption of vitamin E within LDL, β-carotene becomes an effective antioxidant, particularly under the conditions of low pO_2, which have been demonstrated in the inflamed rheumatoid joint . We have isolated LDL from matched rheumatoid plasma and synovial fluid using discontinuous density gradient ultracentrifugation. Furthermore, we compared the visible absorption spectra of isolated LDL samples as an indication of relative concentrations of β-carotene. β-Carotene was shown to be depleted in synovial fluid LDL samples when compared to matched serum LDL (n=5).

The observation that LDL from rheumatoid synovial fluid is depleted of b-carotene supports the concept that oLDL formed within the arthritic joint may contribute to the pathogenesis of inflammatory joint disease.

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E 119 THE INTRACELLULAR SIGNALLING PATHWAY FOR PDGF-STIMULATED MIGRATION OF VASCULAR SMOOTH MUSCLE CELLS REQUIRES OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II.

Edward G. Lakatta, Rebecca R. Pauly, Claudio Bilato, Jeffrey Fredman, Michael T. Chin, Tatsuo Suzuki* and Michael T. Crow. NIA-NIH, Baltimore, MD 21224 and *Nagoya City Med. Sch., Nagoya, JAPAN.

The migration of proliferating vascular smooth muscle cells (VSMCs) in vitro toward the chemoattractant, PDGF, is suppressed after serum removal, which leads to cell-specific differentiation. in differentiated VSMCs. We have observed differences in intracellular signalling between differentiated and proliferating VSMCs that may account for this behavior. When stimulated with PDGF, both proliferating and differentiated cells upregulate the expression of c-fos and MCP-1 (JE), but only proliferating cells activate calcium/calmodulin-dependent protein kinase II (CamKinase II), detected with an antibody specific for phosphorylation at Thr-286. Blocking either calcium-calmodulin interactions (50 μM W7) or the activation of CamKinase II (10 μM KN62) blocked the PDGF-induced migration of proliferating VSMCs by more than 90%, while inhibitors of protein kinase C had no significant effect on migration. Pretreatment of differentiated cells with the calcium ionophore, ionomycin (1 μM) or endothelin (10-100 nM) (which also increase intracellular calcium) resulted in an 84 ± 6% return to the migration rate of proliferating VSMCs. This return was also blocked by CamKinase inhibitors and unaffected by inhibitors of PKC. Finally, forced expression in differentiated cells of constitutively-activated CamKinase II significantly increased their migration. These results indicate that CamKinase II activation is required for VSMC migration and that differences in its activation by PDGF (possibly via phospholipase Cγ) between proliferating and differentiated VSMCs accounts for the suppression of migration in the differentiated phenotype.

E 120 FOAM CELL DEVELOPMENT CAN BE STIMULATED BY A PROINFLAMMATORY CHEMOTACTIC S100 PROTEIN, Wendy Lau, Jannine Devery and Carolyn L. Geczy, Heart Research Institute, 145 Missenden Road, Camperdown, NSW 2050, Australia.

The inflammatory process is an important influence in atherogenesis. Monocyte recruitment is regulated by chemotactic factors and the expression of adhesion molecules. Stimulatory influences on macrophage lipid accumulation have been studied *in vitro*, but there have been few studies of this nature *in vivo*.

Our laboratory has characterised a murine S100 protein termed CP-10 (MW 10kD) with potent chemotactic activity for myeloid cells. Intraperitoneal injection of CP-10 into mice caused a sustained inflammatory reaction over 24h, characterised by the recruitment of activated neutrophils and monocytes, as determined by differential staining, flow cytometric analysis and electron microscopy. The newly recruited monocyte/macrophages expressed increased levels of the adhesion molecule Mac-1.

Inflammatory monocyte/macrophages recruited by CP-10 *in vivo* accumulated increased cholesteryl esters in response to acetylated (Ac)-LDL, both *in vivo*, and *in vitro*, as determined by HPLC and Oil Red O staining. In contrast, CP-10 did not increase lipid accumulation by resident peritoneal macrophages *in vitro*. CP-10-elicited, Mac-1^{high} monocyte/macrophages accumulated significantly more cholesteryl esters when cultured with AcLDL in adherent compared to nonadherent conditions. Ligation of Mac-1 with an activating antibody in nonadherent conditions increased cholesteryl ester accumulation in CP-10-elicited macrophages to the same degree as cells cultured in adherent conditions. Thus, CP-10 or related S100 proteins may play an important role in the inflammatory process of atherogenesis by stimulation of myeloid cell recruitment, activation and foam cell development.

E 121 **Human High Density Lipoprotein Stimulates the Production of Endothelin-1 From Cultured Endothelial Cells,** Ellis R Levin, Ren-Ming Hu, Eric Chuang, Bruce Prins, Harrison JL Frank, Ali Pedram, and Moti Kashyup. Departments of Medicine and Pharmacology, University of California, Irvine, Irvine, CA 92717 and the Long Beach Veterans Hospital, Long Beach, CA 90822

High density lipoprotein (HDL) levels in the blood inversely correlate with the incidence of cardiovascular disease. The protective effect of HDL is probably related to its ability to efflux cholesterol from the cell. It is also possible that HDL interacts with vasoactive peptides implicated in the development of vascular diseases. We determined the effects of HDL on endothelin-1 (ET-1) production and secretion from cultured bovine aortic endothelial cells (BAEC). HDL produced a highly significant, dose-related stimulation of endothelin secretion, beginning by 2 hours of incubation and persisting for 8 hours. The stimulation was as great as 240% of control, and was significant even at very low levels of HDL (1μg/ml). HDL also comparably and significantly stimulated the production of pre-pro ET-1 and ET-1, determined by translation studies in the BAEC. In contrast, HDL had no effect on steady state mRNA levels (by protection assay), transcript degradation (mRNA half-life studies) or transcription (nuclear run-on) in the BAEC. To determine the protein moiety of the HDL complex which was responsible for ET-1 secretion, we found that the Apo-AI apoprotein comparably augmented ET-1 secretion and translation, compared to HDL. The stimulation of ET-1 by HDL(or Apo-AI) was dependent on protein kinase C activation. Our results indicate that low concentrations of HDL potently stimulate the production and secretion of ET-1, a powerful vasoconstrictor and mitogen for the vascular smooth muscle cell. This stimulation uniquely occurs at the level of translational control, and not transcription. The interaction of HDL with ET-1 may play a role in the regulation of vasomotor tone and the proliferation of smooth muscle, induced by endothelin.

E 122 VASCULAR SMOOTH MUSCLE CELLS STABLY OVER EXPRESSING CONSTITUTIVELY ACTIVE $G\alpha_{i2}$ RESEMBLE THE CONTRACTILE PHENOTYPE, X.M. Li, P. Tsai, L.E. Heasley, E.D. Weider, and R. A. Nemenoff, Division of Renal Diseases and Hypertension, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO.

Vasoconstrictors such as vasopressin (AVP) induce hypertrophy in vascular smooth muscle cells (VSMC). AVP also causes elevations of the smooth muscle isoform of α-actin (SM-α-actin), which has been used as a marker for the contractile phenotype. In VSMC vasoconstrictors have been shown to stimulate phosphoinositide-specific phospholipase C through a pertussis toxin insensitive G-protein, probably G_q. However, receptors which signal through G-proteins are capable of interacting with more than one G-protein. To examine the role of G_{i2} in the physiologic responses of VSMC, cells isolated from rat aorta were stable transfected with a constitutively active mutant of the α subunit of G_{i2} which has impaired GTPase activity (α_{i2}Q205L). Clones overexpressing α_{i2} were selected by immunoblotting with specific antisera. VSMC stably overexpressing α_{i2}Q205L polypeptide grew much more slowly than wild type cells or cells transfected with a control plasmid (NEO), in contrast to earlier studies in fibroblasts where overexpression of α_{i2}Q205L transforms the cells. AVP-induced increases in intracellular Ca^{2+} and activation of mitogen-activated protein kinase were similar in both cells lines. Levels of SM-α-actin were examined by immunoblotting. Cells transfected with α_{i2}Q205L showed a marked elevation (4-5 fold) in levels of SM-α-actin compared to NEO. VSMC exist in two phenotypic states: proliferative and contractile. Contractile cells are characterized by low proliferative rates and enhanced levels of muscle-specific proteins including SM-α-actin. Thus VSMC stably overexpressing constitutively active α_{i2} manifest at least two important characteristics of the contractile phenotype. These studies suggest that G_{i2} couples to post-receptors signalling pathways distinct from Ca^{2+} and MAP kinase which regulate differentiation in VSMC .

E 123 THE EFFECT OF PROBUCOL AND CHTA ON ENDOTHELIAL CELL ELAM-1 EXPRESSION, George E. Makroglou, Howard A. I. Newman, Deborah A. Knight, Daniel D. Sedmak, and Donald T. Witiak, Department. of Pathology, College of Medicine, The Ohio State University, Columbus OH 43210 and College of Pharmacy, University of Wisconsin, Madison, WI 53706

The pathogenesis of atherosclerosis is a complex and chronic disease characterized by extensive mononuclear infiltrates into the arterial intima. The induction of endothelial cell adhesion molecules during atherogenesis or inflammation may be considered a manifestation of endothelial cell dysfunction. These pathological conditions induce hyperactive endothelial surface sites for leukocyte binding which may lead to transendothelial migration. Probucol, 4-4'-(isopropylidene-dithio)bis(2,6-di-tertbutylphenol), and the experimental antilipidemic agent 4-(4-chlorophenyl)-2-hydroxytetronic acid (CHTA), hydrophobic antioxidants with free radical scavenging capabilities, were tested for inhibition of Endothelial Adhesion Molecule 1 (ELAM-1) expression in human umbilical endothelial (HUVE) cell cultures. ELAM-1 expression is measured with a biotin-avidin ELISA protocol and flow cytometry. Confluent HUVE cells, passages 6 - 7, were exposed to various concentrations of antioxidants for 24 hours followed by a coincubation with lipopolysaccharide (LPS) for 7 hours. Unlabeled murine anti ELAM-1 IgG_1 is the primary monoclonal antibody used (Caltag Laboratories) with the Vectastain ABC ELISA kit (Vector Laboratories). The ELISA color development substrate used was 2,2-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) ABTS. Indirect flow cytometry uses GAM-FITC(Coulter) as the second antibody. LPS, in the range of 0.10 to 100 ng/mL, induces ELAM-1 in a dose-dependent manner with initial saturable expression at 10 ng/mL for ELISA; and 30 ng/ml with fluorescence. To standardize comparisons between the two methodologies 30 ng/mL LPS concentrations were used for ELAM-1 induction. With both techniques probucol in the range of 10-100 μM exhibited a concentration dependent inhibition of ELAM-1 expression which maximized at 30%. CHTA(1-10 μM) showed a similar concentration-dependent inhibition of ELAM-1 expression maximizing at 40% and gradually decreasing with a complete loss of inhibitory activity at 100 μM. Since both Probucol and CHTA exhibited inhibitory effects on HUVE ELAM-1 expression they may play a significant role in atherogenesis-related decreased mononuclear binding and infiltration .

E 124 HUMAN ENDOTHELIAL CELLS TREATED BY OXIDIZED LOW DENSITY LIPOPROTEINS EXPRESS P-SELECTIN AND BIND MONOCYTES. J.LMcGregor, J. Murphy, M-P, Reck, V.Gebuhrer. INSERM Unit 331, Faculty of Medicine Alexis Carrel, Pasteur Institut of Lyon, France. Stanford Medical school, division of Hematology (S161), Stanford, CA 94305, USA.

Recruitment of monocytes to the subendothelium vascular space appears as the first stage of atherogenesis. It is now well established that oxidized low density lipoproteins (LDL) can induce endothelial cell (EC) activation and hence monocytes adhesion. However, the role of endothelial cell adhesive receptors implicated in the adhesion of monocytes, following an insult by oxidative LDL, remains to be elucidated. This study has investigated the effect of native or oxidized LDL on the expression of P-, E-selectin and PAF. Native LDL (N-LDL) was oxidized by incubation with endothelial cells (EC-LDL) or with copper (Cu-LDL) or left with culture medium as a control (C-LDL). Expression of P-selectin was assayed with an anti-P-selectin monoclonal antibody (MoAb) (LYP20). Results show that EC-LDL and Cu-LDL induce the expression of P-selectin by human endothelial cells (HUVEC) after 2 hours activation. This induction of P-selectin by LDL at low concentrations (20ug/ml) is directly related to the state of their oxidation (as measured by TBARS and conjugated dienes). P-selectin expression was sustained for a period of over an hour in LDL-activated endothelial cells in contrast to thrombin-or histamin-activated endothelial cells whose P-selectin levels drops whithin 15 to 20 min following induction. Results obtained in this study show that P-selectin expressed by oxidized LDL-treated endothelial cells are involved in mediating the adhesion of monocytes or U937 cells. LYP20 inhibited the binding of these monocytes whereas WEB 2086, a PAF receptor antagonist, did not. The absence of E-selectin expression, following LDL-treatment, could considerably reduce neutrophil recruitment in the early stages of atherogenesis. P-selectin expression, induced by oxidized LDL, is most probably implicated in the initial stages of atheroma plaque formation.

E 125 **Temporal and Spatial expression of mRNA, latent, and active TGF-ß isoforms during ovine excisional and incisional wound repair,** Heather McMullen,[1] Michael T. Longaker[1]. Raphael C. Cabrera[1], John W. Siebert[1], and Leslie I. Gold[2], Departments of Pathology and Surgery, NYU Medical Center, NY, NY 10016.

The multifunctional nature of Transforming Growth Factor-ß (TGF-ß) influences many aspects of tissue repair. TGF-ß is involved in neovascularization and growth regulation of cellular constituents of the wound and since it also is a potent chemoattractant for inflammatory cells and fibroblasts, it is important in the recruitment of these cells in the inflammatory phase of repair. Although, TGF-ß is responsible for the increased production of collagen and other matrix components critical for tissue remodeling, we have proposed that scarring after wounding reflects the prolonged presence of TGF-ß. TGF-ß is synthesized as a latent molecule, which is activated following release from a precursor complex. TGF-ß isoforms (ß1, ß2, and ß3) have been shown to be differentially expressed in the processes of embryogenesis, carcinogenesis, and wound repair. The goal of these studies was to rigorously examine the temporal and spatial expression of TGF-ß mRNA compared with the presence of both the latent and active TGF-ß isoform throughout excisional and incisional wound repair (1-21 days) to try to further elucidate the role of TGF-ß in the repair process. Isoform specific anti-peptide antibodies were used to detect the latent and mature isoforms of TGF-ß, by immunohistochemical localization, and specific digoxygenin labeled riboprobes to detect TGF-ß isoform mRNA, by *in situ* hybridization.

There was a striking increase in immnunoreactivity for all three mature active TGF-ß isoforms in both the epidermis, dermis (granulation tissue), and epidermal appendages one day after wounding, which peaked at day five, and slowly subsided until day 21, latent TGF-ß isoforms however, showed only a slight increase in these same structures of the skin. Mature TGF-ßs demonstrated both a marked cellular and extracellular distribution, whereas latent TGF-ß isoforms showed only very slight matrix association. Both latent and mature immunoreactive TGF-ß was observed in a dense band of PMNs and macrophages in the granulation tissue. Most notably, there was a complete absence of both the latent and mature proteins in the migrating epithelium until day 7, when re-epithelialization was completed for the excisional wounds. In contrast, the epidermis at the wound margin, was highly immunoreactive for all TGF-ßs and although TGF-ß mRNA was not found in the unwounded epidermis, there was marked mRNA expression, confined only to the most proximal section of the wound margin. Conversely, PCNA (marker for cell division) was observed in the distal portion of the wound margin and overlapped with TGF-ß immunoreactive cells. Thus, although TGF-ß inhibits the growth of keratinocytes, it is expressed independently of PCNA. TGF-ß mRNA was also present in hair follicles and fibroblasts subjacent to the wound.

E 126 GROWTH FACTOR ANTAGONISTS IN VASCULAR DISEASE: EFFECT OF SURAMIN ON SMOOTH MUSCLE CELL PROLIFERATION *IN VITRO* AND NEOINTIMAL FORMATION *IN VIVO*, Christopher J. Molloy, Helen Weber, Pam Ferrer*, Tonya Jenkins-West*, Maria Valentine*, David S. Taylor and Maria L. Webb, Depts. of Cardiovascular Biochemistry and Pharmacology*, Bristol-Myers Squibb Pharm. Res. Inst., Princeton, NJ 08543

Cultured rat aortic smooth muscle (RASM) cells and a rat carotid balloon-injury model were used to evaluate the effects of specific agents on vascular smooth muscle cell proliferation. Suramin (SRM) was selected as a model compound in these studies since it exhibits unique properties as a growth factor antagonist. In RASM cell cultures we found that SRM (10-100 μM) inhibited the mitogenic effects of both PDGF and basic FGF. SRM also blocked the "delayed" mitogenic effects stimulated by angiotensin II (AII), endothelin-1 (ET), or α-thrombin in RASM cells (IC_{50}~10μM). In signal transduction studies, suramin (100 μM) inhibited phosphatidylinositol hydrolysis (PH) stimulated by PDGF or basic FGF, but had no significant effect on PH stimulated by AII, ET, thrombin, or a thrombin receptor-derived activating peptide. SRM also failed to inhibit increases in growth factor gene expression induced by AII in the RASM cells. In contrast, SRM effectively blocked the mitogenic activity present in conditioned medium samples derived from AII-stimulated cells. These data indicate that SRM may antagonize growth factors secreted in an autocrine fashion by VSMC in response to certain agonists. In the rat carotid balloon-catheter injury model of neointimal hyperplasia, SRM (25 μg/kg/IP every 2 d) significantly inhibited lesion formation (~30% decrease in lesion/media area). Taken together, these results indicate that growth factor antagonists may serve as effective antiproliferative agents in disease models of vascular smooth muscle cell proliferation.

E 127 SELECTIVE INHIBITION OF PLATELET-DERIVED GROWTH FACTOR (PDGF) RECEPTOR BINDING AND PDGF-STIMULATED BIOLOGICAL ACTIVITY IN VITRO AND INHIBITION OF INTIMAL LESION FORMATION IN VIVO BY 2-BROMOMETHYL-5-CHLOROBENZENESULFONYLPHTHALIMIDE. Deborra E. Mullins, Fozia Hamud, Robin Reim and Harry R. Davis, Jr. Schering-Plough Research Institute. Kenilworth, New Jersey 07033-0539.

The proliferation of vascular smooth muscle cells (SMC) is a key event in the development of atherosclerotic lesions and in the restenosis of arteries following angioplasty. Polypeptide growth factors are potent SMC mitogens in vitro and are believed to be involved in SMC proliferation in vivo. Strong data exist linking platelet-derived growth factor (PDGF) activity to human atherosclerosis. However, no low molecular weight antagonists of this growth factor have been discovered to date. We have identified a compound, 2-bromomethyl-5-chlorobenzenesulfonylphthalimide (SCH 13929), which inhibits binding of ^{125}I-PDGF BB to cell surface receptors in a dose-responsive manner, with an IC_{50} of 1 μM. This compound has little effect on the binding of ^{125}I-epidermal growth factor (EGF), ^{125}I-basic fibroblast growth factor (bFGF), or ^{125}I-endothelin to specific cell surface receptors. Exposure of cultured SMC or fibroblasts to SCH 13929 inhibits PDGF BB- and PDGF AA-stimulated mitogenesis but not EGF- or bFGF-stimulated mitogenesis. PDGF BB-stimulated proliferation of SMC is also inhibited dose-responsively by exposure to SCH 13929. Chemotaxis assays indicate that SCH 13929 inhibits PDGF-stimulated directional migration and suggest that the compound interacts with PDGF rather than with the receptor. The compound displays no detectable cytotoxicity in cultured cells, as determined by morphological observation, trypan blue staining, and release of lactate dehydrogenase activity. Unilateral balloon catheter-deendothelialization of the carotid artery in Sprague-Dawley or spontaneously hypertensive rats was used to evaluate the ability of the compound to inhibit vascular SMC hyperplasia in vivo. Oral administration of SCH 13929 (100mg/kg/day) resulted in significant inhibition of lesion formation. These results suggest that SCH 13929 may be a useful tool for understanding the role of PDGF in intimal lesion formation.

E 128 OXIDIZED LDL BINDS TO CD36 IN HUMAN MONOCYTE-DERIVED MACROPHAGES AND TRANSFECTED CELL LINES, Andrew C. Nicholson, David P. Hajjar, S.A. Frieda Pearce, Kenneth B. Pomerantz and Roy L. Silverstein, Departments of Pathology, Medicine and Biochemistry, Cornell University Medical College, New York, NY 10021

Accumulating evidence strongly implicates oxidized LDL in the pathogenesis of atherosclerosis. Identification of receptor mediated pathways of oxidized LDL uptake is, therefore, central to an understanding of foam cell development. CD36 is an 88-kD transmembrane glycoprotein expressed on platelets, monocytes, and microvascular endothelium which has been implicated as a putative receptor for oxidized LDL. It also functions as an adhesion receptor for collagen, thrombospondin (TSP) and P. Falciparum-infected RBC. We initially evaluated CD36/oxidized LDL interactions in murine NIH-3T3 cells stably transfected with human CD36 cDNA. Oxidized LDL bound to CD36-transfected cells in a saturable manner. Specific binding, internalization, and degradation of oxidized LDL was increased 4-fold in CD36 transfected cell lines relative to sham-transfected lines. Binding of oxidized LDL to 3T3/CD36 cells was also inhibited by a panel of anti-CD36 antibodies and by soluble CD36, but not TSP. Specificity of binding was demonstrated by the equivalent binding of LDL and acetylated LDL to control and CD36-transfected cell lines. We also demonstrated that 50% of the specific binding of oxidized LDL to human monocyte-derived macrophages was inhibited by anti-CD36 monoclonal antibodies. The epitope(s) on oxidized LDL recognized by CD36 is undefined, but likely involve the apoprotein, since binding of oxidized-HDL was only minimally increased in CD36-transfected cell lines. Finally, CD36/glutathione-S-transferase fusion proteins were used in an attempt to identify regions of CD36 which are involved in oxidized LDL binding. These findings will also be discussed. These data confirm that CD36 functions as an oxidized LDL receptor and suggest that CD36 may play an important role in the accumulation of lipids by human macrophages and subsequent foam cell development during atherosclerosis.

E 129 OXYRADICALS, A MEDIATOR OF HYPERCHOLESTEROLEMIC ATHEROSCLEROSIS: EFFECT OF PROBUCOL, Kailash Prasad, Jahwar Kalra and Paul Lee. Departments of Physiology and Pathology, College of Medicine and Royal University Hospital, Saskatoon, Sask. S7N 0W0, Canada.

We investigated the effects of high cholesterol diet in the presence and absence of probucol on the genesis of atherosclerosis, the blood lipid profile, aortic tissue lipid peroxidation product malondialdehyde (MDA), and aortic tissue chemiluminescence (CL) a marker for antioxidant reserve in rabbits. Three groups each of 10 rabbits were studied: group I, regular rabbit chow; group II, as I + cholesterol (1%), and group III, as I + cholesterol (1%) and probucol 0.5 gm/kg/day). Blood concentrations of triglyceride (TG), total cholesterol (TC), HDL-C, LDL-C, VLDL-C were measured at monthly intervals for 4 months. The aorta was removed at the end of the protocol for assessment of atherosclerotic changes (gross and microscopic), MDA concentration and CL. TC, LDL-C, LDL-C/HDL-C ratio increased in all the groups except group I. HDL-C decreased in groups II and III but remained unchanged in group I. There was a decrease in HDL-C and VLDL-C components and an increase in the LDL-C components of total cholesterol in groups II, III. Probucol did not appreciably affect the changes in lipid profile except that it decreased HDL-C significantly. Aortic tissue MDA and CL increased in groups II and III to a similar extent. Atherosclerotic changes were similar in groups II and III. Histological changes were practically similar in groups II, III. The increased levels of aortic MDA and CL, were associated with development of atherosclerosis. Ineffectiveness of probucol in 1% cholesterol-fed rabbits was associated with its inability to reduce MDA and increase antioxidant reserve. These findings support the hypothesis that oxygen free radicals are involved in the genesis and maintenance of hypercholesterolemic atherosclerosis.

E 130 GENERATION OF THE LIPID SECOND MESSENGER MOLECULE, PHOSPHATIDIC ACID (PA) IS INVOLVED IN SIGNAL TRANSDUCTION FOR PDGF, VEGF AND FGF-INDUCED MITOGENESIS Glenn C. Rice, Paul A. Brown, Andrius Kazlauskas, Jack W. Singer, James A. Bianco, and Stuart Bursten, Cell Therapeutics, Inc., Seattle, WA 98119 and National Jewish Center for Immunology, Denver, CO 80206

Phosphatidic acid (PA) and its precursor lysophosphatidic acid (lyso-PA) are potent intracellular signaling lipids which have been shown to directly activate atypical protein kinase C species and alternative kinases, and when added exogenously, are potent mitogens and induce expression of several protoncogenes and growth factors. In addition, PA can function as an intracellular second messenger molecule by dephosphorylation to 1,2 sn-diacylgycerol (DAG) by the enzyme phosphatidate phosphohydrolase (PAPh). We demonstrate, using high performance liquid chromatography (HPLC) and fast atom bombardment mass spectrometry that PDGF and FGF induce synthesis of large amounts of PA within 30 sec in stimulated mouse Balb-3T3 cells, human stromal cells (HSC) and in human smooth muscle cells (SMC). CT-3501 is representative of a class of novel small molecule synthetic compounds which block production of platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF) - induced PA. Since large amounts of lyso-PA accumulate following addition of CT-3501, the mechanism is most likely inhibition of lysophosphatidate acylCoA:acyl transferase (LPAAT), which catalyzes the transfer of acyl CoA from lyso-PA, rather than PA synthesis via DAG kinase. Addition of CT-3501 to PDGF, VEGF or FGF-stimulated 3T3 cells, HSC, SMC, or human endothelial cells inhibits proliferation with an IC-50 between 10-100 nM. CT-3501 also inhibits PDGF-induced mitogenesis of hepatoma cells transfected with PDGF receptor tyrosine phosphorylation-site mutants unable to bind either PLC-γ1 or PI3-kinase. Both PLC-γ1 and PI3-kinase are independent downstream mediators of PDGF mitogenesis. CT-3501 does not inhibit generation of IP3, calcium mobilization, or receptor autophosphorylation stimulated by PDGF. These data indicate that PA is likely involved in the multiple mitogenic signaling pathways induced by PDGF, VEGF and FGF, and that CT-3501 may represent a novel class of compounds that selectively inhibit only the proliferative signal transduction of PDGF, VEGF and FGF.

E 131 ANALYSIS OF SECRETION AND EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR IN CULTURED ENDOTHELIAL CELLS FROM STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT

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Hypertension is a risk factor for atherosclerosis, and causes significant vascular changes. Endothelial cells have been recognized to play a crucial role in the vascular remodeling observed with hypertension through the synthesis and release of several growth-regulatory molecules. Our studies demonstrate that endothelial injury induced by hypertension initiate specific vascular changes as well as arteriosclerosis.

In this study, we characterized the endothelial cell-derived growth factors from Spontaneously hypertensive Rat from a Stroke-Prone Strain (SHRSP) and of Wistar Kyoto Rats (WKY), respectively. We found that platelet-derived growth factor (PDGF)-B chain constituted a major portion of the mitogenic activity of the conditioned media of aortic endothelial cells from both animals. Further, Northern analysis demonstrated that the expression of PDGF-B chain was enhanced in cultured aortic endothelial cells of SHRSP. This enhanced expression of PDGF-B chain may relate to the genesis of vascular disease associated with hypertensive conditions.

E 132 THE EXPRESSION OF HYALURONAN (HA) AND THE HA RECEPTOR, RHAMM, IN EXPERIMENTAL MYOCARDIAL INFARCTION Rashmin C. Savani, Chao Wang, Robert Stern and Eva A. Turley. Department of Pediatrics and Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada R3E 0V9, and Department of Pathology, Univ. of California at San Francisco, CA, USA.

Myocardial infarction (MI) is a disorder that in humans is frequently characterized by macrophage infiltration and fibrosis of the myocardial wall. Rupture of this weakened wall is a frequent cause of death in these patients. The hyaluronan (HA) receptor RHAMM (Receptor for HA-Mediated Motility) has been implicated in growth factor- and ras-mediated locomotion, and in migratory responses of smooth muscle cells and macrophages to injury. Expression of HA in the myocardium is known to increase during experimental MI. We therefore investigated the expression of RHAMM and HA following experimental myocardial injury. Male Sprague-Dawley rats were treated intraperitoneally with 85 mg/kg of isoproterenol causing subendocardial ischemia. Serum samples from the rats were obtained prior to harvest of the hearts at various timepoints following injury. Histologically, gross myocardial fiber abnormalities were evident by 3-6 hours. By four days after injury, macrophages and neutrophils accumulated in areas of the injured myocardium. By 7 days, fibrosis was noted in the injured areas. The concentration of HA in the serum rose 100-fold (from 34 to 3500 μg/L) 12 hours after injury and returned to control levels by 1 week after wounding. Immunocytochemistry of uninjured tissue revealed constitutive staining for RHAMM in the subendocardial regions of both ventricles. By 24 hours, and reaching a maximum at 4 days after injury, the inflammatory cells that accumulated in the injured myocardium stained strongly for RHAMM. By 7 days, RHAMM accumulated in the extracellular matrix in fibrotic areas. Immunoblots of extracted heart proteins showed two increases in the expression an 84 kDa isoform of RHAMM, first coinciding with the observed increase in serum HA at 6-12 hours, and a second increase coinciding with the inflammatory infiltration. Rats treated with normal saline did not develop myocardial fibrosis and did not show any changes in the expression of RHAMM or HA. These data may explain, in part, the efficacy of early hyaluronidase treatment in reducing the severity of experimental myocardial fibrosis.

E 133 CORONARY SMOOTH MUSCLE CELL GROWTH AND ANGIOTENSIN II. Laura Saward and Peter Zahradka, Department of Physiology, Division of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, University of Manitoba, 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6

The restriction of smooth muscle cell proliferation by angiotensin converting enzyme inhibitors has generally been attributed to a reduction in angiotensin II levels. For this reason, angiotensin II has been described as an activator of vascular smooth muscle cell growth and, as such, may have a key role in the pathogenesis of vascular diseases such as atherosclerosis and hypertension. The cellular actions of angiotensin II, in particular its vasoactive effects, are mediated primarily by the AT_1 receptor. While the function of the AT_2 receptor has not been defined, its high level of expression in fetal tissues indicates it may be essential for angiotensin II-induced growth. To evaluate the relative contribution of the AT_1 and AT_2 receptor subtypes to growth stimulation by angiotensin II, both binding of ^{125}I-angiotensin II and RNA synthesis were measured in a primary cell culture of porcine coronary artery smooth muscle cells. The binding studies demostrated that receptors for angiotensin II were present on these cells. Competition with the receptor subtype specific antagonists Losartan (AT_1) and PD123319 (AT_2) established the predominance of AT_1 receptors on coronary artery smooth muscle cells. Since ribosomal RNA gene transcription is one of first processes activated when cell growth is stimulated, ^{3}H-uridine incorporation was considered ideal for monitoring the transition from the contractile to the synthetic smooth muscle phenotype that occurs following mitogen stimulation. Angiotensin II (10^{-6} M) stimulated RNA synthesis by approximately 180% when cells were maintained in a defined medium containing insulin. Although the AT_1 receptor is present in a substantially higher proportion, only the AT_2 antagonist inhibited the angiotensin II-mediated stimulation of RNA synthesis. The effective intervention by PD123319 suggests it may operate as a valuable agent in treating the progression of cardiovascular diseases such as atherosclerosis that result from an increase in smooth muscle cell growth. Support for these studies was provided by a grant to P.Z. from NSERC Canada and a studentship to L.S. from MRC Canada.

E 134 APOLIPOPROTEIN E MODULATES LOW DENSITY LIPOPROTEIN RETENTION BY LIPOPROTEIN LIPASE ANCHORED TO SUBENDOTHELIAL MATRIX, Uday Saxena, Erika Ferguson and Charles L. Bisgaier, Atherosclerosis Pharmacology, Parke-Davis Pharmaceutical Research, Division of Warner Lambert Co., Ann Arbor, MI, 48105

Lipoprotein lipase (lipase), a key enzyme in lipoprotein triglyceride metabolism, has been shown to markedly increase LDL retention by subendothelial matrix. In the present study, we examined the role that lipoprotein and matrix components play in retention of LDL by lipase anchored to the subendothelial matrix (SEM). Lipase addition to SEM increased LDL retention by 66-fold. Scatchard analysis of LDL binding to lipase-containing matrix yielded an association constant of 12nM. Exogenous addition of matrix components, heparan sulfate and dermatan sulfate (i.e chondroitin sulfate B), reduced LDL retention by > 90%. These glycosaminoglycans (GAGs) also reduced lipolytic activity assiciated with the matrix , suggesting that lipase was released from its binding sites on the matrix. In contrast, other matrix components (collagen, fibronectin, vitronectin and chondroitin sulfate A) did not affect LDL retention. Thus , heparan sulfate and dermatan sulfate function to anchor lipase to the SEM. The effects of apoE and apoA-I were also examined. Preincubation of SEM with apoE, followed by washing, did not affect subsequent lipase nor its ability to retain LDL. However, direct addition of apoE alone or in combination with phospholipid liposomes decreased lipase-mediated LDL retention in a concentration-dependent fashion. Addition of apoA-I had no effect. Thus in these studies apoE functions to displace LDL bound to lipase, but not lipase anchored to the matrix. To further examine the physiologic implications of this process, we assesed the ability of human apoE-rich and apoE-poor high density lipoproteins (HDL) to displace LDL from matrix-anchored lipase ApoE-rich HDL reduced LDL retention dramatically (86% at 2.5 ug/ml). In contrast, apoE-poor HDL, at the highest concentration evaluated (400 ug/ml), decreased LDL retention by only 32%. Overall, these data suggest apoE and specifically apoE containing HDL reduce the lipase-mediated retention of LDL by SEM. This observation, in part could explain the protective effects of apoE and apoE containing HDL against atherosclerosis.

E 135 RESTENOSIS OF PREEXISTING ATHEROSCLEROTIC LESIONS: A REPEAT BALLOON INJURY MODEL IN HYPERCHOLESTEROLEMIC RABBITS. M. Skinner, K. Whiting, M. Rosenfeld, E. Raines, and R. Ross, Department of Pathology, University of Washington, Seattle, WA.

Restenosis following balloon angioplasty of human atherosclerotic lesions occurs in ~40% of cases. The restenotic lesions develop quickly relative to primary atherosclerosis and the pathogenesis is unknown. To assess the changes that occur following balloon injury to atherosclerotic arteries a repeat injury model was developed. Atherosclerotic lesions were induced in the aortas of NZ white rabbits using a combination of balloon injury to the aorta via the right femoral artery and an atherogenic diet consisting of 0.2% cholesterol and 5% peanut oil. Twelve weeks after the initial balloon injury, an identical repeat injury was performed via the left femoral artery. To assess the response to injury, animals were killed before (primary lesions) and 1, 3, 7, 15 and 35 days after repeat injury. Lesions were analysed by histology and immunocytochemistry to determine the cellular and extracellular composition of the primary lesions and the changes that occured following repeat injury to these lesions. Primary lesions had significant intimal thickening, were usually eccentric and resembled human fibrous plaques with the presence of intimal smooth muscle cells (SMCs), macrophages and occasional T lymphocytes. SMCs tended to form a superficial fibrous cap and macrophages were associated with an underlying core. In these lesions a subpopulation of cells, predominantly macrophages, were positive for proliferating cell nuclear antigen (PCNA). Following repeat injury, partial dissection of the intima away from the underlying media was observed in several cases. Three days after repeat injury there was an ~3-fold increase in the percentage of total intimal cells that were PCNA positive. These cells were predominantly SMCs and were particularly found in the shoulders of eccentric lesions and in the opposite wall. By day 7 after repeat injury a second layer of intima was observed which was most obvious by day 15. This second intimal layer was less fibrous, was proteoglycan rich and contained SMCs and macrophages. The SMCs of the second neointima tended to form a fibrous cap with macrophages more prominant in the deeper layers. These results characterize the response of preexisting lesions of atherosclerosis to balloon injury in the rabbit. A knowledge of the timecourse and the characteristics of such repeat injury induced lesions may help in the design of future experiments to investigate the process of restenosis.

E 136 ENDOTHELIUM-DERIVED NO MODULATES MONOCYTE ADHESION TO THE VESSEL WALL, Philip S. Tsao, Leslie M. McEvoy, Helmut Drexler, Eugene C. Butcher and John P. Cooke, Division of Cardiovascular Medicine and Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

Exogenous nitric oxide (NO) inhibits monocyte-endothelial cell (M-EC) adhesion *in vitro*. We have shown that chronic administration of the NO precursor (L-arginine) normalizes NO-dependent vasodilation, and markedly inhibits atherogenesis in an animal model. We hypothesize that this anti-atherogenic effect is due to augmentation of endothelial-derived NO which inhibits M-EC interaction. Accordingly, NZW rabbits were fed normal chow (Cont), normal chow + the NOS inhibitor, nitro-arginine (NA), a high cholesterol diet (Chol), or a high cholesterol diet supplemented with the NO precursor (Arg). After 2 weeks, the thoracic aorta was harvested, opened longitudinally, and fixed with the endothelial surface exposed to culture medium containing WeHi78/24 cells, a monocytoid cell line ($2x10^6$ cells/ml in modified HBSS medium). After incubation with the mononuclear cells for 30 min on a rocking platform, the aortic segments were washed repeatedly to remove non-adherent cells and adherent cells counted by epifluorescent microscopy. M-EC binding was significantly increased in Chol ($p<.001$, v Cont) as well as in NA ($p<0.05$, NA v Cont). By contrast, M-EC binding in Arg significantly reduced in comparison to Chol ($p<.05$, Arg v Chol).

Thus, the enhanced monocyte adhesion induced by hypercholesterolemia is abrogated by the NO precursor. Conversely, inhibition of NO synthesis markedly augments monocyte binding. The results are consistent with our hypothesis that NO is an endogenous anti-atherogenic molecule.

E 137 INDUCTION OF ARTERIAL SMOOTH MUSCLE PROLIFERATION BY EXCESS MEMBRANE CHOLESTEROL.

T.N. Tulenko, R.P. Mason and M. Chen. Department of Physiology, Medical College of Pennsylvania., Philadelphia, PA 19129.

The ability of free (unesterified) cholesterol (FC) to alter cellular growth rates was examined in rabbit aortic smooth muscle cells (SMC) in culture. In the 2^{nd}-4^{th} passage, the cells were growth arrested in serum-free medium for 3-5 days, followed by incubation for 2 days with FC/phospholipid (FC/PL) liposomes in a 2:1 molar ratio. This incubation resulted in a 4-5 fold increase in cell number and was accompanied by a 70% increase in cell FC content and similar increase in membrane FC content. FC-enrichment also increased $^{45}Ca^{++}$ influx through a dihydropyridine (DHP)-sensitive pathway. The proliferative response to FC was similar to that obtained with PDGF (8 nM) and greater (2x) than that obtained with FGF (30 nM). The FC-induced proliferative response and $^{45}Ca^{++}$ influx was inhibited by amlodipine (1.0 pM) when administered simultaneously with FC-enrichment. X-ray diffraction of plasma membranes isolated from FC-enriched and atherosclerotic SMC demonstrated marked enrichment with FC and a marked increase in membrane bilayer width (up to 20%). Incubation of FC-enriched and atherosclerotic SMC with human LDL_3 reduced membrane cholesterol content and reversed the increased proliferative, structural and calcium permeability alterations. Taken together, these data indicate that excess membrane FC increases proliferative activity and calcium permeability in SMC by inducing a membrane structural defect in arterial SMC, and may explain, in part, the "injury" to SMC in situ and the early proliferative response in atherogenesis. Supported by NIH Grant HL-30496 and an AHA predoctoral award to M. Chen.

E 138 A SPECIFIC INHIBITOR OF PHOSPHATIDYLINOSITOL 3-KINASE, Chris J. Vlahos, William F. Matter, and Raymond F. Brown. Cardiovascular Research, Lilly Research Laboratories, Indianapolis, IN 46285-0403

Phosphatidylinositol (PtdIns) 3-kinase is an enzyme implicated in growth factor signal transduction by associating with receptor and nonreceptor tyrosine kinases, including the platelet-derived growth factor (PDGF) receptor. PtdIns 3-kinase is thought to play an important role in mitogenesis; mutants of the PDGF-receptor that lacks the PtdIns 3-kinase binding site fail to exhibit increased DNA synthesis and cell division while other PDGF responses are normal. Therefore, inhibitors of PtdIns 3-kinase may be useful in preventing mitogenesis associated with cellular growth stimuli such as in restenosis and atherosclerosis. Quercetin, a naturally occuring bioflavinoid was found to inhibit PtdIns 3-kinase with an IC50 of 1.3 μg/ml (3.8 μM); inhibition appears to be directed at the ATP binding site of the kinase. Analogs of quercetin were also investigated as PtdIns 3-kinase inhibitors, with the most potent ones exhibiting IC50's in the range of 1.7-8.4 μg/ml. In contrast, genistein, a potent tyrosine kinase inhibitor of the isoflavone class, did not inhibit PtdIns 3-kinase significantly (IC50 > 30 μg/ml). Since quercetin had previously been shown to inhibit other PtdIns and protein kinases, flavinoid analogs were developed that inhibit PtdIns 3-kinase without affecting PtdIns 4-kinase or protein kinases. One such compound, LY294002 (2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one), completely and specifically abolished PtdIns 3-kinase activity (IC50 = 0.43 μg/ml; 1.4 μM) but did not inhibit protein kinases or PtdIns 4-kinase. Analogs of LY294002 demonstated a very selective structure-activity relationship, with slight structural changes causing marked decreases in inhibition. Since PtdIns 3-kinase appears to be centrally involved with growth factor signal transduction, the development of specific inhibitors against the kinase could potentially be beneficial in the treatment of proliferative diseases.

E 139 **APOLIPOPROTEIN E: NOVEL INHIBITOR OF ENDOTHELIAL AND TUMOR CELLS PROLIFERATION,** **Tikva Vogel, Neng-hua Guo, Rachel Guy, Nina Drezlich, Henry C. Krutzsch, Diane A. Blake, Amos Panet, and David D. Roberts*, Institutes of Health, Bethesda, MD 20892 (T.V., N.G., H.C.K., D.D.R.); Department of Biochemistry, Meharry Medical College, Nashville, TN 37208 (D.A.B.); and BioTechnology General, Ltd., Rehovot, Israel(T.V., R.G, N.D., A.P.).**

Recombinant human apolipoprotein E3 (apoE), purified from *E. coli*, inhibited the proliferation of several cell types, including endothelial cells and tumor cells in a dose- and time-dependent manner. ApoE inhibited both de novo DNA synthesis and proliferation assessed by increase in cell number. Maximal inhibition of cell growth by apoE was achieved under conditions were proliferation was dependent on heparin-binding growth factors. Thus, at low serum concentrations (0%-2.5%) and in the presence of basic fibroblast growth factor (bFGF), the proliferation of bovine aortic endothelial (BAE) cells was stimulated several fold. bFGF dependent proliferation was dramatically inhibited by apoE with an IC_{50} ≈50nM. However, under conditions where cell proliferation was mainly serum-dependent, apoE addition had also suppressed growth, but the inhibitory activity was relatively lower (IC_{50} ≈ 500nM). ApoE also inhibited growth of bovine corneal endothelial cells, human melanoma cells, and human breast carcinoma cells. The IC_{50} values obtained with these cells were generally 3 to 5 times higher than with BAE cells. Inhibition of cell proliferation by apoE was reversible and dependent on the time of apoE addition to the culture. In addition, apoE inhibited the chemotactic response of endothelial cells that were induced to migrate by a gradient of soluble bFGF. Inhibition of cell proliferation by apoE may be mediated both by competition for binding to proteoglycans and by a cellular antiadhesive activity of apoE. The present results demonstrate that apoE is a potent inhibitor of proliferation of several cell types, and suggest that apoE may be effective in modulating angiogenesis, tumor cell growth, and metastasis.

E 140 ICAM-1: A COSTIMULATORY FACTOR IN T CELL ACTIVATION BY AUTOLOGOUS OR ALLOGENEIC CMV-INFECTED ENDOTHELIAL CELLS, WJ Waldman, NC Alampi, DA Knight, EH Huang, CG Orosz, DD Sedmak, The Ohio State University College of Medicine, Columbus, Ohio 43210

Cytomegalovirus (CMV) has been associated with atherogenesis and transplantation-associated arteriosclerosis, both of which intimately involve the endothelium, a common site of infection by this virus *in vivo*. In an effort to elucidate potential mechanisms for these associations, we have previously demonstrated 1) that CMV-infected endothelial cells (EC) potently activate both allogeneic and autologous, CMV-seropositive donor-derived $CD4^+$ T cells, and 2) that T cells thus activated produce quantities of IFNγ sufficient to induce generalized HLA class II expression on nearby uninfected EC. Although these findings suggest a scenario in which the virus might initiate a cascade of intravascular immunopathogenic events, the mechanism by which purified $CD4^+$ T cells are initially activated by EC/CMV remains a curiosity, since these stimulator populations are uniformly HLA class II-negative. We have recently discovered that ICAM-1, a potential costimulatory factor in the activation process, is substantially upregulated on EC/CMV. To determine the contribution of this adhesion molecule to the activation responses we have observed, autologous or allogeneic EC/CMV-driven $CD4^+$ T cell proliferation was measured by [^{3}H]thymidine incorporation in 6-day microculture assays in the presence of blocking mAb specifically reactive with ICAM-1. Results indicate that proliferative responses are attenuated by 50-80% by anti-ICAM-1 at concentrations in the range of 1-5 μg/ml, but unaffected by equal concentrations of irrevelant isotype matched control antibody. We conclude from these studies that ICAM-1 expressed on CMV-infected EC provides a powerful costimulatory signal in the activation of autologous or allogeneic $CD4^+$ T cells, and may play a similar role in other endothelial-initiated immunopathogenic interactions.

E 141 CELL SURFACE ASSEMBLY OF LIPOPROTEIN (a) IN PRIMARY CULTURES OF BABOON HEPATOCYTES, Ann L. White and Robert E. Lanford, Southwest Foundation for Biomedical Research, San Antonio, TX 78228

Lipoprotein (a) [Lp(a)] is a modified form of low density lipoprotein (LDL) in which apolipoprotein (apo) B100 is disulfide-linked to the high molecular weight glycoprotein, apo(a). Apo(a) is synthesized by the liver and is highly homologous to plasminogen, possessing multiple copies of a plasminogen kringle 4-like domain. We have established a primary baboon hepatocyte system for the analysis of Lp(a) morphogenesis. Using this system, we previously demonstrated that apo(a) is synthesized as a lower molecular weight precursor which is processed to a higher molecular weight mature form. Mature apo(a) was associated with the cells for a prolonged period of time before secretion. Assembly of Lp(a) occurred after secretion and a proportion of apo(a) remained in a free form in the culture medium. In the current study we demonstrate that mature apo(a) can be released into the medium by incubation of hepatocytes with 6-amino hexanoic acid (AHA) at 4°C, suggesting that mature apo(a) is bound to the cell surface via its lysine-binding kringle domains. Proline and lysine also released apo(a) from the cell surface and, like AHA, inhibited the interaction between apo(a) and apoB. In add-back experiments, apo(a), but not Lp(a), bound to the cell surface and could be released by the addition of LDL. Addition of LDL resulted in recovery of apo(a) at Lp(a) density. We therefore propose that apo(a) is secreted as a free protein which immediately binds to the cell surface via its kringle domains. Assembly of at least some Lp(a) occurs at the cell surface, with the association of apo(a) and apoB reducing the affinity of apo(a) for its 'receptor' and resulting in release of Lp(a) into the culture medium.

E 142 THE POSSIBLE ROLE OF HEAT SHOCK PROTEIN 65/60 IN THE DEVELOPMENT OF ATHEROSCLEROSIS, Georg Wick, Roman Kleindienst, Cornelia Seitz and Qingbo Xu, Institute for Biomedical Aging Research, Austrian Academy of Sciences, Rennweg 10, 6020 Innsbruck, Austria

We have previously provided evidence that humoral and cellular immune reactions against a certain type of stress proteins, the so called heat shock proteins (hsp) 65/60, seem to play an important role in the development of atherosclerosis.

Stress proteins or heat shock proteins are phylogenetically highly conserved. Thus, mycobacterial hsp 65 shows an over 50 % homology with its mammalian counterpart, hsp 60. Hsp 60 is a mitochondrial protein that would seem to be accessible only if endothelial cells were deteriorating. We now, however, present evidence that one of several monoclonal antibodies against hsp 60 also reacts with an epitope on the surface of living arterial endothelial cells. Furthermore, application of various forms of stress (cytokines, H_2O_2, high temperature, etc.) to endothelial cell monolayers or organ cultures of aortic intima of experimental animals and humans not only resulted in the expression of hsp 60 but concomitantly also induced the expression of adhesion molecules (intracellular adhesion molecule 1 - ICAM 1, vascular cell adhesion molecule 1 - VCAM 1), providing the prerequisites for the interaction of specific T cells with the endothelium and subsequent establishment of inflammatory foci in the intima. This type of early intimal inflammatory foci has also been observed in microscopically unaltered portions of the aorta and peripheral arteries, and even occasionally in veins. Based on these observations we have tentatively formulated the concept that - in analogy to the mucosa-associated lymphoid tissue (MALT) - a vascular-associated lymphoid tissue (VALT) may also exist that serves to monitor the inner surface of our vascular tree, similar to MALT being responsible for the monitoring of our internal mucosal surface. Similar to the hsp 65-induced atherosclerosis in normocholesterolaemic rabbits, these early inflammatory processes seem to be still reversible. However, when additional risk factors come into effect, such as high blood pressure, virus infections, toxins, chemically altered LDL, etc., classical atherosclerotic lesions develop which are then progressive and irreversible. Most recently, we were able to demonstrate a significant correlation of the presence of antibodies to hsp 65 in the sera of a large number of clinically healthy persons with the presence of sonographically demonstrable atherosclerotic lesions in their carotid arteries. The occurrence of these antibodies showed a significant positive correlation with age, the male sex, high blood pressure, smoking, but, surprisingly, not total serum cholesterol or LDL levels. Furthermore, these antibodies cross-react with human hsp 60.

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E 143 LIPID OVERLOAD OF VASCULAR SMOOTH MUSCLE CELLS IN ALLOGRAFTS: DOES HUMAN CYTOMEGALOVIRUS PLAY A DIRECT ROLE? Janet E. Wilson, Todd J. Kendall, James M. Gulizia, Gray Malcom, Stanley J. Radio, Reinhard Kandolf, Maria R. Costanzo, Sheldon L. Thieszen, Bruce M. McManus, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada V6T 1Y6

Chronic rejection is expressed explicitly as vascular disease in solid organ allografts. The arteries of all solid organ grafts including heart, kidney, liver and pancreas are involved in a similar process of atheroarteritis. We have studied in-depth the degree of atheromatous (intra- and extra-cellular lipid) deposition in arteries of heart allografts, as well as liver and pancreas grafts. In the 46 heart allografts we have studied thus far by protocol, utilizing light and electron microscopy (transmission and scanning), histochemistry and immunohistochemistry, and biochemical analysis, there is a ***marked*** increase in arterial wall lipids in comparison to coronary site-matched, donor-age comparable coronary arterial segments from trauma victims (Pathobiological Determinants of Atherosclerosis in Youth Study). Lipid content (μg/mg dry defatted weight) and concentration (μg/cm^2 intimal surface area) are strongly correlated statistically with luminal narrowing and cumulative cyclosporine dose. Excess lipids localize as lipoprotein aggregates in the extracellular space and in smooth muscle foam cells. In light of our observations, and prior evidence that herpes family viral infections can alter smooth muscle cell lipid metabolism, we undertook molecular characterization of human cytomegalovirus (HCMV) in allograft coronary artery walls. Of 36 allografts evaluated by PCR with primers specific for exon 4 IE gene, positivity was observed in 6 samples on more than 1 occasion. By in situ hybridization with probes to IE and late gene regions, 4 allograft coronary segments of 23 studied were positive. The presence of HCMV nucleic acids did not correspond with biochemically-determined arterial wall lipid content or to digitized luminal narrowing in perfusion-fixed vessels. In situ hybridization probes did not specifically localize within medial or intimal smooth muscle cells or endothelial cells. Indeed, in situ positivity was most common in perivascular myocardium and adventitia. These studies mitigate against the hypothesis that direct infection of vascular smooth muscle cells may alter their lipid metabolism and in this fashion contribute to the ultimate expression of transplant arteriopathy.

E 144 COMBINATORIAL INTERACTIONS BETWEEN AP-1 AND ETS-DOMAIN PROTEIN CONTRIBUTE TO THE DEVELOPMENTAL REGULATION OF THE MACROPHAGE SCAVENGER RECEPTOR GENE, Hong Wu, Karen S. Moulton, Andrew Horvai, Shreya Parikh, Christopher K. Glass, Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0656

The macrophage scavenger receptor gene is transcriptionally activated during monocyte macrophage differentiation and serves as a model to investigate molecular mechanisms that control this developmental process. In this report, we have identified three genomic regulatory elements that are required for transactivation of the scavenger receptor gene in the THP-1 monocytic leukemia cell line following treatment with the phorbol ester, TPA. Each of these regulatory elements contain a consensus or near-consensus binding site for members of the AP-1 gene family, while the two most active elements also contain juxtaposed binding sites for ets-domain transcription factors. We demonstrate that TPA treatment results in a marked and prolonged increase in AP-1 binding activity on these elements, which can be accounted for almost entirely by c-*jun* and *jun*B. These proteins in turn form a ternary complex with a factor that binds to the ets-recognition motif. Several indirect lines of evidence indicate that ets-2 represents a component of this ternary complex. The combined expression of c-jun, ets-2 and a constitutive form of ras result in synergistic increases in transcription from promoters containing the scavenger receptor regulatory elements, suggesting that these elements represent downstream targets of the m-csf receptor.

E 145 STRUCTURE OF THE GENES ENCODING MEMBERS OF THE PLATELET GPIb-V-IX SYSTEM, Mayumi Yagi, Mark J. Hickey, and Gerald J. Roth, Seattle VA Medical Center (151), Seattle, WA 98108.

The adhesion of platelets to the vascular subendothelium is the initiating event in hemostasis. Under conditions of arterial shear, von Willebrand factor mediates the interaction of platelets with endothelial cells. The platelet receptor for von Willebrand factor is a complex consisting of four subunits, glycoproteins (gp) Ibα, Ibβ, V, and IX. GpV and IX are noncovalently associated with the disulfide-linked Ibαβ heterodimer. Although these four subunits are encoded by separate genes located on different chromosomes, they share several structural features. All four proteins contain a leucine-rich glycoprotein repeat structure flanked by conserved amino acid sequences. The expression of all four subunits is diminished in Bernard-Soulier syndrome, a genetic disease characterized by deficient platelet adhesion, abnormally large circulating platelets, and thrombocytopenia.

cDNA and genomic clones for the genes encoding gpIbα , Ibβ, V, and IX have been isolated. Similarities in the structure of the genes reflect the similarities in protein structure and expression. The open reading frame and 3' untranslated region (UTR) of the genes are encoded in a single exon. An intron junction is present within a few nucleotides of the initiation codon. The putative promoter regions of gpIbα and IX, as well as other platelet protein genes, lack TATAA and CCAAT motifs but contain potential binding sites for GATA and *ets* transcriptional factors. These results, as well as work in progress on the gpIbβ and V genes, suggest that the proteins have a common evolutionary origin and may share regulatory pathways.

E 146 PROSTAGLANDINS AND CORONARY SMOOTH MUSCLE CELL GROWTH. Lorraine Yau, Karen Harris and Peter Zahradka, Department of Physiology, Division of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, University of Manitoba, 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6

It is of particular interest that the growth stimulatory effects of both angiotensin II (AngII) and bradykinin (BK) potentially involve both prostaglandins E_2 (PGE_2) and I_2 (prostacyclin, PGI_2). With this in mind, the direct effects of PGE_2 and/or PGI_2 on coronary smooth muscle cells were examined. Porcine coronary artery smooth muscle cells (SMC), generated by an explant method from the isolated left anterior descending artery of pig heart, were prepared in D-MEM containing 20% FBS, 1% antibiotic/antimycotic. All studies were conducted after a single passage, since a low passage number was critical to minimize the change in smooth muscle characteristics that can occur with time in culture. SMC were differentiated by placing them for seven days in serum-free D-MEM media supplemented with ITS (insulin, transferrin, selenium), however, addition of growth medium induced entry into the synthetic state as determined by DNA synthesis. Since ribosomal RNA gene transcription is one of the first processes activated when cell growth is stimulated by angiotensin II, ^{3}H-uridine incorporation into rRNA was used to monitor whether prostaglandins can also influence growth. While PGI_2 did not stimulate or depress rRNA synthesis, addition of PGE_2 stimulated the rate of rRNA synthesis. In contrast, an increase in rRNA synthesis was not observed when both agents were added together. PGI_2 may thus be able to block the action of PGE_2 to induce rRNA synthesis. These data support the hypothesis that the stimulation of rRNA synthesis by AngII may involve the action of prostaglandins as part of the signal transduction pathway associated with this aspect of cell growth. Support for these studies was provided by grants to P.Z. from NSERC Canada and the Manitoba Health Research Council, and a studentship to L.Y. from MRC Canada.

Transcriptional Regulation in Vascular Cells; Genetically Modified Animals

E 200 MODULATION - INDUCED EXPRESSION OF SERUM RESPONSE FACTOR IN VASCULAR SMOOTH MUSCLE CELLS. James K. Belknap, Phillip E. Schwartz, Mary C. M. Weiser, and Richard A. Majack. Departments of Pediatrics and Cell and Structural Biology, University of Colorado Health Sciences Center, Denver, CO

Serum response factor (SRF) is a MADS box transcription factor which, upon activation of cells by growth factors, binds the serum response element (SRE) to activate transcription of c-*fos*, c-*myc*, and a variety of other mitogen-regulable genes. In cultured cells, SRF is believed to be ubiquitously expressed and has been classified as an immediate early gene due to its rapid upregulation in response to growth factors. Because of the necessary presence of this transcription factor for cellular replication, we investigated the expression of SRF in rat SMC, and, in particular, its regulation in response to SMC modulation. Using oligonucleotide primers based on the human SRF sequence and RT-PCR, a 420 bp DNA fragment was amplified from cultured adult rat SMC. DNA sequencing of this PCR product revealed a >95% identity with human SRF. Because the SRF sequence has only been characterized, to date, from *Xenopus* and human tissues, we screened a cDNA library, prepared from cultured adult rat SMC, with the 420 bp PCR-derived fragment and isolated and sequenced a rat SRF cDNA clone. In addition, RT-PCR was used to assess the regulable expression of SRF in SMC *in vivo* and *in vitro*. While SRF expression was easily detectable in cultured sparse or confluent SMC, no SRF expression could be detected in the intact adult rat aorta. SRF expression by vascular SMC therefore appeared to occur only under culture conditions, suggesting that SRF may be induced as a function of SMC "modulation" to a replication-competent phenotype. We therefore isolated SMC from intact adult aortas (negative for SRF expression) by enzyme dispersion under serum free conditions; the isolated SMC expressed readily detectable levels of SRF mRNA. These results suggest that SRF is not constitutively expressed in rat aortic SMC *in vivo*, but is induced upon removal of the SMC from their *in vivo* environment. The results of these enzyme dispersion experiments indicate that interactions with the extracellular matrix may be important determinants of SRF expression in vascular SMC. The data suggest that "modulation" of SMC from a contractile (non-replicative) to a replication-competent phenotype may be associated with the induced expression of specific transcription factors required for cellular growth. The induced expression of SRF by modulated SMC may therefore be a key event in the acquisition of a replication-competent state.

E 201 OPPOSITE IN VITRO AND IN VIVO REGULATION OF HEPATIC APO A-I GENE EXPRESSION BY RETINOIC ACID. Didier Branellec [1], Laurence Berthou [1], Bart Staels [1], Irène Saldicco [1], David Parrott [2], Jean-Charles Fruchart [3] and Patrice Denèfle [1]. [1] RHONE-POULENC RORER SA, Vitry-sur-Seine, FRANCE; [2] RHONE-POULENC RORER Ltd., Dagenham, UK and [3] Institut Pasteur de Lille, Lille, FRANCE.

Decreased plasma concentrations of high density lipoprotein (HDL) cholesterol have been associated with an increased risk for the development of coronary heart disease. Apo A-I and apo A-II are the two major protein constituents of HDL. Recent epidemiological as well as transgenic animal-based studies suggest that apo A-I and apo A-II may play a differential role in the protective effect of HDL on atherogenesis. In view of these data, agents which could selectively up-regulate apo A-I, and not apo A-II, gene expression and production might be of interest in the treatment of atherosclerosis.

The pharmacological potential of retinoids to modulate apo A-I and apo A-II gene expression and production was studied both *in vitro* in the human HepG2 hepatic cell line and in primary cultures of rat hepatocytes, and *in vivo* in the rat. Treatment of HepG2 cells with *all-trans* retinoic acid (RA) doubled apo A-I mRNA within 24 hr and protein secreted in the culture medium after 48 hr. The induction of apo A-I mRNA by RA was completely blocked by actinomycin D, suggesting that RA acts at the transcriptional level. Interestingly, adding RA to primary cultures of rat hepatocytes also raised apo A-I mRNA in a dose- and time-dependent manner as well as apo A-I protein secretion. Similar changes in apo A-I mRNA were observed with *9-cis* RA.

In contrast, *in vivo* hepatic apo A-I mRNA levels already decreased after one single administration of RA at 10 mg/kg and remained low after prolonged treatment as well as at a higher dose whereas Apo A-I plasma levels did not change. Furthermore RA treatment did not affect apo A-II mRNA or protein secretion substantially, either *in vitro* (HepG2 cell line, primary culture of rat hepatocytes) or *in vivo*.

In conclusion, the results from these studies demonstrate that treatment with RA selectively induces apo A-I, and not apo A-II expression *in vitro*, but not *in vivo*. These results therefore point to additional regulatory effects of RA on apo A-I gene expression *in vivo* and render the utility of retinoic acid and its derivatives in the treatment of atherosclerosis questionable.

E 202 CONSTRUCTION OF A YEAST ARTIFICIAL CHROMOSOME CONTIG ENCOMPASSING HUMAN FIBROBLAST GROWTH FACTOR 1 (FGF-1) GENE. Ing-Ming Chiu, Edward C. Gilmore, Yang Liu and Robert A. Payson. Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

The region surrounding the human fibroblast growth factor 1 (FGF-1) locus on chromosome 5q31 is of particular interest since it represents a critical region consistently lost in acute nonlymphocytic leukemia (ANLL) or myelodysplastic syndrome (MDS) patients who have a demonstrable deletion of the distal portion of the long arm of chromosome 5. It is proposed that an ANLL/MDS leukemia suppressor gene resides on 5q31. We have previously shown that the gene is most likely localized between FGF1 and PDGFRB/CSF1R loci. The region has also been linked to three other genetic diseases, Treacher Collins Syndrome, diastrophic dysplasia and limb-girdle muscular dystrophy, by linkage analysis. Here we describe yeast artificial chromosomes (YAC) spanning 780 kbp around the FGF-1 gene. Six YAC clones were isolated from a human YAC library and their restriction enzyme maps were determined. The overlap of the clones with each other and with FGF1 cosmid and phage clones were characterized. Three of the YAC clones were found to contain the entire aFGF gene which spans more than 100 kbp. Proximal and distal ends of several of these YAC clones were isolated for further overlap cloning. The proximal ends of both Y2 and Y4 were localized to previously isolated FGF1 DNA by sequence analysis. The distal ends of these two clones also hybridized to a human-hamster hybrid containing chromosome 5 as the only human genetic material. These results suggest that these YAC clones represent colinear DNA around the FGF1 locus. None of the YAC clones were found to contain the CD14 and GRL genes, the closest known proximal and distal markers (relative to the centromere) to the FGF-1 gene, respectively. This contig is useful for the overlap cloning of the 5q31 region and for reverse genetic strategies for the isolation of disease genes in the region. Moreover, the three YAC clones that contain the entire FGF-1 gene in cloned forms can be introduced into transgenic mice in order to study the tissue-specific regulation of its four distinct promoters.

E 203 PROMOTER SWITCHING OF FGF-1 GENE EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS IN RESPONSE TO SERUM AND PHORBOL ESTER. Maqsood A. Chotani[1], Jeffrey A. Winkles[3] and Ing-Ming Chiu,[1,2] [1]Program in Molecular, Cellular & Developmental Biology, [2]Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, and [3]Laboratory of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rockville, MD 20855

Our lab has previously identified three variants of fibroblast growth factor 1 (FGF-1) mRNA. Recently, a fourth variant of FGF-1 mRNA (designated aFGF 1.D) has been identified and partial cDNA isolated. Our results indicate expression of this mRNA in fibroblasts and vascular smooth muscle cells. Using RNase protection assay we have determined multiple transcription start sites for this -1D promoter. A single strand nuclease sensitive region has also been identified in this -1D region that may have implications in transcriptional regulation of this promoter. Furthermore, treatment of cultured saphenous vein smooth muscle cells with serum or tumor promoter, phorbol 12 myristate 13-acetate (PMA), leads to a significant induction of FGF-1 transcripts. We have identified FGF-1 gene promoters that are upregulated by serum. This transient induction is seen as early as three hours after addition of serum, and points to the FGF-1 gene as being an early response gene. The protein kinase C dependent signalling pathway seems to be involved in this induction. Overall, this phenomenon implicates that these transcripts may have a role in vascular smooth muscle cell hyperplasia associated with atherosclerosis, inflammatory settings, wound healing, tissue repair, and neovascularization events and processes via autocrine and paracrine mechanisms. Also, our results suggest a "promoter switching," i.e., normal growing cells utilize promoter -1D, but in response to serum, "switch" to different promoters. Electrophoretic Mobility Shift Assays will be performed to identify regulatory elements involved in the induction of these promoters. Given the important biological properties of FGF-1, our findings point to regulation of the gene for this growth factor via multiple promoters. This regulation is at the transcriptional as well as the splicing level. These promoters may respond to different physiological conditions and stimuli, in reference to the cell type or tissue milieu, resulting in ultimate production of FGF-1.

E 204 DEVELOPMENT OF AN IN VIVO SMOOTH MUSCLE CELL PROLIFERATION ASSAY USING IMAGE ANALYSIS

Ernst de Reeder, Esther van Vugt, Ralph Bakens, Jacques van de Broek, Ad van Disseldorp, Frans van Mensvoort, Dick Meuleman, Department of Vascular Pharmacology, Organon International bv., The Netherlands.

Smooth muscle cell (SMC) proliferation is a key event in the development of restenosis after angioplasty. To develop compounds that inhibit SMC proliferation a series of consecutive in vitro and in vivo models is needed. Various in vitro models have been described. In vivo, the development of intimal thickening after endothelial denudation is often used as a model. The measurable response in this model is a combination of extra cellular matrix synthesis, SMC proliferation and migration. Our aim was to develop an vivo assay in which only the effect upon SMC proliferation can be measured. We aimed for an histological assay using automatic image analysis and a minimum number of histological sections. To perform measurements, development of a double staining method to discriminate proliferating and non-proliferating SMC was a prerequisite. Proliferation was induced in the rat carotid artery (CA) by endothelial denudation with a balloon catheter. Proliferating cells were labelled by continuous administration of bromodeoxyuridine (Brdu). After 48 hours the CA was isolated, fixed and embedded in glycolmethacrylate. To determine optimal sample size, series of transverse and longitudinal sections were measured. Proliferating cells were demonstrated with anti-Brdu antibodies. Non-proliferating cells were stained with Feulgen staining or by making use of anti-DNA antibodies. Discrimination between non-proliferating and proliferating SMC was better by the combination of histological staining and anti-BRDU antibodies then by double labeling with anti-Brdu and anti DNA antibodies. Double labeling was only possible when the histological staining preceded the immunohistochemical method. Similar results were obtained for both transverse and longitudinal sections. To obtain a reliable estimate of the percentage proliferating SMC a number of 1500 to 2000 SMC should be counted. Most efficiently this is done by measuring a 0.6-0.8 cm long longitudinal section through the centre of the CA. It is concluded that by using image analysis and optimalization of histological sampling and staining in vivo proliferation can be quantified rapidly.

E 205 EVALUATION OF RECOMBINANT ADENOVIRUS VECTORS FOR GENE THERAPY OF LIPOPROTEIN DISORDERS.

Patrice P.Denèfle[1], Steve Hughes[2], Emmanuelle Vigne[3], Michel Perricaudet[3], Patrick Benoit[1] and Edward Rubin[2]
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HDL and its major associated apolipoprotein, apoAI, has been demonstrated in several studies to exert antiatherogenic properties regardless of the genetic and environmental etiology contributing to heightened atherosclerosis susceptibility. In order to evaluate the feasibility of an intervention at the genetic level on such a complex disease, we selected to develop new recombinant adenoviral vectors which were designed so as to achieve high levels of expression of human apolipoprotein AI (apoAI), the core protein of high density lipoproteins (HDL).

Different adenoviral vectors were constructed and tested *in vitro* on various cell-lines so as to compare their relative strength. Two vectors, which expressed apoAI under the control of either the CMV or the LTR-RSV promoters were then prepared for animal studies.
The animal model in which we performed the assay is the mouse line C57Bl/6, which develops pre-atherosclerotic lesions upon induction by an hypercholesterolemic diet. Since apoAI is extremely abundant in the plasma (1.3 g/l in humans), the protocol was also developed in apoAI knock-out mice (AI-KO) so as to measure the net increase in total HDL in animals with extremely low HDL and completely devoid of apoAI-containing HDL.
After a single-dose intravenous injection of viral preparations (performed either in the tail or eye vein), which corresponds to approximately 10^{10} pfu, the plasma levels of human apoAI were found surprisingly high (0.2 to 15 mg/dl) and lasted for several weeks with a plateau of three weeks. The LTR-RSV adeno construct proved much more stable than the CMV construct in terms of levels and stability of the recombinant apoAI expression. A second injection performed at day 11 was also shown to further raise apoAI plasma levels.
HDL levels were significantly increased in the treated versus inbred control animals. In treated apoAI-KO mice, gradient gel electrophoresis analysis revealed a lipoprotein profile which was similar to that of human apoAI transgenic animals, with a characteristic bimodal density distribution of HDL corresponding to human apoAI containing lipoprotein particles in transgenic mice.
Following these very encouraging results, an atherosclerosis regression study on C57Bl/6 mice with pre-developed aortic lesions is currently underway.

E 206 INHIBITION OF E-SELECTIN GENE TRANSCRIPTION THROUGH A cAMP DEPENDENT PROTEIN KINASE PATHWAY.

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Cytokines induce the expression of the cellular adhesion molecules E-selectin, VCAM-1 and ICAM-1 on endothelial cells as part of the inflammatory response. We investigated the role of phosphorylation in the regulation of gene expression of these molecules. We show here that expression of these surface proteins is differently affected by increasing intracellular cAMP. Forskolin and forskolin analogues decrease E-selectin and VCAM-1 gene transcription induced by IL-1 and TNFα, but they increase induced ICAM-1 expression. The effect of forskolin is abolished by the protein kinase A inhibitors H89 and H88, suggesting that repression is mediated by phosphorylation. We demonstrate by nuclear run-on assays and mRNA half-life analysis that the cAMP repression of the E-selectin gene occurs via transcription inhibition. Although activated NF-κB is necessary to mediate the cytokine inducibility of the gene, activation of this factor is not affected by increased levels of cAMP. We found that the minimal promoter sequence (-383 bp) necessary to confer cytokine inducibility is sufficient to permit the forskolin effect in transfected endothelial cells. Since the E-selectin promoter lacks a consensus CREB site, we are presently studying promoter mutants to localise a region responsible for the forskolin effect.

E 207 REGULATION OF THE p55 and p75 TNF RECEPTORS IN HUMAN SYNOVIAL MICROVASCULAR ENDOTHELIAL CELLS BY INTERFERON GAMMA. ROLE IN THE UPREGULATION OF ICAM-1 EXPRESSION. Mary E. Gerritsen and William W. Carley, Miles Inc. West Haven CT. 06516.

Recent studies by this laboratory have shown that treatment of human synovial endothelial cells (HSE) with TNF elicits minimal upregulation of ICAM-1, in contrast to the response in human umbilical vein endothelial cells (HUVE). Co-incubation of TNF with IFNγ synergistically increases ICAM-1 expression in HSE and HUVE. To elucidate the molecular mechanisms of the heterogeneity in TNF responsiveness in HSE versus HUVE, we compared basal and IFNγ treated HSE and HUVE TNF receptor number, affinity and type. Both cell types predominantly expressed the p55 type TNF receptor. HSE cells exhibited more TNF binding sites/cell (2200) than HUVE (1200), although the apparent affinity of HSE (K_D=120 pM) was lower than HUVE (K_D=66 pM). IFNγ induced a dose-dependent, biphasic increase in TNF binding sites on HSE requiring several hours to appear, reaching maximum at 8-10 hrs, then declining to basal levels at 24 hr. Crosslinking antibodies directed against the p55 (but not p75) receptor mimicked the effects of TNF and the synergism with IFNγ on ICAM-1 expression in HSE. Northern analyses indicated that the mRNAs for both p55 and p75 receptor in HSE were upregulated by IFNγ. In conclusion, this study indicates that the minimal upregulation of ICAM-1 in HSE (compared to HUVE) is not a consequence of a major difference in TNF receptor number, type or affinity. However, these studies do implicate a role for the upregulation of TNF receptors in the synergistic actions of IFNγ on TNF stimulated ICAM-1 expression.

E 208 MECHANICAL STRAIN ENHANCES TRANSCRIPTION OF THE PDGF-A GENE IN VSM CELLS VIA AN ELEMENT IN A 92bp MINIMAL PROMOTER

Harlan E. Ives, Tucker Collins, and Emily Wilson, Cardiovascular Research Institute, University of California, San Francisco, and Dept. of Pathology, Harvard Univ., Boston, MA

Cyclic mechanical strain (1 Hz) induces up to a 10-fold increase in DNA synthesis in cultured vascular smooth muscle (VSM) cells through secretion of the A- and B- chains of PDGF. We previously showed that strain induces mRNA for the PDGF-A gene. To study the effect of strain on transcription of the PDGF-A gene, neonatal rat VSM cells were transiently transfected with a PDGF-A full length promoter-CAT construct and with various promoter truncation-CAT constructs. 24h after transfection, cells were exposed to strain for 24h, lysed and CAT activity was measured. Strain enhanced CAT expression with the full length (-890bp) promoter by 2-3 fold. Basal transcription and ratio of strain/static transcription responses were as follows:

Construct (bp)	Fold basal activity	Strain Static activity
-890	1.0	2.4
-643	1.0	2.0
-262	0.2	4.8
-92	1.8	1.6
-41	0.0	0.0

All constructs tested except the 41 bp promoter fragment contained sufficient information to yield basal transcription and a response to mechanical strain. The 92bp minimal promoter contains 3 SP1 sites and a WT-1 site, the site of interaction with the Wilm's tumor protein and egr-1. Current work is aimed at determining which of these sites is required for the response to mechanical strain.

E 209 ACTIVATION OF TGF-β IS DEPRESSED IN THE SERA AND IN THE VESSELS OF TRANSGENIC APO(a) MICE. Paul Kemp, James Metcalfe, *Alex Liu, *Richard Lawn and David Grainger, Department of Biochemistry, University of Cambridge, CB2 1QW U.K. and *Division of Cardiovascular Medicine, Stanford University, CA 94305

In vitro observations on human aortic vascular smooth muscle cells (VSMCs) have shown that lipoprotein(a) and its major protein component, apo(a), inhibit the activation of plasminogen to plasmin and hence the proteolytic activation of latent TGF-β produced by the cells. Since TGF-β is an autocrine inhibitor of the proliferation of the cells, the effect of Lp(a) is to promote proliferation by relieving the inhibition caused by TGF-β. If TGF-β and Lp(a) have similar functions *in vivo*, TGF-β may be a key regulator of VSMC proliferation in lesion development. To test this hypothesis we have used a transgenic mouse model in which human apo(a) is expressed. The apo(a) mice rapidly develop intimal lesions on a high fat diet and die prematurely of coronary artery stenoses. We have used novel ELISAs to determine active and total TGF-β in serum and we have also developed immunofluorescence assays to measure active TGF-β, total TGF-β and plasmin in sections of mouse arteries. The total amount of TGF-β is the same in the sera of apo(a) mice and in littermate control mice, whereas the proportion of active TGF-β is approximately 30% of the total in the apo(a) mice compared with over 90% active TGF-β in control mice. In fluorescence microscopy studies of sections of the aortic wall from apo(a) mice, we have found that the amounts of active TGF-β are 60 to 80% lower than in litter mate controls, whereas the total amounts of TGF-β are very similar. Furthermore, co-staining the sections for plasmin showed that the amounts of plasmin are ~80% lower than in controls although the amounts of plasminogen are similar. The relationship between active TGF-β and apo(a) level predicted from the *in vitro* studies is strongly supported by the transgenic model.

E 210 PRODUCTION OF TGF-β BY HUMAN VASCULAR SMOOTH MUSCLE CELLS. Heidi Kirschenlohr, David Grainger, *Peter Weissberg and James Metcalfe, Department of Biochemistry, University of Cambridge, CB2 1QW U.K. and *School of Clinical Medicine, University of Cambridge, CB2 2QQ, U.K.

When human vascular smooth muscle cells (VSMCs) are obtained by explanting pieces of aorta and culturing the cells which migrate from the tissue, the cells are predominantly spindle-shaped and proliferate with a doubling time of ~35 hours. Addition of TGF-β (10ng/ml) to the cells increases the population doubling time to ~42 hours. Addition of tamoxifen (5μM) to the cells decreases the rate of proliferation to a similar extent to TGF-β and the effect of tamoxifen is fully reversed by the addition of anti-TGF-β antibody. TGF-β decreases the rate of proliferation of the cells by specifically extending the G_2 phase of the cell cycle. In all of these properties the human VSMCs are similar to the properties we have previously described for rat aortic VSMCs prepared by enzyme dispersal. By contrast, when human aortic VSMCs are prepared by the same enzyme dispersal technique as rat aortic cells, their properties are markedly different. The cells proliferate very slowly with a population doubling time of ~71 hours, are predominantly large cells lacking the characteristic spindle-shaped morphology of the explant cells and they do not form 'hills and valleys' at confluence. The population doubling time of these cells is unaffected by the addition of TGF-β or tamoxifen, whereas addition of anti-TGF-β antibody reduces the population doubling time to ~45 hours. The enzyme-dispersed cells therefore produce active TGF-β and inhibit proliferation by an autocrine mechanism. The enzyme-dispersed cells retain amounts of smooth muscle-specific myosin heavy chain similar to those in the freshly isolated cells, but the explant cells rapidly lose smooth muscle-specific proteins. However, addition of TGF-β to the explant cells prevents de-differentiation. Overall, the responses of human and rat VSMCs to TGF-β are very similar.

E 211 NITRIC OXIDE MAY REGULATE PROLIFERATION AND FIBRONECTIN DEPOSITION IN HYPOXIC PULONARY ARTERY SMOOTH MUSCLE CELLS. Machelle Manuel, Shewan M. Aziz, Sharmen F. Tofiq, Harry S. Nick*, Jack W. Olson, and Mark N. Gillespie, Division of Pharmacology and Experimental Therapeutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536, and *Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, FL 32610.

Chronic hypoxia causes remodeling of the pulmonary circulation leading to structurally-based increases in vascular resistance. One of the effectors of the hypoxic response is the pulmonary artery smooth muscle cell (PASMC), which may be a source of connective tissue proteins that accumulate in the medial arterial layer during hypertensive vascular remodeling. Signal transduction mechanisms linking hypoxic exposure to alterations in PASMCs are not well delineated. Recently, however, observations in both intact lungs and cultured vascular smooth muscle cells suggest that hypoxia may elevate nitric oxide (NO) synthesis. In the current experiments, we tested the hypothesis that NO mediates accumulation of fibronectin and decreases in proliferation evoked by hypoxia in cultured bovine PASMCs. Relative to PASMCs grown in standard culture conditions (culture medium Po_2>100 torr), cells exposed to hypoxia (Po_2=18-22 torr) failed to proliferate and deposited increased amounts of fibronectin in the culture media. Treatment with the NO synthase inhibitor, L-NAME, engendered a modest proliferative response in hypoxic cells and, importantly, abolished the hypoxia-induced accumulation of fibronectin. Addition of L-arginine to the media reversed these effects of L-NAME. The source of NO did not appear to be the inducible form of NO synthase inasmuch as the mRNA transcript for the enzyme could not be detected by Northern analysis in either normoxic or hypoxic PASMCs. The mRNA transcript for fibronectin was decreased in hypoxic cells relative to controls; this change was prevented by L-NAME and reversed by L-arginine. These observations suggest that hypoxia causes NO synthesis in PASMCs, possibly by activating the constitutive NO synthase, and that NO may act by an autocrine mechanism to regulate fibronectin deposition and decrease cell proliferation.

E 212 PROTEIN MARKERS OF LESION DEVELOPMENT IN THE VESSELS OF TRANSGENIC APO(a) MICE, James Metcalfe, Paul Kemp, *Alex Liu, *Richard Lawn and David Grainger, Department of Biochemistry, University of Cambridge, CB2 1QW U.K. and *Division of Cardiovascular Medicine, Stanford University, CA 94305

We have used a transgenic mouse model in which human apo(a) is expressed to examine changes in protein expression patterns during lesion development. The apo(a) mice rapidly develop intimal lesions on a high fat diet and die prematurely of coronary artery stenoses. We have stained the lesions for several protein markers which are preferentially expressed by vascular smooth muscle cells (VSMCs) when proliferating in culture.

Recent studies by Schwartz and colleagues have shown that osteopontin (OP) is expressed more strongly by proliferating VSMCs *in vitro*. We have stained sections of aorta from transgenic apo(a) mice and normal litter mate controls for OP using the monoclonal antibody MPIIIB10. Within 4 weeks of initiating the high fat diet, focal areas of the aortic wall stained strongly for OP in the apo(a) transgenic mice, but not in control mice. The staining was limited to the luminal surface, and covered 40-60% of the vessel circumference. The underlying media did not stain significantly with this antibody. By 9 weeks after initiating the high fat diet, the area staining with the OP antibody had increased, although the staining remained focal. The OP staining was co-localised with strong staining for the apo(a) transgene product. Although the luminal surface was most strongly stained, the underlying media also stained significantly.

Another antibody (MAb20), which recognises the zeugmatin gene product, also selectively stains proliferating VSMCs in culture. This antibody stained the sections from the apo(a) transgenic mice, but not the control mice, in the same regions as the OP antibody. However, the staining with MAb20 was limited to the luminal surface even at 9 weeks after the high fat diet was initiated.

E 213 ALTERATION IN BOVINE PULMONARY ARTERY SMOOTH MUSCLE CELL (BPASMC) GLUTATHIONE (GSH) DURING HYPOXIA IS ASSOCIATED WITH AN ALTERATION IN FIBRONECTIN RELEASE, Peter E. Morris*, Shewan M. Aziz**, Mark N. Gillespie**, Division of Pulmonary Medicine* and Division of Pharmacology & Experimental Therapeutics**, University of Kentucky, Lexington, KY 40536

Hypoxia causes remodeling of the pulmonary circulation that is dependent, in part, on alterations of vascular smooth muscle cells. Smooth muscle cells have been noted as a source for many connective tissue proteins, including fibronectin. GSH is a widely distributed tripeptide exerting its antioxidant activity through a network of enzymes. Also, it has been postulated that the GSH system is involved in cell proliferation. To determine whether the effect of hypoxia on fibronectin release by BPASMC, was associated with alteration in intracellular glutathione (GSH), *in vitro* studies of BPASMC were conducted under normoxic (21% oxygen) and hypoxic (3% and near 0% oxygen) conditions for a period of 24 hours. GSH was determined by a modification of the fluorometric method of Hissan and Hilf. BPASMC fibronectin release into culture media was determined by ELISA. BPASMC, under normoxic conditions, demonstrated 7.7 nmoles GSH per 10^6 cells, whereas cells incubated at 3% oxygen contained 5.0 nmoles GSH per 10^6 cells and those cells incubated at near 0% oxygen revealed 3.1 nmoles GSH per 10^6 cells. Fibronectin release by BPASMC into the culure media is expressed as absorbance/mg protein. The value of fibronectin release increased with reduction in the incubating oxygen concentration. The normoxic cells' fibronectin value was 1.44, compared to 0.94 at 3% O2 and 0.76 at 0% O2. To further illuminate the relationship between GSH and fibronectin accumulation under hypoxic conditions, parallel studies were performed with the addition of N-acetylcysteine (NAC) to the culture media. NAC has previously been shown to be deacetylated intracellularly, allowing cysteine to become available for GSH synthesis. Cells incubated with NAC (5mM, 25mM, 50mM) did show an increase in GSH concentration above baseline values; however, the trend in fibronectin accumulation in hypoxic conditions was not reversed, but accentuated by the augmentation of GSH above baseline values. Furthermore, BPASMC incubated with NAC at normoxic conditions demonstrated an increment in fibronectin accumulation, as well as a decrease in cell number and cell protein concentration. These *in vitro* studies suggest that exposure of BPASMC to hypoxic conditions or incubation with NAC, produced reduction and elevation of intracellular GSH, respectively. As well, these deviations of GSH from control values were associated with an increase in fibronectin accumulation suggesting perturbations of BPASMC's GSH may accompany alterations in the cell's proliferative state such as release of fibronectin.

E 214 APO E-DEFICIENT MICE DEVELOP LESIONS OF ALL PHASES OF ATHEROSCLEROSIS THROUGHOUT THE ARTERIAL TREE, Yutaka Nakashima[1], Andrew S. Plump[2], Elaine W. Raines[1], Jan L. Breslow[2] and Russell Ross[1], [1]Department of Pathology, University of Washington, Seattle WA 98195, [2]Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York NY 10021

Initial description of apo E-deficient transgenic mice demonstrated the development of severe hypercholesterolemia due to probable delayed clearance of large atherogenic particles from the circulation. Examination of these mice demonstrated foam cell accumulation in the aortic root and pulmonary arteries by ten weeks of age. In the present study, the animals were fed either chow or a high fat, Western-type diet and examined at ages ranging from six to forty weeks. Gross examination by dissection microscopy revealed a predilection for development of lesions in the aortic root, at the lesser curvature of the aortic arch, the principal branches of the aorta, and in the pulmonary and carotid arteries. Monocyte attachment to endothelial cells was observed by light and electron microscopic examination at six weeks, the earliest time point examined. Foam cell lesions develop as early as eight weeks, and after fifteen weeks advanced lesions (fibrous plaques) were observed. The latter consist of a fibrous cap containing smooth muscle cells surrounded by connective tissue matrix that covers a necrotic core with numerous foamy macrophages. Mice fed the Western-type diet generally had more advanced lesions than those fed a chow diet. The apo E-deficient mouse contains the entire spectrum of lesions oberserved during atherogenesis and is the first mouse model to develop lesions similar to those in humans. This model should provide numerous opportunities to study the pathogenesis and therapy of atherosclerosis in a small, genetically defined animal.

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E 215 *Abstract Withdrawn*

E 216 SERUM, PMA, AND TESTOSTERONE REGULATE EXPRESSION OF AN ACIDIC FIBROBLAST GROWTH FACTOR (FGF-1) TRANSCRIPT. Robert A. Payson[1], Stephen E. Harris[2], and Ing-Ming Chiu[1], Department of Internal Medicine[1], The Ohio State University, Columbus, OH 43210 and Department of Medicine[2], University of Texas, San Antonio, TX 78249

The human fibroblast growth factor 1 (FGF-1) is a potent mitogen for mesoderm-derived cells such as fibroblasts, smooth muscle cells, and endothelial cells. It also stimulates the growth of cells of ectodermal origin such as prostate epithelial cells. This implicates FGF-1 as a growth factor in the pathogenesis of atherosclerosis and in prostate cell growth and malignancy. There now appears to be at least four 5' non-coding exons for the human FGF-1 gene. These exons and their associated promoters confer tissue-specific expression. Here we show that expression of the FGF-1 1.C transcript is upregulated by testosterone in the androgen-dependent prostate carcinoma cell line LNCaP. In contrast, FGF-1 is expressed in the absence of testosterone in PC-3 cells, an androgen-independent prostate carcinoma cell line. No detectable levels of FGF-1 1.C are present in brain or kidney as ascertained by RNase protection analysis. We further show that FGF-1 promoter 1.C is activated in PC-3 cells treated with either serum or PMA following serum starvation. These data suggest that: 1) FGF-1 is at least one growth factor that replaces the need for testosterone in steroid-independent prostate cancer; and 2) serum mitogens may function at least in part through protein kinase C to induce FGF-1 expression in PC-3 cells. The AP-1 binding site, known to be involved in the transcriptional response of cells to phorbol ester, is present twice 5' of the 1.C start site. Also located 5' is a putative androgen response element (ARE). We are currently investigating the functions of these putative *cis*-acting sequences using luciferase reporter gene assays.

E 217 SYNERGISTIC ACTION OF INSULIN-LIKE GROWTH FACTOR-I AND BASIC FIBROBLAST GROWTH FACTOR IN CULTURED VASCULAR SMOOTH MUSCLE CELLS, T.J. Reape, J.M. Kanczler, K.G. Burnand*, J.P.T. Ward and C.R. Thomas, Departments of Medicine and *Surgery, U.M.D.S., St. Thomas's Hospital, London SE1 7EH, U.K.

Vascular smooth muscle cell (VSMC) proliferation is a predominant feature of the response of the arterial wall to injury, local synthesis of growth factors playing a major role in its induction. We have investigated the effects of two growth factors implicated in intimal thickening after vascular injury, basic fibroblast growth factor (bFGF) and insulin-like growth factor-I (IGF-I), on aortic rabbit VSMC in culture. Incorporation of ^{3}H-thymidine into DNA was measured 24 hours after stimulation of VSMC with IGF-I and/or bFGF. We found that IGF-I, at a concentration of 10 ng/ml (12.8 nM) caused a 1.6 fold increase in DNA synthesis in rabbit SMC. bFGF, which is known to be a potent mitogen for VSMC, caused a 9.6 fold increase in DNA synthesis at a concentration of 0.25 ng/ml (139 pM). However, when IGF-I was added in combination with bFGF, at the same concentrations, DNA synthesis was stimulated to an extent that is more than additive i.e. 28.4 fold compared to 11.2 fold. This synergistic response was also evident using lower concentrations of bFGF. IGF-II, together with bFGF, failed to elicit a synergistic response. Northern blot analysis revealed that IGF-I (10ng/ml) increases mRNA levels for bFGF flg receptor as early as 1 hour after stimulation and levels remain elevated for at least 24 hours (4.1 and 6.4 fold increase, respectively). In conclusion IGF-I, whilst being a relatively weak mitogen for vascular SMC itself, appears to be capable of up-regulating flg receptor mRNA expression, thereby making VSMC more responsive to the actions of bFGF.

E 218 TRANSCRIPTIONAL REGULATION OF THE HUMAN E-SELECTIN GENE, Ulrike Schindler, Vijay Baichwal and Steven L. McKnight, TULARIK, Inc., 270 E. Grand Ave., South San Francisco, CA 94080

The endothelial-leukocyte adhesion molecule (ELAM-1, E-selectin), a cell surface glycoprotein, mediates the adhesion of neutrophils and plays a key role in the inflammatory response. Expression of the ELAM-1 gene is induced by various cytokines (including TNFα and IL-1) and is restricted to endothelial cells. Both, cytokine-induced and cell-type specific expression are mediated at the transcriptional level and 200 bp upstream of the transcriptional start site are sufficient for the induction by cytokines. However, very little is known about the requirements for the cell-type specific expression. We used site-directed mutagenesis and transfection assays to identify promoter elements required for cytokine induction. In addition to the previously characterized NF-KB site we have found another region of the promoter that is essential for TNFα induced gene expression. In order to locate regions that are involved in the cell-type specific expression of the ELAM-1 gene we mapped DNaseI hypersensitive sites in human umbilical vein endothelial cells (HUVEC) and HeLa cells. Hypersensitive sites have been identified that are cell-type specific and/or whose presence is dependent on exposure of cells to TNFα.

E 219 **IDENTIFICATION, CLONING AND CHARACTERIZATION OF A NOVEL GENE, ALLOGRAFT INFLAMMATORY FACTOR-1 (AIF-1), UPREGULATED IN RAT CARDIAC ALLOGRAFTS WITH TRANSPLANT ARTERIOSCLEROSIS**, Ulrike Utans, Robert J. Arceci,* Yukari Yamashita, and Mary E. Russell, Cardiovascular Biology Laboratory, Harvard School of Public Health, and *Dana-Farber Cancer Institute, Boston, MA 02115

Chronic rejection of cardiac allografts is manifested by early and persistent infiltration of inflammatory cells and allograft arteriosclerosis. To isolate potential mediators of chronic rejection, we used the differential RNA display technique in a rat model of heterotopic cardiac transplantation (Lewis to Fisher) that produces monocyte/macrophage infiltration and arteriosclerotic lesions similar to those observed in human allografts. The comparison of gene expression levels in cardiac allografts (7 and 14 days after transplantation) with those in syngrafts (Lewis to Lewis) or host hearts has resulted thus far in the identification of 12 differentially regulated transcripts in allograft tissue. Full-length cDNA clones (651 bp) have been obtained for one of these transcripts, allograft inflammatory factor-1 (AIF-1). The predicted amino acid sequence of the longest open reading frame (147 amino acids) contains a 12-amino acid region similar to the EF-hand (Ca^{2+}-binding) domain. The AIF-1 clone specifically hybridized to RNA isolated from allografts (7, 14, 28, and 75 days after transplantation) and spleens by Northern analysis. Semiquantitative PCR studies showed elevated AIF-1 transcript levels in splenic and peritoneal macrophages, balloon-injured carotid arteries with neointimal thickening, and allografts. By in situ hybridization AIF-1 transcripts localized to areas of mononuclear cell infiltration in cardiac allografts. The localization within cardiac allografts of a mononuclear-cell subset expressing AIF-1 transcripts suggests that AIF-1 may act as an inflammatory mediator in chronic rejection and arteriosclerosis.

E 220 THE GROWTH-RELATED TRANSCRIPTION FACTOR OCT-1 IS EXPRESSED AS A FUNCTION OF VASCULAR SMOOTH MUSCLE CELL MODULATION. Mary C.M. Weiser, Phillip E. Schwartz, Nicole A. Grieshaber, and Richard A. Majack, Department of Pediatrics and Cell and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262.

Vascular smooth muscle cells (SMC) undergo a phenotypic "modulation" associated with the initiation of DNA synthesis and cellular proliferation. The molecular changes accompanying this phenotypic switch, which occurs following vascular injury and when SMC are placed in culture, remain largely unknown. A family of octamer-binding ("Oct") transcription factors have been shown to be selectively expressed in replicating cells. Oct-1, the prototype member of this gene family, is believed to be ubiquitously expressed and is known to be responsible for histone gene transcription. The expression of this factor, therefore, is necessary but not sufficient for cell replication. We have studied the expression of Oct-1 and related factors in SMC *in vivo* and *in vitro*. Nuclear proteins binding to the consensus octamer-binding site were demonstrated using gel shift analysis with a DNA fragment containing the octamer motif. One of these proteins was identified as Oct-1 using a specific antibody. Oct-1-specific oligonucleotide primers were used to amplify a 403 bp PCR product from cDNAs prepared from cultured SMC. DNA sequencing of this PCR product revealed a >98% homology with human Oct-1. Using RT-PCR coupled with Southern blot hybridizations, Oct-1 transcripts were readily detectable in growing sparse and confluent SMC, in growth-inhibited sparse and confluent SMC, and in confluent SMC growth-arrested in the presence of heparin. However, Oct-1 transcripts could not be detected in SMC samples isolated from normal, uninjured adult rat aortas. The expression of Oct-1 by vascular SMC therefore does not appear to be significantly regulated by growth state; rather, Oct-1 expression appears to be induced during the *in vivo* to *in vitro* transition. When the aortic media from adult rats was enzyme-dispersed (under serum-free conditions) into single cells, Oct-1 transcripts became readily detectable even before plating, suggesting that disruption of cell:matrix interactions is sufficient to induce Oct-1 expression. Enzyme digestion of the aortic media in the presence of heparin, an inhibitor of SMC replication, prevented the digestion-induced expression of Oct-1. Our results suggest that Oct-1 may be a marker for SMC modulation and provide further evidence that heparin-like molecules may act *in vivo* to suppress specific molecular events associated with SMC modulation. Most importantly, the data demonstrate that the modulation of SMC into replication-competent cells may involve the acquired expression of specific transcription factors required for cellular growth.

Cytokines and Their Inhibitors

E 300 ESTROGEN RECEPTOR IN VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS, Francis Bayard, Simone Clamens, Georges Delsol and Jean Charles Faye, Department of Endocrinology, CHU Rangueil, 31400, Toulouse, France.

Beside their "lipoprotein mediated" effects, estrogens inhibit the initiation of atherosclerosis by a direct effect at the level of the arterial wall. The mechanisms may involve suppression of uptake and/or degradation of LDL, modification of vascular tone and blood flow characteristics. Estrogen binding has been reported in the vascular tissues of human as well as other species. However neither estrogen receptor protein itself (ER) nor its messenger ARN (ARN_m) have been characterized in pure population of vascular cells. We have undertaken such characterization in aortic human and rat smooth muscle cells as well as in bovine aortic endothelial cells from primo-secondary cultures.

ER has been characterized by immunohistochemistry using 2 monoclonal antibodies which recognize epitopes localized in the A/B domain of the molecule (aa 118 to 140). In contrast to uterine or breast cancer cells where ER is localized in the nucleus, ER has a predominant cytoplasmic localisation in the 3 populations of cells, even in presence of estradiol added to the culture medium for 1 h at 37°. Studies of estradiol metabolism by these cells show the presence of the 17β-dehydrogenase enzyme but with predominant reductive activity. This peculiar behaviour does not appear to result from a specific maturation of the ARN_m. By choosing oligonucleotides from exon 2, 5 and 8 sequences and taking care of species specificity, RT-PCR followed by restriction enzyme analysis shows that the complete ARN_m is expressed in these cells. The transcriptional activity of this receptor on endogenous as well as on reporter genes is presently under investigation.

E 301 MOLECULAR CHARACTERIZATION OF A FAMILY OF LIGANDS FOR EPH-RELATED TYROSINE KINASES. M. Patricia Beckmann, Douglas P. Cerretti, Peter Baum, Tim Vanden Bos, Carl Kozlosky, Laura James, Terry Farrah, Tamy Hollingsworth, Tony Pawson+, Eugene Maraskovsky, Fred Fletcher and Stewart D. Lyman. Immunex Corporation, Seattle, WA and +Samuel Lunenfeld Research Institute, Toronto, Canada.

Characterization of a ligand for the elk tyrosine kinase receptor was undertaken using a soluble form of the receptor. Screening of a human placenta cDNA library using a two step direct-expression strategy allowed isolation of a cDNA clone which encodes a 346 amino acid transmembrane protein capable of binding to the elk receptor molecule. Northern blot analysis of human tissues showed that the mRNA size for this elk binding protein is 3.5 kb and is expressed in placenta, heart, lung, liver, skeletal muscle, kidney, pancreas, and stimulated peripheral blood T cells. The mRNA is also induced in human umbilical vein endothelial cells following TNF stimulation. The DNA sequence of this factor is novel, but shares similarity with other recently isolated cDNAs from TNF-induced endothelial cells and a human T cell line. We have termed these molecules LERKs (Ligands for Eph-Related Kinases). These ligands may affect growth or differentiation of cells in the brain since this is a rich source of the elk receptor. While several LERKs bind the elk receptor, promiscuity in binding to other elk-related receptors will be discussed.

E 302 PROLIFERATIVE ANGIOPATHY IN THE BRAIN INDUCED BY THE CEREBRAL OVEREXPRESSION OF INTERLEUKIN-6 IN TRANSGENIC MICE, Iain L. Campbell, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Cytokines, including interleukin-6 (IL-6), possess a spectrum of neuroregulatory actions and have been hypothesized to play a pathogenetic role in a variety of CNS diseases including MS, AIDS encephalopathy and Alzheimer's disease. To test this hypothesis further, transgenic (tg) mice were developed in which the expression of IL-6 was targeted to astrocytes in the brain using a glial fibrillary acidic protein (GFAP) genomic expression vector. IL-6 tg mice displayed neurologic disease (runting, tremor, ataxia and seizure), neurophysiologic impairment and a spectrum of neuropathologic changes (including neurodegeneration, astrocytosis and neovascularization). Cerebrovascular changes were particularly striking and progressed to a proliferative angiopathy which was most marked in the cerebellum with considerable distortion of the granular cell layer and white matter tracts. Immunostaining and in situ hybridization for von Willebrand factor (vWf) confirmed the dramatic increase in blood vessel formation in the cerebellum and also to a lesser extent in other brain regions. In addition, absolute levels of vWf protein and RNA were apparently increased in cerebrovascular endothelial (CE) cells indicating expression of this gene was also modulated in IL-6 tg mice. Consistent with the histopathologic features of cerebral angiogenesis, [^{3}H]-thymidine autoradiography revealed nuclear incorporation in numerous CE cells of IL-6 tg mice but not their normal littermates. Coincident with proliferative angiopathy, significant deposition of Ig (particularly in the cerebellum) suggested the integrity of blood brain barrier was compromised in IL-6 tg mice. Proliferative angiopathy and increased vWf gene expression were not observed in the brains of tg mice with cerebral overexpression of the cytokine IFN-α, indicating some specificity for the CE cell response to IL-6. Interestingly, tg mice with pancreatic islet cell overexpression of IL-6 did not show evidence of islet angiogenesis, suggesting CNS specific factors may be responsible for the cerebral proliferative angiopathy in response to IL-6. The gross structural and functional cerebrovascular changes in the IL-6 tg mice may impact negatively on CNS function and could in part account for the neurologic impairment in these animals. This novel genetic model should provide valuable insights into the molecular and cellular aspects of angiogenesis and CE cell function in the CNS.

E 303 TRANSGENIC MICE EXPRESSING HUMAN INTERLEUKIN-1ß EXHIBITED RUNTED PHENOTYPE AND PATHOLOGY IN VARIOUS TISSUES, Howard Y. Chen, Myrna E. Trumbauer, Christina C. Grabiec, Kathryn J. Hofmann*, Hui Zheng, Karla A. Stevens, Dalip Sirinathsingji+, Lex H.T. Van der Ploeg and Alan R. Shaw*. Merck Research Laboratories, P.O. Box 2000, Rahway NJ. 07065, , Merck Research Laboratories*, West Point, Pa 19486 and Neuroscience Research Center+, Terlings Park, Harlow Essex, CM20 2QR England

To study the effects of overexpression of IL-1ß in various tissues, an active form of human (h) IL-1ß cDNA with the mouse IL-1ra signal peptide sequence was linked to the mouse metallothionein-1 promoter and used to produce transgenic mice. Of 26 transgenic founders created, 11 were sick and severely runted by two weeks of age. Histological examination of two mice showed pathology in many of the tissues examined, including liver, kidney, pancreas, lung, heart, leg joint, vertebrae and brain. Of particular interests are arthritis in the joints and meningoencephalitis, vasculitis, and necrosis in the brain with infiltration of lymphoid cells. In two other mice that were moribund and necropsied, the major pathology was found only in the central nervous system with lymphocytic infiltrations. To detect expression of the transgene in various tissues, we employed an RNase protection assay to measure the h IL-1ß mRNA. IL-1ß expression was high in liver and kidney, consistent with the tissue specificity of metallothionein-1 promoter. Additionally, an RT-PCR assay was employed to detect h IL-1ß as well as several endogenous mouse RNA species. This comparison revealed expression of the IL-1ß transgene and endogenous mouse IL-6 and IL-1ß mRNA in the liver. Another assay involving *in situ* hybridization of the h IL-1ß mRNA in the brain of transgenic mice was performed, revealing expression of the transgene in the cerebellum and brain stem regions. These results demonstrate that transgenic mice expressing h IL-1ß can be a useful model for studying inflammatory diseases.

E 304 M-CSF SELECTIVELY ENHANCES SCAVENGER RECEPTOR EXPRESSION AND FUNCTION IN MURINE MACROPHAGES. Willem J.S. de Villiers, Iain P. Fraser, Derralyn A. Hughes, Anthony G. Doyle and Siamon Gordon, *Sir William Dunn School of Pathology, Oxford University, South Parks Road, Oxford, OX1 3RE, England*

The macrophage scavenger receptor (MSR) has been implicated in foam cell formation in atherogenesis and factors regulating its expression and function are of interest. Serum cholesterol levels in patients treated with macrophage-colony stimulating factor (M-CSF) have been noted to be reduced; this effect may be mediated by increased MSR activity. We tested this hypothesis in murine macrophages (Mϕ) using a recently developed monoclonal antibody to murine MSR (Fraser, et al. Nature 364(1993):343). Immunoblots of lysates from Biogel-elicited peritoneal Mϕ (BgPMϕ) treated with human M-CSF for 48 hours showed an increase in MSR protein whilst class II MHC and F4/80 antigen expression were unaffected. Immunoprecipitation of MSR from metabolically labelled cells confirmed this and suggested that, in addition to increasing the synthesis, M-CSF could also prolong the half-life of the synthesized receptor. Upregulation by M-CSF of cell surface expression of MSR was also confirmed by flow cytometry. This effect was blocked by the addition of antibody to murine M-CSF. Semi-quantitative RT-PCR assays showed that M-CSF increases MSR expression transcriptionally and both the type I and II forms of the receptor were elevated. The significance of these differences in levels of MSR expression were related to functional MSR studies. The ability of BgPMϕ to endocytose and accumulate AcLDL (uptake of DiI-AcLDL) and to adhere to tissue culture plastic in the presence of EDTA were significantly enhanced after treatment with M-CSF; correlating with M-CSF-mediated upregulation of MSR protein and message levels. Vascular endothelial cells and smooth muscle cells produce M-CSF in response to a range of stimuli; we suggest that increased MSR expression and function by Mϕ may be an important mechanism by which these cellular interactions contribute to atherogenesis.

E 305 INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1ra) LACKS ONE OF THE TWO RECEPTOR BINDING SITES ON IL-1ß, BASED ON STRUCTURE/FUNCTION ANALYSIS.

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IL-1ra, an IL-1 family member, binds with high affinity to the type I IL-1 receptor (IL-1RI), blocking IL-1 binding, but not inducing an IL-1 like response. To investigate why IL-1ß and IL-1ra have such different biological activities, we determined the crystal structure of IL-1ra and performed site-directed mutagenesis to identify residues involved in binding to IL-1RI. The crystal structure of IL-1ra was solved to 2.75Å resolution. IL-1ra is similar in overall topology to IL-1ß. Both molecules have 12 ß-strands; six ß-strands pack in an antiparallel manner to form a ß-barrel, which is closed at one end by the other 6 strands. Site directed mutagenesis of IL-1ß by our group and others has revealed the presence of two discrete sites involved in binding to IL-1RI. One of these sites is also present in IL-1ra based on analysis of muteins for competitive receptor binding and for antagonist activity in an IL-1 bioassay. This site consists of Arg-11, His-30, Leu-31 and Gln-32 of IL-1ß, and Trp-16, Tyr-34, Leu-35 and Gln-36 of IL-1ra. We find that the other site on IL-1ß, located around residues Lys-92 and Lys-93 on the opposite side of the molecule from site 1, is absent in IL-1ra, since mutagenesis of the IL-1ra residues in this area does not significantly reduce its affinity for the receptor or its biological activity. Interestingly, this second site corresponds to the open end of the ß-barrel, where the major structural difference is seen between IL-1ra and IL-1ß. The presence of both receptor binding sites on IL-1ß may be necessary for its agonist activity.

E 306 ROLES OF MCP-1, TNF-α, AND ICAM-1 IN GLUCAN INDUCED PULMONARY VASCULITIS IN THE RAT. Craig M. Flory, Peter A. Barton, Michael L. Jones, Bradford F. Miller, and Jeffrey S. Warren. Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109.

The intravenous infusion of particulate yeast cell wall glucan into rats results in the synchronous development of angiocentric pulmonary granulomas that are composed almost entirely of monocytes and macrophages. Northern hybridization analysis of whole rat lungs reveals a biphasic increase in monocyte chemoattractant protein-1 (MCP-1) mRNA expression. Monocyte chemoattractant protein-1 mRNA reaches peak levels at 1 and 6-24 hours after glucan infusion. Analyses of bronchoalveolar lavage (BAL) fluid and whole lung extracts (by Western blot) revealed that both MCP-1 activity and MCP-1 protein expression peak at 48 hours. In situ hybridization and immunohistochemical analyses revealed that components of the bronchial and vascular walls are responsible for the early rise (1 hour) in MCP-1 mRNA and protein expression while granuloma-associated alveolar macrophages are the predominant source of MCP-1 later (6-24 hour) in the evolution of these lesions. Intravenous or intratracheal administration of anti-MCP-1 antibody at the time of glucan infusion results in a significant reduction in both the number and size of pulmonary granulomas at 48 hours. Glucan-induced pulmonary granuloma formation is accompanied by an early (6 hours) rise in BAL fluid tumor necrosis factor-alpha (TNF-alpha) activity. Since it is known that TNF-alpha can induce MCP-1 production in a variety of cell types, we sought to determine the potential regulatory role played by TNF-alpha in this model. A single infusion of anti-TNF-alpha antibody at the time of glucan infusion (time 0) markedly reduced MCP-1 mRNA levels at 1 and 6 hours, but not at later time points, and there was no effect on granuloma size or number measured at 48 hours. When multiple infusions of anti-TNF-alpha antibody were administered over a 24 hour period (0-24 hours), MCP-1 mRNA was reduced through 24 hours, and there were significant reductions in BAL fluid MCP-1 activity and in granuloma formation at 24 and 48 hours. These data suggest that TNF-alpha plays a regulatory role in glucan-induced pulmonary granulomatous vasculitis through the modulation of MCP-1. Granuloma formation was also inhibited following the intravenous infusion of antibodies to ICAM-1, suggesting that integrin-mediated adhesion plays an important role in granuloma formation in this model.

E 307 INTERLEUKIN-10 IN HUMAN ARTERIES AND VEINS, Sheila E. Francis [1,2], Hazel Holden [1], Cathy M. Holt [2], Geoffrey H. Smith [2], Gordon W. Duff [1] Sections of Molecular Medicine [1] and Cardiac Surgery [2], University of Sheffield. Sheffield U.K.

Interleukin 10 (IL-10) is a cytokine first associated with type 2 helper T cells but is now known to be produced by other cell types including monocytes, macrophages and B cells. Within the immune system, IL-10 has been shown *in vitro* to inhibit synthesis of numerous inflammatory mediators including IL-1, IL-6, IL-8 and TNF. It also inhibits the production of the type 1 helper cell lymphokines, interferon gamma and IL-2 and thus, suppresses cell-mediated immunity. In addition to intimal smooth muscle cell proliferation, coronary artery disease has a significant inflammatory component and therefore, IL-10 may be a good candidate gene in the study of occlusive vascular disease. To test this hypothesis, first we have performed reverse transcription/polymerase chain reaction (RT-PCR) analysis with human IL-10 specific oligonucleotide primers using RNA from human arteries and veins. The identity of the PCR products were confirmed by cloning, sequencing and Southern Blotting. IL-10 mRNA was then localised by *in situ* hybridisation using biotinylated oligonucleotide probes and IL-10 protein was detected by immunolocalisation with an IL-10 MAb IgG1 (Genzyme).

Our preliminary findings indicate that the IL-10 gene is expressed in arteries but not in veins. IL-10 mRNA was detected in all arteries tested (internal mammary artery and coronary artery, n=7) but not in freshly isolated saphenous vein (n=4). All vessels tested showed similar levels of mRNA for PDGF A chain, PDGF B chain and IL-6 ruling out an explanation based on the reduced cellularity of veins compared with arteries. IL-10 mRNA was observed in the endothelial layer of the arteries with some weaker staining in specific areas of the intima and media . IL-10 protein was also localised to these regions.

These results suggest that IL-10 may have a role to play in limiting inflammation and smooth muscle cell proliferation within the walls of high pressure vessels and raises an important new issue in the cellular biology of such tissues.

E 308 INFLUENCE OF IL-8 ISOFORMS ON BOVINE NEUTROPHIL MIGRATION *IN VITRO*. Galligan CG, Coomber BL. Department of Biomedical Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1.

Interleukin 8 (IL-8) is a potent neutrophil activating peptide that is produced by mitogen stimulated T cells, cytokine activated monocytes, fibroblasts, neutrophils and endothelial cells. Five different biologically active amino acid variants exist. The predominant N-terminal variant produced by endothelial cells is the 77 amino acid form; whereas, most other cells produce the 72 amino acid form. The two forms have different activities *in vitro*. Costar transwell inserts (pore size 3μm) were used in a modified Boyden chamber assay to assess the effects of human recombinant IL-8_{77} (hrIL-8_{77}) and hrIL-8_{72} on bovine neutrophil migration *in vitro*. 10^5 bovine neutrophils isolated from the peripheral blood were added to the upper well, and Hanks' balanced salt solution (HBSS), human recombinant IL-8_{77}, human recombinant IL-8_{72}, zymosan activated serum (ZAS) or lipopolysaccharide (LPS) was added to the lower well. The chamber was incubated for 30 minutes. The filters were fixed, stained with a Wrights' Giemsa stain and mounted on coverslips. The number of cells on the top and bottom surface of the filter was assessed with light microscopy. The HBSS induced a minimal amount of neutrophil migration. The remaining experiments were, therefore, expressed as a percentage of this negative control. 20% ZAS induced a significant increase in the migration of these cells through the filter. Neither form of IL-8 induced significant changes in neutrophil migration over a range of 10-3000 ng/ml. LPS also did not induce neutrophil chemotaxis. Therefore, bovine neutrophils will migrate in the presence of complement fragments generated by zymosan activated serum but not in the presence of either hrIL-8_{77}, hrIL-8_{72} or LPS *in vitro*.

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E 309 EXOGENOUS tat PROTEIN ACTIVATES CENTRAL NERVOUS SYSTEM-DERIVED VASCULAR ENDOTHELIAL CELLS, Florence M. Hofman, Albion D. Wright, David Hinton, Flossie Wong-Stahl* and Sharyn Walker**, Department of Pathology, University of Southern California School of Medicine, Los Angeles, CA 90033; *University of California, San Diego, CA 92093; **Department of Pediatrics, Childrens Hospital of Los Angeles, CA 90027

Neurological abnormalities in AIDS patients have been associated with the focal breakdown of the blood brain barrier (BBB), suggesting disease abnormalities in endothelial cell structure and function. This raises the question whether circulating HIV infected cells release factors which could activate endothelial cells, initiating breakdown of the BBB and thereby enhancing viral entry into the central nervous system (CNS). The tat protein, an HIV gene product which functions as a transactivator for HIV replication, has been shown to be secreted extracellularly by virally infected cells. In order to determine the potential role of tat in the dissemination of HIV into the CNS, we examined the effect of tat protein on human CNS derived endothelial cells in vitro. Results show that the tat protein induces E-selectin expression within 4 hours of treatment, as determined by immunochemistry and flow cytometry. This HIV derived protein also induces IL-6 production, using the B9.9 bioassay; IL-6 has been shown to increase endothelial cell permeability. Furthermore, low concentrations of tumor necrosis factor (TNF), a cytokine reported to be produced by HIV infected cells, and tat protein act synergistically in the production of IL-6. These data suggest that the extracellular tat protein secreted or released by HIV infected cells, alone or in combination with TNF many contribute to leukocyte binding to CNS microvessels, transmigration into the CNS, and the eventual change in permeability of the blood brain barrier leading to neurological disease.

E 310 DETECTION OF IL-1 IN HUMAN ATHEROSCLEROSIS
Cathy M Holt, Johanna Armstrong, Pat Gadsdon, Sheila E Francis, Geoffrey*H Smith. Sections of Cardiac Surgery and Molecular Medicine*, University of Sheffield, Sheffield, UK

Interleukin-1 (IL-1) is a candidate cytokine that may be involved in the pathogenesis of atherosclerosis. It is a known mitogen for VSMC, is involved in leucocyte adhesion, the formation of new blood vessels and is capable of inducing other growth regulatory factors such as PDGF. We have studied the release of IL-1ß from segments of human coronary artery maintained in serum free organ culture and performed immunolocalisation of IL-1ß on plastic sections of artery. The cellular source of IL-1ß was subsequently defined by staining for macrophages and endothelial cells, and the detection of proliferating cell nuclear antigen (PCNA) was determined by PC-10 immunostaining.
Segments of atherosclerotic human coronary artery were obtained from the explanted hearts of transplant recipients immediately following removal from the patient (n=8). Segments were opened out and maintained in serum free organ culture for 24 hours. Conditioned medium was harvested and IL-1ß detected by ELISA (Cistron). Separate segments of artery were fixed in acetone or buffered formalin and subsequently processed for plastic or paraffin sections. Plastic sections were stained for IL-1ß using a monoclonal antibody (Genzyme). Macrophages, endothelial cells and PCNA positive cells were detected using antibodies to CD-68, von Willebrand factor antigen and PCNA.
IL-1ß was detected by ELISA in conditioned medium obtained from cultured segments of coronary artery. Furthermore, immunostaining revealed that IL-1ß was present within capillaries and small vessels of the adventitia and also within areas of the intima. This staining was shown to be present within the endothelial cells and macrophages, some of which were positive for PCNA.
In conclusion, we have demonstrated the release and immunolocalisation of IL-1ß in human coronary artery. These observations provide support for the involvement of this cytokine in vascular cell proliferation and other immune mediated mechanisms ocurring in the pathogenesis of atherosclerosis.

E 311 ENDOTHELIAL INHIBITION OF LPS-STIMULATED TNF RELEASE IN WHOLE BLOOD: ENHANCEMENT BY ATHEROSCLEROSIS IN A PIG MODEL, Donald S. Houston, Craig W. Carson, Gary L. Ferrell, Charles T. Esmon, Oklahoma Medical Research Foundation and Howard Hughes Medical Institute, Oklahoma City, OK 73104
Earlier studies have indicated that cultured human umbilical vein endothelial cells can inhibit the endotoxin-stimulated release of tumor necrosis factor-α (TNF; assayed by a double monoclonal-antibody ELISA) from whole blood. To be sure this effect was not merely an artifact of cultured endothelial cells, segments of pig aorta were placed in a template device so that the intimal surface formed the bottom of a series of wells. Heparinized human blood was placed in the wells, stimulated with LPS, and incubated 4 hours. TNF release was inhibited in wells containing aortic segments with intact endothelium compared with wells containing segments from which the endothelium had been mechanically denuded. The inhibitory effect was not prevented by treatment of the wells with aspirin to block the formation of inhibitory prostaglandins. We hypothesized that this anti-inflammatory endothelial function might be altered early in atherosclerosis. Weanling pigs were raised for six months on either a control diet or a high-cholesterol diet (supplemented with 15% beef tallow and 1.5% cholesterol). At the time of sacrifice, mean ($\pm$ s.e.m.) serum cholesterol levels were 127$\pm$23 mg/dl in control and 743$\pm$108 mg/dl in supplemented groups. Aortic segments showed typical changes of early atherosclerosis in the latter pigs. Aortas were assayed for their ability to inhibit TNF release in whole blood as above. In both groups, TNF release was significantly inhibited by segments with intact endothelium compared with endothelium-denuded segments. The endothelium-dependent inhibitory activity was significantly greater in the pigs on a high-cholesterol diet. This enhanced inhibitory effect may represent a compensatory response of the endothelium in the face of other pro-inflammatory events that accompany the atherosclerotic process.

E 312 **INHIBITION OF IFNγ-MEDIATED ENDOTHELIAL HLA DR INDUCTION BY IFNβ PRODUCED BY CMV-INFECTED ENDOTHELIAL CELLS,** DA Knight, S Chaiwiriyakul, WJ Waldman, DD Sedmak, The Ohio State University College of Medicine, Columbus, Ohio 43210
Cytomegalovirus (CMV) has been associated with atherogenesis and transplantation-associated arteriosclerosis, both of which represent chronic pathogenic processes involving the endothelium, a common site of infection by this virus *in vivo*. It is tempting to postulate that low-grade immunopathologic responses chronically sustained by CMV persisting in the endothelium may represent a contributing etiologic factor in the development of these lesions. In our ongoing efforts to elucidate mechanisms of viral immune evasion, we have demonstrated that CMV-infected EC (EC/CMV) do not express HLA class II antigens, and furthermore, are refractory to their induction by IFNγ. We now show that EC/CMV produce quantities of IFNβ sufficient to inhibit the HLA DR-inducing effects of IFNγ on uninfected EC. Uninfected human umbilical vein endothelial cells (HUVEC) were treated with IFNγ alone or in combination with supernatants generated by uninfected EC or EC/CMV, then flourocytometrically analyzed for the expression of HLA DR. Results indicated that soluble factor(s) present only in the infected EC supernatant reduced IFNγ-induced endothelial DR expression by 60-70% (mean fluorescence intensity). Additional experiments demonstrated that the inclusion of monoclonal antibody specifically reactive with IFNβ effectively neutralized the IFNγ-inhibiting activity, completely restoring HLA DR expression in these cultures. As further confirmation of the identity of the IFNγ-inhibiting factor, IFNβ was detected at a concentration of 240 IU/ml in EC/CMV-generated supernatants by ELISA, while this cytokine was undetectable in uninfected cell supernatants. These findings suggest an additional mechanism by which CMV might evade immune surveillance, thereby fostering the establishment of a state of persistence, and possibly promoting the initiation and maintenance of local immune-mediated damage which may contribute to the pathogenesis of athersclerosis.

E 313 BETAGLYCAN IS A DUAL REGULATOR OF TGF-β ACCESS TO THE SIGNALING RECEPTORS, Fernando López-Casillas and Joan Massagué, Cell Biology and Genetics Program and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Betaglycan, also known as the TGF-β type III receptor, is a membrane-anchored proteoglycan that presents TGF-β to the type II signaling receptor, a transmembrane serine/threonine kinase. The betaglycan extracellular region, which can be shed by cells into the medium, contains an N-terminal domain related to endoglin and a C-terminal domain related to uromodulin, sperm receptors Zp2 and 3, and pancreatic secretory granule GP-2 protein. Using a panel of deletion mutants, we found that the TGF-β binding function of betaglycan resides in the N-terminal endoglin-related region and that the ability of betaglycan to present TGF-β to receptor II does not require the uromodulin-related region or the cytoplasmic region, but requires membrane anchorage. Furthermore, soluble recombinant betaglycan acts as potent inhibitor of TGF-β binding to membrane receptors and of TGF-β effects. The results suggest that release of betaglycan into the medium converts this enhancer of TGF-β action into a TGF-β antagonist.

E 314 GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HUMAN RANTES. Ji Lu; Bing Li; Thomas J. Schall and K. Jin Kim. Genentech Inc., South San Francisco, CA 94080.

RANTES is a member of the chemokine family. RANTES may play an important role in inflammatory diseases by attracting T cells and eosinophils to the site. To have a better understanding of the biological role and structure-function relationship of RANTES, we generated and characterized monoclonal antibodies (mAb) to RANTES. Lymphoid cells of BALB/C mice immunized with recombinant human RANTES emulsified in MPL/TDM were fused with P3/X63-Ag8U1 myeloma cells. Five mAbs were selected for their high binding capacities to RANTES in ELISA. All these mAbs bind to RANTES but not to MIP-1a, IL8, MGSA, PF4 and thromboglobulin in ELISA. The predominant isotype of these mAbs is IgG2a. Three of the five mAbs were shown to block the binding of ^{125}I-RANTES to transfected 293 cell line (J1-81) expressing the RANTES receptor. Further, these blocking mAbs were able to neutralize RANTES' ability to induce intracellular Ca^{++} release from a human monocytic cell line, THP-1, by flow cytometry analysis. All these mAbs were able to capture ^{125}I-RANTES in the radioimmunoprecipitation assay. The affinities of these mAbs were determined by the Scatchard plot analysis: Kd's range from 10^{-9} to 10^{-12} M.

Currently we are performing epitope analysis and investigating antagonist activities of these mAbs on the chemotactic activities of RANTES *in vitro* as well as *in vivo*.

E 315 A UNIQUE VIRAL ANTI-INFLAMMATORY PROTEIN, SERP-1, REDUCES INTIMAL HYPERPLASIA IN CHOLESTEROL FED RABBITS AFTER ANGIOPLASTY, Alexandra R. Lucas, Liying Lui, Joanne Macen, Weidong Yan, Patric N. Nation, Grant McFadden, Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G-2B7

The recurrence of atherosclerotic arterial occlusion after angioplasty, restenosis, is a significant problem, occurring in 30-50% of patients. The inflammatory response to endothelial injury produces intimal hyperplasia and connective tissue deposition. SERP-1 is a poxvirus encoded serine protease inhibitor with known anti-inflammatory properties. SERP-1 was over expressed from vaccinia vector; a parallel preparation of vaccinia vector lacking SERP-1 and phosphate buffered saline served as controls. We examined the effect of SERP-1 infusion in 16 cholesterol-fed New Zealand rabbits (R). Each R had balloon angioplasty induced endothelial denudation of the abdominal aorta via femoral cut down under general anesthetic. The site of balloon induced injury was infused with either SERP-1 or one of the controls via Wolinsky infusion catheter (USCI) immediately after angioplasty. 9R had SERP-1infusion (SR), 5R had vaccinia vector control infusion (CVR), and 2R had saline control infusion (CR). Contrast aortography was recorded before and after angioplasty and at 4 wks follow up just prior to sacrifice. The aorta was then removed for histologic examination. Angiography at 4 wks demonstrated a relative increase in the abdominal aorta diameter with SERP-1 infusion (SR 5.1±0.7mm, CR 3.1±0.46mm, $p<0.01$) measured by electronic calibrator. There was a significant decrease in the intimal fatty proliferative area and plaque thickness with SERP-1 infusion on morphometric analysis (Plaque area SR 0.037±0.016mm^2, CVR 0.516±0.163mm^2, CR 1.049±0.429, $p<0.0017$; Plaque thickness SR 0.094±0.033mm, CVR 0.435±0.224mm, CR 0.835±0.345mm, $p<0.0017$). SERP-1 infusions decreased intimal hyperplasia only at the site of infusion. There was no significant difference between measured plaque area or intimal thickness after control infusions of either vector or saline infusion ($p<0.1934$). Areas of intimal hyperplasia were selectively stained for smooth muscle cells using immunohistochemistry. Preliminary data indicates decreased plaque complexity after SERP-1 infusion; fewer smooth muscle cells, staining positive for actin, were detected in fibrous cap structures after SERP-1 infusion on comparison with vector or saline infusion. This is the first example of the use of a virus encoded anti-immune protein in a clinical application. Conclusion: Anti-inflammatory treatment with SERP-1 protein dramatically decreased intimal fatty cellular proliferation.

E 316 **BIFUNCTIONAL EFFECTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (BP-1) ON MITOGENIC RESPONSES OF RAT VASCULAR SMOOTH MUSCLE CELLS (RASMC).**

AS MOTANI, GN COX*, MJ McDERMOTT*, EE ÄNGGÅRD & GAA FERNS.

The William Harvey Research Institute, London, UK.

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We have demonstrated an inhibitory effect of BP-1 on IGF-1 -mediated growth of RASMC. Cells were challenged with IGF-1/BP-1 and ^{3}H-thymidine uptake was measured over 24hr. Data were expressed as fold stimulation relative to basal (mean±sem). Maximal response to IGF-1 observed in these cells was 2.48±0.3 fold (EC_{50}=0.18±0.04nM; n=5). Co-incubation of BP-1 with IGF-1 (2.6nM) resulted in inhibition of the IGF-1 -mediated response (IC_{50}=2.84±1.4nM, n=5). In a separate, immortalised cell line of rat embryonic SMC (A7r5 clone), no response to IGF-1 was observed. In these cells, BP-1 inhibited basal ^{3}H-thymidine uptake (E_{max}=50±3%; IC_{50}=11.3±4.67nM; n=3), measured over 36 hr. These results suggested a possible autocrine role for IGF-1 in this cell line, although Blat et al *(1992, Growth Factors 6:65-67)* have reported that IGFBP-28(BP-1) is able to inhibit stimulation induced by serum proteins different from IGF-1 or -2 in transformed mouse fibroblasts. However, in support of our hypothesis, we were able to demonstrate an interaction between platelet poor plasma (PPP) and BP-1 in these cells, as previously reported by Clemmons et al *(1990, J Cell Physiol 145:129-135)*. BP-1 (3.9pM) potentiated ^{3}H-thymidine uptake in the presence of 1% PPP (E_{max}=11±3%, n=4). Further, BP-1 also potentiated the mitogenic response to platelet releaseate (0.1% of 10^8platelets/mL preparation), which was maximal at 0.39nM of BP-1 (48±11%, n=8 determinations). In conclusion, our observations of the inhibitory effect of BP-1 in A7r5 cells and the potentiating effect of a platelet- and plasma-derived factor on BP-1 -mediated responses suggests that A7r5 cells may respond to IGF-1 in an autocrine manner.

BP-1 was a gift from Synergen. We are grateful to ONO Pharmaceutical Co Ltd, Japan & the British Heart Foundation for financial support of this project.

E 317 DIFFERENTIAL REGULATION OF E-SELECTIN AND ICAM-1 EXPRESSION BY TNF ON HUVEC, Marian T. Nakada, Donna Souders, Susan H. Tam and John Ghrayeb, Dept. of Molecular Biology, Centocor, Inc., Malvern, PA 19355 USA

Endotoxin and cytokines have been shown *in vitro* to increase the expression of E-selectin and ICAM-1 on human umbilical vein endothelial cells (HUVEC). E-selectin expression upon TNF challenge peaks at approximately 4 hours and returns to near baseline levels by 24 hours. ICAM-1 expression on HUVEC also peaks at 4-8 hours after TNF addition, but unlike E-selectin, its expression remains high beyond 24 hours. A neutralizing anti-TNF monoclonal antibody, Centocor CA2 (chimeric anti-human TNF IgG1), was used to study the mechanism by which TNF regulates both E-selectin and ICAM-1 expression. Addition of anti-TNF (10 μg/ml) to HUVEC which had been stimulated with TNF (50 U/ml) for 4 hours enhanced the rate of disappearance of E-selectin, whereas neutralization of TNF after the same 4 hour stimulation did not decrease ICAM-1 expression at later time points. Levels of mRNA for E-selectin and ICAM-1, measured by Northern blot analysis appear to parallel levels of protein expression, suggesting that E-selectin and ICAM-1 protein levels are controlled at the mRNA level. These data provide support for the notion that E-selectin is expressed transiently in acute inflammation and that its regulation requires the continued presence of activator. Neutralizing antibodies to activating cytokines may be effective therapeutic tools to block E-selectin mediated inflammation. In contrast, ICAM-1 expression once it is induced is more prolonged and continues to remain high in the absence of activator. ICAM-1 mediated processes may be better inhibited by antibodies directed against ICAM-1 itself or to its co-receptor LFA-1.

E 318 CAP37, AN INFLAMMATORY MEDIATOR THAT PROMOTES BOTH MONOCYTE CHEMOTAXIS AND LEUKOCYTE ADHESION TO ENDOTHELIAL CELLS

H. Anne Pereira, Pete Moore, Kimberley Sachen and Paula Grammas, Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Activation of endothelial cells and the subsequent adherence of mononuclear cells are critical early events in atherogenesis. The identification and characterization of factors that regulate these processes are important in developing appropriate therapies. CAP37 (Cationic Antimicrobial Protein of mol. wt 37 kD), is a multifunctional protein isolated from the granules of human neutrophils and platelets with important implications in host defense and inflammation. We have recently demonstrated that CAP37 is a potent and specific mediator of monocyte chemotaxis. The objective of this study was to investigate the ability of CAP37 and a number of synthetic peptides based on the native CAP37 sequence to activate endothelial cells and to promote adherence of leukocytes to endothelial cells. Our studies indicated that native CAP37 activated endothelial cell protein kinase C (PKC), a second messenger enzyme, in a time dependent and dose dependent fashion. CAP37 was also capable of causing mono mac 6 cells (a human monocytic cell line), to adhere to cultured rat cerebral endothelial cells. The CAP37 peptides were similarly tested for chemotactic activity, ability to activate PKC, and to promote leukocyte adherence. Our findings indicated that peptides that mediated monocyte chemotaxis also activated endothelial cells. The results suggest that CAP37, is an important mediator responsible for recruiting monocytes. Furthermore, CAP37 could be critical in the formation of early atheromatous plaques due to its ability to both promote monocyte chemotaxis as well as monocyte adherence to endothelial cells. (Supported in part by USPHS grants AI28018 and NS30457, and American Cancer Society IM 699).

E 319 EFFECTS OF IL-13 ON ENDOTHELIAL AND MESOTHELIAL CELLS: SELECTIVE INDUCTION OF VCAM-1 AND STIMULATION OF IL-6 PRODUCTION, Giuseppe Peri, Sergio Bernasconi, Francesca L. Sciacca, Cristian Matteucci, Mario Conni, Marina Sironi and Alberto Mantovani, Istituto Mario Negri Milan, Italy

The present study was designed to explore the interaction of IL-13 with vascular endothelial cells (EC). In vitro exposure to IL-13 of human unbilical vein EC induced surface expression of vascular adhesion molecule-1 (VCAM-1). At optimal concentrations (10-15 ng/ml) and exposure times (24h), IL-13 was a 2 to 3 fold less effective inducer of VCAM-1 than IL-1, used as reference EC activator. When IL-13 was combined with IL-1, almost additive induction of VCAM-1 was observed. Induction of VCAM-1 was selective in that E-selectin and intercellular adhesion molecule -1 (ICAM-1) were unaffected. IL-13 caused a modest reduction of IL-1 induction of E selectin and ICAM-1. Surface expression of VCAM-1 on IL-13 treated cells was associated with mRNA induction (as assessed by northern analysis and RT-PCR), with predominance of transcripts encoding the 7 Ig domain form of this molecule. In agreement with previous reports, IL-13 inhibited cytokine prodiction un human monocytes. In contrast, IL-13 was a week inducer and an amplifier (in concert with IL-1) of IL-6 expression in EC. Mesothelial cells, which share properties with EC and regulate the traffic and function of leukocytes in serosal cavities, were stimulated to express VCAM-1 and IL-6 by IL-13. Thus, IL-13 elicits a spectrum of responses in vascular endothelium remarkably similar to that of IL-4 and IL-10. Interaction of these cytikines with vascular endothelial may play an important role in the induction and expression of Th2-dependent responses.

E 320 STRETCH-INDUCED SELECTIVE SECRETION AND ACTIVATION OF TRANSFORMING GROWTH FACTOR BETA (TGF-ß) ISOFORMS BY MESANGIAL CELLS (MC); POSSIBLE ROLE IN GLOMERULAR SCLEROSIS. Bruce L. Riser, Janet Grondin, Stephanie Ladson-Wofford, Pedro Cortes, and Robert G. Narins, Dept. of Medicine, Henry Ford Hospital, Detroit, Michigan.

While glomerular hypertension is causally related to increased mesangial matrix deposition and the development of glomerular sclerosis, the mechanisms were unknown. However, we recently showed that MC stretch accompanying the increased pressure in isolated glomeruli stimulates matrix accumulation. In this study, we have examined the isoforms of TGF-ß produced by MC, specifically in response to stretch, to determine whether this known modulator of MC matrix synthesis might mediate this process. Rat MCs were cultured on elastin-coated flexible bottom plates then subjected to cyclic stretching (average elongation of 7%) for 3 days. The medium was then examined for TGF-ß by a cell growth inhibition assay using an antibody specific for TGF-ß1 and another specific for TGF-ß 2. Total TGF-ß (latent plus active) was determined following activation of the latent component by acid treatment. MC cultures (24h collection) contained active TGF-ß 1 that increased 3.3-fold following stretch (control 592 ± 267 inhibitory units (IU)/10^6 cells; stretched 1,970 ± 510 IU/10^6 cells, P<0.01). Total TGF-ß 1 increased 3.6-fold in response to stretch (control, 3,608 ±1,808 IU/10^6 cells; stretched, 12,876 ± 3,711 IU/10^6 cells, P<0.02). MCs also secreted TGF-ß 2. However, the active component did not change with stretch (control, 1,589 ± 1,519 IU/10^6cells; stretch, 1,512 ± 1,007 IU/10^6cells) nor did the total (control, ± 3,584 ± 5,264 IU/10^6 cells; stretch, 3,138 ± 5,578 IU/10^6 cells).

In conclusion, these results demonstrate that MCs produce at least two isoforms of TGF-ß. Under these conditions, stretching selectively increases the secretion of TGF-ß 1 without changing the level of TGF-ß 2. These data also indicate that while TGF-ß 1 and TGF-ß 2 are secreted at similar levels, a higher proportion of TGF-ß 2 exists in the active state. Mechanical strain-induced increase in this cytokine may mediate the increased mesangial matrix accumulation of progressive glomerulosclerosis.

E 321 INFLAMMATORY CYTOKINES INDUCE AIDS-KAPOSI'S SARCOMA-DERIVED CELLS TO RELEASE BASIC FIBROBLAST GROWTH FACTOR, Felipe Samaniego, Robert C. Gallo and Barbara Ensoli. Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20892.

AIDS-associated Kaposi's sarcoma (AIDS-KS) is a proliferative disease of vascular origin characterized by lesions of spindle-shaped cells and angiogenesis. As a group, homosexual males have frequent infections and signs of immune stimulation. We have shown that inflammatory cytokines (IL-1ß, TNFα and IFNγ) present in conditioned media of activated immune cells (ACM) stimulate the growth of AIDS-KS cells that can be blocked by anti-basic fibroblast growth factor (bFGF) antisera. Here we show that ACM induces AIDS-KS cells to release bFGF. Individual cytokines (IL-1ß, TNFα and IFNγ) reproduce the release of bFGF and, in combination, these cytokines stimulate the synthesis of bFGF in AIDS-KS cells. Extracellular bFGF is found as a soluble and as a bound protein. Gentle trypsinization of AIDS-KS cells releases the bound bFGF which is effective in stimulating the growth of H-UVE cells, demonstrating that trypsin-released bFGF remains bioactive. These findings suggest that inflammatory cytokines induce AIDS-KS to switch intracellular stores of bFGF to extracellular bFGF, the location which renders bFGF effective for growth stimulation of AIDS-KS cells as well as endothelial cells.

E 322 COTREATMENT WITH TGF-β AND ANGIOTENSIN-II OR α-THROMBIN INDUCES A SYNERGISTIC GROWTH RESPONSE IN CULTURED SMOOTH MUSCLE CELLS.

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Transforming growth factor-β (TGF-β), angiotensin-II (Ang-II) and α-thrombin (Thr) have been implicated in mediating SMC growth following vascular injury. These factors have been shown to promote growth of cultured SMC; however, interactions between these factors have not been examined. Previously, we have shown that cotreatment with TGF-β enhances growth responses to epidermal growth factor, basic fibroblast growth factor and platelet-derived growth factor-BB. These growth factors activate receptors with primarily tyrosine kinase activity, and it is not known whether TFG-β will enhance growth responses to factors (e.g. Ang-II, Thr) which activate G-protein coupled receptors. The purpose of these studies was to examine the effect of cotreatment with TGF-β and Ang-II or Thr on SMC growth. SMC isolated from SHR were grown to confluence, growth arrested in serum-free medium and treated. Results demonstrated that TGF-β, Ang-II or Thr stimulated an 11-, 3- or 77-fold increase, respectively, in ^{3}H-thymidine incorporation measured 48-72 hours after treatment. Cotreatment with TFG-β and Ang-II or Thr elicited a 24- or 146-fold increase in ^{3}H-thymidine incorporation, respectively. Cell counts performed 7 days after treatment demonstrated that treatment with TFG-β or Thr stimulated a 30% or 240% increase in cell proliferation. In contrast, cotreatment with TGF-β and Thr resulted in a 410% increase in SMC proliferation. In a separate experiment, treatment with TGF-β or Ang-II had no effect on cell proliferation, whereas cotreatment with TGF-β and Ang-II resulted in a 50% increase in cell number over 7 days. Taken together, these results demonstrate that TGF-β has synergistic effects on SMC growth in combination with Ang-II or Thr, factors which activate G-protein coupled receptors.

E 323 MICROVASCULAR EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR (PDGF) B-CHAIN AND PDGF ß-RECEPTORS, Sundberg, C. Ljungström M. Lindmark, G. Gerdin, B. Rubin, K. Departments of Physiological Chemistry, and Surgery. University of Uppsala, Biomedical Center, Box 575, S-751 23 Uppsala, SWEDEN

The expression of PDGF β-receptors, and PDGF B-chain in human healing wounds and colorectal adenocarcinoma was investigated. Frozen sections were subjected to double immunofluorescence staining using monoclonal antibodies specific for pericytes, endothelial cells, laminin, as well as PDGF β-receptors and PDGF B-chain. Stained sections were analyzed by computer aided imaging processing which allowed for a numerical quantification of the degree of co-localization of the investigated antigens. In six tumor specimens, PDGF β-receptors co-localized with a marker for pericytes, but not with a marker for endothelial cells in microvessles. Qualitatively the same pattern was obtained in the two investigated healing wounds. PDGF B-chain did not co-localize with markers for endothelial cells or pericytes, but was, however, frequently found juxtaposed to the microvasculature. A large proportion of the PDGF B-chain expressing cells could be identified as macropahges, present in healing wounds and colorectal adenocarcinoma. In the latter, cells tentatively identified as tumor cells also expressed this growth factor. Our results suggest that PDGF B-chain can be involved in the regulation of microvascular pericytes and a possible paracrine stimulation of pericytes by macrophages and/or tumor cells.

E 324 MONOCLONAL ANTIBODIES (mAbs) TO THE NK1 REGION OF HUMAN HEPATOCYTE GROWTH FACTOR (HGF) BLOCK HGF ACTIVITY. Kelly Tabor, Nathalie A. Lokker, Paul J. Godowski and K. Jin Kim. Genentech, Inc., South San Francisco, CA 94080.

HGF, a potent mitogen for parenchymal liver cells, is a heterodimer composed of an α chain containing four kringle domains as well as a β chain that resembles a serine protease. We have shown that the N-terminal end first kringle domain (NK1) of the α chain has been shown to be the primary receptor binding site of HGF. To investigate the possibility that mAbs to NK1 would inhibit HGF activities, BALB/c mice were immunized with recombinant NK1 from *E. coli*. cells. Immune lymphoid cells were fused with P3/X63-Ag8U1 myeloma cells. We characterized 13 hybridomas which secreted mAbs with high binding capacity to recombinant human HGF (rhuHGF) in ELISA. Four mAbs were able to block 53-77% of the ^{125}I-rhuHGF binding to human lung carcinoma A549 cells, at a 1: 400 molar ratio of rhuHGF to mAb. Further, these mAbs showed a significant inhibition of the proliferative response of rat hepatocytes induced by rhuHGF. These blocking mAbs were able to immunoprecipitate 95-100% of ^{125}I-rhuHGF at a 1: 40 molar ratio. The predominant immunoglobulin isotypes of our anti-NK1 mAbs are IgG2a or IgG2b and two mAbs were able to recognize denatured HGF in the western blot analysis.

Future studies with these mAbs include affinity determination and the epitope(s) analysis with NK1 variant proteins.

E 325 CHARACTERIZATION OF MONOCYTE CHEMO-ATTRACTANT PROTEIN-1-PRODUCING CELLS IN ATHEROSCLEROTIC LESIONS BY DOUBLE IMMUNO-HISTOCHEMICAL STAINING Motohiro Takeya, Teizo Yoshimura, Edward J Leonard, and Kiyoshi Takahashi, Second Department of Pathology, Kumamoto University School of Medicine, Kumamoto 860, Japan, and Immunopathology Section, Laboratory of Immunobiology, NCI-FCRDC, Frederick, MD 21702, USA.

Monocyte chemoattractant protein-1 (MCP-1) is considered to play an important role in the early event of monocyte infiltration into arterial wall in atherogenesis. Recent literatures demonstrated MCP-1 production in atherosclerotic lesions using in situ hybridization and immunohistochemistry. However, precise characterization of MCP-1-producing cells was not completed yet. In the present study we performed double immunohistochemical staining with an anti-MCP-1 monoclonal antibody and one of the antibodies against macrophages, smooth muscle cells, or endothelial cells to identify the nature of MCP-1 positive cells. In both of diffuse intimal thickening and fatty streak lesions endothelial staining of MCP-1 was observed in about two-third of the cases. Subendothelial macrophages were strongly stained for MCP-1 in all fatty streak lesions. In atheromatous lesions subendothelial macrophages were still positive for MCP-1, whereas endothelial cell were mostly negative. A few smooth muscle cells in the intima were positive for MCP-1 at this stage. From these results it is considered that the cell populations positive for MCP-1 are different in early and advanced atherosclerotic lesions. Endothelial cells and subendothelial macrophages are considered to be the major source of MCP-1 in early atherosclerotic lesions.

E 326 EXPRESSION OF HUMAN INTERLEUKIN 1 RECEPTOR ANTAGONIST (IL-1RA) IN TRANSGENIC MICE, Myrna E. Trumbauer, Howard Y. Chen, Christina C. Grabiec, Kathryn J. Hofmann*, Hui Zheng, Lex H.T. Van der Ploeg and Alan R. Shaw*, Department of Genetics and Molecular Biology, Merck Research Laboratories, P.O. Box 2000, Rahway NJ 07065 and Department of Virus and Cell Biology*, Merck Research Laboratories, West Point, Pa 19486

To study the physiological effects of overexpressing human IL-1 ra in the brain, transgenic mice were produced using a gene construct encoding human IL-1ra linked to the human Thy-1 promoter, which is specifically expressed in the mouse brain. Six transgenic founders were generated which appeared normal. Elevated levels of h IL-1ra were detected in the brain and plasma of the transgenic mice using immunoassay. Occasionally, a runted phenotype could be observed in some of the transgenic progeny. This finding awaits future studies including a detailed histological study of the mouse brain.

E 327 A MENINGITIS-SPECIFIC ANTIGEN, DEFINED BY A RUBELLA MONOCLONAL ANTIBODY, CONTAINS hMCP-1& 3 SEQUENCES

D. Van Alstyne and M.R. Wilson, Global Tek, Inc., Blaine, WA. 98231-0880

Rubella Virus (RV) is known to cause a mild meningomyelitis. Whole virus was employed to construct monoclonal antibodies (Mab's) to RV. One of these, designated RV1, was characterized using Western Blot analyses of whole virus. RV1 was found to bind to the 45 Kd glycosylated, membrane-associated E2 protein and to the 30 Kd core protein. The Macintosh DNA and Protein ("Macintosh DNASIS Pro") Sequence Analysis System was employed to define Homologous Antigenic Sequences (HAS's) in both the envelope and core proteins. Each homologous sequence is composed of seven amino acids.

The RV1 Mab was found to cross-react with proteins in several other meningitis-causing agents including: **Hemophilus Influenzae, Neisseria meningitidis, Streptococcus pneumoniae,** and **Listeria monocytogenes** (which together account for more than 85% of all bacterial meningitis in the United States), and HIV1. A family of HAS's was found to be common to all of the above-mentioned infectious agents.

All etiological agents of meningitis studied here express HAS's on their surfaces, and infect monocytes/macrophages, prior to entering the central nervous system (CNS). These highly conserved, meningitis-specific HAS's have also been identified in 2 variants of the human Monocyte Chemoattractant Factor (hMCP-1 and hMCP-3). This factor is thought to recruit lipid-scavenging monocytes implicated in "foamy cell"and plaque formation near fatty streaks in arteries. These same lipid-scavenging monocytes may also routinely enter the lipid-rich CNS to perform maintenance functions related to normal turn-over and repair. Therefore, the meningitis-related function of HAS's may be to recruit these specific monocytes for subsequent infection, thereby ensuring the entry of the infectious agents into the CNS.

E 328 GUINEA PIG, AS AN ANIMAL MODEL TO STUDY THE ROLE OF NEUTROPHIL ATTRACTANT PROTEIN-1 (NAP-1/IL-8) AND MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1), Teizo Yoshimura, Immunopathology Section, Laboratory of Immunobiology, NCI-FCRDC, Frederick, MD 21702

NAP-1 and MCP-1 are cytokines that belong to a family of proteins characterized by their small molecular weights and location of four half-cystines. NAP-1 is chemotactic for neutrophils but not for monocytes. In comparison, MCP-1 is a potent chemoattractant for monocytes but not for neutrophils. Both NAP-1 and MCP-1 have been detected in inflammatory lesions including atherosclerosis. To investigate the role of NAP-1 and MCP-1 in vivo, animal models are necessary. MCP-1 has been purified or cloned from baboon, bovine, sheep, rabbit, guinea pig, rat, and mouse. NAP-1 has been cloned from pig, sheep, rabbit, but not from rat or mouse. In the present study NAP-1 cDNA was cloned from guinea pig spleen cells stimulated with Con A. The cDNA comprised 1433 base pairs with an open reading frame which encoded for a 101 amino acid protein. Guinea pig NAP-1 (gpNAP-1) had 70% amino acid sequence similarity to human NAP-1 (huNAP-1). Nucleotide sequence similarity within the coding region was 75%. To confirm its biological activity in guinea pig, recombinant gpNAP-1 was expressed in COS-7 cell, then purified. N-terminal sequence analysis gave two different N-termini at position 23 (Met) or 24 (Val). The two proteins showed their peak activity for guinea pig neutrophils at the concentration of 1 μg/ml (10^{-7} M). Despite its high similarity to huNAP-1, the responsiveness of human neutrophils to gpNAP-1 was minimum. Recombinant gpNAP-1 caused strong neutrophil infiltration after intradermal injection into guinea pig skin. Since guinea pig is classified as a rodent, it was of interest to know whether huNAP-1 cDNA hybridizes to genomic DNA of other rodents such as mouse or rat. Under low stringency conditions, huNAP-1 cDNA hybridized to human, rabbit and guinea pig DNA, but not to mouse or rat DNA. The apparent absence of a NAP-1 gene in mouse or rat makes this chemoattractant unique among the members of the protein family. Since both NAP-1 and MCP-1 have been found in guinea pig, guinea pig may be a good model to study the role of NAP-1 and MCP-1 in vivo.

Adhesion Pathobiology: Anti-Inflammatory Strategies; Pathophysiologic Aspects of Proteases and Their Inhibitors

E 400 C-JUN INHIBITS TNFα MEDIATED TRANSACTIVATION OF VASCULAR CELL ADHESION MOLECULE-1 (VCAM-1) PROMOTER, Mushtaq Ahmad, Nobuyuki Marui, R. Wayne Alexander and Russell M. Medford, Division of Cardiology, Department of Medicine, Emory University, Atlanta, GA 30322

Diverse inflammatory signals, such as the cytokine TNFα, activate endothelial cell VCAM-1 gene expression through an oxidation-reduction (redox) sensitive mechanism that likely involves the redox sensitive activation of an NF-κB like inducible transcriptional regulatory protein. Activation of the important inducible transcriptional factor AP-1 (fos/jun heterodimer) is also redox sensitive but in a manner strikingly distinct from that of NF-κB. As the VCAM-1 promoter contains both AP-1 and NF-κB DNA binding sites, this raises the possibility that these two factors may interact to modulate VCAM-1 gene expression. To test this hypothesis, the VCAM-1 promoter containing both κB and AP-1 elements attached to CAT reporter gene was transiently transfected into bovine aortic endothelial (BAE) cells and treated with TNFα, an activator of NF-κB. As expected, TNFα significantly induced the basal activity of the promoter. However, co-transfection of a c-jun expression vector alone blocked the TNFα mediated transactivation of the promoter. Interestingly, TNFα transactivation of the VCAM-1 promoter was unaffected if both c-jun and c-fos expression vectors were co-transfected. This suggests that c-jun homodimer or its heterodimer with a bZIP motif containing factor other than c-fos may serve to inhibit cytokine activation of the VCAM-1 promoter. Thus, cytokine activation of VCAM-1 gene expression may be regulated through the interaction of distinct redox sensitive transcriptional regulatory factors.

E 401 STRUCTURE/FUNCTION STUDIES OF P-SELECTIN GLYCOPROTEIN LIGAND, Karen M. Barone, Deborah Pittman and Gray Shaw, Genetics Institute Inc. 87 Cambridge Park Drive, Cambridge, MA 02140

Recently, by an expression cloning approach, we have isolated a cDNA from HL-60 cells encoding a functional ligand for P-selectin, PSGL-1 (P-Selectin Glycoprotein Ligand). The encoded amino acid sequence reveals a novel, mucin-like protein with putative extracellular, transmembrane and cytoplasmic domains. The extracellular domain contains three potential N-linked glycosylation sites, a region of 15 decameric repeats having potential O-linked glycosylation sites, and 3 potential tyrosine sulfation sites. In order to ascertain which of these structural elements are required for binding to P-Selectin, we have generated a DNA construct encoding a soluble form of PSGL-1, sPSGL.T7, comprising most of the extracellular domain with 46 amino acids fused at the carboxyl-terminus. This construct was co-expressed in COS cells with an expression plasmid encoding a 1,3/1,4 fucosyltransferase enzyme, required for the generation of functional ligand. Glycosidase digestions of the resulting COS cell produced sPSGL.T7 were performed to determine the type of carbohydrates present and their role in P-selectin interaction. PAGE analysis after neuraminidase, N-glycanase and O-glycanase confirmed that the glycoprotein contains sialic acid, as well as N-linked and O-linked carbohydrates. After both O-glycanase and neuraminidase treatment, sPSGL.T7 failed to bind P-selectin. These results and the results of specific mutants will be presented.

E 402 A MODEL FOR E-SELECTIN LIGAND BINDING: ANALYSIS OF CRITICAL AMINO ACID RESIDUES BY SITE-DIRECTED MUTAGENESIS, Pamela J. Beck, B. Mitch Reveille, Stephen Tapp, Dee Scott, and Timothy P. Kogan, Departments of Molecular Biology and Chemistry, Texas Biotechnology Corporation, Houston, Texas 77030

A molecular model of the binding of sialyl Lewis^x [sLe^x: Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc] and E-selectin has been developed based on the X-ray crystallographic structure of an oligomannose oligosaccharide bound to the rat mannose binding protein. In this model, the sLe^x fucose residue is coordinated to a calcium ion, while the sialyl carboxylate is positioned between the sidechains of arginine-97 and lysine-99. To further investigate the role of amino acid residues that form the E-selectin carbohydrate binding pocket, a series of mutants were made, expressed, and their ligand binding capacity assessed. These results are presented and discussed in terms of this newly proposed 3-dimensional structure for the E-selectin lectin binding domain.

E 403 TELOMERE LENGTH AS A BIOMARKER FOR CELL TURNOVER IN NORMAL AND ATHEROSCLEROTIC TISSUE FROM HUMANS, Edwin Chang and Calvin B. Harley, Department of Cell Biology, Geron Corporation, Menlo Park, California, 94025

Repeated denudation of the endothelium and consequent senescence of intimal cells may contribute to atherognesis. Our previous investigations have shown that telomeres, the ends of eukaryotic chromosomes, shorten with *in vitro* and *in vivo* age of human skin fibroblasts (Harley et al., Nature **325:** 458(1990), Allsopp et al., PNAS **89:**10114(1992)). We subsequently investigated the role of telomere length as a biomarker of cellular turnover in tissues implicated in atherogenesis. Telomere lengths were assessed by Southern analysis of terminal restriction fragments (TRF) in *Hinf*1/*Rsa*1-digested genomic DNA. Mean TRF length decreased with *in vitro* age in endothelial cell cultures from human umbilical veins (slope (m)=-190 base pairs (bp)/Population Doubling (PD), P=0.01), iliac arteries (m=-120bp/PD, P=0.05) and iliac veins (m=-180bp/PD, P=0.05). When mean TRF length was assessed for endothelial cell cultures from donors of different ages but of the same passage, there was a statistically significant decrease for iliac arteries (m=-102 bp/yr,P=-0.01) but not for iliac vein (m=-47bp/yr,P=-0.14). This is consistent with increased cellular turnover *in vivo* of endothelial cells from iliac artery. Preliminary data also show a significant decrease in mean TRF length of intimal (P=0.03) and medial (P=0.05) tissue as a function of donor age thus demonstrating cell turnover and/or expansion *in vivo* of primary vascular tissue. Intimal tissues from the internal thoracic artery showed consistently longer telomere lengths than intimal tissue from the iliac artery. In general, mean TRF lengths of medial tissues from plaque regions were shorter than those from non-plaque regions. Thus telomere length may be a biomarker for the replicative history and capacity of intimal and medial tissues and thus can be used to examine vascular cell turnover in atherogenesis.

E 404 DIVERSE PROLIFERATIVE AND SYNTHETIC RESPONSES OF DISTINCT SMOOTH MUSCLE CELL POPULATIONS IN THE ADULT ARTERIAL MEDIA. Maria G. Frid, Julie D. Wohrley, Edward C. Dempsey and Kurt R. Stenmark, Univ. of Colorado Health Sci. Center, Denver, CO 80262

Based on differential expression of smooth muscle (SM) variants of contractile and cytoskeletal proteins we have recently shown that at least three distinct smooth muscle cell (SMC) populations, progressing along different developmental pathways, can be identified in vivo within the media of adult and developing bovine arteries. Further, we described the existence of differential responses of these unique SMC populations in the neonatal main pulmonary artery during the development of hypoxic pulmonary hypertension: two cell populations exhibited marked increases in proliferation and tropoelastin mRNA expression, while one SMC population demonstrated no response. The purpose of this study was to determine if multiple and unique SMC populations from the adult bovine arterial media could be identified and maintained in culture, and, if so, to assess whether they would exhibit differential growth and synthetic responses to mitogenic stimuli and hypoxia. In primary explant cultures we observed two morphologically distinct cell populations, one with spindle-shaped cell morphology and the other more epithelioid in shape. Utilizing cloning methods, enriched cultures of these cell populations were obtained and evaluated for expression of smooth muscle markers and growth response to serum stimulation. The spindle-shaped cells grew very slowly (doubling time 60.5 $\pm$ 5.8 hrs) and expressed α-SM-actin, calponin and SM-myosin. In contrast, cells with epithelioid morphology were much faster growing (doubling time 28.8 $\pm$ 4.3 hrs), reached higher saturation density and expressed some α-SM-actin and calponin but no SM-myosin. These characteristics were maintained in culture over at least 4-5 passages. The proliferative response (^{3}H-thymidine incorporation) to PMA, PDGF, IGF-1 and hypoxia was markedly increased in the cell population with more epithelioid morphology. Interestingly, the slow growing SMC population expressed more tropoelastin mRNA than the other population. These findings demonstrate that multiple distinct SMC populations exist within the normal adult bovine arterial media and that at least two SMC populations exhibiting distinct morphological, proliferative and synthetic characteristics, can be obtained and maintained in culture. Future in vitro studies of these SMC populations may provide insight into mechanisms controlling vascular SMC responses to injury.

E 405 MACROPHAGES IN VEIN GRAFT INTIMAL HYPERPLASIA, J.R. Hoch, V.K. Stark and D.A. Hullett, Department of Surgery, University of Wisconsin School of Medicine, Madison, WI 53792

Intimal hyperplasia (IH) is responsible for the early failure of arterialized vein grafts. Cytokines elaborated by infiltrating macrophages and vascular wall cells are postulated to modulate this process. Our studies identified macrophage infiltration into vein grafts over time and also probed, for the first time, for gene expression of cytokines associated with macrophages.

Methods: Epigastric vein to common femoral artery bypass grafts were performed in male Lewis rats (350-450g) using standard microsurgical techniques. Grafts were snap frozen in OCT at various time points (4d, and 1, 2, 4, 8, 12 w). 8μ sections of proximal regions were prepared for immunoperoxidase staining using monoclonal antibody for macrophages (ED1). Spleen and epigastric vein served as positive and negative controls, respectively. Antibody specificity was confirmed by staining with IgG isotype standard. RNA was extracted, reverse-transcribed and utilized in PCR experiments using primers for MCP1/JE and IL1α.

Results: Neointimal growth was apparent by 1w and continued to increase until 4w, stabilizing by 12w. The proximal perinanastomotic region consistently displayed the most dramatic neointimal proliferation. Immunohistochemistry showed macrophage infiltration in the adventitia and media by 4d, and expansion into the lumen by 1w. Macrophages continued to be present in the lumen and adventitia through 8w; by 12w, only a few adventitial macrophages were detected. Macrophages were always seen adjacent to actively proliferating areas of IH. Control epigastric veins contained no macrophages. PCR experiments revealed the expression of MCP1/JE at all time points, with upregulation at 4d-4w. Also, transcripts of IL1α were demonstrated up to 4w, after which there were none detected.

Conclusions: Macrophage recruitment into bypass vein grafts occurs early, coinciding with IH development. Although macrophages initially play a role in the inflammatory process, they remain after inflammation has subsided and substantial IH has developed. Our results suggest that macrophages infiltrate vein grafts at actively proliferating sites and may exert autocrine and paracrine effects on the IH process via their cytokines.

E 406 INCREASE IN P-SELECTIN IN THE ENDOTHELIUM OVERLYING HUMAN ATHEROSCLEROTIC PLAQUES: COEXPRESSION WITH ICAM-1. Ruth R Johnson-Tidey, Robin N Poston, Department of Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT, UK.

P-selectin (previously known as GMP140) is an adhesion molecule present within small vessel endothelial cells which is rapidly translocated to the cell membrane on activation, where it mediates endothelial-leukocyte interactions. Immunohistochemical analysis of human atherosclerotic plaques has shown strong expression of P-selectin in the overlying endothelium. It was not however present in normal arterial endothelium, and very little was found in inactive fibrous plaques. Colour image analysis was used to quantitate the degree of P-selectin expression in the endothelium, and demonstrated a highly significant increase in atherosclerotic compared to normal areas. TNF has been shown previously to induce a similar increase in cultured endothelial cells, and moreover to allow surface expression (1). It is therefore possible that plaque cytokines are stimulating the endothelium in atherosclerosis.

Our previous work has shown an increase in ICAM-1 in atherosclerotic endothelium (2). A correlation study between the expression of P-selectin and ICAM-1 in multiple 160 micron segments of atherosclerotic endothelium reveals a strong positive correlation. These results suggest that the selective and cooperative expression of P-selectin and ICAM-1 may be involved in the recruitment of monocytes into sites of atherosclerosis.

1) A Weller et al. J Biol Chem 1992, 267:15176
2) R Poston et al. Amer J Pathol 1992, 140:665

E 407 INTERACTION OF INTEGRINS WITH OSTEOPONTIN MEDIATES ADHESION AND CHEMOTAXIS OF VASCULAR CELLS IN VITRO Lucy Liaw, Manuela Almeida, Michael P. Skinner, Stephen M. Schwartz, and Cecilia M. Giachelli, Department of Pathology, University of Washington, Seattle, WA 98195

We previously cloned rat osteopontin as a gene distinguishing smooth muscle cells (SMC) cultured from immature rat aortas versus adult-derived SMC. In addition, osteopontin was re-expressed in the neointima of injured vessels, suggesting that osteopontin may be regulated both developmentally and during wound repair of vessels. We investigated whether the temporal expression of osteopontin reflects functions pertaining to development or remodelling of the vasculature. Functional studies with osteopontin showed that it is a chemotactic factor for SMC and promotes adhesion of both endothelial cells and SMC. Chemotaxis of SMC in a Boyden-type chamber was induced by osteopontin with half-maximal effects observed at 77 nM. Adhesion of vascular cells to osteopontin substrates was dose dependent, and half maximal when solutions containing 7nM and 30nM osteopontin were used to coat plastic wells for endothelial cells and SMC, respectively. Adhesion was cation dependent and inhibited by a GRGDSP peptide, suggesting the involvement of integrin receptors. Studies with integrin antagonists showed that multiple integrin heteodimers can interact as osteopontin receptors, including $\alpha_V\beta_3$, $\alpha_V\beta_5$ and at least one β_1 integrin. Our findings demonstrate that osteopontin may be functionally important as an adhesive and chemotactic molecule for vascular cells during processes including development and remodelling after injury. In addition, the ability of multiple integrin receptors to interact with osteopontin may allow for different signalling mechanisms in responding cell types, or an additional level of regulation at the level of receptor availability.

E 408 TROPOELASTIN EXPRESSION AND CELL REPLICATION ARE TEMPORALLY DISTINCT MARKERS OF SMC PHENOTYPE DURING DEVELOPMENT AND AFTER ARTERIAL INJURY. J.K. Belknap, P.E. Schwartz, M.A. Reidy*, and R.A. Majack. Department of Pediatrics and Cell and Structural Biology, University of Colorado Health Sciences Center, Denver, CO, and *Department of Pathology, University of Washington, Seattle, WA.

Vascular smooth muscle cells (SMC) appear to modulate from an adult contractile phenotype to a more fetal, synthetic/replicative phenotype during disease states (e.g. atherosclerosis), after experimental injury, and following cell culture. We have used a double-labeling technique utilizing *in situ* hybridization for tropoelastin message and immunocytochemistry for bromodeoxyuridine (BrdU) to characterize the relationship between SMC replication and elastin expression during aortic development (day 13 embryo-2 month adult) and following balloon catheter injury of the carotid artery (2 days-6 weeks after injury). During development, both SMC and endothelial cells express elastin message. Hybridization signal in SMC was detectable as early as embryonic day 13 and persisted uniformly throughout the vessel wall until 1-2 months post partum. Elastin expression increases during fetal life (day 19-20) when SMC replication rates fall, and continues during early postnatal life when SMC replication has decreased dramatically. Small groups of adventitial cells continue to express elastin messages in the 2- and 3-month aorta. The data indicate that no correlation exists between SMC replication and elastin expression during rat aortic development. In balloon-injured carotids, SMC replication preceded elastin expression in both the tunica media and in the developing neointima. In the tunica media, SMC replication was observed at 2 days, and at 7 days elastin expression was observed *throughout* the vessel wall. In the neointima, significant elastin expression was not observed until 14 days, following the substantial cell replication observed at day 7. Few cells incorporating BrdU showed significant elastin expression. Our results suggest that cell proliferation and elastin expression are separate, independently regulated, temporally distinct phases in the vascular response to injury. The data suggest a model whereby, in the injured vessel, adult SMC revert to an undifferentiated phenotype characterized solely by cell replication, followed by a later "fetal" elastogenic phenotype.

E 409 SPECIFIC ADHESION OF U937 MONOCYTE-LIKE CELLS TO THE INTIMA OF HUMAN ATHEROSCLEROTIC PLAQUES. Robin N Poston, Ruth R Johnson-Tidey, Department of Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT, UK.

The adhesion of U937 cells to cryostat sections of human atherosclerotic plaques was assessed in a modified Stamper-Woodruff assay. The cells in medium were incubated with the sections on a rotating table for a range of temperatures, times and concentrations. Adherent cells were fixed with paraformaldehyde, and the sections were counter-stained with haematoxylin. Adhesion was assessed by counting cells over the intima of atherosclerotic and control areas of the artery wall, and likewise for the endothelium.

At 4°, as originally described for the assay, negligible binding was seen. However at 20 or 37°, with a 40' incubation, U937 cells attached in large numbers over most regions of atherosclerotic intima. Binding to normal intima was much less. In 13 atherosclerotic lesions, 16.1 $\pm$ 10.2 cells bound / high power field, compared to 1.3 $\pm$ 1.1 in 6 normal arteries, $p < 0.008$. Endothelial binding was at a similar intensity as in the bulk of the intima, and was increased in atherosclerotic over normal areas, $p < 0.003$. Occasional U937 cells were seen flattened on the endothelial surface. Preliminary results suggest that the binding of blood monocytes is similar.

Inhibition studies showed that chelation of divalent cations with EGTA or EDTA gave complete inhibition of adhesion, and demonstrated a dose dependent inhibition by the peptide GRGDS, containing the RGD sequence, but not by a non-RGD peptide.

These results give functional evidence for the involvement of adhesion phenomena in atherosclerosis, and suggest that an integrin- mediated interaction is promoting the accumulation of monocytes/ macrophages in the plaque.

A patent application has been filed relating to this work.

E 410 EXPRESSION OF FGFR2 ISOFORMS DURING AMPHIBIAN LIMB REGENERATION. Matthew L. Poulin, Kevin M. Patrie, Mary Jane Botelho, Roy A. Tassava and Ing-Ming Chiu,
Departments of Molecular Genetics and Internal Medicine, The Ohio State University, Columbus, OH, 43210.

Two splicing isoforms of FGFR2 have been cloned from a newt (*Notophthalamus viridescens*) limb blastema cDNA library. Sequence analysis revealed that we have isolated both the *bek* and KGFR variants of FGFR2. These two variants differ only in the second half of the last of their three Ig-like domains. The expression patterns of these variants during limb regeneration have been determined by *in situ* hybridization. During the pre-blastema stages of regeneration, FGFR2 expression is observed in the basal layer of the wound epithelium and in the cells of the periosteum. The wound epithelial hybridization was observed using the KGFR specific probe while the *bek* specific probe hybridized to mRNA in the cells of the periosteum. As regeneration progresses to the blastema stages, FGFR2 expression continues to be observed in the basal layer of the wound epithelium with additional hybridization seen in the blastema mesenchyme closely associated with the bisected bones. The *bek specific hybridization pattern* observed at this stage corresponds to the mesenchymal hybridization. Interestingly, there is hybridization of the KGFR specific probe in the blastema mesenchyme as well. In the differentiation stages of regeneration, the mesenchymal expression of FGFR2 becomes restricted to the cells of the condensing cartilage and later to the perichondrium. The expression patterns of KGFR and *bek* suggest that they have distinct roles in limb regeneration. Further investigation regarding the potential sources of the FGF ligands will help establish the role that FGFs and FGFRs play in limb regeneration.

E 411 MATRIX-DEGRADING METALLOPROTEINASES ARE DIFFERENTIALLY EXPRESSED DURING THE MENSTRUAL CYCLE IN HUMAN ENDOMETRIUM: A MODEL SYSTEM FOR GLANDULAR TISSUE DEVELOPMENT. William. H. Rodgers, Lynn. M. Matrisian, and Kevin. G. Osteen. Departments of Pathology and Obstetrics and Gynecology, The University of Alabama at Birmingham, Birmingham, AL 35294

Matrix-degrading metalloproteinases (MMPs) are a tightly regulated family of enzymes that degrade multiple components of the extracellular matrix and basement membrane. MMPs likely play important roles in growth and development, wound healing, and in pathological conditions such as arthritis and invasion and metastasis of tumors.

Using *in situ* hybridization, Northern hybridization, immunohistochemistry and radioimmunoprecipitation, the expression of MMPs has been determined in the cycling human endometrium, an adult organ that undergoes dramatic growth, remodeling, and destruction during the menstrual cycle. The mRNA for the MMP matrilysin was highly expressed in the glandular epithelium during the menstrual phase, while the expression of stromelysins-1,2,3, interstitial collagenase, and the 92 Kd gelatinase was limited to the stromal component during this phase of the cycle. Matrilysin and stromelysin-3 were expressed in the epithelial and stromal compartments, respectively, of proliferative endometrium. None of these MMPs were expressed during the progesterone-dominated secretory phase, suggesting that progesterone had a repressive effect on the expression of these enzymes. The mRNA for the 72 Kd gelatinase was detected in the stromal compartment throughout the menstrual cycle. Stromal cells had qualitative and quantitative differences in MMP RNA signals. These observations were confirmed *in vitro* by radioimmunoprecipitation of MMP proteins and *in situ* hybridization of explant cultures where progesterone inhibited the synthesis of matrilysin protein and mRNA.

The promoter of the human matrilysin gene has several transcription elements that may be involved in the negative regulation, either direct or indirect, by progesterone. These results suggest that the steroid hormones may regulate endometrial remodeling and tissue destruction during menstruation, at least in part, by virtue of their ability to alter metalloproteinase expression. The study of MMP expression in endometrium may provide unique insight into basic endocrine and paracrine mechanisms thought to regulate the menstrual cycle and into the role played by MMPs in glandular tissue development in general.

E 412 Cloning and Upregulation of Gal/GalNAc Macrophage Lectin in Rat Cardiac Allografts with Arteriosclerosis *Mary E. Russell,*‡§ Ulrike Utans,* Africa F. Wallace,* Peng Liang,* Morris J. Karnovsky,‡ Lauri R. Wyner,‡ and Tarn Chi**Cardiovascular Biology Laboratory, Harvard School of Public Health; ‡Department of Medicine, Harvard Medical School,Boston, Massachusetts 02115*

The hallmark of transplant arteriosclerosis is early and persistent monocyte/macrophage infiltration in the neointima of transplanted vessels. To characterize potential mediators associated with this process, we devised a PCR-based differential cloning strategy that identified a cDNA fragment induced in Lewis to F344 rat cardiac allografts compared with Lewis cardiac syngrafts. The full-length cDNA (1.4 kb) isolated from a rat cardiac allograft cDNA library is 99% homologous to Gal/GalNAc macrophage lectin, a highly specific cell-surface carbohydrate receptor. Our full-length cDNA probe hybridized in Northern blot analysis with total RNA from all 8 cardiac allografts studied but not with host hearts, hearts from syngeneic transplantations, or 8 other organs rich in resident macrophages. A PCR assay with ^{32}P-dCTP was developed to measure normalized Gal/GalNAc lectin transcript levels (using G3PDH as a control). There was a significant allograft-specific increase in transcript levels at days 7, 14, 28, and 75 compared with paired F344 host hearts (subject to same circulation but normal on histologic examination), day-0 hearts, and day-14 Lewis syngrafts (P<0.008, N=4 at each time point). Cardiac allograft levels were high compared with those in paired host spleens (the major source of inflammatory cells) (P<0.0001), indicating the localized nature of Gal/GalNAc lectin induction. Transcripts were increased in inflammatory (thioglycolate ellicited) but not resting or splenic macrophages. This specific and localized gene induction within the cardiac allograft links Gal/GalNAc macrophage lectin to chronic rejection, as a possible mediator of macrophage infiltration.

E 413 Expression Cloning of a Functional Glycoprotein Ligand for P-Selectin Dianne Sako*, Xiao-Jia Chang*, Karen M. Barone*, Gloria Vachino*, Gray Shaw*, Trudi M. Veldman*, Kevin M. Bean*, Tim J. Ahern*, Bruce Furie+, Dale A. Cumming*, and Glenn R. Larsen*. *Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA. 02140 and + Center for Hemostasis and Thrombosis Research, Division of Hematology and Oncology, New England Medical Center and the Department of Biochemistry, Tufts University School of Medicine, Boston, MA. 02111.

The initial adhesive interactions between circulating leukocytes and endothelia is mediated, in part, by P-selectin, an integral membrane glycoprotein found in secretory granules of platelets and endothelial cells. Previous studies have shown the human promyeloid cell line (HL-60) to contain a receptor for P-selectin which is sensitive to treatment with protease or glycosidases that liberate either sialic acid or fucose residues. We now report the expression cloning of a functional ligand for P-selectin from an HL-60 cDNA library. The predicted amino acid sequence reveals a novel mucin-like protein with putative extracellular, transmembrane, and cytoplasmic domains that exhibits no homology to Glycam-1, a previously cloned ligand for L-selectin. The extracellular domain contains 3 potential N-linked glycosylation sites and a unique internal region comprised of 15 decameric repeats. Binding of transfected COS cells to P-selectin is calcium-dependent and can be inhibited by neutralizing monoclonal antibodies to P-selectin. Transfected COS cells express a major ligand species of apparent MW of 220 kD as observed by non-reducing SDS-PAGE. Under reducing conditions, a single species of approx. 110 kD is observed. Northern analysis indicates that a predominant 2.5 kb mRNA and a minor 4kb mRNA are expressed in a variety of human cell types which exhibit binding to P-selectin. A soluble ligand construct, when *expressed in COS cells, also mediates P-selectin binding* in a calcium-dependent, antibody-inhibitable fashion.

E 414 DIFFERENTIAL EXPRESSION OF TENASCIN ISOFORMS BY *NEONATAL, AND ADULT NEOINTIMA AND MEDIAL AORTIC* SMOOTH MUSCLE CELLS

Behrooz G. Sharifi, David W LaFleur, James S. Forrester, Stephen M. Schwartz, James A. Fagin, Division of Cardiology, Cedars-Sinai Medical Center, Los Angeles, CA and Department of Pathology, University of Washington, Seattle WA.

Tenascin, one of the component of the extracellular matrix is present in isoforms which are developmentally regulated. To understand the functional activity of tenascin isoforms, we cloned and sequenced the alternatively spliced region of rat tenascin, a domain which exhibits anti-adhesive activity. RNA was extracted from PDGF-BB treated cells, reversed transcribed, and amplified with well conserved primers bracketing the alternatively spliced domains of human and mouse tenascin, and the products sequenced. Three major bands were seen: 1727, 633, and 361 bp products. The 1727 bp product has 86% homology to the unspliced region of human tenascin, and contains 5 complete fibronectin type III (FN III) repeats. Four and five FN III repeats are removed in the novel intermediate (633) and small (361) bp products, respectively. ASMC isolated from neonatal (9-days old) rats as well as adult neointima prominently express the small and intermediate tenascin isoforms. In contrast, the ASMC isolated from the medial layer of adult rat aorta express all three tenascin isoforms. This suggests that, as far as tenascin is concerned, *neointimal* ASMC may be similar to neonatal ASMC. Since tenascin isoforms differentially interact with cells and matrix, and FNIII repeats have been implicated in the down-regulation of focal adhesion points, the differential expression of tenascin isoforms by neonatal, and adult medial and neointimal ASMC may be important in the development of a less adhesive matrix environment favoring growth and/or cell migration.

E 415 Expression of Novel 400 kD Laminin *Chains by Mouse Endothelial Cells*

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The laminin family of proteins are the main constitutents of basement membranes. One well characterized isoform is isolated from Engelbreth-Holm-Swarm (EHS) tumour and contains a 400 kD A-chain, a 215 kD B1-chain, and a 205 kD B2-chain. However, the in vivo distribution of this laminin isoform is limited. In particular, the expression of the A-chain mRNA and protein, compared to B1 or B2-chains, is restricted. This is the case for most endothelial cells. The present study presents evidence that this may be due to the synthesis of novel laminin chains by various endothelial cells.

Immunoprecipitated laminin revealed two 200 kD chains, a 400 kD chain and nidogen. The 200 kD bands were identified as the laminin B1 and B2-chains. However, polyclonal anti-laminin sera and various A-chain specific MAbs failed to react with the 400 kD band in immunoblotting, immunoprecipitation or immunofluorescence. PCR, as well as Northern analysis using three different cDNAs, failed to detect the mRNA for the laminin A-chain in most cells studied. Only in one cell line (SVEC) a 10 kb mRNA was detected by Northern analysis using a cDNA specific for the 3´ coding region of the laminin A-chain. cDNAs specific for the central and 5´ coding region of the A-chain did not show any reaction.

The results demonstrate the existence of two novel 400 kD laminin chains which complex with the B1 and B2-chains to form specialized basement membranes in mouse endothelial cells.

E 416 ANTIGEN PRESENTATION BY VASCULAR SMOOTH MUSCLE CELL CLONES. Jill Suttles*, Robert W. Miller*, and Carolyn F. Moyer#. *Department of Biochemistry, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614 and the #Department of Comparative Medicine, Tufts University School of Veterinary Medicine, 200 Westboro Rd. North Grafton, MA 01536.

The infiltration of monocytes and T lymphocytes at atherosclerotic lesions resembles a chronic inflammatory condition, and suggests that immunological events (i.e., antigen presentation) occur during development of the disease. Although vascular smooth muscle cells (VSMC) have been shown to be capable of class II histocompatibility antigen expression and cytokine production, the functional significance of these capabilities and the extent to which VSMC contribute to vascular inflammation is unclear. We have evaluated the ability of cloned murine VSMC to present antigen to histocompatibility matched, antigen specific Th1 and Th2 cell lines. IFNγ primed cloned VSMC were antigen pulsed and evaluated for their ability to activate the ovalbumin specific Th1 cell line J6.19, or the conalbumin specific Th2 line D10.G4. Incubation of either Th1 or Th2 cells with antigen pulsed VSMC resulted in the formation of T cell-VSMC conjugates accompanied by morphological changes in both cell types. Although the T cells display characteristics of activated cells (including IL-2 receptor expression) when in contact with antigen pulsed VSMC, they are unable to progress through cell cycle. Fixation of the antigen-pulsed VSMC's eliminates the inhibitory signal - resulting in extensive proliferation of the T cells in an antigen specific fashion, with Th2 responding more efficiently to antigen presented by VSMC than did Th1. The inhibitory effect of unfixed VSMC on Th cell cycle progression could not be abrogated with indomethacin or an inhibitor of the generation of reactive nitrogen intermediates, suggesting that prostaglandin synthesis and/or nitric oxide production are not solely responsible for the inhibition of proliferation. Our results suggest that the T cell-VSMC interaction occurring during antigen presentation is complex, resulting in the activation of both cell types.

E 417 COMPARISON OF MESENCHYMAL AND NEURAL CREST-DERIVED VASCULAR SMOOTH MUSCLE CELLS ISOLATED FROM THE CHICK EMBRYO AORTA

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In the chick embryo aorta, a mixture of two smooth muscle cell (SMC) types is present in a proximal-distal gradient. The ascending aorta (4th arch) is composed of ectomesenchymal SMC (ECT) that originate in the neural crest and migrate to the developing outflow tract arteries. In contrast, the wall of the abdominal aorta is formed by mesenchymal SMC (MES) that derive from lateral plate mesoderm. Based on this anatomical distribution, we have isolated MES and ECT SMC from the E-14 chick embryo aorta. We propose that, in addition to common properties characteristic of their SMC identity, the two SMC types have intrinsic differences that may confer distinct roles during large vessel wall formation and repair.

We found that both SMC types express similar levels of desmin, calponin and α-SM actin, thus confirming their SMC identity. ECT outgrowth from 4th arch explants was more rapid and less serum-dependent than MES outgrowth from abdominal aortic explants. Moreover, confluent ECT (P1 to P3) exhibited increased DNA synthesis (4-fold, at 24h) to TGF-β_1 (0.01-10 ng/ml) while MES had no response or were growth-inhibited by TGF-β_1. Mitogenic responses to PDGF-AA, -BB, bFGF and EGF were similar in the two SMC types. We also asked if MES and ECT express different cell-cell adhesion properties that might influence how they organize within the vessel wall. ECT and MES were prelabelled with fluorescent dyes DiI and DiO, mixed together at 37°C with gentle rocking and aliquots retrieved at intervals between 1-24h. The vast majority of aggregates consisted of ECT only or MES only, consistent with expression of lineage-specific cell-cell adhesion molecules. Studies are underway to explore this possibility. SMC diversity in vivo might be maintained by expression of lineage-specific transcription factors. We have employed RT-PCR and primers that span the conserved homeodomain of *GHox* genes to obtain five amplified bands from ECT cells. DNA sequence analysis indicates that ECT express *GHox-8* and *GM-Hox*. RNA blot analysis showed that *GHox-8* and *GM-Hox* are expressed at similar levels in MES and ECT. The identity of the other bands is being determined.

A better understanding of lineage-dependent properties of MES and ECT SMC will provide insight into their interactions during the formation and repair of the artery wall.

Strategies to Modify Gene Expression

E 500 SUBPOPULATIONS OF B & T LYMPHOCYTES EXPRESS CD11B/CD18 AND ARE CAPABLE OF CD11B/CD18 MEDIATED ADHESION, Andrew, D.P. and E.C. Butcher. Department of Pathology, Stanford University School of Medicine, Stanford CA 94305.

In this report we examined expression and function of CD11b/CD18 on mononuclear cells. Large subpopulations of T and B cells expressed CD11b/CD18, although at 10 fold lower levels than that detected on neutrophils. The NK cells expressed high levels of CD11b/CD18 and had a unique phenotype; CD45RO-ve CD45RA+ve CD29int CD11ahigh CD44low, while subsets of NK cells expressed CLA and L-Selectin. On activation with PMA NK cells upregulated CD11b/CD18, but no upregulation was apparent on T and B lymphocytes. Expression of CD11b/CD18 on T cells did not correlate with known memory or activation markers such as CD45RO and CD11c/CD18 and showed wide variation between individuals (5-82%). Large fluctuations were also seen in one individual over one month, but not over a single day. Therefore, CD11b/CD18 on T cells, unlike other CD molecules fluctuates widely in expression.

Inspite of the low levels of CD11b/CD18 expression observed on lymphocytes, we were able to demonstrate CD11b/CD18 mediated adhesion of PMA activated lymphocytes to BSA coated plastic. CD11b/CD18 lymphocytes were greatly enriched in the adherent fraction and all adherent lymphocytes were CD11ahigh (memory) lymphocytes. The adhesion of PMA activated lymphocytes to BSA coated glass was blocked by MAbs to CD18 and CD11b, but not by anti-CD11a MAbs. Therefore, CD11b/CD18 may have some role in lymphocyte function in vivo. Also due to the heterogeneous expression of homing receptors L-Selectin and CLA by NK cells, we feel that NK cells may differ in their trafficking properties, as has been found for memory and naive T cells.

E 501 PURIFICATION OF A NOVEL COBRA VENOM PROTEASE THAT CLEAVES THE VON WILLEBRAND FACTOR RECEPTOR ON HUMAN PLATELETS AND THE P-SELECTIN RECEPTOR ON NEUTROPHILS, Robert K. Andrews, Christopher M. Ward, Lindsay C. Dunlop and Michael C. Berndt, Vascular Biology Laboratory, Baker Medical Research Institute, Prahran, Australia, 3181

The initial adhesion of blood platelets and neutrophils to the blood vessel wall are crucial steps in hemostasis and inflammation. Unactivated circulating platelets adhere to the glycoprotein von Willebrand Factor (vWF) incorporated in the extracellular subendothelial matrix of damaged blood vessels leading to a hemostatic platelet aggregate. In the inflammatory response, neutrophils interact with activated endothelial cells lining blood vessels and roll along the endothelial surface prior to their migration through the vessel wall to the site of tissue damage. Each of these processes is controlled by specific cell surface receptors. The vWF receptor on platelets is a heterodimeric transmembrane glycoprotein complex, Ib-IX. Endothelial cells activated by agonists such as thrombin rapidly express on their surface a granule membrane glycoprotein, P-selectin, which binds to an as yet unidentified receptor on neutrophils and related cell lines.

We have isolated a calcium-dependent protease from the venom of the Mozambiquan cobra, *Naja mocambique mocambique,* that completely abolished both vWF-platelet interaction and P-selectin-neutrophil interaction. The protease purified by heparin-affinity chromatography and gel filtration was 55,000 in molecular weight. It potently inhibited vWF-dependent platelet aggregation, but did not inhibit platelet aggregation induced by other agonists. The protease was found to specifically cleave the extracellular vWF binding domain of glycoprotein Ib between Glu-282 and Asp-283 within a highly negatively-charged peptide sequence believed to be critical for binding to vWF. Glycoprotein Ib was the only substrate detectable on the platelet surface. Treatment of neutrophils with the protease also abolished binding of P-selectin which was associated with cleavage of one major glycoprotein from the cell surface. Neither vWF nor P-selectin were substrates. In addition to allowing further molecular definition of the von Willebrand Factor receptor, this highly selective protease may provide the basis for defining the structure of the neutrophil P-selectin receptor.

E 502 MATRIX METALLOPROTEINASE EXPRESSION AFTER CAROTID ARTERIAL INJURY IN THE RAT, Michelle P. Bendeck and Michael Reidy, Department of Pathology, University of Washington, Seattle, WA 98195

Balloon catheter injury to a rat carotid artery induces medial smooth muscle cells to replicate and migrate to form an intimal lesion. Recent studies have shown that smooth muscle cell migration makes an important contribution to intimal thickening; when smooth muscle cell migration is inhibited independently of replication, intimal lesion formation is retarded. In an attempt to investigate mechanisms of smooth muscle cell migration, we have examined production of matrix metalloproteinases (MMP's) after two types of injury in the rat carotid artery. Using gelatin zymography, we have shown induction of a lytic band with MW ~ 88 kDa after balloon catheter injury. This 88 kDa band is not present in uninjured control vessels, and activity decreases beyond 6 days after injury; we believe this to be the active form of 92 kDa gelatinase/type IV collagenase (MMP-9). A lytic band of MW~238 kDa is expressed in a similar manner to the 88 kDa band. There were also lytic bands at MW's of 70 and 62 kDa constitutively present in the carotid artery. All activities were inhibited by treatment with EDTA or 1,10-phenanthroline, suggesting the enzymes were metalloproteinases. Northern blots with RNA from vessels harvested at various times after injury were probed with a cDNA for rat 92 kDa gelatinase (supplied by Dr. Birkedal-Hansen). 92 kDa gelatinase mRNA expression was induced as early as 6 hours after injury, and mRNA levels remained high for one week. In contrast, 72 kDa gelatinase mRNA (probe supplied by Dr. Goldberg) was constitutively expressed in unmanipulated carotids, and there was a decrease in expression after injury. Using another arterial injury procedure (filament denudation) where migration is significantly retarded, we observed decreases in 88 kDa and 238 kDa activity when compared with arteries subject to balloon catheter injury. These results suggest that the 88 kDa and 238 kDa gelatinases are involved in vascular smooth muscle cell migration and neointimal formation after injury.

E 503 THE NO PRECURSOR DOWN-REGULATES MONOCYTE ADHESIVENESS IN HYPERCHOLESTEROLEMIC ANIMALS, John P. Cooke, Leslie M. McEvoy, Helmut Drexler, Philip S. Tsao and Eugene C. Butcher, Department of Pathology and Division of Cardiology, Stanford University School of Medicine, Stanford, CA 94305

We have previously demonstrated that chronic administration of the nitric oxide (NO) precursor, L-arginine, markedly inhibits atherogenesis in rabbits. We hypothesize that this anti-atherogenic effect is due to augmentation of endothelial and/or monocyte-derived NO. To examine this possibility, NZW rabbits were fed normal chow, a high cholesterol diet, or a high cholesterol diet supplemented with arginine. After 2 weeks, peripheral blood mononuclear cells were isolated and incubated with cultured endothelial monolayers plated in multi-chamber slides for 30 min on a rocking platform. Slides were washed once to remove nonadherent cells and adherent cells counted by microscopy. Mononuclear cell binding was significantly increased in cholesterol-fed animals compared to control. *In contrast, adhesion of mononuclear cells isolated from cholesterol plus arginine-fed animals was reduced to near normal levels.* To determine if the effect of the NO precursor to reduce monocyte adhesiveness was due to down-regulation of any of the known adhesion pathways, FACS analysis was performed using MAb to all of the known adhesion molecules mediating monocyte-EC binding; no alteration in the treated group was observed by FACS. Thus, hypercholesterolemia-induced hyper-adhesiveness of mononuclear cells is attenuated by the NO-precursor arginine and may account for its anti-atherogenic effect. This effect does not appear to be mediated through any of the known adhesion pathways.

E 504 MOLECULAR AND FUNCTIONAL PROPERTIES OF VE-CADHERIN [7B4/CADHERIN-5] A NOVEL ENDOTHELIAL SPECIFIC CADHERIN, Elisabetta Dejana, Ferruccio Breviario, Monica Corada, Luis Caveda, Ines Martin-Padura and M.G.Lampugnani, Istituto di Ricerche Farmacologiche Mario Negri,Milano, Milano, Italy.

VE-cadherin [7B4/cadherin-5] is a novel endothelial specific cadherin localized at intercellular junctions. Sequence analysis revealed that besides many homologies with the other human cadherins it also presents important differences at the cytoplasmic and extracellular domains.In order to directly investigate the functional role of this molecule we transfected the full-length VE-cadherin cDNA in CHO cells.The transfected molecule presented similar characteristics as compared to the natural molecule in endothelial cells: it reacted with several VE-cadherin mAbs, presented the same molecular weight, selectively concentrated at intercellular junctions and codistributed with a-catenin.

In addition VE-cadherin tranfectants displayed an epithelioid morphology reminescent of the cobblestone morphology of endothelial cells.Transfected VE-cadherin presented adhesive properties, some of which in common with the other members of the cadherin family.It mediated homophilic, calcium dependent, aggregation and cell to cell adesion; it decreases intercellular permeability to high molecular weight molecules and it reduces cell migration rate across a wounded area.These data support the concept that VE-cadherin may exert a relevant role in endothelial cell biology participating in vascular injury repair, angiogenesis and permeability control.

E 505 LIPOPOLYSACCHARIDE BINDING AND MONOCYTE-CHEMOTAXIS BY HBP, A NEUTROPHIL GRANULE-DERIVED CATIONIC PROTEIN, IS INDEPENDENT FUNCTIONS, Hans Flodgaard and Charlotte Goriche, Biopharmaceuticals Research, Novo Nordic, Bagsværd, Denmark.

HBP/CAP37/Azurocidin is a multifunctional protein isolated from the granules of human neutrophils with important implications in host defence and inflammation. HBP mediates reversible contration of fibroblasts and endothelial cell monolayers and induces protein C kinase activation in endothelial cells. HBP is a powerful mediator of monocyte chemotaxis and binds to endotoxin. The binding to endotoxin was studied using ^{125}I labeled HBP binding to LPS immobilized on microtiter plates. Schatchard analysis unveil a K_{ass} = 0.8 x 10^9 M^{-1} and the binding was competitive inhibited with excess of polymyxin B in accordance with a K_{ass} for the latter of 10^7 M^{-1}. A recently discovered antibacterial 15-kDa protein from rabbit neutrophils compete with a K_{ass} of the same magnitude as HBP. Monocyte chemotaxis was studied in Boyden chambres using freshly isolated human monocytes. The HBP mediated chemotaxis was in the nmolar range and was not inhibited by excess LPS addition but was inhibited by heparin which on the other hand do not inhibited the LPS binding of HBP. These results indicate that the LPS bind domaine and the monocyte binding domaine in HBP are independent. Recently is has been shown that a synthetic peptide based on residues 20 - 44 in HBP mimics the lipopolysaccharide binding and antibiotic action (1).

1. Pereira AH, Erdem I, Pohl J & Spitznagel JK,Proc. Natl. Acad. Sci.,90,4733, 1993.

E 506 LEUKOCYTE ADHESION TO CYTOKINE-ACTIVATED ENDOTHELIUM IS MORE THAN THE INTERACTION OF ADHESION MOLECULES

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The extravasation of the different leukocyte types into a site of inflammation is a tightly controlled process. Most interestingly, granulocytes, monocytes and lymphocytes utilize similar adhesion mechanisms, but are nevertheless able to migrate into a locally inflamed site with different kinetics. This finding suggests the existence of distinct adhesion control mechanisms to regulate the activity of the adhesion molecules themselves.

We have characterized a number of monoclonal antibodies that interfere with the adhesion of granulocytes and monocytes to mouse endothelial cells in vitro. One panel is directed against endothelial cell selectins, whereas another panel is interfering with the adhesion of granulocytes in a more complex way. We suggest the existence of a novel class of cell surface molecules that have anti-adhesive effects. In addition, the expression of the mouse E-selectin on the endothelial cell surface does not show the same kinetics as the adhesion of U937 cells to this cytokine activated mouse endothelium, suggesting a regulation of adhesion beyond the expression of the adhesive selectins themselves. The implications of this model will be discussed.

E 507 SULFATION REQUIREMENT OF ENDOTHELIAL LIGANDS FOR L-SELECTIN, Stefan Hemmerich & Steven D. Rosen, Department of Anatomy and Program in Immunology, University of California, San Francisco, CA 94143-0452

L-selectin as recombinant chimeric protein specifically stains high endothelial venules of murine peripheral and mesentheric lymphnodes (LN) and has been shown in LN-lysates to bind in a Ca^{2+}-dependent fashion at least three glycoproteins of 50, 90 and 200 kDa (Sgp50, Sgp90 & Sgp200) that contain sialic acid, galactose and sulfate. Both Sgp50 and Sgp90 are sialomucins; the former has been cloned and designated GlyCAM-1, while the latter was identified as a sulfated glycoform of CD34 (Baumhüter *et al., Science*, in press). Imai *et al.* (*Nature* **361**, 555-557) have shown earlier by using chlorate as metabolic inhibitor of sulfation, that in addition to the well established sialic acid requirement sulfate is neccessary for ligand activity of GlyCAM-1. We have extended this finding also for Sgp90 and Sgp200. Furthermore we are currently investigating the sulfated carbohydrate structure recognized by L-selectin and the adhesion-blocking monoclonal antibody MECA-97 (Streeter *et al., J. Cell Biol.* **107**, 1853-1862).

E 508 INACTIVATION OF LYSOSOMAL PROTEASES BY OXIDIZED LDL IS PARTIALLY RESPONSIBLE FOR ITS POOR DEGRADATION BY MACROPHAGES, Henry F Hoff, George Hoppe, and June O'Neil, Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195

Deficient processing in macrophages of apoB in oxidized low density lipoprotein (Ox-LDL) by lysosomal proteases has been documented by several groups and attributed, in large part, to substrate modification, especially protein crosslinking. We considered that inactivation of lysosomal proteases by Ox-LDL could also be responsible for this deficient degradation. When mouse peritoneal macrophages (MPM) were pre-incubated for 5 hr at 37°C with unlabeled monomeric (m-), aggregated (aggr-) Ox-LDL, LDL, acetyl LDL, vortex-aggregated LDL (aggr-LDL), or opsonized latex beads, only the two forms of Ox-LDL (aggr. more than m.) inhibited the subsequent degradation of all of the ^{125}I-labeled forms of the above lipoproteins (LP) over 3 hr. The degradation of ^{125}I-acetyl LDL was reduced 30% after pre-incubation with aggr-Ox-LDL relative to values in untreated cells. However, when ^{125}I-acetyl LDL was aggregated by vortexing, its degradation was reduced by 95%. No reduction of internalization of any labeled LP was found by pretreatment of cells with Ox-LDL. Extracts of MPM pre-incubated with aggr-Ox-LDL demonstrated lower protease activity toward native LDL than control cells. Furthermore, pre-incubation of a mixture of cathepsin B and D for 3 hr with aggr-Ox-LDL but not with aggr-LDL or LDL inhibited enzyme activity for cathepsin B substrate. These results suggest that Ox-LDL can directly inactivate lysosomal proteases. This may be partially responsible for reduced degradation of Ox-LDL in macrophages.

E 509 C5a AND TNF-α INDUCE ALTERATIONS IN THE EXPRESSION OF β_2 INTEGRINS AND L-SELECTIN ON HUMAN NEUTROPHILS THROUGH A COMMON CALCIUM/CALMODULIN-DEPENDENT PATHWAY. Mark A. Jagels and Tony E. Hugli. The Scripps Research Institute, La Jolla CA 92037.

We have investigated the intracellular regulation of L-selectin and β_2 integrin expression on polymorphonuclear leukocytes (PMN) upon activation by either C5a or TNF-α. Levels of CD18 and L-selectin were measured by flow cytometry in both whole blood and isolated cell preparations using the fluorescent labeled antibodies IB4 and DREG-200, respectively. Cells were treated with inhibitors of known signal transduction pathways for 15 min. at 37° prior to stimulation for 15 min. with either C5a or TNF-α. Our results suggest a dependence on intracellular calcium in response to each stimulus, derived either from intracellular release or influx of extracellular Ca^{++}. Calmidazolium and W-7, inhibitors of calmodulin-dependent processes, suppressed C5a-induced increases in cell surface CD18 by 40 -60%, and calmidazolium suppressed the shedding of L-selectin by approximately 35%. TNF-α-induced responses were similarly affected. Ethanol and diphosphoglycerate, purported inhibitors of phospholipase D, inhibited modulation of both molecules by up to 90%. Erbstatin analog, tyrphostin-51 and genistein, inhibitors or tyrosine kinases, were without effect on either up-regulation of CD18 or shedding of L-selectin. H-7 and calphostin C, which inhibit activation through protein kinase C, slightly augmented both of these responses. These results suggest that C5a and TNF-α induce changes in expression of L-selectin and β_2 integrins through a common calcium, calmodulin and phospholipase D dependent pathway.

E 510 IDENTIFICATION OF NEW MOLECULES INVOLVED IN POLYMORPHONUCLEAR TRANS-EPITHELIAL MIGRATION, R. Owen Lockerbie, Lynn Courtney, Kristin Ziegler, Joel Hayflick and Mike Gallatin, Cell Adhesion Group, ICOS Corporation, Bothell, WA 98021

Migration of polymorphonuclear cells (PMN) across endothelial and epithelial cell barriers in response to chemotactic stimuli is an essential component in inflammatory diseases in several tissues. While the complex events related to PMN transendothelial migration are being widely addressed, those related to transepithelial migration have received less attention. To better understand the interactions between PMN and tissue epithelium, we have developed a transmigration assay in which PMN migrate across intact monolayers of human epithelial cells in response to a chemoattractant gradient. We show that PMN transepithelial migration is CD18 dependent, but does not involve any of the known members of the intercellular adhesion molecule (ICAM) family, thereby distinguishing this mode of migration from that across endothelium. Monoclonal antibodies raised against lung epithelial cells recognize a cell surface associated species at approximately 100KDa and significantly block formyl peptide induced PMN transepithelial migration. The antibodies also markedly block PMN adhesion to monolayers of lung epithelial cells. This species is broadly expressed on human tissue epithelium, but shows no cytokine inducible regulation, and is not expressed on resting leukocytes or human umbilical vein endothelial cells. Our results provide evidence for the presence of novel epithelial molecules that may play a role in the pathophysiology of inflammmatory disease states.

E 511 MAST CELLS REGULATE ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION, Hong Meng, Jackie Marchese and Barry Gruber, Department of Medicine and Dermatology, SUNY at Stony Brook, Stony Brook, NY 11794

Vascular endothelial cells (EC) play an active role at the site of inflammation by displaying adhesion molecules along the luminal surface which bind to their ligands (or counter-receptors) on circulating leukocytes prior to their emigration into the extravascular tissue. Mast cells (MC) are often found in close proximity to microvasculature. To investigate the possible role of MCs inducing leukocyte adhesion to vascular EC, MC supernatants (MS) were prepared by calcium ionophore (A23187, 500ng/ml)-induced degranulation of murine MCs and freshly isolated rat peritoneal MCs. MS was monitored for a regulatory effect on adhesion molecule expression in human dermal microvascular endothelial cells (HDMEC) and human umbilical vein endothelial cells (HUVEC). Cell surface expression of VCAM-1 and ICAM-1 was determined using monolayer cell culture ELISA and FACS analysis. Unstimulated HDMEC and HUVEC expressed lower basal levels of VCAM-1 as compared to ICAM-1. Incubation of HDMEC and HUVEC with MS resulted in prominent increases of both ICAM-1 and VCAM-1 expression, noted as early as 4 hours. Maximal levels were attained by 16 hours followed by a general decline, still significantly higher than control level at 48 hours. Dose-dependency of ICAM-1 and VCAM-1 expression, using increasing dilutions of MS, was observed with both types of ECs. The percentage increase of ICAM-1 expression in HDMEC was significantly higher than observed in HUVEC. In contrast, VCAM-1 expression was more responsive in HUVEC versus HDMEC. To evaluate the function of these two EC adhesion molecules, T lymphocytes isolated from normal human donors were used in a cell adhesion assay. Both ICAM-1 and VCAM-1 functional expression increased significantly after exposure to MS as reflected by increased T cell binding; this could be inhibited by neutralizing antibodies to ICAM-1 and VCAM-1. Steady-state levels of mRNA for VCAM-1 in HDMEC and HUVEC were determined in Northern blot analysis. MS augmented VCAM-1 mRNA levels in both EC types, although more pronounced in HUVEC. These studies indicate that MCs may play a very important role in the induction of an inflammatory response by releasing factors which upregulate EC adhesion molecules and enhance leukocyte binding. Furthermore, it is suggested that the cell surface expression of ICAM-1 and VCAM-1 and their mRNA levels are differentially regulated in ECs derived from different sized vessels following exposure to MC products. Preliminary data suggests that MC-derived TNFα may be in part responsible for these observations.

E 512 NEUTRALIZATION OF TUMOR NECROSIS FACTOR-α REDUCES EXPRESSION OF VASCULAR ADHESION MOLECULES AND ASSOCIATED INTIMAL THICKENING IN RABBIT CARDIAC ALLOGRAFTS, Silvana Molossi, Nadine Clausell, Suvro Sett and Marlene Rabinovitch, Division of Cardiovascular Research, The Hospital For Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8.

Expression of adhesion molecules, i.e., vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), occurs in myocardium and coronary arteries (CA) of cardiac allografts. The presence of these molecules on the activated endothelium promotes adhesion and transendothelial migration of inflammatory cells. Tumor necrosis factor (TNF)-α increases the expression of these molecules but the influence of cyclosporine A (CsA) remains controversial. We therefore compared, in cholesterol-fed rabbits following heterotopic cardiac transplantation, the effects of neutralizing TNF-α using TNF-α soluble receptor (sr) and the influence of CsA on the expression of VCAM-1 and ICAM-1 in the CA. We also investigated the effect of TNFsr on the expression of TNF-α itself. The immunohistochemical studies were performed using monoclonal antibodies. The animals were fed a cholesterol-rich diet and received either CsA (n=5), saline (n=5), or TNFsr (2.5/mg/kg s.c.) (n=6). The rabbits were sacrificed at 9 or 10 days after cardiac transplantation. All donor hearts showed grade III rejection, except the CsA group which had only grade I rejection. All host vessels were negative for the expression of adhesion molecules as well as TNF-α. In the saline-treated group, donor vessels showed intense positive immunostaining for ICAM-1 and VCAM-1. However, in donor vessels from both the CsA-and the TNFsr-treated groups there was minimal endothelial expression of ICAM-1 and VCAM-1 associated with decreased presence of T cells and macrophages on the endothelium, as well as reduced intimal thickening. In addition, the expression of TNF-α was decreased. Thus, TNFsr appears to interfere selectively with the immune-inflammatory reaction decreasing the vascular changes associated with graft arteriopathy. This is associated with expression of adhesion molecules and TNF-α on the endothelial surface. Cyclosporine A, however, causes generalized suppression of the immune-inflammatory response and this includes the development of arterial lesions within this experimental time frame.

E 513 INDUCTION OF HUMAN ENDOTHELIAL CELL ICAM-1 AND E-SELECTIN GENE EXPRESSION BY THE CARDIAC GLYCOSIDE OUABAIN, Asahiko Oguchi, and Russell M. Medford, Department of Medicine, Emory University Atlanta, GA 30322

Dysregulation of intracellular ionic homeostasis is a characteristic feature of hypertensive vascular disease while endothelial cell expression of adhesion molecules such as ICAM-1 characterize vascular inflammatory processes such as atherosclerosis. As hypertension is a risk factor for atherosclerosis, we have tested a hypothesis that links endothelial cell adhesion molecule gene expression with ion mediated signals. Human umbilical vein endothelial (HUVE) cells were treated with ouabain, a cardiac glycoside that specifically inhibits the plasmalemmal Na^+,K^+-ATPase (NAKA) resulting in altered intracellular Na^+, K^+ and Ca^{++} homeostasis. Ouabain (1μM) alone induced both ICAM-1 and E-selectin mRNA accumulation that was first detected at 5 hours for both genes and peaked at 20 hours for ICAM-1 and 7 hours for E-selectin. Preliminary quantitative analysis of the response revealed peak levels that were approximately 2-5% for E-selectin and approximately 20-30% for ICAM-1 compared with tumor necrosis factor-α (TNF-α, 100U/ml). Although both ouabain and TNF-α induced E-selectin mRNA with similar transient kinetics, ouabain induced a gradual induction of ICAM-1 mRNA accumulation over 20 hours compared with the more rapid response by TNF-α. These results suggest that NAKA mediated alterations of intracellular ionic milieu caused by ouabain, such as increased Na^+ and Ca^{++}, may directly induce ICAM-1 and E-selectin gene expression in HUVE cells.

E 514 EXPRESSION OF ADHESION MOLECULES AND CYTOKINE GENES IN AORTIC ADVENTITIAL CHRONIC INFLAMMATION ASSOCIATED WITH ADVANCED ATHEROSCLEROSIS (CHRONIC PERIAORTITIS), Dinah V. Parums and Lisa A. Ramshaw, Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, LONDON W12 ONN, U.K.

A spectrum of chronic inflammation is seen associated with human advanced atherosclerosis when the aortic media is thinned ('chronic periaortitis'). Lymphoid follicles are common ('aortic associated lymphoid tissue',-AALT). Immunohistochemistry demonstrated a predominance of B lymphocytes surrounded by CD4-positive T lymphocytes and macrophages; MHC Class II molecule expression was abundant as was IL-2 and IL-4 receptor expression; B and T cell proliferation was seen within the lymphoid follicles.
Immunohistochemical analysis of cytokine-dependent adhesion molecules showed strong anti-VCAM-1 staining associated with B cell aggregates, with moderate staining of vessels in lymphoid follicles; strong anti-ELAM-1 staining was confined to endothelial cells in the aortic media and adventitia; anti-ICAM-1 staining was extensive on endothelium. VCAM-1 and ICAM-1 expression paralleled the degree of inflammation.
RNA was extracted from six fresh surgical specimens of atherosclerotic aortic aneurysm wall showing a spectrum of chronic periaortitis. PCR assisted amplification of mRNA was carried out for interleukin (IL)-1, IL-2, IL-4, tumour necrosis factor (TNF)-α, interferon (IFN)-γ with β-actin as an internal control. All except TNF-α were detected in the six atherosclerotic aortas. RNA dot blots were hybridized with ^{32}P-labelled cytokine and control probes. The bound radioactivity was measured using a flat-bed scintillation counter. These quantitative hybridization measurements supported the PCR data and revealed increasing cytokine expression with increasing severity of inflammation.
These findings reveal the active nature of chronic inflammation associated with human advanced atherosclerosis (chronic periaortitis) and reveal its progressive potential.

E 515 THE MIGRATION OF VASCULAR SMOOTH MUSCLE CELLS THROUGH A RECONSTITUTED BASEMENT MEMBRANE REQUIRES 72 KD TYPE IV COLLAGENASE AND IS SUPPRESSED BY CELLULAR DIFFERENTIATION. Rebecca.R. Pauly, Robert Monticone, Claudio Bilato, Edward Lakatta, and Michael T. Crow. Lab. of Cardiovascular Science, NIA, NIH, Baltimore, MD 21224.

The migration of vascular smooth muscle cells (VSMCs) is a key event in the development of many vascular diseases and involves the local degradation and remodelling of extracellular matrix (ECM) separating the media and neointima. We have used an in vitro assay to study VSMC migration through a defined ECM barrier of reconstituted basement membrane (BM). Cultured VSMCs maintained in a proliferating or undifferentiated state migrated through the BM barrier toward a chemoattractant (PDGF BB), while the migration of serum-starved/differentiated VSMCs was less than 20% ($p<0.001$) that of proliferating cells. A peptide that mimics the inhibitory propeptide region of all matrix metalloproteinases (MMPs) blocked migration of proliferating VSMCs through the barrier by more than 80% ($p<0.005$), but did not significantly affect migration that occurred in the absense of the barrier. Antisera capable of neutralizing the activity of the 72 kD type IV collagenase (MMP-2) also inhibited migration through the barrier by more than 80% ($p<0.005$). Northern blotting and zymographic analyses indicate that MMP2 is expressed and processed to an active form by VSMCs. Furthermore, the large reduction in migration seen in serum-starved/differentiated VSMCs was associated with a decrease in MMP-2 activity to less than 5% of that measured in proliferating VSMCs. These results demonstrate that VSMC migration through a BM barrier involves the production and regulation of a specific MMP and suggests that this ability may normally be suppressed in the vessel by conditions that support VSMC differentiation and/or growth arrest.

E 516 FLOW CYTOMETRIC ANALYSIS OF CELL SURFACE ADHESION MOLECULE EXPRESSION ON TNFα-, PMA-, AND INTERFERONγ-STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS TREATED WITH PD 144795, Sheila K. Sanders, Jeffrey B. Marine and Sheila M. Crean, Immunopathology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

Flow cytometric analysis was used to determine the effect that the endothelial cell activation inhibitor, PD 144795 (5-methoxy-3-(1-methylethoxy)benzo[b]thiophene-2-carboxamide 1-oxide) had on the TNFα or PMA (4 hr) upregulated surface expression of three adhesion molecules: E-selectin, a member of the lectin-like protein family, and two members of the immunoglobulin gene superfamily, ICAM-1 and VCAM-1. These three adhesion molecules are transiently expressed on cytokine-activated endothelium and are responsible for the recruitment of leukocytes to sites of inflammation. In addition, we looked at the effect PD 144795 had on the upregulation of the histocompatibility antigens HLA class I and HLA class II. HLA class I upregulation was observed with TNFα and interferonγ treatment at 24 hr while HLA-DR expression was observed at 24 hr with interferonγ treatment only. Interferonγ also induced the upregulation of ICAM-1 at 24 hr. PD 144795 had the following effects: 1) it significantly inhibited the 4-hr TNFα- and PMA-induced upregulation of E-selectin, ICAM-1 and VCAM-1, 2) it completely inhibited the TNFα-induced VCAM-1 expression at 24 hr and significantly inhibited the TNFα-induced ICAM-1 expression at 24 hr, 3) it enhanced the PMA-induced expression of E-selectin at 24 hr, compared to the inhibition at 4 hr, 4) it completely inhibited the upregulation of HLA class I induced by TNFα and interferonγ, 5) it completely inhibited the expression of HLA-DR induced with interferonγ, and 6) it had no effect on the interferonγ-induced upregulation of ICAM-1. Thus, we have identified a small molecule, PD 144795, that has the ability to inhibit cell surface adhesion molecule expression on activated HUVEC and which may be useful for modulating the inflammatory response.

E 517 THE ANTIINFLAMMATORY EFFECTS OF PD 144795, AN INHIBITOR OF ADHESION LIGAND EXPRESSION, Denis J. Schrier, Mark E. Lesch, Clifford D. Wright, Godwin C. Okonkwo, Martin P. Finkel and Kathleen Imre, Immunopathology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

PD 144795 (5-methoxy-3-(1-methylethoxy)benzo[b]thiophene-2-carboxamide 1-oxide) is a potent inhibitor of neutrophil adhesion, as well as adhesion ligand expression and IL-8 release by activated endothelial cells. Using a human umbilical vein endothelial cell monolayer and an ELISA-based detection system, PD 144795 prevented the expression of E-selectin and ICAM-1 on TNFα-stimulated cells with IC_{50}s of 2.0 and 3.4 μM, respectively. The compound also prevented PMA-induced expression with IC_{50}s of 2.2 and 3.8 μM, respectively. These results have been confirmed by flow cytometric analysis (see companion abstract). PD 144795 also inhibited IL-8 release by TNFα-stimulated endothelial cells (IC_{50} = 4.3 μM). The compound was much less effective against PMA-induced IL-8 release (IC_{50} = 61 μM). To investigate the antiinflammatory effects of PD 144795, the compound was evaluated orally in a variety of animal models including thioglycollate peritonitis, reverse passive arthus and streptococcal cell wall arthritis. The compound, at doses of 0.6-2.0 mg/mouse significantly inhibited neutrophil influx into the peritoneal cavity of mice that had been challenged 2 hours earlier with thioglycollate. Previous studies with ligand specific antibodies had shown that this model is MAC-1, LFA-1, ICAM-1 and MEL-14 dependent. PD 144795 also inhibited accumulation of neutrophils and exudate into the pleural cavity in the rat arthus model. The compound inhibited neutrophil accumulation at a slightly lower dose (10-30 mg/kg) than the dose required to inhibit exudate (30-100 mg/kg). PD 144795, at 10 mg/kg, significantly reduced the transient arthritic response induced in rats injected intraarticularly with streptococcal cell walls. The compound was ineffective against the chronic phase of the response induced by intraperitoneal administration of the antigen. These results provide further evidence that pharmacologic inhibition of adhesion ligand expression and IL-8 biosynthesis is an effective strategy for the development of novel antiinflammatory agents.

E 518 ACQUIRED LEUKOCYTE ADHESION DEFICIENCY IN ACUTE LEUKEMIA: HIGH LEVELS OF THE SHED FORM OF L-SELECTIN (sL-SELECTIN) INHIBIT ADHESION OF LEUKEMIC BLAST CELLS TO THE VASCULAR ENDOTHELIUM. Olivier Spertini, Patrizia Callegari, Anne-Sophie Cordey, Jacques Hauert, Jean Joggi,* Vladimir von Fliedner,* Marc Schapira. Division of Hematology, University of Lausanne, CHUV and Ludwig Institute for Cancer Research,* Lausanne, Switzerland.

L-selectin mediates leukocyte rolling along vascular endothelium. The interaction of L-selectin with its endothelial ligands is inhibited by some anti-L-selectin monoclonal antibodies (mAbs), certain sulfated carbohydrates and the shed form of L-selectin (sL-selectin), formed by proteolytic cleavage of the extracellular region of L-selectin. Since little information is available on the mechanisms that regulate the traffic of blast cells out of the bloodstream, we evaluated the role of L-selectin and its cleaved form in modulating the adhesion of blast cells to endothelium. sL-selectin concentration was measured by ELISA in plasma from 151 patients with acute leukemia. The mean (±1 SD) plasma level of sL-selectin among 100 healthy individuals was 2.1±0.7 μg/ml. This value was increased (>2 SD above the mean, $P < 0.001$) in 63% of 58 patients with acute lymphocytic leukemia (ALL) (range: 0.1 to 98 μg/ml) and in 59% of 93 patients with acute myeloid leukemia (AML) (range: 0.7 to 68 μg/ml). Repeated measurements in 24 patients showed normal range levels in 16/16 patients in complete remission and increased levels in 8/8 patients with therapy-resistant acute leukemia or leukemia relapse. Observations made with aleukemic patients indicated that plasma sL-selectin reflected the total mass of blast cells in the body. Thus, two patients who had no detectable blasts in peripheral blood had increased sL-selectin levels (5.2 and 17.4 μg/ml, respectively) and >50% of blasts in the bone marrow. sL-selectin was characterized by Western blotting and epitope mapping. Plasma samples from 6 patients with ALL contained a sL-selectin form with a M_r ranging from 60,000-90,000 whereas a M_r 70,000-95,000 form was detected in 8 AML plasma. Epitope mapping with mAbs demonstrated that L-selectin shedding was accompanied by conformational changes of its epidermal growth factor-like domain. A functional role for L-selectin was supported by its ability to almost completely inhibit L-selectin-dependent adhesion of blasts to activated endothelium. Thus, high levels of sL-selectin induce an acquired leukocyte adhesion deficiency state in L-selectin$^+$ acute leukemia. Furthermore, sL-selectin may be a useful marker for evaluating leukemia activity.

E 519 HOMOLOGOUS SELECTIN PEPTIDES BLOCK NEUTROPHIL ADHESION *IN VITRO* Susan H. Tam, Marian T. Nakada, George A. Heavner, Robert Weber, Marian Kruszynski and John Ghrayeb, Dept. of Molecular Biology and Dept. of Peptide Chemistry, Centocor, Inc., Malvern, PA 19355 USA

Molecular details of the interaction between selectin and carbohydrate ligands are poorly understood. To gain insight into the critical selectin binding sites, we synthesized overlapping peptides to E-, P- and L-selectin sequences and performed structure-function studies. Previously, we have shown that peptides corresponding to regions 23-30, 54-63, and 70-79 of the N-terminal lectin domain of P selectin inhibit neutrophil adhesion to P-selectin. Here we present evidence that peptides corresponding to regions 109-118 of E-, P- and L-selectin also inhibit neutrophil adhesion. Results show differences in the potency of peptides derived from E-selectin with $IC_{50} \sim 6\ \mu M$, P-selectin with $IC_{50} \sim 35\ \mu M$, and L-selectin with $IC_{50} \sim 5\ \mu M$. We confirm that lysines at positions 111 and 113 are important for P-selectin interaction (consistent with results of Lasky et al), and also that conservative amino acid substitutions at the lysine residues do not alter functional activity. Both cyclic and acyclic peptides from this region have been investigated. The ability of peptide analogues of this region to block neutrophil adhesion to cytokine activated HUVECs and E-selectin transfected COS cells will be presented. Overall results should elucidate critical sites necessary for selectin dependent neutrophil adhesion and may prove useful in the development of anti-inflammatory drugs.

E 520 SELECTINS AND CD18 DEPENDENT NEUTROPHIL FUNCTIONS, Mathew Vadas, Debbie Cooper, Carolyn Butcher and Michael Berndt*, Hanson Centre for Cancer Research, Institute of Medical & Veterinary Science, Adelaide, South Australia, 5000; *Baker Medical Research Institute, Prahran, Victoria, 3181, Australia.

Neutrophils fail to or weakly recognise and phagocytose unopsonised zymosan (UZ) particles. Activation by TNF has been shown to increase recognition (rosetting) as well as phagocytosis of UZ (Klebanoff SJ *et al*, J Imm 136:4220, 1986). We now show that both purified platelet derived P-selectin (Ps) and Ps or E-selectin (Es) expressed on CHO cells also increases phagocytosis of UZ from 14.55±4.04% to 30.2±5.78% ($p < 0.002$). This Ps stimulated phagocytosis is inhibited by antibodies to Ps and CD11b and CD18. Treatment of neutrophils with thermolysin has been shown to inhibit their binding to Ps (Erban J *et al*, J Biol Chem 267:11104, 1992). Thermolysin also inhibits Ps but not TNF enhanced phagocytosis. Furthermore, Ps induces in PMN an epitope on β_2 integrin (P24) associated with activation (Dransfield I & Hogg N, EMBO J 8:3759, 1989). The effect of Ps is rapidly reversible in keeping with the rapid off rate of this molecule and is due to its effect on neutrophils rather than UZ. In summary we show that Ps induces an activation epitope on CD11/18 and function that involves this molecule. We suggest that Ps may directly influence β_2 integrins.

E 521 ENDOTHELIAL CELL FERRITIN PROTECTS AGAINST OXIDIZED LOW-DENSITY-LIPOPROTEIN MEDIATED CYTOTOXICITY. GM Vercellotti, MB Juckett, J Balla, G Balla, HS Jacob. University of Minnesota, Minneapolis, MN 55455.

A recent Finnish study (Circulation 86:1,1992) suggests increased body iron, reflected by elevated serum ferritin, increases myocardial infarction risk. This is coherent with our recent report that endothelium (EC), if briefly exposed to heme, accumulates iron and becomes hypersusceptible to oxidant injury. Paradoxically, more prolonged heme exposure renders EC resistant to oxidants by inducing Fe-sequestering ferritin. Heme also catalyzes oxidation of LDL which becomes EC cytotoxic. We wondered if heme's ability to induce EC ferritin might also serve to limit its own fostering of lipid-mediated vascular damage. Indeed, porcine aortic EC when pretreated 16 hrs before with a pulse of 10μM hemin--thereby increasing cellular ferritin 10-fold--become completely resistant to injury induced by LDL (200μg/ml) oxidized by hemin (10μM)/H_2O_2 (50μM) (6.0 ± 0.8 % ^{51}Cr release vs 41.4 ± 1.2 % in controls). Protective induction of ferritin depends on iron exposure, as Fe-pyridoxal-isonicotinoyl-hydrazone identically induced ferritin synthesis, and concomitantly prevented EC cytotoxicity by oxidized LDL ($p < 0.001$). Ferritin is the critical cytoprotectant, since addition of exogenous apoferritin to EC also prevents oxidized LDL-mediated EC injury. Moreover, the H-chain ferroxidase activity of ferritin is essential for the protection: cells loaded with hr-H-chain, but not with a mutant lacking ferroxxidase activity (PNAS 88:770,1991), resist oxidized LDL damage. In-vivo relevance of these findings is suggested by examination of ferritin content of atherosclerotic human plaques. In autopsy specimens, significant ferritin immunoreactivity was noted in plaques but not normal aorta. We conclude: serum ferritin levels may be analogous to hemoglobin A_{1c} in diabetics--assaying cumulative Fe exposure and concomitant risk of oxidant vascular damage. But ferritin itself, by sequestering free Fe, is cytoprotective against vascular toxic oxidant assault such as provided by oxidized LDL.

E 522 SPECIFIC PHARMACOLOGICAL INHIBITION OF IL-1/TNF-INDUCED 92 kDa TYPE IV COLLAGENASE (MMP-9) Reitha Weeks, James McMillan, Glenn C. Rice, David Lovett Department of Medicine, University of California, San Francisco 94141 and Cell Therapeutics Inc., Seattle, WA 98119

It is now recognized that the members of the matrix metalloproteinase supergene family play major roles in the degradation of extracellular matrices during development and for basal matrix turnover by differentiated tissues. In addition, enhanced secretion of these enzymes, particularly the 72 kDa and 92 kDa Type IV collagenases, is an important component of the tissue response to inflammatory cytokines such as Interleukin 1 (IL-1) and Tumor Necrosis Factor (TNF) as well as bacterial endotoxin (LPS). Augmented secretion of these enzymes has also been shown to correlate with the ability of cancerous cells to metastasize. Recent studies from our laboratories have defined a novel IL-1, TNF and LPS signaling pathway resulting from the rapid activation of lysophosphatidate acyl CoA:acyltransferase (LPAAT) and phosphatidate phosphohydrolase (Amer. J. Physiol., 1992, 262:C328; J. Biol., Chem., 1991, 266:20732). The activation of this pathway yields distinctive species of phosphatidic acid, which are critical for subsequent cellular activation. Specific small molecule competitive inhibitors of LPAAT have been developed and used to probe the significance of this pathway for the induction of IL-1, TNF or LPS mediated gene products. The 92 kDa Type IV collagenase was chosen as a model inflammatory cytokine-regulated gene, using the human leukemic cell line, THP-1. Incubation of THP-1 cells with IL-1, TNF or LPS resulted in a rapid induction of 92 kDa Type IV collagenase with little increase in constitutive expression of the 72 kDa collagenase. CT-2519, representative of the LPAAT inhibitor class of small molecules, selectively blocked induction of IL-1, TNF or LPS-induced expression of the 92 kDa collagenase with nearly complete inhibition at 10 μM without effect on steady state levels of the 72 kDa Type IV collagenase. Northern blot analysis demonstrated the absence of 92 kDa Type IV collagenase transcripts in TNF, IL-1 or LPS-induced THP-1 cells treated with CT-2519, suggesting CT-2519 was in fact inhibiting at a pretranslational point. Pharmacologic modulation of cytokine-dependent gene products may offer an important alternative therapeutic approach to the treatment of inflammatory and neoplastic disorders.

E 523 **TARGETED DISRUPTION OF THE MURINE E-SELECTIN AND VCAM-1 GENES**

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Cell adhesion molecules expressed on endothelial cells mediate the trafficking of leukocytes during normal immune responses and during the development of inflammatory diseases. E-selectin is a member of the selectin family of adhesion molecules and is exclusively expressed on activated endothelial cells. E-selectin mediates the binding of a number of leukocytes including neutrophils and monocytes. VCAM-1 was originally identified in activated endothelial cells as a transmembrane protein containing seven Immunoglobulin (Ig)-like domains which mediated adhesion to a number of different leukocytes. In contrast to E-selectin, VCAM is expressed in a number of cell types in both a basal and inducible manner.

In order to dissect out the roles of E-selectin and VCAM-1 in development and in the development of disease we are attempting to create mice deficient in their expression by targeted homologous recombination in ES cells. The E-selectin and VCAM genes were disrupted in ES cells and chimeric mice produced. Mice homozygous for the disrupted E-selectin allele were produced at the expected mendelian frequency from heterozygous crosses indicating that the E-selectin gene is not required for mouse development. These mice are currently being analyzed for the effects of E-selectin deficiency on leukocyte trafficking. In contrast to E-selectin, most VCAM-1 deficient mice die during development, indicating that VCAM-1 is required for mouse development. Interestingly, a single mouse homozygous for the VCAM-1 gene disruption has been identified indicating that VCAM-1 expression is not absolutely essential for development. Further characterization of the E-selectin and VCAM-1 deficient mice will be presented.

E 524 HUMAN PROTEIN C INHIBITS SELECTIN-MEDIATED CELL ADHESION : ROLE OF UNIQUE FUCOSYLATED OLIGOSACCHARIDE. S. Betty Yan, Robert B. Hermann and Brian W. Grinnell, Cardiovascular Research and Supercomputer Applications and Molecular Design, Eli Lilly and Company, Indianapolis, IN 46285-1543.

The human anticoagulant factor, Protein C, is a plasma glycoprotein that has reported anti-ischemic and anti-inflammatory properties. To explore potential mechanisms for these reported activities, we examined the effect of Protein C on the process of cell adhesion to vascular endothelial cells, which plays a critical role during inflammatory responses. We show that both human plasma-derived and recombinant Protein C inhibit E-selectin-mediated cell adhesion. This effect was not mediated through the serine protease activity of Protein C, but through its carbohydrates. Using oligosaccharides isolated from recombinant human Protein C, we show that a unique structural determinant

{GalNAcβ(1→4)[Fucα(1→3)]GlcNAcβ(1→•)}, named PC-293, inhibits adhesion with a potency greater than that of the natural ligand for E-selectin, the sialylated Lewis X antigen. Our data suggest the potential mechanism for the reported anti-inflammatory effects of Protein C, and a potential utility of this anti-thrombotic in the treatment of inflammation.

E 525 CYTOSKELETAL INTERACTION OF E-SELECTIN THROUGH ITS CYTOPLASMIC DOMAIN AND ITS ROLE IN NEUTROPHIL ADHESION, Masayuki Yoshida, Anthony Rosenzweig, Nitzan Resnick, and Michael A. Gimbrone,Jr., Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115

E-selectin is a member of the selectin family of adhesion molecules, which is expressed on the surface of activated vascular endothelium (EC), and appeared to play an important role in inflammation. Recently, we have found that E-selectin becomes associated with the EC cytoskeleton during PMN adhesion (Westlin and Gimbrone, *FASEB J.* 6: A1888, 1992). To analyze the role of the cytoplasmic domain of E-selectin in this process, we generated a cytoplasmic truncated (ΔCYTO) form by site-directed in vitro mutagenesis of the wild type (WT) cDNA, and transfected it into COS-7 cells. The surface expression of both WT and ΔCYTO constructs was evaluated by FACS analysis with an antibody specific to E-selectin. PMNs were allowed to adhere to COS-7 cells expressing comparable surface levels of both constructs under non-static conditions (64rpm rotation, 18-23°C) in RPMI-1640+1%FCS. Both the WT and ΔCYTO transfectants showed similar levels of PMN adhesion. The association between E-selectin and cytoskeleton during PMN adhesion was examined. COS-7 transfectants were extracted with non-ionic detergent in the presence of protease inhibitors before, and after, PMN adhesion, and the soluble and insoluble (cytoskeletal) fractions from samples were Western blotted with E-selectin antibodies. Significant amount of E-selectin were detectable in the insoluble (cytoskeletal) fraction of WT transfectants. In contrast, no E-selectin was detected in the insoluble (cytoskeletal) fraction of ΔCYTO transfectants. These data suggest that the cytoplasmic domain of E-selectin mediates interaction with the endothelial cytoskeleton during PMN adhesion, but the initial attachment of PMNs does not appear to depend upon this E-selectin-cytoskeleton interaction. (Supported by NHLBI grant P01-HL-36028)

E 526 L-SELECTIN PROMOTES NEUTROPHIL ADHESION TO FIBRONECTIN AND FIBRINOGEN UNDER CONDITION OF FLOW

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L-selectin plays a crucial role in leukocyte rolling, which is a prerequisite of firm adhesion and leukocyte emigration in postcapillary venules *in vivo*. In an *in vitro* flow chamber, human polymorphonuclear leukocytes can adhere to fibronectin and fibrinogen at shear rates ranging from 0.2 to 1.0 dyn/cm^2. A function-blocking monoclonal antibody to human L-selectin (LAM1-3, 1:250 dilution of ascites) inhibited neutrophil adhesion at 1.0 dyn/cm^2 by approximately 80% but had no significant effect at less than 0.5 dyn/cm^2. A binding control antibody (LAM1-12, 1:250 dilution of ascites) had no effect at any shear rate. In order to exclude unspecific effects of antibody binding, we investigated adhesion of the human pre-B cell line, NALM6, to fibronectin under the same conditions. NALM6 cells do not express L-selectin but VLA-4 integrin, a receptor for fibronectin. NALM6 cells transfected with cDNA encoding for human L-selectin (clone NALM6-IF4), were able to adhere to fibronectin at 1.0 dyn/cm^2, while non-transfected NALM6 cells showed very little adhesion. LAM1-3 but not LAM1-12 antibody blocked approximately 80% of NALM6-IF4 adhesion to fibronectin at 1.0 dyn/cm^2. Neither mAb influenced adhesion of non-transfected NALM6 to fibronectin at any shear rate. These data show that L-selectin confers the ability to adhere to fibronectin at shear rates relevant in microvesssels *in vivo*. Granulocytes appear to require functional L-selectin to bind to fibronectin and fibrinogen under conditions of higher flow.

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E 600 INTRACORONARY LIPOSOME MEDIATED GENE TRANSFER OF HUMAN HLA-B7 INTO MOUSE ISOGRAFTS AT THE TIME OF CARDIAC TRANSPLANTATION PRODUCES ACUTE CELLULAR REJECTION, Alistair I. Fyfe, Abbas Ardehali, Hillel Laks, Aldons J. Lusis, Divisions of Cardiology and Cardiovascular Surgery and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The period between harvest and implantation of solid organ transplants provides a unique opportunity for gene transfer to modify the subsequent immunological response. We have demonstrated gene transfer of a luciferase reporter into functioning mouse heart transplants. In order to determine if an immunologically important protein could be transferred into vascularized cardiac transplants, C57BL/6 mouse hearts (n=3) were harvested and 30ug HLA-B7 DNA + 30ug cationic liposomes were infused into the aortic root and coronary arteries. After one hour the heart was transplanted heterotopically into the abdomen of C57BL/6 recipients. After 12 days transplanted and recipient hearts were removed for histologic examination. All hearts functioned until the time of harvest. Histology showed moderate cellular rejection in HLA-B7 transfected isografts with widespread mononuclear cell infiltrates and myocyte necrosis. These changes were not detectable in recipient hearts or luciferase transfected cardiac isografts. Gene transfer and expression of an immunologically relevant molecule into functioning vascularized mouse heart transplants is associated with the development of acute cellular rejection. This model should allow the development of gene based therapies in solid organ transplantation to modify the immune response.

E 601 **NOVEL GENE PRODUCT DELIVERY SYSTEM UTILIZING HUMAN APOLIPOPROTEIN E TRANSDUCED CAPILLARY ENDOTHELIAL CELLS IN A THREE-DIMENSIONAL MATRIX**, Scott P. Kennedy, *Jonathan D. Smith, Willis V. Burton, Jeremy P. Springhorn, Stephen, P. Squinto, #Joseph A. Madri and George B. Zavoico, Departments of Cardiovascular Biology and Molecular Development, Alexion Pharmaceuticals, New Haven, CT 06511, *Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York, NY 10021, # Department of Pathology, Yale University School of Medicine, New Haven, CT 06510

Capillary endothelial cells(CEC) represent an ideal vehicle for gene therapy. CECs have a large surface area that is located strategically at the blood-surface interface, enabling secretion of gene products directly into circulation. *In vitro*, CECs can be grown in three-dimensional(3-D) collagen gels where they form capillary vessel-like structures in response to transforming growth factor beta(TGF-β). To determine if a 3-D CEC gel could provide an efficient vehicle for gene product delivery, rat CEC were grown in a 3-D collagen gel, incubated with TGF-β, and implanted subcutaneously into rats. An angiogenic response to the gel was evident after 1 week and CEC gels were recoverable at least 5 weeks post-implantation. Histological analyses revealed vessel-like lumens containing erythrocytes deep within the gel suggesting a physical connection between implanted and existing capillaries. To determine if genetically modified CEC could function in a 3-D CEC collagen gel, CEC were retrovirally transduced with human Apolipoprotein E(ApoE). In humans, ApoE-deficiency results in an abnormal plasma lipoprotein pattern and an increased risk of atherosclerosis. Thus, ApoE replacement by gene therapy may have significant clinical potential. Northern blots of transduced CEC showed the appropriate size ApoE viral transcript. Transduced CEC grown in 2 or 3-D culture secreted similar levels of immunoreactive ApoE gene product into culture media as determined by western blotting. We are currently evaluating if the gene product of transduced 3D-CEC implants is secreted into the circulatory compartment and is detectable in the serum of the recipient.

E 602 HIGH LEVEL GENE TRANSFER TO HUMAN ARTERIAL SMOOTH MUSCLE CELLS USING ADENOVIRUS-TRANSFERRIN-POLYLYSINE/DNA COMPLEXES. Joel M Kupfer, Guoging Liu, Johan Tran, Vishva Dev, Frank Litvack, and James Forrester Division of Cardiology, Cedars-Sinai Medical Center, Los Angeles, CA 90048

Transfer of DNA to vascular smooth muscles cells using chemical or physical means is inefficient and results in low levels of expression. Recombinant viruses offer an attractive means of achieving efficient gene transfer, which will be necessary for application of gene therapy to cardiovascular diseases. Retroviruses have been used, but often lack predictable results, and are associated with neoplastic transformation. Adenoviruses, have recently gained widespread attention as gene transfer vectors because they can be produced in high titer, do not require cell replication to achieve infection, and are not associated with neoplastic transformation in humans. It has recently been demonstrated that coupling of adenovirus to polylysine/DNA complexes results in efficient transfer of DNA into CHO and epithelial cells. In this system the adenovirus functions to disrupt the endosomes, freeing the DNA to be translocated to the nucleus. To determine whether this vector system can be applied to cardiovascular diseases we used this system to transfect human vascular smooth muscle cells (hSMCs). Adenovirus was coupled to transferrin-polylysine/ plasmid SV40CAT (CAT reporter gene driven by SV40 promoter),and exposed to cultured hSMCs. CAT assay was performed 24 hours later using phase-separation method. Levels of expression were compared to plasmid alone versus lipofectin-mediated transfection. Results were as follows.

Transfection Method	CAT ASSAY (CPM/UG cell protein)
control	32.3±5.1
plasmid alone	27.7±2.4
lipofectin-mediated transfection	483±13
Adenovirus-Mediated	12118±110

Using the E.coli β-galactosidase gene, driven by the CMV promoter, as the reporter gene we next compared transfection efficiencies. Lipofectin-mediated transfer resulted in <10% efficiency, where as adenovirus-mediated transfer ($5x10^9$ particles/ml) resulted in nearly 100% efficiency after 1 hour of exposure. When human transferrin was removed from the complexes, transfection efficiency was reduced. We conclude that adenovirus-transferrin-polylysine/DNA complexes offer an attractive means of achieving high level expression in hSMCs. These cells poses receptors for adenovirus, and transferrin augments transfection efficiency. In. vivo studies are currently in progress.

E 603 AUTOCRINE/PARACRINE RENIN ANGIOTENSIN AS A DETERMINANT OF VASCULAR STRUCTURE: A GENE TRANSFER EXPERIMENTAL APPROACH, Ryuichi Morishita, Gary H Gibbons, Kristin E Ellison, Lunan Zhang, Yasufumi Kaneda*, Toshio Ogihara*, Victor J Dzau. Falk Cardiovascular Research Center, Stanford Univ., Stanford, CA, *Osaka University Medical School, Osaka, Japan.

Vascular remodeling in hypertension involves an increase in vascular cell growth. The process of vascular hypertrophy is associated with increased expression of angiotensin converting enzyme (ACE) activity within the vasculature. Previously, we demonstrated that the vascular renin angiotensin system (RAS) modulates vascular smooth muscle cell growth in vitro via the autocrine/paracrine production of angiotensin (Ang) II using a highly efficient viral-mediated gene transfer method (HVJ). However, the relative role of the autocrine/paracrine RAS vs. the endocrine RAS as the mediator of vascular hypertrophy in vivo is unknown. To define the role of the vascular RAS as a determinant of vascular structure in vivo, we transfected the angiotensin converting enzyme (ACE) cDNA into intact rat carotid arteries by the HVJ method (15 minute intraluminal incubation). At 3 days after ACE gene transfection, we detected increased ACE activity in vessels that was localized in the medial layer by immunohistochemistry. The increase in vascular ACE activity was associated with a parallel increase in DNA synthesis as assessed by BrdU index and DNA content. This increase in BrdU labeling was abolished by the administration of an Ang II receptor specific antagonist (DuP 753). The induction of vascular hypertrophy was evident by an increase in wall : lumen ratio at 2 weeks after transfection. Similarly, total vascular protein content was increased in ACE-vector transfected vessels as compared to control vector transfected vessels. **Conclusion:** In summary, this study provides the first demonstration that increased local expression of ACE within the vessel wall promotes autocrine/paracrine Ang II generation and results in Ang II mediated vascular hypertrophy in the intact animal.

E 604 HIGHLY-EFFICIENT IN VIVO GENE TRANSFER INTO INJURED VASCULAR WALL CELLS USING REPLICATION-DEFECTIVE RECOMBINANT ADENOVIRUS, Hikaru Ueno, Li Jian Jun, Pan Yan, Kenji Sadamatsu, Yasunori Sugiyabu and Akira Takeshita, Department of Cardiology, Kyushu University School of Medicine, Fukuoka, 812 Japan

It has been reported that a recombinant gene can be delivered to a specific site of artery *in vivo* by means of infection with a retroviral vector or *in situ* transfection of DNA-liposome complexes. The principal problem associated with these methods to date is their low efficiency of gene transfer (less than 0.1% of cells were transfected). We tested a recombinant adenoviral vector for *in vivo* gene transfer into the balloon-injured arterial wall in animal models. We prepared a replication-defective recombinant adenovirus containing the *E. coli lacZ* gene encoding β-galactosidase (β-Gal) *under* SRα promoter. Almost 100 % cells in cultures of vascular smooth muscle cells (VSMC) of bovine, rabbit and canine origins as well as endothelial cells of porcine and bovine origins showed high level expression of β-Gal after 60 min exposure with the recombinant adenovirus. Total β-Gal activity in VSMC infected with the virus was 500 times higher than that in cells transfected with DNA-liposome complexes 7 d after transfection. The infection did not alter cell shapes and expression of smooth muscle myosin heavy chain gene. In canine and rabbit models, we achieved high level expression of β-Gal assessed by X-Gal staining in balloon-injured as well as intact iliofemoral arteries by *in vivo* gene transfer through a double-balloon catheter. The expression of β-Gal sustained more than 3 weeks. Our delivery system allowed us to introduce the gene repetitively to the same site, and we confirmed that multiple attempts enhance the efficiency of gene transfer *in vivo*. The promoter-*lacZ* sequence was not detected in other tissues by PCR analysis. Since adenoviral vector is able to transfer and express the exogenous gene in non-dividing cells as well as proliferating cells with this high efficiency, it is promising to deliver a recombinant gene to the vessel wall efficiently enough to consider as a therapeutic means. Currently dominant-negative mutations of growth factor receptors are being introduced to the injured arterial segment of intact animals to explore the possibility to prevent restenosis after coronary interventions.

Late Abstract

INHIBITION OF INTIMAL HYPERPLASIA BY ANTISENSES C-MYC AND C-MYB IS MODULATED BY THE DURATION OF THE OLIGONUCLEOTIDES DELIVERY.

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One of the major side effects following arterial injury is neointimal hyperplasia composed of proliferating and migrating smooth muscle cells (SMC). Several investigators have shown that proliferation of SMC *in vitro* may be mediated in part through the expression of c-myc and c-myb proto-oncogenes. Although both proto-oncogenes are involved in this process, they have different time course of expression. We studied whether local vascular delivery of antisense oligonucleotides affects the neointima formation following catheter injury of the rat carotid artery. Antisense c-myc and c-myb oligonucleotides were embedded in pluronic gel or ethylene-vinyl acetate copolymer (EVAc). These polymers were then applied to the adventitial aspect of the artery and the rats were sacrificed 14 days after angioplasty. The pluronic gel provides a rapid and transient delivery of the oligonucleotides (within 2 hrs) whereas the EVAc polymer provides a constant and sustained delivery (over 14 days) of the oligonucleotides. The application of antisense c-myb with pluronic gel or EVAc reduced the accumulation of SMC in the intima by 90 and 72% as compared to scrambled controls. However, the antisense c-myc reduced restenosis by 94% only when applied with EVAc polymer and not in pluronic gel. These results suggest that 1) both c-myb and c-myc are involved in neointima formation and 2) inhibition of neointimal formation can be achieved by matching kinetics of target gene expression to the mode of inhibitor delivery.