PERSPECTIVES IN INFLAMMATION, NEOPLASIA AND VASCULAR CELL BIOLOGY

Thomas Edgington, Russell Ross and
Samuel Silverstein, Organizers
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Molecular Biology of Cell Surface Receptors

0330 INDUCIBLE RECEPTORS FOR ADHESIVE PROTEINS ON PLATELETS. Edward F. Plow, Mark H. Ginsberg, Research Institute of Scripps Clinic, La Jolla, California, and Gerard Marguerie, INSERM U-143, France.

Four proteins—fibrinogen (Fg), fibronectin (Fn), von Willebrand Factor (vWF), thrombospondin (TSP), which share the features of being large, glycosylated and multimeric, directly mediate or modulate the platelet adhesive reactions of attachment and spreading on subendothelial matrices and the formation of platelet aggregates. Using fibrinogen as a model, we have shown that this protein does not bind to resting platelets with high affinity. In contrast, when platelets are stimulated with a wide variety of physiologic and pharmacologic stimuli, specific binding sites for Fg come available. This interaction occurs with an affinity of 2 μM^{-1}, and 20,000-80,000 Fg molecules are bound per platelet. Four distinct steps have been delineated during this interaction: a) encounter of the platelet with an agonist; b) induction of the Fg receptor; c) reversible binding of Fg to the cell surface; and d) stabilization of platelet-bound Fg in a non-dissociable state. Platelet aggregation occurs as a result of reversible Fg binding, and stabilization of bound Fg renders platelet aggregates less suceptible to dissociation. Reversible binding is divalent ion dependent and is the rate determining step of the interaction, whereas EDTA does not dissociate Fg bound in its stabilized state. Fg binding is mediated by specific peptide sequences within the molecule, and one site has been localized to the extreme C-terminal of the gamma chain. Peptides of 10-12 amino acids from this region prevent fibrinogen binding by a mechanism consistent with that of competitive antagonism. These peptides do not prevent receptor induction or dissociate stabilized Fg from the platelet. The concept of inducible receptors is not restricted to Fg. Thrombin also stimulates platelet binding of Fn, vWF and TSP. The fact that Fn and vWF binding is inhibited by the same set of Fg gamma chain peptides suggests that a common mechanism may be involved in these interactions. In the case of Fn, it has also been possible to define a discrete platelet recognition specificity. RGDS, a region of Fn involved in fibroblast attachment, inhibits Fn binding to platelets. Thus, the interactions of these adhesive proteins with platelets provide examples of inducible receptor systems. The platelet is an excellent model cell for defining recognition specificities within these adhesive proteins and for ultimate identification of their cellular receptors.

Cellular Receptors and Growth Factors

0331 MOLECULAR CHARACTERIZATION OF BOVINE AND HUMAN BRAIN FIBROBLAST GROWTH FACTORS. Peter Böhlen, Andrew Baird, Fred Esch, and Denis Gospodarowicz*. Laboratories for Neuroendocrinology, The Salk Institute, La Jolla, CA 92037 and *Cancer Research Institute, University of California, San Francisco, CA 94143.

Fibroblast growth factor (FGF) has been purified to homogeneity from bovine and human brain by tissue extraction at pH 4.5, cation-exchange chromatography, Heparin-Sepharose affinity chromatography and reverse-phase HPLC. Using the proliferation of bovine aortic endothelial cells in culture as a bioassay, four forms of FGF were isolated from bovine brain. The quantitatively major form possesses the amino-terminal sequence Pro-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala-Phe-Pro-Pro-Gly which is identical to that of bovine pituitary FGF (Böhlen et al, PNAS 81, 5364, 1984). Furthermore, antibodies raised against the amino-terminal synthetic nonapeptide recognize all four FGF forms. This and other experimental evidence (molecular weight ~ 16 kD, amino acid composition and pI ~ 9.6) suggests that basic bovine brain FGFs are closely related in structure to pituitary FGF. Using the same methodology, one form of FGF was isolated from human brain. Human FGF is, by the above criteria, very similar to bovine FGF. The amino terminal sequences are identical except for a replacement of Asp^{6} by a Glu residue in human FGF. When bovine brain was extracted at pH 7 five forms of FGF were isolated. Of those one form is not related to pituitary FGF (no crossreactivity with antibodies, different amino acid composition). Further characterization of this FGF form will establish whether it is related to the acidic FGF (Thomas et al. PNAS 81, 357, 1984).

0332 FIBRINOGEN(fg)-ENDOTHELIAL CELLS(EC)INTERACTION,Elisabetta Dejana,Lucilla Languino,Giovanna Balconi,Nadia Polentarutti,Alberto Mantovani,Gerard Marguerie.Mario Negri Institute,Milan,Italy and INSERM U 143,Le Kremlin-Bicetre,France.

Cultured human and bovine EC migration was studied using the Boyden chamber tecnique.Highly purified human fg in the lower compartment of the chamber caused a time and concentration dependent migration of EC across filters.Trypsin or plasmin digestion of fg with specific Fab fragments completely abolished fg-induced EC migration.Dyalisis of fg did not modify fg-induced migration.Ion exchange chromatography on DEAE cellulose showed that only the fraction associated with fg was able to induce EC migration.Plasma obtained from healthy donors induced EC migration but plasma from an afibrinogenemic patient was completely ineffective.Plasmin degradation fragments D and E of M.W. 100,000 and 50,000 respectively did not induce EC migration but fragment E caused dose related inhibition of fg-induced migration Direct interaction of 125I-fg with EC was observed.Binding was time dependent,specific,saturable and inhibited by monospecific Fab fragments directed to fg .Purified fragment E inhibited binding by 44% but fragment D was completely ineffective.Autoradiography of the display of EC-bound 125I on polyacrylamide gel showed the constitutive B_{β} and γ chains of the fg molecule with a partial loss of the A_{α} chain.The three chains were present in the extract of 125I-fg present in the supernatant at the end of the binding experiment.Thus fg is able to induce EC migration and to specifically associate with these cells.

0333 A PROLIFERATION DEPENDENT CYTOTOXIC PEPTIDE
L. David Dion, Larry R. Smith, and J. Edwin Blalock
University of Texas Medical Branch, Galveston, Texas 77550

We have isolated and purified a 2100 dalton peptide which is specifically cytotoxic for proliferating cells. We have been able to isolate this peptide from pituitary, spleen, and brain tissues. Mouse kidney and muscle and human peripheral lymphocytes did not have detectable levels. The peptide has been purified by size exclusion on Bio-gel P-4, ion exchange on carboxymethyl cellulose, and affinity chromatography. Following this purification procedure, the peptide elutes as a single peak on RP-HPLC. The cytotoxic action of this peptide has been demonstrated in vitro against primary and established cell lines derived from human, monkey, mouse and hamster and thus is not species or transformation specific. The cytotoxic effect is, however, dependent on cell proliferation. When serum depleted (0.5% serum), quiescent cultures of mouse embryo fibroblasts are treated with the cytotoxic peptide, the cells are resistant to the cytotoxic effect. Duplicate cultures stimulated overnight with 10% serum are killed within 24 hours of treatment. After exposure to the peptide, susceptible cells become committed to die within two hours with the mean time to death occurring by 4 hours after treatment. The proliferation dependence of the cytotoxic activity can also be demonstrated using mitogen stimulated mouse spleen cultures. This peptide may represent a new class of immunoregulatory and/or antineoplastic molecules.

0334 THE PRESENCE OF INTERLEUKIN-1 IN NORMAL HUMAN STRATUM CORNEUM. Lorise C. Gahring and Raymond A. Daynes. Dept. of Pathology, University of Utah School of Medicine, Salt Lake City, Utah 84132.

Interleukin-1 (IL-1) is a 15 kd polypeptide hormone that plays an integral part in the generation of both immune and inflammatory responses. Pyrogenicity, neutrophil chemotaxis, lymphocyte activation and chemotaxis, muscle proteolysis and the production of acute phase proteins are all effects induced by the action of IL-1 on various anatomical sites including the brain, bone marrow, lymphoid tissue, muscle and liver. Two known producers of IL-1 are activated macrophages plus the keratinocytes of the skin. While macrophages are of mesodermal origin, keratinocytes are of ectodermal origin and potentially serve as a major source of this hormone following perturbation of the skin. Keratinocytes form in the germinative basal layer, differentiate, and enucleate to eventually form the outermost layer of the skin known as the stratum corneum (SC). Based on the information that the precursors of cells found in the SC are active producers of IL-1, plus reports of the profound inflammatory nature of this material implanted intradermally, we questioned whether IL-1 could be isolated from SC obtained from normal human volumteers. Extraction of normal SC with a physiologic saline solution yielded a tremendous amount of activity, as measured by the thymocyte co-stimulatory assay. To eliminate the possibility that this co-stimulator activity was do to a bacterial contaminate, SC was aseptically taken from a newborn human immediately following cesarian section delivery. A significant amount of IL-1 was found in an extract derived from this sterile source. Stratum corneum-derived IL-1 1) eluted off a sizing column with an approximate molecular weight of 15 kd, and 2) had three isoelectric point forms which separated on a chromofocusing column. By these physiochemical characteristics, the SC-derived IL-1 was found to be indistinguishable from macrophage and keratinocyte derived IL-1. Further, biologic effects known to be induced by IL-1, such as an elevation in core body temperature, neutrophilia, and an increase in plasma levels of acute phase proteins, were all induced by the injection of SC-derived IL-1 into endotoxin non-responsive mice. Our studies show that IL-1, a major pro-inflammatory mediator, is present in normal SC.

0335 PURIFICATION OF TUMOR-DERIVED ANGIOGENESIS FACTORS, Michael Klagsbrun, Judah Folkman, Dennis Lund, and Yuen Shing, The Children's Hospital, Boston, MA 02115
Capillary development is a complex phenomenon that includes among its components the migration and proliferation of capillary endothelial (CE) cells. Growth factors capable of stimulating the proliferation of CE cells in vitro at concentrations of 1-10 ng/ml were isolated from a transplantable rat chondrosarcoma and from a human hepatoblastoma carried in nude mice. The growth factors were purified to apparent homogeneity by a combination of BioRex 70 cation exchange chromatography and heparin-sepharose affinity chromatography. Analysis by HPLC TSK 2000 size exclusion chromatography and SDS PAGE indicated that the tumor-derived growth factors had a molecular weight of about 18,000. The tumor-derived growth factors were capable of inducing angiogenesis in vivo on the chorioallantoic membrane (CAM) of the 9-day old chick embryo; the yolk sac membrane of the 4-day old chick embryo and in the rat cornea. In the yolk sac membrane and CAM bioassays about 500 ng (500 units) of growth factor stimulated a strong angiogenic response within 24 hours in over half the eggs tested. Histological examination showed the presence of numerous vessels streaming towards the implantation site without any inflammatory response. About 10-20 ng (10-20 units) of tumor-derived growth factor were implanted into the corneal pocket and after six days, vessels were seen sprouting from the limbus towards the implant in 7 of the 8 corneas tested. Although capillary cell development and growth is a complex multi-step process, our results suggest that a single CE cell growth factor is sufficient to induce the total angiogenic response.

0336 **AN IMPROVED ASSAY FOR THE DETECTION OF INTERLEUKIN 1,** James W. Larrick, Michael Doyle, Leah Brindley, Cetus Immune Research Labs, Palo Alto, CA, 94303.
Interleukin 1 (IL-1) comprises a family of proteins derived from mononuclear phagocytes and other cells with a variety of functions, including enhancement of T cell proliferation to mitogens and antigens, augmentation of B cell responses, mediation of fever and the acute phase response, stimulation of fibroblast growth and secretion of collagenase. We describe an overnight, highly sensitive assay for detection of IL-1. A thioguanine resistant mutant of murine lymphoma cell line LBRM-33 was selected. When this cell line is incubated with low concentrations of PHA and IL-1 it produces interleukin 2 (IL-2). IL-2 dependent HT-2 cells are cocultured with the LBRM cells to measure the released IL-2. Prior to the addition of tritiated thymidine to the cocultured cells, hypoxanthine and azaserine are added to the cells to metabolically block DNA synthesis of the mutant LBRM cells. The results of this highly sensitive 24-hour IL-1 assay will be presented.

0337 MURINE MARROW STROMAL CELL PRODUCTION OF A CSF-1 DEPENDENT GROWTH FACTOR, P.J. Quesenberry, Z. Song, R. Shadduck and A. Waheed, University of Virginia School of Medicine, Charlottesville, VA 22908 and University of Pittsburgh, Montefiore Hospital, Pittsburgh, PA 15213
Murine long term marrow cultures provide a model for the stromal regulation of hemopoiesis. We have isolated a cell line (TC-1) from the adherent stromal cells in murine marrow liquid culture. Conditioned media (CM) from the line contains from 384 to 638 units of CSF-1 per ml as assayed by radioimmunoassay. The CM stimulates giant (over 1 mm in diameter) macrophage colonies at 14 days of culture. This colony formation is blocked by antibody to CSF-1 and is not seen with up to 4545 units pure CSF-1 per ml of culture. CSF-1 can be separated from a separate synergistic activity by anti-CSF-1 antibody column chromatography. The run-through fraction from this separation has no intrinsic colony stimulating activity, but in the presence of CSF-1 induces giant macrophage colonies. Stimulation of giant macrophage colony growth by TC-1 CM was not blocked by antisera to interleukin-3. This CM was also capable of producing secondary adherent murine marrow cell lines which after 3-4 weeks of passaging produced conditioned media supporting their own growth and stimulating giant macrophage colonies. The TC-1 CM was negative for reverse transcriptase assay and high speed pelleting did not remove activity from TC-1 supernatants. Whether the cell line inducing activity and the giant macrophage activity are identical await further biochemical characterization. The stromal derived synergistic activity may be a regulator of early hemopoietic cell differentiation.

0338 BIOLOGIC ACTIVITY OF PDGF-RELATED SEQUENCES EXPRESSED IN YEAST, Elaine W. Raines, Jim Kelly, Mark Murray, and Russell Ross, University of Washington and ZymoGenetics, Seattle, WA 98195

The transforming gene of simian sarcoma virus encodes a protein, $p28^{sis}$, whose predicted sequence is homologous to one of the two distinct chains of the platelet-derived growth factor (PDGF) isolated from platelets. This homology begins at residue 67 and appears to extend through residue 175 of the predicted $p28^{sis}$ sequence. In an attempt to determine whether this protein sequence was sufficient to mimic the biological activity of PDGF, plasmids were constructed to direct synthesis of varying portions of $p28^{sis}$ in the yeast Saccharomyces cerevisiae. Constructions spanning residues 1-226 and 67-226 of the $p28^{sis}$ sequences result in expression of proteins that are mitogenic for monolayer cultures of 3T3 cells and are capable of competing with ^{125}I-PDGF for binding to the PDGF receptor. The mitogenic activity of both constructions can be completely blocked by preincubation with anti-PDGF antibodies. Analysis of the mitogenically active proteins expressed in yeast, by gel filtration on Sephacryl S-200 in 1.0 N. acetic acid, revealed multiple species with varying molecular weights. In the case of the polypeptide spanning residues 67-226, approximately 60% of the mitogenic activity elutes with an apparent molecular weight greater than 200,000 (presumably multimers or highly glycosylated products); approximately 25% with an apparent molecular weight of 40,000 (consistent with the molecular weight of a dimerized molecule); and 3% with an apparent molecular weight of 16,000 (the approximate molecular weight of a single chain). Combined, these data directly demonstrate that the $p28^{sis}$ sequence homologous to PDGF is sufficient to induce a mitogenic response and suggest that a single chain molecule that is not disulfide bonded to another chain (as determined by gel filtration) is also capable of stimulating a mitogenic resonse.

0339 SECRETORY CELLS FROM MOUSE BONE MARROW STROMA SUPPORT EARLY B CELL GROWTH, Debra Robertson and Owen N. Witte, UCLA, Los Angeles CA 90024

Our laboratory has previously described the in vitro long term growth of murine pre B and B lymphocytes. These B cell populations are dependent for growth and viability on an adherent cell layer derived from the mouse bone marrow stroma. We have isolated and cloned this stromal cell which supports the long term B cell growth. The cells are irregular in shape with large numbers of dendritic processes which interconnect the cells. Ultrastructural analysis shows that these cells unlike other hematopoietic cells contain numerous, large cytoplasmic granules. The granules are osmiphilic and appear to be contained in large vacuoles. Histochemical and biochemical analysis show that the cells are devoid of phenotypic markers found on other hematopoietic accessory cells. These results suggest that these stromal cells may share a commom lineage with other granulated exocrine cells found outside the bone marrow.
We have shown the B cell growth activity is secreted by the adherent cells into the culture supernatant. This activity can be detected by measuring the uptake of ^{3}H-thymidine by B cells. Partial purification of this activity shows that this activity is a basic, trypsin senstitve polypeptide. Gel filtration studies showed that the activity elutes in a single peak at a molecular weight of 12,000 daltons. (SDS-PAGE= 16KD)

0340 CHARACTERIZATION OF GROWTH FACTOR AND APOLIPOPROTEIN PRECURSORS, J. Scott*, T. Knott*, L. Rall[7] and G. Bell[7], *MRC Clinical Research Center, Harrow, UK and [7]Chiron Corporation, Emeryville, CA 94608, USA.

We have recently characterized the precursors for epidermal growth factor (EGF), nerve growth factor (NGF) and human insulin-like growth factors (IGF) I and II from cDNA clones. EGF precursor is infered to be a protein of 133 kd with at least 7 EGF-like peptides and a membrane spanning domain encoded in the precursor. EGF mRNA is found at high levels in the mouse submaxillary gland and kidney. In the submaxillary gland low molecular weight immunoreactive EGF is produced, whereas in the kidney the high molecular weight precursor is not processed. IGF I and II precursors are predicted to have NH_2 terminal signal peptides linked directly to the mature protein and COOH terminal extensions of 35 and 89 amino acids residues respectively. The precursor for apoprotein (apo) AII has a signal peptide and a five residue propeptide. The gene for apo AII has also been characterized and shown to have a similar intron/exon structure to apo AI and apo E indicating a close evolutionary relationship. The apo AII gene is on human chromosome 1q in a conserved linkage group with genes specifying renin, antithrombin III and one subunit of the basement membrane laminin. Comparative gene mapping indicates that there is a high density lipoprotein regulator locus in this grouping.

0341 ACTIVATED MACROPHAGES SECRETE A PDGF-LIKE MOLECULE, Kentaro Shimokado, Elaine W. Raines, and Russell Ross, University of Washington, Seattle, WA 98195.
Peripheral human blood monocytes, activated to become macrophages by agents such as endotoxin, or activated peritoneal or lung macrophages, all produce a growth factor (MDGF) in vitro that has been shown to be mitogenic for many mesenchymal cells including endothelial cells. Medium conditioned by macrophages has been partially purified by chromatography on G-100 in 1.0 N acetic acid. A fraction with a molecular weight of approximately 30,000 can be isolated from both human alveolar and peritoneal macrophages which is mitogenic for 3T3 cells and that competes with ^{125}I-PDGF for binding to the PDGF receptor. Mitogenic activity and competition for binding to the PDGF receptor can be blocked by preincubation of the G-100 fraction with anti-PDGF antibody. Lack of recognition by anti-PDGF IgG of nonpurified macrophage-conditioned medium in earlier studies was apparently due to the presence of binding protein(s) which, after removal during partial purification of MDGF, resulted in the observation that much of the growth factor activity released by activated macrophages is due to secretion by the macrophages of a PDGF-like molecule.

0342 UTERINE EGF RECEPTORS: CHARACTERIZATION AND INDUCTION BY ESTROGEN, George M. Stancel and Venkat R. Mukku, Univ. of Texas Medical School, Houston, Texas 77025. Uterine membranes contain high affinity (K_d=0.36 nM), saturable binding sites for ^{125}I labelled epidermal growth factor (EGF). The binding of ^{125}I-EGF is specific since it is abolished by excess unlabelled EGF but not by excess unlabelled insulin, fibroblast growth factor or multiplication stimulating activity. Incubation of ^{125}I-EGF with uterine membranes, followed by chemical cross-linking with disuccinimidyl suberate and detergent extraction reveals a major species of specifically bound EGF (MW = 170,000) and a minor species (MW = 150,000) visualized by autoradiography of SDS gels after electrophoresis of the extracts. In detergent solubilized preparations EGF also stimulates the phosphorlation of major (MW = 170,000) and minor (MW = 150,000) species of identical molecular weight. The increased phosphorylation produced by incubation of membrane extracts with EGF occurs largely at tyrosine residues, as indicated by phosphoamino acid analysis. These results indicate that the rat uterus contains high affinity EGF binding sites with the properties expected of EGF receptors. Treatment of ovariectomized female rats with physiological doses of estradiol leads to a 2-3 fold increase in levels of measurable EGF receptors as determined by ^{125}I-EGF binding and cross-linking experiments. EGF receptor levels increase between 6-12 hours after hormone treatment, and remain elevated up to 24 hours after estrogen administration. These results suggest that EGF may be involved in regulating estrogen mediated uterine growth.

0343 LEUKEMIA DERIVED GROWTH FACTOR(LDGF)-LIKE ACTIVITY IN THE CYTOPLASM OF HUMAN MALIGNANT LYMPHOID CELL LINES, C.H.Uittenbogaart and D.J.Anisman, UCLA School of Medicine, Los Angeles, CA 90024
We have previously shown that several human T leukemia cell lines produce an autostimulatory growth factor(LDGF). This growth factor differs from Interleukin-2 in biological and physicochemical properties. Autostimulatory factors may be secreted and interact with growth factor receptors at the cell surface or activate the receptor intracellularly. In order to determine if an LDGF-like activity was present in the cytoplasm of T leukemia cells, we studied the effect of a cytoplasmic filtrate of the malignant T lymphoid cell lines MOLT-4f, CCRF-CEM AND CCRF-HSB-2 on these cell lines and on the B lynphoblastoid cell line BJAB and the myeloid cell line KG-1. All the cell lines were cultured long-term in serum-free medium(AT-IMDM). Cell filtrates of the T lymphoid cell lines did contain an autostimulatory growth promoting activity and also stimulated the growth of the other T lymphoid cell lines. These cell filtrates did not contain growth promoting activity for the B lymphoblastoid cell line BJAB or for the myeloid cell line KG-1. Cell filtrates of malignant T lymphoid cell lines contain a LDGF-like autostimulatory growth factor activity which may be important for the independent proliferation of T leukemia cells.

Growth Factors and Their Generation

0344 PARTIAL PURIFICATION OF MACROPHAGE ANGIOGENESIS FACTOR AND ITS RELATIONSHIP TO MACROPHAGE GROWTH FACTORS, M.J. Banda, A.G. Rice, and B. Halliday. Laboratory of Radiobiology and the Department of Surgery, University of California, San Francisco.

An angiogenesis factor (AF) has been partially purified from serum-free culture medium conditioned by 12-14 day old rabbit bone marrow derived macrophages. Macrophages were induced to express AF by hypoxic (2% oxygen) culture conditions (1). No immunologic or inflammatory agents, which can stimulate macrophages to release AF (2), were used. The conditioned medium was dialyzed, lyophilized, and reconstituted to 1% of the original volume with 10mM acetic acid. Size exclusion HPLC of the acid soluble fraction resolved a peak with angiogenic activity from a peak with mitogenic activity for Balb/3T3 and rabbit fibroblasts (RF), suggesting that macrophage AF is not the macrophage derived growth factor, MDGF, (3). At this stage, the AF peak contained slight activity for rabbit brain capillary endothelial cells (RBCE). Further fractionation by anion exchange HPLC, resulted in a peak of macrophage AF that had no mitogenic activity but stimulated the growth of capillaries in the corneal implant assay and the directed migration of RBCE and RF. At this level of purification, angiogenic and migratory activity was detectable at 1-10 ng/ml. The UV absorption spectrum was consistent with that of a peptide or protein. The macrophage AF eluted with a retention time similar to that of AF purified from rabbit wound fluid, which is also a nonmitogenic chemotactic factor for capillary endothelial cells (4). When mixed in equal proportions, the macrophage AF and the wound fluid AF eluted as a single peak. Macrophages are required for wound healing (5), and these data suggest that one of their essential functions is to regulate angiogenesis during the reparative stage of healing.

1. Knighton, D.K., T.K. Hunt, H. Scheuenstuhl, B.J. Halliday, Z. Werb, and M.J. Banda. 1983. Science 221:1283-1285.
2. Polverini, P.J., R.S. Cotran, M.A. Gimbrone, Jr., and E.R. Unanue. 1977. Nature (London) 269:804.
3. Martin, B.M., M.A. Gimbrone, Jr., E.R. Unanue, and R.S. Cotran. 1979. J. Immunol. 126:1510.
4. Banda, M.J., D.K. Knighton, T.K. Hunt, and Z. Werb. 1982. Proc. Natl. Acad. Sci. U.S.A. 79:7773.
5. Leibovitch, S.J., and R. Ross. 1975. Am. J. Pathol. 78:71.

0345 ENDOGENOUS SOURCES OF PLATELET-DERIVED GROWTH FACTOR-LIKE MOLECULES: POSSIBLE ROLES IN VASCULAR BIOLOGY, Daniel F. Bowen-Pope, Department of Pathology, University of Washington, Seattle, WA 98195.

Release of platelet-derived growth factor (PDGF) from platelets has been postulated to stimulate at least some of the cell proliferation seen at sites of tissue damage, both beneficially (wound healing) and perniciously (during formation of atherosclerotic lesions). PDGF-like molecules (PDGFc) which bind to the PDGF receptor and are recognized by anti-serum against PDGF may also be synthesized by vessel wall cells themselves under certain circumstances: **1)** Cultured arterial endothelial cells secrete several mitogens, one of which is a PDGFc (1). Release of endothelial cell PDGFc is greatly stimulated by exposure of the cells to concentrations of thrombin which could be achieved at sites of activation of the coagulation cascade, e.g., at sites of vascular injury (Harlan and Bowen-Pope, unpublished). Arterial endothelial cells do not themselves respond to PDGF, but since they are separated from smooth muscle cells (SMC) in vivo by only a few microns, it is possible that this endothelial cell-derived PDGFc acts in a "paracrine" mode to stimulate proliferation and/or synthesis of connective tissue elements by underlying or nearby SMC. **2)** Cultured aortic smooth muscle cells (SMC) from two-week-old rats accumulate mitogenic levels of PDGFc in their culture medium (2). In this case, PDGFc is the only detectable mitogen produced by these cells (when assayed on 3T3 cells). SMC obtained from adult rat aortae secrete 150-fold less PDGFc. Since vascular SMC are responsive to PDGF, it is possible that the PDGFc produced by the pup SMC is functioning as an "autocrine hormone" to stimulate the cells which produce it. This is supported by the observation that the number of cell-surface PDGF receptors is greatly reduced in pup SMC (consistent with occupation and downregulation by endogenously synthesized PDGFc) and by the observation that pup SMC show a reduced dependence on exogenous mitogens for proliferation (3). It is possible that autocrine production of PDGFc contributes in vivo to the rapid proliferation of SMC in pup rat aortae. **3)** Preliminary evidence suggests that autocrine production of PDGFc can be reactivated in adult vessels under certain conditions. Balloon catheter deendothelializaton of the rat carotid artery stimulates an approximate doubling of the number of SMC in the vessel wall within two weeks (4). When SMC are isolated from the intima of a two-week-old lesion, they are found to secrete more PDGFc, and express fewer PDGF receptors, than do SMC from an uninjured carotid (Walker, L.N., Bowen-Pope, D.F. and Reidy, M.A., unpublished). The above observations suggest that synthesis of PDGFc by cells of the vessel wall could play roles in stimulating growth during both normal development and response to injury.

(1) DiCorleto, P.E. and Bowen-Pope, D.F., PNAS 80:1919-1923. (2) Seifert, R.A., Schwartz, S.M., and Bowen-Pope, D.F., Nature, In press. (3) Bowen-Pope, D.F., and Seifert, R.A., Cancer Cells (eds.: Feramisco, J., Ozanne, B., and Stiles, C.) Cold Spring Harbor Press, CSH, NY, In press. (4) Clowes, A.W., Reidy, M.A., and Clowes, M.M., Lab. Invest. 49:324, 1983.

0346 GROWTH FACTORS FROM PLATELETS AND MACROPHAGES: POSSIBLE ROLES IN REPAIR AND DISEASE. Russell Ross, Kentaro Shimokado, Elaine W. Raines, and Daniel F. Bowen-Pope, University of Washington, Seattle, WA 98195.
Both platelets and macrophages have been shown to be involved in the genesis of the lesions of experimentally induced atherosclerosis. Inhibition of platelets results in inhibition of experimental lesion formation. Both platelets and macrophages are also involved in wound repair, and the latter in the proliferative fibrotic responses associated with virtually all chronic inflammatory reactions ranging from chronic granulomatous disease to rheumatoid arthritis. Upon exposure to collagen or thrombin, platelets release platelet-derived growth factor (PDGF), a growth factor also secreted by numerous cells including endothelium and many transformed cells. Platelets contain a number of growth factors, including an epidermal growth factor-like molecule and transforming growth factors, all of which will induce the growth of many different cells and which may play a role in both normal as well as abnormal repair. Platelets also contain numerous chemotactic factors, including PDGF, platelet factor 4, beta thromboglobulin, and 12-HETE. Many of the cellular activities induced by PDGF can be modulated in vitro, and potentially in vivo, by binding proteins present in plasma and possibly in some tissues, and by local alteration and metabolism. Upon activation, peripheral blood monocytes become macrophages, and synthesize and secrete a growth factor that has recently been shown to be a major source of a mitogen that competes for binding to the PDGF receptor. Both mitogenesis and competition for binding are abolished by anti-PDGF IgG, demonstrating that activated macrophages produce a PDGF-like molecule. Thus, genetic expression of PDGF formation by different cells may represent a relatively ubiquitous response through activation of gene(s) controlling the expression of endogenously derived PDGF. Intercellular reactions between platelets and/or macrophages, and appropriately responsive cells, elicits a proliferative response, probably due to PDGF release which in many instances supports tissue repair and which in others may result in manifestations of disease.

Cellular Responses to Growth Factors

0347 REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR, Paul J. Bertics, Wolfgang Weber, Claude Cochet, and Gordon N. Gill, Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, La Jolla, CA 92093
The epidermal growth factor (EGF) receptor is a transmembrane glycoprotein possessing EGF-stimulable protein-tyrosine kinase activity. On EGF stimulation, the receptor rapidly undergoes a self-phosphorylation reaction at tyrosine residues located primarily in the extreme carboxyl-terminal region of the protein (1). Using enzymatically active EGF receptor purified by immunoaffinity chromatography, we have characterized the self-phosphorylation reaction as an intramolecular process exhibiting a Km for ATP (0.2 μM) which is 10-fold lower than that calculated for the phosphorylation of exogenous substrates (2). When the tyrosine kinase activity of the EGF receptor was measured in relation to its self-phosphorylation state, it was observed that self-phosphorylation enhances the ability of the receptor to catalyze the phosphorylation of exogenous substrates. Furthermore, analysis of the dependence of the tyrosine kinase activity on ATP concentration yielded hyperbolic kinetics when plotted in double reciprocal fashion, indicating that the substrate, ATP, is also an activator of the enzyme. It thus appears that self-phosphorylation in the carboxyl-terminal region of the receptor may remove some inhibitory/competitive constraint so that exogenous substrates can have greater access to the substrate binding region of the enzyme.

In addition to self-phosphorylation, the EGF receptor can also be phosphorylated by the calcium- and phospholipid-dependent protein kinase C (3,4). This phosphorylation of the EGF receptor results in decreased self-phosphorylation at tyrosine residues both in vivo and in vitro and in decreased EGF-stimulated tyrosine kinase activity in vivo.

The EGF receptor is thus a key metabolic enzyme regulated by phosphorylation. Self-phosphorylation enhances tyrosine kinase activity, whereas protein kinase C-catalyzed phosphorylation depresses enzyme activity. Because these two phosphorylations account for only a fraction of the total phosphate present in the EGF receptor labeled in vivo (3), other protein kinases can apparently phosphorylate the receptor and these may exert additional controls on the activity of the EGF receptor/kinase.

1. Downward, J., Parker, P. and Waterfield, M. D. (1984) Nature 311, 483-485.
2. Weber, W., Bertics, P. J. and Gill, G. N. (1984) J. Biol. Chem., in press.
3. Cochet, C., Gill, G. N., Meisenhelder, J., Cooper, J. A. and Hunter, T. (1984) J. Biol. Chem. 259, 2553-2558.
4. Iwashita, S. and Fox, C. F. (1984) J. Biol. Chem. 259, 2559-2567.

0348 EARLY EVENTS IN FIBROBLASTS EXPOSED TO PLATELET-DERIVED GROWTH FACTOR
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Although the amino acid sequence of the platelet-derived growth factor (PDGF) receptor is not known, it seems likely that it will resemble the epidermal growth factor receptor and contain a domain with recognizable homology to the *src* family of tyrosine-specific protein kinases. Others have shown that the PDGF receptor becomes phosphorylated at tyrosine when cell membranes, or intact cells, are exposed to PDGF. We have examined the phosphoproteins of cells treated with PDGF for the presence of phosphotyrosine. Five phosphoproteins of 41-45 kDa and approximately neutral isoelectric point contain phosphotyrosine and phosphoserine. In some, but not all, mitogenically-responsive cell types, PDGF also induces the phosphorylation of an abundant protein of the cortical cytoskeleton known as p36. Phosphorylation of the 41-45 kDa proteins is maximal within 5 min of PDGF addition and then decreases, correlating with occupation of the cell surface receptors with ligand. The same proteins are phosphorylated in response to other mitogenic agents, including, in appropriate cell types, serum, EGF, FGF, the insulin-like growth factors, alpha-TGF, thrombin and some tumor promoters. Activation of serine/threonine-specific protein kinase C by tumor promoters can thus stimulate protein phosphorylation at tyrosine, although the identity of the tyrosine protein kinase involved is unknown. We have also found, paradoxically, that protein kinase C can inactivate one tyrosine protein kinase, the EGF receptor protein kinase.

One other rapid response to PDGF is the accumulation of cytoplasmic mRNA transcribed from the c-*fos* gene. The amount of this mRNA is maximal at 20 min and decreases thereafter. Nuclear proteins specified by this gene can also be detected in the first 30 min of PDGF treatment. Since cells constitutively expressing this proto-oncogene exhibit uncontrolled replication, transient expression of the c-*fos* gene induced by PDGF may be important for the mitogenic response. Unlike the phosphorylation of the substrate proteins we have identified, c-*fos* protein synthesis is not noticeably increased by exposure of resting fibroblasts to EGF. Therefore we propose that some other protein substrate for the PDGF receptor protein kinase, that is not phosphorylated by the EGF receptor protein kinase, might mediate the nuclear response to PDGF.

0349 CELLULAR INTERACTIONS IN THE RESPONSE OF VASCULAR VESSEL WALL CELLS TO INJURY,
Stephen M. Schwartz, Department of Pathology, University of Washington, Seattle, WA 98195. The bulk of our attempts at understanding proliferative responses of the vessel wall has been given to the role of factors exogenous to the vessel wall. This has included factors derived from platelets and factors derived from leukocytes. At the same time, however, there have been a number of sources of evidence suggesting that the primary mechanisms responsible for growth control of the two critical cells, endothelial cells, and the smooth muscle cells, may be factors intrinsic to the wall.

For the endothelial cell, the central observation may be simply that the cell is able to regenerate while bathed in the normal plasma present *in vitro*. This, combined with the general ability of cultured endothelial cells to grow in the absence of known mitogens, has led to the conclusion that endothelial cell growth is controlled by some form of cell-cell interaction. Direct evidence for this, however, depends upon identification of some mechanism able to control growth at a molecular level. Recent studies from our laboratory have identified a membrane protein, endothelial growth inhibitory protein (EGIP), which has this ability. Addition of EGIP to sparse endothelial cells mimics the effects of contact inibition of growth, as well as movement.

While smooth muscle cell growth has generally been attributed to the presence of exogenous growth factors, growth of these cells in response to denuding injuries continues long after the period of active platelet release. Recent cell kinetic studies from this laboratory imply that this may be due to the continued proliferation of cells that have been induced to enter the growth fraction by some as yet undefined event occurring at the time of injury. One suggestion as to the nature of this event comes from studies of fetal smooth muscle cells. These studies, done in collaboration with Dr. Daniel Bowen-Pope, show that fetal smooth muscle cells are themselves capable of producing a mitogen, will proliferate in the absence of exogenous growth factor, and the mitogen they produce appears to be platelet-derived growth factor. A combination of these data in cell culture with the cell kinetic data obtained in the animals suggest that induction of a similar population may be important for the continued proliferation of vessel walls responding to denudation.

0350 CONTRIBUTIONS OF DENDRITIC CELLS AND IL-2 TO THE FUNCTION OF T LYMPHOCYTES. K, Inaba A. Granelli-Piperno, and R. Steinman. The Rockefeller Univ. New, N.Y. 10021

The growth of T lymphocytes is mediated by T cell growth factor, or interleukin-2 (IL-2). Both IL-2 and its receptor have been identified biochemically and were subsequently cloned. The capacity to release and to respond to IL-2 are properties of large "activated" T cells, or T lymphoblasts; small resting T cells are quiescent. The induction of IL-2 release and responsiveness is controlled by the interaction of T cells with other "antigen-presenting" cells rather than by additional soluble factors.

This presentation will consider the distinct contribution of dendritic cells to the initiation of T cell growth. Much of the data comes from the primary mixed leukocyte reaction or MLR. The dendritic cell is the principal stimulatory cell in the murine, rat and human MLR. Purified B lymphocytes and macrophages, which express the requisite transplantation antigens, are weak or inactive. The conditioned medium from dendritic-T cell cocultures contains abundant IL-2, but these media do not induce the proliferation of resting T cells. Instead, it appears necessary for DC to physically interact with T cells. The clusters can be isolated by velocity sedimentation and are greatly enriched in antigen-specific T blasts. Helper blasts are selected by depleting contaminating cytolytic cells with anti-lyt-2 antibody andcomplement. The helper cells are responsive to exogenous IL-2 doubling with a generation time of 14-18 hrs. In contrast to resting T cells, the sensitized blasts release IL-2 and proliferate in response to antigen presented by most leukocytes including dendritic cells, B cells and macrophages. Stimulatory capacity correlates roughly with the level of Ia antigens. Therefore most leukocytes can present antigen to sensitized T cells, but dendritic cells are required to trigger resting lymphocytes.

Sensitized T blasts not only grow when exposed to IL-2. They also released B cell stimulating factors which are a distinct class of lymphokines that mediate the growth and differentiation of antibody secreting cells. The experiment is to add IL-2 to irradiated helper T blasts for 3 hrs, and then observe that the conditioned medium is a rich source of helper factor. No helper activity is seen with IL-2 alone, conditioned medium from unchallenged T blasts alone, or a mixture of the two.

Therefore IL-2 is a critical mediator of T cell function. Responsiveness to IL-2, particuarly in the case of helper T cells, is induced by dendritic cells. Once activated, the T cells can release IL-2 and helper factors in response to antigen presented by many cell types.

Cellular Response to Growth Factors in Oncogenes

0351 FIBROBLAST GROWTH FACTOR (FGF) IN CHONDROSARCOMA: INHIBITION OF TUMOR GROWTH WITH ANTI-FGF ANTIBODIES. Andrew Baird, Pierre Mormede and Peter Böhlen, Laboratories for Neuroendocrinology, The Salk Institute, La Jolla, Ca.

Transplantable rat and mouse chondrosarcomas contain an FGF-like growth factor that is recognized by antisera raised against the amino-terminal sequence of bovine pituitary FGF (Böhlen et al., PNAS 81:5364, 1984). Like pituitary FGF, chondrosarcoma-derived FGF is a cationic polypeptide with a molecular weight of 16-18 kD that is retained on heparin-sepharose affinity columns and has a similar retention behavior on HPLC. It is conceivable that FGF is identical to chondrosarcoma-derived growth factor (Shing et al., Science, 223:1296, 1984). C57/BL mice, treated with the globulin fraction of normal rabbit serum and innoculated i.m. with cells prepared from EHS (mouse) chondrosarcoma, developed tumors in 28 days that had a mean weight of 1.70 ± 0.26 g (SEM, N=9). When treated with the globulin fraction of antiserum to FGF, tumor growth was decreased to 0.73 ± .21 g (SEM, N=11, p<.01), which could be directly correlated with the titer of antibody obtained in each animal. These results demonstrate that FGF is present in chondrosarcomas; furthermore, the inhibition of tumor growth by antibodies suggests a role for FGF in this process. The mechanism of action (eg. via inhibition of neovascularization or via inhibition of growth due to lack of growth factor) is at present unknown.

0352 MOUSE MAMMARY TUMOR VIRUS TRANSCRIPT LEVEL REGULATED BY MOUSE LPS GENE IN TRANS.
Jean K. Carr, Vicki L. Traina-Dorge and J. Craig Cohen, Louisiana State University, New Orleans, LA 70112

Expression of mouse mammary tumor virus (MMTV) in the lactating mammary glands of uninfected mice varies between strains of mice in a manner independent of the proviral content. Statistical analysis of this variability identified one genetic locus which was associated with high MMTV RNA: the Lps locus on chromosome 4 of the mouse. The Lps locus mediated the mouse's response to lipopolysaccharide (LPS) in the responder mouse while mice with the deficient allele are incapable of responding. It was hypothesized that the Lps gene product regulated the level of MMTV RNA in the lactating mammary gland and this was tested by injection of LPS-responder mice with LPS. The level of MMTV-specific transcripts increased from 4 to 12 fold following LPS injection, but the increase was due only to one of the possible MMTV transcripts: the 1.6 kilobase (kb) transcript containing the LTR open reading frame (orf) sequence. The level of MMTV-specific transcripts in the lactating mammary glands of uninfected mice, then, is regulated by a cellular gene which is not linked to any viral coding sequences and therefore must act _in trans_.

A likely mechanism for the action of LPS is the release of glucocorticoids which immediately follow LPS injection. Glucocorticoids are positive regulators of MMTV transcription and injection of glucocorticoids also resulted in the induction of the 1.6 kb transcript. Adrenalectamized mice were injected with LPS and died of toxic shock within 20 minutes, but in that time the 1.6 kb transcript was induced, suggesting that the LPS effect is independent of glucocorticoids.

0353 PDGF-LIKE ACTIVITY IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS (MPD), Mario Romano, Piera Viero, Tiziano Barbui, Maria Benedetta Donati and Andreina Poggi, Mario Negri Institute, Milano, Department of Haematology, Bergamo, Italy.

Myelofibrosis is a frequent complication in patients with Myeloproliferative Disorders (MPD). Abnormalities of blood platelets and bone marrow megacaryocytes have been described in these patients. Intramedullary release of Platelet Derived Growth Factor (PDGF) might stimulate fibroblast hyperproliferation and secretion in bone marrow. Platelets, as a consequence, might be defective in PDGF. We studied PDGF-like activity in samples of whole derived serum (WBS), platelet rich plasma derived serum (PRS) and platelet poor plasma derived serum (PDS) from 14 patients affected by MPD and 7 normal individuals. PDGF-like activity was measured as increase of 3H thymidine incorporation by NIH 3T3 cells. A dose response curve of pure PDGF was used as reference. Significant differences were found in WBS and PRS mitogenic activity: PDGF-equivalent levels in PRS were 7.9±1.6 ng/ml in normals and 3.9±0.4 ng/ml in patients with MPD ($p<0.05$), whereas no differences were found in PDS activity. These data suggest that circulating platelets from patients with MPD do have reduced PDGF activity either for a depletion of PDGF in the bone marrow or due to circulation of an abnormal peptide.

0354 SMOOTH MUSCLE CELL MITOGENIC RESPONSES TO PLATELET-DERIVED GROWTH FACTOR AND SEROTONIN: EFFECTS OF CALCIUM ANTAGONISTS, Robert N. Saunders and Georgina M. Nemecek, Platelet Department, Sandoz Research Institute, E. Hanover, N.J. 07936

Bovine aortic smooth muscle cells in vitro respond to platelet-derived growth factor (PDGF, 2-40 ng/ml) with increased incorporation of [^{3}H]thymidine into DNA. The mitogenic response was linear with respect to growth factor concentration when the cultures were incubated with 10-28 ng of PDGF/ml. Smooth muscle cells exposed to 0.1 nM to 100 μM serotonin (5-HT) also showed enhanced DNA synthesis. The observed mitogenic effect of 5-HT was maximal above 1 μM and showed an ED_{50} of approximately 50 nM. The increase in smooth muscle cell [^{3}H]thymidine incorporation in response to 1 μM 5-HT was comparable to that seen with 16 ng PDGF/ml, the ED_{50} for the growth factor. Nifedipine, diltiazem and verapamil were ineffective inhibitors of 5-HT or PDGF-induced increases in smooth muscle cell DNA synthesis (IC_{50}s > 50 μM). However, TMB-8 showed an IC_{50} of 14 μM for inhibition of the smooth muscle cell mitogenic response to PDGF. These data demonstrate that 5-HT at concentrations which are comparable to those found _in vivo_ has a significant mitogenic effect on smooth muscle cells _in vitro_. The results of this investigation additionally raise the possibility that certain calcium antagonists could be used to modulate cellular responses to mitogens.

0355 DIACYLGLYCEROL AND INOSITOL 1,4,5 TRIS-PHOSPHATE IN NORMAL AND RAS-TRANSFORMED CELLS

Laurie F. Stein and Lewis Cantley, Harvard University, Cambridge, Massachusetts 02138

Ras-transformed and non-transformed rodent cells were labelled in culture to isotopic equilibrium with ^{3}H-glycerol and cellular lipids were extracted and analysed by thin layer chromatography. Levels of diacylglycerol, inositol 1,4,5 trisphosphate and other lipids were examined under various conditions of cell density, serum stimulation and serum deprivation. The results suggest that alterations in the relative levels of these membrane components may be important during normal cell growth and contact inhibition and that the ras (p21) transforming protein may act by affecting these levels.

0356 BIOLOGICAL RESPONSES AND ARACHIDONIC ACID METABOLISM IN MURINE TUMOR CELLS,

James Varani and Suzanne E.G. Fligiel, The Univ. of Michigan, Ann Arbor, MI 48109

Recent studies have shown that several types of higher eukaryotic cells, in addition to the well-studied leukocytes, are capable of chemotactic responses. The cellular and molecular basis of the chemotactic response in non-leukocytic cells has not been delineated. In this study we examined the biological responses in a line of murine fibrosarcoma cells to three different ligands - 12-O-tetradecanoyl phorbol acetate (TPA), laminin and fibronectin. Arachidonic acid metabolism was also examined under the same conditions. TPA stimulated a rapid but transient adherence response and a motility response which was primarily chemotactic. With TPA stimulation, there was a rapid but transient release of arachidonic acid from prelabeled phospholipid pools and metabolism of the released arachidonic acid. The major lipoxygenase metabolites produced were 5-monohydroxieicosatetraenoic acid (5-HETE) and 12-HETE. Prostaglandins (PG) produced include PGE_2, 6-keto $PGF_{1\alpha}$, and $PGF_{2\alpha}$. In contrast to TPA, both laminin and fibronectin stimulated a slow, sustained adherence response in the fibrosarcoma cells. These ligands also stimulated random migration and haptotaxis. In regards to arachidonic acid metabolism, there was a slow release of arachidonic acid from prelabeled cells and conversion into lipoxygenase and cyclooxygenase products. Thus, with all three ligands the kinetics of the biological responses and the kinetics of arachidonic acid metabolism were consistent with one another and allow for the speculation that, as in leukocytes, the metabolism of arachidonic acid may be related to the induction of biological function.

0357 IN VITRO PI KINASE ACTIVITY ASSOCIATED WITH POLYOMA MIDDLE T,

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The transforming gene product of polyoma virus, middle T, is a membrane protein which is known to associate with c-src in immunoprecipitates from polyoma infected cells. The recent observation that v-src and v-ros can phosphorylate phosphatidyl- inositol (PI) and the potential physiological significance of this activity prompted us to investigate the possibility that the middle t-c-src complex can also phosphorylate PI. We have found that immunoprecipitates made from polyoma infected cells with anti-polyoma serum have PI kinase activity, whereas immunoprecipitates made from uninfected cells or with preimmune serum lack this activity. We have also analyzed a variety of polyoma mutants for in vitro PI kinase activity, and found a strong correlation between mutant transforming activity and PI kinase activity. Immuno- precipitates made with a monoclonal antibody to c-src from polyoma infected cells also have PI kinase activity, whereas anti c-src immunoprecipitates from uninfected cells or cells infected with a transformation defective polyoma mutant lack this activity, indicating that the presence of c-src in the immunoprecipitate complex is not sufficient for PI kinase activity, but rather that a viral transforming function is also required. These results suggest that the transforming activity of middle t may be mediated at least in part through effects on phosphoinositide metabolism.

0358 AMINO TERMINAL DELETIONS IN pp60^{src} DEFINE A REGION CRITICAL FOR CELL TRANSFORMATION, Victoria W. Wilkerson and J.T. Parsons, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Site-directed mutagenesis has been used to generate a collection of mutant viruses bearing deletions within the 5' half of the src gene, the gene which encodes the transforming protein of Rous sarcoma virus. The mutant src proteins which result contain deletions spanning the region from amino acid residue 82 through 227. Viruses containing the deleted src genes exhibit a range of phenotypes, from non-transforming (deletions of amino acids 82-169, 102-174, 111-197), temperature sensitive for transformation (deletions of residues 169-225, 173-227), or transforming (deletion of residues 92-128). These mutant src proteins retain kinase activity, and cells infected with each of the mutant viruses contain a tyrosine phosphorylated form of the 34K phosphoprotein, a putative substrate for pp60^{src}. These deletions define a region critical for pp60^{src} transforming activity. Further deletion mapping, together with biochemical investigation of the mutant src proteins, are being used to probe the structure and function of this region.

Vascular Biology

0359 SOME NEGATIVE AND POSITIVE CONTROLS IN VASCULAR INJURY AND REPAIR, Morris J. Karnovsky, Richard L. Hoover, and John J. Castellot, Department of Pathology, Harvard Medical School, Boston, MA 02115

In the pathogenesis of arteriosclerosis, proliferation of smooth muscle cells (SMC) is a key early step. We have shown that heparin inhibits the growth of SMC both in vivo and in vitro. We have also demonstrated that cultured endothelium secretes a highly antiproliferative heparin species. This activity of heparin has the following characteristics: 1) it is specific for SMC and related cell types, 2) heparin is the most potent glycosaminoglycan, 3) anticoagulant and non-anticoagulant heparin are equally effective, and 4) the effect is reversible. Structure-function studies demonstrated that the smallest fragment of heparin which still retained growth inhibitory activity was a pentasaccharide sequence containing a critical 3-O-sulfate; this oligosaccharide had no anticoagulant activity. We have also identified a heparin derivative (N-desulfated, re-N-acetylated heparin) which has lost its anticoagulant but not its antiproliferative activity. Cell cycle kinetic analysis revealed that heparin blocked SMC in late G_1, thus ruling out a direct interaction with platelet-derived growth factor. Two other effects of heparin on SMC metabolism have been observed which share all the properties of the antiproliferative activity: 1) the induction of a 35K dalton protein which is secreted into the medium, and 2) the inhibition of nucleoside uptake. Taken together, the above results suggest that heparin may play an important physiological role in growth regulation in the vascular wall, and that non-anticoagulant, antiproliferative heparin species may be useful in preventing SMC proliferation following vascular surgery. We have also examined the properties of macrophages elicited from normal and hypercholesterolemic rats. When tested in a monolayer adhesion assay, hypercholesterolemic macrophages were more adhesive than normal cells to plastic and to monolayers of SMC; attachment to endothelial monolayers was the same. Conditioned media from both types of macrophages were chemotactic for SMC. However, the chemoattractant activity of hypercholesterolemic macrophages was 3-fold greater than that of normal cells. These same conditioned media caused an increase in the growth of SMC, as measured by cell counting assays. The mitogenic effect of hypercholesterolemic macrophages was 2-3 fold greater than that of normal macrophages. The proliferative response of SMC to macrophage conditioned medium was blocked by heparin. Finally, macrophages from hypercholesterolemic rats produced $>$ 40% more superoxide when challenged with PMA than normal macrophages. The data suggest that macrophages may play a key role in mediating vascular injury and subsequent SMC proliferation. These results may provide a causal link between hyperlipidemia, the disordered lipid metabolic, and the reaction to injury, mechanisms underlying atherogenesis.

0360 ENDOTHELIAL CELL PROTEASES: INDUCTION BY ANGIOGENESIS FACTORS. Daniel B. Rifkin, Department of Cell Biology, New York University Medical Center, New York, NY 10021.

During the initial stages of angiogenesis, the basement membrane overlying the capillary is degraded by specific proteases. We have hypothesized that these proteases are plasminogen activator (PA) and collagenase produced by the invading endothelial cells in response to specific angiogenesis factors. We have found that capillary, but not large vessel, endothelial cells respond to three different angiogenic preparations by secreting increased amounts of PA and collagenase. These three preparations, bovine retinal extract, mouse adipocyte conditioned medium, and associate from a human hepatoma, all stimulated enzyme production in a dose responsive manner. The protease inducing activity from the human hepatoma has been partially purified using ion exchange and gel permiation chromatography. It has an apparent molecular weight of 20,000 daltons, is heat and pH label, non-dialyzable, and has a pI of 5.6. The most purified fractions not only increase protease production but also stimulate endothelial motility and chemotaxis as well as cell division.

Molecular Assembly at the Cell Surface in the Initiation and Propagation of Effector Pathways

0361 CELLULAR PATHWAYS AND SIGNALS REQUIRED TO INDUCE CELL SURFACE ASSEMBLY AND INITIATION OF THE COAGULATION PROTEASE CASCADE. Thomas S. Edgington, Research Institute of Scripps Clinic, La Jolla, California 92037

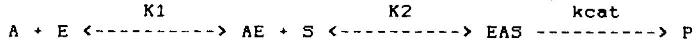
Whereas some cells constitutively synthesize and express on their surface tissue factor (TF), a protein which initiates the cell surface assembly of the extrinsic coagulation protease cascade, cells of other selected differentiation express TF only following appropriate signals. Cells of the monocyte lineage can be induced by a variety of signals to synthesize TF, a recognition coupled response implicated in the in vivo activation of coagulation in response to a variety of stimuli. Four pathways have been identified which induce TF biosynthesis and cell surface expression. Three pathways require T lymphocytes as the recognitive unit for the appropriate stimuli. Selected T cell clones when triggered by stimulus produce the appropriate matrix of signals that induce TF in monocytes. Pathway I is very rapid and occurs in response to bacterial lipopolysaccharide, immune complexes, and some syngeneic transformed cells. The Pathway I T instructor cell induces the TF gene by a genetically restricted non-lymphokine mediated pathway, and gene induction at the level of the monocyte can be suppressed by PGE_2. Pathway II is slower and occurs in response to class II MHC products, e.g. DR in the human. The T instructor cell is stimulated to produce an 18 kd protein (MPIF) which in turn directly induces TF biosynthesis in monocytes. The Pathway III response to antigen is slow and appears to include more than one phenotype of T instructor cell. Both lymphokine producing and non-lymphokine contact mediated T instructor cell clones have been identified. The signals that induce TF have not yet been defined at the molecular level. There may be an additional pathway (IV) whereby monocyte TF is induced in primed cells by selected stimuli in the absence of T instructor cells. Each of these mechanisms involves a coupled set of responses differing significantly in mechanistic detail but culminating in the induction of the same gene product. Both direct contact mediated signals and soluble signals capable of inducing TF have been identified. Elucidation of the molecular sequences promises to provide information regarding the principles of coupled gene induction as an event necessary to initiation of the assembly of the extrinsic coagulation cascade on cell surfaces, a response underlying thrombogenesis and inflammation.

0409 PROTHROMBINASE: A MODEL FOR ENZYME BOUND COMPLEXES IN BLOOD COAGULATION, Kenneth G. Mann, Michael M. Nesheim, and Paula B. Tracy, University of Vermont College of Medicine, Burlington, VT 05405.

The term "prothrombinase" refers to the complex composed of the enzyme Factor Xa, the cofactor protein Factor Va, calcium ions and phospholipid, which is thought to be responsible for the physiological conversion of the zymogen prothrombin to the enzyme thrombin. Although Factor Xa is sufficient for prothrombin activation, the cofactors increase the rate of thrombin generation approximately 300,000 fold at plasma concentrations of enzyme and substrate. Both the enzyme (Factor Xa) and the substrate (prothrombin) of this reaction are vitamin K-dependent proteins, each of which possess γ-carboxyglutamic acids at the NH_2-terminals of their respective peptide chains. As a consequence of these residues, these proteins can, in the presence of calcium, bind membranes containing acidic phospholipids. The cofactor for the reaction, Factor V, is a rod-like single chain protein with a molecular weight of 330,000. It possesses three binding sites for calcium, one of which has $K_d < 10^{-8}$ M. Factor V is best described by the term "procofactor". Treatment of Factor V with thrombin results in a number of discrete peptide cleavages and in the generation of the active cofactor (Factor Va), which is at least 400 times more active than the procofactor. Both Factor V and Factor Va bind to phosphatidylcholine-phosphatidylserine vesicles, and to blood platelets. In contrast to the vitamin K-dependent proteins, Factor V binding to membranes is metal ion and ionic strength independent. The activated cofactor binds to acidic phospholipid containing vesicles with approximately ten times the affinity of either prothrombin or Factor Xa and binds to a limited number of sites on the platelet membranes with a dissociation constant of 10^{-10} M. We are examining the participation of each of the three proteins; phospholipid and platelet membranes; and calcium ions in the generation of thrombin from prothrombin. Our approaches involve equilibrium binding measurements of each of the species in the reaction system, using light scattering, fluorescence, and sedimentation techniques, and studies of the conversion of prothrombin to thrombin using both synthetic substrates and prothrombin. In the latter case, the fluorescent inhibitor, DAPA, has been used as a probe. The results of our studies thus far indicate that the substrate (prothrombin) is not an intrinsic component in the formation of the complex catalyst. Further, these studies indicate that Factor Va functions, in part, by partitioning Factor Xa to the surface of the phospholipid vesicle or to the platelet membrane surface. Using the composite of kinetic and equilibrium data, the rate enhancement observed for prothrombinase can be modeled in terms of partitioning and enzyme to the vesicle surface, and by enhancement of the catalytic efficiency of Factor Xa.

0362 ORDERED ADDITION: A KINETIC MECHANISM FOR THE LOCALIZATION OF THE TISSUE FACTOR PATHWAY OF COAGULATION, Yale Nemerson, Department of Medicine, Mt. Sinai School of Medicine of CUNY, New York, N.Y. 10029 and Rodney Gentry, Department of Mathematics and Statistics, University of Guelph, Guelph, Ontario N1G2W1.

Blood coagulation can start via a catalytically active zymogen, factor VII (Zur, M., Radcliffe, R., Oberdick, J., and Nemerson, Y., J. Biol. Chem. 257, 5623, 1982). This zymogen exhibits a complete dependence upon an essential activator, tissue factor, a membrane-bound glycoprotein not usually associated with blood cells. Implicitly, the reason blood does not clot continuously is due to an anatomical barrier separating factor VII from tissue factor. On complexing with tissue factor, factor VII manifests coagulant activity by proteolytically activating two other zymogens, factors IX and X. The product of the latter, factor Xa, cleaves factor VII to its 120-fold more active 2-chain form, factor VIIa. To maintain blood fluidity, this enzymic complex must remain localized. The kinetic model we propose for this system has just this property:



where A, activator, is tissue factor; E, enzyme, factor VIIa; and S, substrate, factor IX or X. K1 and K2 are equilibrium dissociation constants and kcat the catalytic rate constant. Experimental data and computer-simulations show Km app to vary as a function of E and A. V max = kcat*(minimum A,E). The latter states that as S goes to infinity, the concentration of the limiting species (probably tissue factor physiologically) would approach zero. That is, high concentrations of substrate literally "lock" factor VIIa in EAS complexes.We show this in two ways: 1) the model states that while resident in EAS, EA cannot dissociate. This implies that S alters E so that it binds more tightly to A. K1 determined under equilibrium conditions = 16 nM; under kinetic conditions K1 < 0.1 nM, thereby showing the effect of S on K1. 2) A derivatized form of factor VIIa rapidly exchanges with active VIIa on tissue factor. Our data show that upon addition of S, little if any VIIa exchanges, thus demonstrating directly the stabilizing effect factors IX and X have on the tissue factor-factor VIIa complex. This property of the ordered-addition model tends to trap active enzyme in localized complexes.

0363 ASSEMBLY OF CYTOLYTIC ATTACK COMPLEXES BY COMPLEMENT AND BY CYTOLYTIC T-LYMPHOCYTES. Eckhard R. Podack, Department of Microbiology/Immunology, New York Medical College, Valhalla, N.Y. 10595

Membrane attack by complement involves the self assembly on target membranes of five hydrophilic proteins (C5b,C6,C7,C8,C9) to an amphiphilic tubular membrane attack complex (MAC) comprising approximately twenty subunits. Assembly of the MAC begins with the proteolytic cleavage of C5. The larger fragment, C5b, associates with C6 and C7 and the forming trimolecular C5b-7 complex undergoes a conformational alteration during which hydrophobic sites become exposed that insert into the hydrocarboncore of the activating membrane. C5b-7 binds one molecule C8 and the C5b-8 complex binds and rapidly polymerizes up to 16 molecules C9. C9 polymerization, mediated by C5b-8 or triggered with isolated C9 in vitro, is asscompanied by a conformational reorganization of C9 resulting in the formation of tubular (cylindrical) transmembrane complexes that appear as ultrastructural membrane lesions upon insertion into artificial or natural membranes. Formation of large membrane channels in complement thus is the function of polymerizing C9[1].

Poly C9 is exceptionally stable to dissociation by detergents, including boiling in SDS, and to proteolytic degradation. This biochemical stability of the poly C9-channel may be necessary to resist proteolytic defense mechanisms of attacked organisms. The amino acid sequence of C9 has been deduced from the nucleotide sequence of C9-cDNA. Despite the large hydrophobic membrane combining site of poly C9, no corresponding continuous hydrophobic segment is found in C9's linear amino acid sequence, supporting the concept of conformational reorganization upon polymerization and transmembrane channel formation.

Lymphocyte mediated cytolysis of tumor cells also is accompanied by the formation of large ultrastructural membrane lesions (160 Å internal diameter) on target cells. These lesions arise on target membranes by polymerization of precursor proteins (perforins) to tubular polyperforin complexes. The precursor perforins are located in the cytoplasmic granules of cloned killer lymphocytes. Granules isolated from cytolytic T-cells and NK-cells mediate potent Ca dependent tumor cell lysis and concomitant assembly of polyperforin complexes. Cytolytic granules contain approximately six major protein bands in the molecular weight range from 12,000 to 75,000[2]. Upon separation of granule proteins by ion exchange chromatography using HPLC , the Ca-dependent cytolytic activity was found to reside in the Mr 75,000 protein which is thus tentatively identified as perforin 1.

A comparison of human complement component C9 (Mr 73,000) and murine cytolytic T-cell perforin 1 (Mr 75,000) reveals striking biochemical similarities sugesting a common evolutionary origin for C9 and perforins.

[1] Podack, E.R., 1984, J. Biol. Chem. 259:8641
[2] Podack, E.R., and Konigsberg, P.J., 1984, J. Exp. Med. 160:695

0364 A NEW ENDOTHELIAL CELL-DEPENDENT PROCOAGULANT PATHWAY, D. Stern, P. Nawroth, D. Handley, jW. Kisiel, Columbia University, NYC, NY 10032.

Although the endothelial cell is considered antithrombogenic, endothelium has recently been shown to participate in procoagulant reactions. In this report cultured bovine aortic endothelial cells are shown to propagate a procoagulant pathway starting with factor XI_a, leading to activation of factors IX, VIII, X, and prothrombin, culminating in fibrinopeptide A clevage from fibrinogen and formation of a fibrin clot. Electron microscopic studies demonstrate fibrin strands are closely associated with the endothelial cells. Endotoxin treated endothelial cells, having acquired tissue factor activity, generated fibrinopeptide A in the presence of factors VII_a, IX, VIII, X, prothrombin and fibrinogen. Factor X activation by factor VII_a and tissue factor expressed by endothelial cells is ten times greater in the presence of factors IX and VIII than in their absence. This indicates that on the perturbed endothelial cell surface, factors IX and VIII do have an important role in the activation of factor X. Addition of platelets (10^8/ml) augmented thrombin formation seen in the presence of endothelium alone by about fifteen-fold. ANtihuman factor V IgG decreased this enhanced thrombin formation in the presence of platelets indicating that factor V from platelets was playing an important role in thrombin formation. These data lead us to propose that endotehlial cells can actively participate in procoagulant reactions. Although platelets can augment thrombin formation by these endothelial cell-dependent reactions, endothelial cells along can lead to formation of a cell-associated fibrin clot. The endotoxin-treated endothelial cell provides a model of the thrombotic state supplying tissue factor to initiate coagulation and propagating the reactions leading to fibrin formation. This endothelial cell-dependent pathway suggests a central role for factors VIII and IX consistent with their clinical importance.

Coagulation, Complement and Other Protease Effector Pathways

0365 *REVERSIBLE STIMULATION OF TISSUE FACTOR EXPRESSION IN CULTURED BOVINE CELLS BY CALCIUM IONOPHORE A23187, Ronald Bach and Daniel B. Rifkin, Mt. Sinai Medical School, New York, NY 10029, and NYU Medical School, New York, NY 10016*

The tissue factor content of confluent monolayers of cultured bovine cells was quantified by immunoassay (TF-ELISA) following extraction of the cells with Triton X-100, and by procoagulant activity (2-stage clotting assay) after cell disruption by freezing and thawing. Pure bovine brain tissue factor in detergent or in phospholipid vesicles composed of 30%PS and 70%PC was used as the standard for the respective assays. Bovine pericytes, MDBK's, and fibroblasts contained 50,000 to 100,000 copies per cell in both assays, whereas smooth muscle cells contained less than 1000 copies per cell and aortic endothelial cells contained no detectable antigen or activity. The hydrolysis of factor X by intact monolayers of pericytes was accelerated by A23187 in a dose dependent fashion in the presence of 5mM Ca++ and 10nM factor VIIa. Maximum stimulation was achieved in less than 15 min. at 10uM A23187. Ionophore treatment resulted in a 2-fold increase in the Vmax and a decrease in the Km for factor X cleavage from 294nM to 81nM. The change in kinetic parameters following A23187 treatment is analogous to that observed with pure tissue factor in phospholipid vesicles when the vesicle charge changes from neutral (100%PC) to acidic (>10%PS). This suggests a mechanism for the ionophore effect whereby A23187 disrupts the asymmetric distribution of phospholipids in the plasma membrane. The stimulated activity decayed back to the basal level in less than 1hr. following removal of the ionophore from the culture medium. The activity could be restimulated by readdition of the ionophore.

0366 DIFFERENTIAL PROTEIN EXPRESSION IN ORGAN-DERIVED ENDOTHELIAL MICROVESSELS AND PARENCHYMA, Paula N. Belloni, Garth L. Nicolson, The University of Texas System Cancer Center,D. Anderson Hospital and Tumor Institute, Houston, Texas 77020·

Interactions between blood borne tumor cells and components of the vascular lumen are important for implantation during metastasis. We have developed a methodology to study glycoproteins on the luminal surface of organ-derived murine endothelium which may effect the metastatic process. Endothelial cell surfaces were radiolabelled in situ by lactoperoxidase catalyzed iodination via intracardiac perfusion. Tissue autoradiography was performed to determine that the localization of the radiolabel was restricted to the luminal vessel surfaces. Protein analysis was performed by SDS-polyacrylamide gel electrophoresis using detergent extracts of vascular trees isolated from brain, thymus, heart, lung, liver and kidney. Most of the radiolabelled proteins were common to all vascular isolates; however, five protein peaks appeared to be differentially expressed in particular organs; brain (135 Kd), lung (55 Kd), liver (230 Kd and 30 Kd), kidney (35 Kd). To determine if differential protein expression is unique to the endothelium or a property of other cells in particular organs, short term cultures of endothelial and parenchma cells from liver and lung were surface labeled and analysed by SDS-PAGE. The labelled protein profiles from liver hepatocytes, sinusoidal cells or endothelial cells were essentially the same but appeared different compared with lung fibroblasts or endothelial cells. These studies suggest that cells derived from a single organ may express a distictive set of proteins which are unique to each organ.

0367 INTERLEUKIN 1 (IL-1) ACTS UPON VASCULAR ENDOTHELIUM TO STIMULATE PROCOAGULANT ACTIVITY AND LEUKOCYTE ADHESION. Michael P. Bevilacqua, Jordan S. Pober, Ramzi S. Cotran, and Michael A. Gimbrone, Jr., Vascular Pathophysiology Laboratory, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

We have studied the effects of IL-1, an important intercellular mediator involved in inflammatory and immunological responses, on cultured human endothelial cells (HEC). Human monocyte-derived IL-1 induced cell surface expression of procoagulant activity (PCA) in endothelial monolayers. This induction was transient (peak 4-6 h) and required protein synthesis. Studies with coagulation factor-deficient plasma, purified coagulation factors, and a goat anti-human apoprotein III antiserum indicated that most, if not all of the IL-1 induced HEC-PCA was tissue factor-like (J. Exp. Med. 160:618, 1984). IL-1 also had a marked effect on endothelial-leukocyte adhesion. Selective pretreatment of HEC monolayers with IL-1 (5u/ml, 4h) led to a 3-4 fold increase in the adhesion of human peripheral blood monocytes, and the human monocyte-like cell line U937, while pretreatment of the leukocytes or inclusion of IL-1 during the adhesion assay had no effect. This IL-1 effect on HEC was concentration and time-dependent and could be largely blocked by cycloheximide or actinomycin D. Similar results were obtained for the adhesion of polymorphonuclear leukocytes and lymphocytes but not of erythrocytes.

These studies define the vascular endothelial cell as a potential target for IL-1-mediated effects. IL-1 stimulation of HEC-PCA and HEC-leukocyte binding may be important in the pathogenesis of a variety of immunological and inflammatory conditions.

0368 RAPID PURIFICATION OF HUMAN FACTOR VIII:C BY HPLC. Steven W. Herring and Charles Heldebrant, Alpha Therapeutic Corp., 5555 Valley Blvd., Los Angeles, CA 90032

Human Factor VIII procoagulant protein (VIII:C) is a plasma protein that participates in the cascade of events leading blood coagulation. It is deficient or defective in patients with Hemophilia A. In vivo Factor VIII:C associates with von Willibrand factor and its multimers to form a high molecular weight particle that can be dissociated into a lower molecular weight form in the presence of high concentrations of salt. We have been able to rapidly purify Factor VIII:C on a large scale using sequential high performance size exclusion chromatography (HPSEC) under first low salt and then high salt conditions. Reconstituted commercial Factor VIII:C concentrate was purified by chromatography on a preparative HPSEC column (Toya Soda, 2.5X60 cm.) in 0.05 M Imidazole buffer, pH 7.35, containing 0.15 M NaCl. Factor VIII:C activity eluted in the void volume in less than 20 min. as a high molecular weight particle well resolved from low molecular weight contaminants. Purification was 25 fold with a yield of greater than 65 percent. Up to 4 grams of Factor VIII concentrate could be purified at one time in this manner. This material was then concentrated and made 0.30 M in CaCl prior to re-chromatography on the same column in a buffer containing 0.30 M $CaCl_2$. Under these conditions Factor VIII:C activity eluted in the included volume of the column at a position corresponding to a molecular weight of several hundred thousand in less than 1 hour and was well resolved from larger protein contaminants. Further purification of Factor VIII:C was accomplished by cation exchange HPLC; the purified material had a specific activity of about 1000 units/mg protein. All steps could be carried out in one day with good yields.

0369 CHEMICALLY MODIFIED HEPARINS AS INHIBITORS OF HEPARAN SULFATE ENDOGLYCOSIDASE FROM METASTATIC TUMOR CELLS. Tatsuro Irimura, Motowo Nakajima and Garth L. Nicolson, Dept. Tumor Biology, The University of Texas - M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

The malignancy of solid tumors can be explained, in part, by their abilities to invade and destroy normal tissues, extracellular matrix and basement membranes. Glycosaminoglycans, especially heparan sulfate (HS), are important constituents in these structures. Recently, we found that HS-degradative activities of metastatic B16 melanoma sublines correlated with their lung colonization abilities (Nakajima, et al. Science 220:611-613, 1983). To characterize HS-degradative activity, we developed a HPLC system for HS degradation products, and found that the activity is due to an endo-β-glucuronidase (heparanase) (Irimura, et al Anal. Biochem. 130:461-468, 1983; Nakajima, et al. J. Biol .Chem. 259:2283-2290, 1984). We found that heparin is a poor substrate and acts as an inhibitor of this enzyme, and we are determining the inhibitory structure. Heparin was converted to pyridinium salt, and sulfate groups on amino residues were removed by limited solvolysis with aqueous DMSO. Simultaneous removal of N- and O-sulfate groups was achieved by prolonged solvolysis in anhydrous methanol. Completely desulfated heparin was sulfated at its amino residues by reaction with triethylamine sulfate in the presence of an alkaline catalyst, to obtain N-sulfated O-nonsulfated heparin. The reaction products gave single bands after cellulose acetate electrophoresis. (^{35}S)HS was isolated from BCE cells in culture, and incubated with a lysate of highly metastatic B16-BL6 melanoma cells in the presence or absence of derivatized heparin, and the reaction products were analyzed by HPLC. The elution profiles of degradation products indicated that the N-sulfate group in heparin plays a significant role in determining inhibitory activity against HS-degradative enzymes.

0370 HUMAN HEPATOMA CELLS SYNTHESIZE FUNCTIONALLY ACTIVE HEPARIN COFACTOR II, Eric A. Jaffe and Douglas M. Tollefsen, Cornell University Medical College, New York, NY 10021

Heparin cofactor II (HCII) isolated from human plasma inhibits thrombin by forming a stable, equimolar complex with the protease. The rate of complex formation is greatly increased by heparin or dermatan sulfate. To determine if the liver synthesizes HCII, we incubated cultured human hepatoma-derived cells (PLC/PRF-5) for 72 h in serum-free medium and then isolated HCII from the post-culture medium using a monospecific rabbit anti-human HCII antiserum coupled to protein A-Sepharose. The immunoisolated HCII was analyzed by SDS-polyacrylamide gel electrophoresis and electrophoretic immunoblotting with anti-HCII. Hepatoma post-culture medium contained a protein of molecular weight = 72 kD that co-migrated with human plasma HCII. HCII was absent from pre-culture medium and post-culture medium from cells treated with cycloheximide. When hepatoma post-culture medium was incubated with thrombin (1 U/ml), covalent complexes of molecular weight = 101 kD were formed in the presence of heparin or dermatan sulfate but not in their absence; these complexes co-migrated with those obtained by incubating thrombin with human plasma under the same conditions. Neither cultured human umbilical vein endothelial cells nor human foreskin fibroblasts synthesized HCII. Thus, cultured human hepatoma cells synthesize and secrete functionally active HCII.

0371 PROCOAGULANT PROPERTIES OF INTERLEUKIN-1 (IL-1). Charles F. Moldow, Anna E. Schorer, Manuel E. Kaplan, University of Minnesota, Minneapolis, MN 55455

Inflammatory stimuli are associated with accelerated consumption of coagulation proteins and local deposition of fibrin thrombi. Endothelial cells (EC) synthesize the procoagulant, tissue factor (TF), in response to the lipid A moiety of LPS, but the TF is intracellular and unavailable to initiate clotting at the blood-vessel wall interface. We and others have reported that the monokine, IL-1, induces TF production by cultured human umbilical vein EC. EC were exposed to IL-1 from either of 2 sources: affinity purified (Genzyme) or IL-1 enriched supernatants from human blood mononuclear cells. Both IL-1 sources contained lymphocyte activating factor (LAF) by murine thymocyte proliferation, and caused febrile responses in rabbits (3.3 pyrogenic doses/ml) but lacked procoagulant (<0.3 TF units/ml), interleukin-2 (cytotoxic T cell assay), or LPS (limulus lysate) activity. TF was measured in disrupted EC (one-stage clotting assay), or *in situ* (factor Xa catalyzed cleavage of a chromogenic tetrapeptide, S-2222). IL-1, at concentrations 10^{-1}-10^{-3} less than those required for minimal LA activity, induced a dose-and-time-dependent (0-3 hrs) increase in TF content (up to 60 times baseline). The EC response was inhibited by cycloheximide (10 ug/ml) but not by polymyxin B (10 ug/ml), and recombinant α-interferon (10^4-10^6 U/ml) was inactive. TF synthesis was dissociable from prostaglandin metabolism. Neither hydrocortisone (1 mg/ml), eicosatetraynoate (ETYA, 5 uM), nor indomethacin suppressed TF production despite blocking EC incorporation of 3H-arachidonate. In contrast to LPS, IL-1 modified intact EC, so that enhanced cleavage of factor X to Xa occurred *in situ* implying that IL-1 induces surface expression of EC TF activity.

0372 HYDROLYSIS OF GLYCOSYLATED AND NON-GLYCOSYLATED FIBRONECTIN BY THE ATP-DEPENDENT PROTEOLYTIC ACTIVITY OF RETICULOCYTES, Sheila A. Newton[1] and Kenneth Olden[1,2], [1]Howard University Cancer Center, Washington, DC 20060 and [2]Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20205

We have obtained evidence in earlier studies that the presence of the oligosaccharide moiety of glycoproteins serves to protect the protein component from attack by a number of proteases. Experiments with the glycoprotein, fibronectin (FN), have shown the non-glycosylated form to be more readily degraded than glycosylated FN *in vitro* by Pronase, thermolysin, trypsin, and chymotrypsin (Bernard *et al*., J. Biol. Chem. 257, 8549, 1982). Recently we have tested the differential sensitivity of normal and non-glycosylated (^{3}H)FN to the ATP-dependent proteolytic activity from rabbit reticulocyte lysate. Although non-glycosylated FN is apparently degraded more rapidly than normal FN by the ATP-independent component of reticulocyte lysate proteinase, the ATP-dependent activity degrades both forms equally well, suggesting the existence of a specific protease-substrate interaction in this system which is unaffected by either the steric interference of or any conformational change induced by the presence or absence of oligosaccharide on the substrate FN. This is in marked contrast to results obtained previously with other proteases. The ATP-dependent activity is sensitive to N-ethylmaleimide, EDTA, hemin, and polylysine, and insensitive to leupeptin or 1,10-phenanthroline. Supported by grant GM-29804 from the National Institutes of Health.

0373 MOLECULAR BASIS OF REACTIVE LYSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) ERYTHROCYTES (E), C.J. Parker, T. Wiedmer, P.J. Sims, and W.F. Rosse, Duke Univ, Durham, NC 27710 and Univ of VA, Charlottesville, VA 22908.

When the alternative pathway of complement (C) is activated in the fluid-phase by activated cobra venom factor (CoFBb), the markedly C-sensitive PNH III E are hemolyzed, whereas the moderately C-sensitive PNH II E are not. Normal (NL) E are insusceptible to reactive lysis, however, NL E treated with aminoethylisothiouronium bromide (AET E) are as susceptible as PNH III E. To investigate the molecular basis of the hemolysis of PNH III and AET E by reactive lysis, we compared binding of C3, C7, C8, and C9 to normal, PNH II, PNH III, and AET E. Following fluid-phase activation of undiluted whole serum complement by CoFBb, the PNH III and AET E bound 15-20,000 molecules/cell of ^{131}I-C9 whereas the PNH II and NL E bound no ^{131}I-C9. The ratio of ^{131}I-C9: ^{125}I-C8 over 5 serum dilutions was ~ 3.3:1 for the PNH III E and ~ 2.5:1 for the AET E. The NL and PNH II E bound no C8. The greater binding of C8 and C9 to the PNH III and AET E was not due to aberrant interactions of C8 with the trimolecular C5b-7 complex since these abnormal cells bound large amounts of ^{131}I-C7 (10-12,000 molecules/cell when undiluted serum was employed) whereas the NL and PNH II E bound no ^{131}I-C7. The greater binding of membrane attack complex constituents to PNH III and AET E was not mediated by cell-bound C3 fragments. Studies of the E membrane glycoproteins demonstrated distinct qualitative differences for PNH III, AET, and PNH II compared to NL. These investigations suggest that NL E (and PNH II E) are resistant to reactive lysis because they have a membrane constituent which inhibits binding of the trimolecular C5b-7 complex. This inhibitory element appears to be abnormal on PNH III and AET E.

0374 HUMAN BLOOD COAGULATION VIA FACTOR VIIa AND VIA FACTOR XIa, Bonnie J. Warn-Cramer and S. Paul Bajaj, University of California at San Diego, La Jolla, CA 92093

During hemostasis, factor IX may be activated by two enzymes: factor XIa and factor VIIa/tissue factor. Furthermore, factor VIIa/tissue factor also activates factor X. To assess potential efficiencies of these enzymes to promote coagulation, we determined kinetic constants for these reactions. Values for activation of factor IX were: K_m = 209 nM, k_{cat} = 11 min^{-1} for factor VIIa; K_m = 330 nM, k_{cat} = 14 min^{-1} for factor XIa. Values for activation of factor X by factor VIIa were: K_m = 206 nM, k_{cat} = 49 min^{-1}. Although two molecules of high mol. wt. kininogen were found to bind per factor XIa dimer (K_d = 34 nM), it did not potentiate the activation of factor IX by factor XIa in the presence or absence of platelets and/or vessel wall. Factor VIIa was found to bind human brain tissue factor with a functional K_d of 110 to 450 pM. Factor VII bound to particulate tissue factor or to the human monocytic cells, U937, was rapidly converted to factor VIIa by traces of factor Xa. Presence of a large excess of unbound factor VII in the system did not inhibit activation of factor X. This suggests that factor VII may not inhibit factor VIIa in systems where factor Xa can be generated. Our kinetic data indicate that factor XIa and factor VIIa activate factor IX with similar efficiencies. However, plasma concentration of the catalytic subunit of factor XI is sixfold greater than that of factor VII. Additionally, at plasma concentrations of factors IX and X, our data predict that only 1/3 of factor VIIa/tissue factor will be available to activate factor IX. These considerations reinforce the concept that generation of factor XIa may be an important initiation event in coagulation.

Receptor Orchestration of Cell Surface Effector Pathways

0375 THROMBOMODULIN: AN EXAMPLE OF CELL SURFACE REGULATION OF PROTEASE FUNCTION, Esmon, C.T., Galvin, J.B., Esmon, N.L., Johnson, A.E., and DeBault, L.E. Thrombosis/Hematology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 (CTE,JBG,NLE,LED), Department of Chemistry, University of Oklahoma at Norman (AEJ), Departments of Pathology (CTE,LED) and Biochemistry (CTE), University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Early studies demonstrated that thrombin plays a critical role in the regulation of blood coagulation through the activation of many coagulation factors required for clot formation and stabilization. In addition, thrombin is a potent platelet activator. Recent studies have demonstrated that thrombin formation can be effectively controlled by thrombin-dependent formation of an anticoagulant enzyme, activated protein C.

Protein C (PC) circulates in plasma as a zymogen which can slowly be activated by thrombin. Complex formation between thrombin and an endothelial cell protein, thrombomodulin (TM) results in an approximate 20,000 fold increase in the rate of PC activation. Perhaps equally important, when thrombin is complexed with TM, thrombin loses its capacity to carry out procoagulant functions. Studies with fluorescent probes in the active site of thrombin suggest that thrombin undergoes a conformational change when it binds to TM that may be responsible for this change in specificity and/or the increased rate of protein C activation.

Immunofluorescence indicates that TM is restricted to the endothelial cells of the vasculature and is distributed in all organ systems of the normal rabbit. Specificity is not absolute, as some lines of malignant cells (of non-endothelial origin) have now been found to express TM.

This leads to the questions, do endothelial cells from different origins activate PC equivalently, and are all TM molecules structurally and functionally identical? Experiments comparing the ability of cells to activate PC and a proteolytically modified PC in which the gla domain (responsible for membrane binding) has been removed, indicate that the endothelial cell contributes a substrate binding site, in addition to TM, to the PC activation complex. Many, but not all, cell lines activate PC preferentially to gla-domainless-PC, although the purified thrombin-TM complex activates the two substrates equivalently. Although we believe all the lines tested synthesize the putative substrate binding site, not all are able to properly link the site to TM. Monoclonal antibody studies have been employed in an attempt to correlate the ability to discriminate substrates and specific epitope(s) on TM. Already apparent is the wide variation in antigenic structure of TM from lines of different origin. These studies are consistent with post-translational modifications of TM which alter antigenicity and may alter cell surface distribution and coupling to the cell surface PC binding site.

0376 THE ROLE OF THE MEMBRANE SKELETON IN TRANS-MEMBRANE SIGNALLING, **V.T. Marchesi, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510.**

Physiologic ligands activate cells by occupying specific receptors on their membrane surfaces. These receptor molecules are designed so that their ligand-binding sites are exposed to the cell exterior while at the same time they are linked to proteins inside the cell through unusual polypeptide segments that are able to span the lipid bilayer.

The proteins that are attached to the inner membrane surface are a special class of cytoskeletal elements composed of oligomeric actin units and actin-binding proteins that both stabilize the lipid bilayer and also modulate the topography of trans-membrane receptor molecules.

The molecular features of the proteins that make up the membrane skeleton of non-nucleated red cells have been studied in most detail, and some provisional ideas as to how they interact have been proposed. Two principal trans-membrane glycoproteins, glycophorin and band 3, are linked to a submembranous skeleton composed of spectrin and actin through two intermediaries, known as ankyrin and protein 4.1. Ankyrin links the beta subunit of spectrin to the cytoplasmic pole of band 3, while 4.1 links spectrin and actin together to the cytoplasmic segment of glycophorin. The latter connection seems to require polyphosphoinositide as a specific lipid cofactor, and in situ phosphorylation reactions also play a modulating role.

Under certain conditions the connections between the spectrin-actin lattice and the overlying membrane can be modified. One consequence of this is a change in the affinity of binding of protein 4.1 to the glycophorin-phospholipid complex. Thus the potential exists for shifts in the topography of surface receptors as a result of re-arrangements of the membrane skeleton. Switching mechanisms of this type may play a general role in cell activation and growth control.

Behavior of Leukocytes and Platelets

0377 T CELL RECOGNITION, Michael J. Bevan, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

T cells recognize and respond to foreign antigens only in conjunction with cell surface products of the major histocompatibility complex (MHC)--the phenomenon referred to as MHC-restriction. The various receptor models that have been proposed to explain this phenomenon will be summarized. Recently, antibodies which detect idiotypic, allotypic or common determinants on the T cell receptor have become available. How these antibodies can be used to address the question of T cell activation will be discussed.

0378 NONENZYMATIC GLYCOSYLATION OF PROTEINS AND THE EFFECTS ON UPTAKE BY MACROPHAGES. Anthony Cerami, Laboratory of Medical Biochemistry, The Rockefeller University, New York, New York 10021

Glucose can react with proteins both *in vivo* and *in vitro* without the participation of enzymes to form specific covalent adducts. The reaction of glucose with hemoglobin to form hemoglobin A_{1c} was the first example to be studied (1), and it has served as a model system in many subsequent investigations. In this reaction, glucose first forms a Schiff base with the amino terminal valine of the hemoglobin molecule. Then, over a period of weeks, the Schiff base adduct undergoes an Amadori rearrangement to form the stable Amadori rearrangement product HbA_{1c}. This reaction is not unique to hemoglobin, *in vivo*. Following Amadori product formation on long-lived proteins, further reactions and rearrangements of the Amadori product give rise to advanced glycosylation endproducts (AGE), which may play an important role in the complications of diabetes and in normal aging as well. In contrast to the Amadori product, the AGE are irreversibly attached to the proteins. Thus, AGE continue to accumulate on long-lived proteins such as collagen, lens crystallins, and myelin, while Amadori products do not (2). The AGE moieties are brown, fluorescent chromophores which can crosslink proteins. We have recently been able to determine the structure of one of the AGE products - 2 furoyl-4(5)-(2-furanyl) - 1-*H*-imidazole (FFI) (3). This compound is of interest since it gives insight how glucose can crosslink proteins intermolecularly. Intermolecular crosslinks induced by glucose have been shown to occur in lens proteins and collagen (4). In addition, formation of AGE on collagen can subsequently trap bystander protein molecules (e.g. IgG, albumin) that had not been exposed to glucose (5). The trapping of proteins on long-lifed molecules may account for accumulated IgG, albumin, and LDL attached to long-lifed collagen molecules.

Recently, we have uncovered another role for AGE products on proteins. The presence of AGE stimulates the uptake and destruction of the modified proteins by macrophages (6). The increased uptake occurred with myelin modified *in vitro* or from aged or diabetic individuals. These results point to the presence of a specific removal system to rid the body of AGE proteins.

1. Koenig RJ, Cerami A: Ann Rev Med 31:29-34, 1980.
2. Brownlee M, Vlassara H, Cerami A: Ann Int Med 101:527, 1984.
3. Pongor S, Ulrich P, Bencsath FA, Cerami, A: Proc Natl Acad Sci USA 81:2684-2688, 1984.
4. Monnier VM, Cerami A: Science 211:491-493, 1981.
5. Brownlee M, Pongor S, Cerami A: J Exp Med 158:1739-1744, 1983.
6. Vlassara, H, Brownlee M, Cerami A: J Exp Med 160:197-207, 1984.

0379 Thrombospondin: Functional Considerations at Cellular Interfaces, R.L. Nachman, R.L. Silverstein, L.L.K. Leung, and P.C. Harpel. Cornell University Medical College New York, NY 10021

Thrombospondin (TSP), a multifunctional alpha granule glycoprotein of platelets, binds fibrinogen, fibronectin, heparin, and histidine rich glycoprotein (HRGP). HRGP complexed with TSP was capable of binding heparin and neutralizing the anticoagulant activity of heparin in plasma. Specific complex formation between TSP and HRGP may play a significant role in regulating thrombotic influences at vessel surfaces. To define further the role of TSP in these reactions, the association of TSP with plasminogen (Plg) was studied. Complex formation of TSP with Plg was detected by rocket immunoelectrophoresis of mixtures of the purified radiolabeled proteins. Significant complex formation of fluid phase Plg with adsorbed TSP was also demonstrated by ELISA. The complex formation was specific, concentration dependent, saturable, inhibited by excess fluid phase TSP, with an apparent K_d=34nM, and blocked by 10 mM EACA. These data imply a role for the high affinity lysine binding site of Plg in mediating the formation of the complex. TSP also formed a trimolecular complex with HRGP and Plg as demonstrated by ELISA as well as rocket immunoelectrophoresis of mixtures of the three proteins. HRGP in the trimolecular complex was functionally active as indicated by binding ^{35}S-heparin as well as the neutralization of the anticoagulant activity of heparin in plasma. Plg in the trimolecular complex also retained functional capability. Tissue plasminogen activator generated significant amounts of plasmin, as measured fluorimetrically, from the TSP-HRGP-Plg complex. No plasmin was generated from the biomolecular TSP-HRGP complex and only minimal amounts from Plg added to other adsorbed proteins such as fironectin or von Willebrand factor. The studies suggest that TSP can serve as a surface for plasminogen activation by tissue activator. Thus TSP can form a functionally active trimolecular complex with HRGP and Plg. Such macromolecular complexes may play a significant role in influencing platelet vessel wall interactions as well as modulating the association of various cells with the extracellular matrix.

Inflammation and Vascular Cell Biology

0380 PLATELET-MEDIATED CYTOTOXICITY AGAINST SCHISTOSOME INDUCED BY ACUTE PHASE SERUM AND PURIFIED CRP, Daniel Bout, Institut Pasteur, Lille, France.

Functions of C' Reactive Protein (CRP), a major acute phase protein, are poorly known. Increased production of such a protein is stimulated as efficiently by autologous damage in the absence of infection as it is by infection. In mouse schistosomiasis, the serum concentration of S amyloid protein which is considered as the major acute phase reactant, was shown to rise sharply and to remain elevated at the time of granulomatous inflammation in the liver. We now report that in the rat, known to be a semi-permissive host for schistosome, the CRP which rises after the lung stage of infection and is at its highest level at the period of warm rejection, interacts with blood platelets. Rat and human platelets activated by acute phase serum or CRP preparation are able to kill schistosomula *in vitro*. CRP activated rat platelets are, furthermore, able to transfer significant protection against schistosomiasis.

0381 SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) INHIBIT THE OXIDATION OF LOW DENSITY LIPOPROTEIN (LDL) BY MONOCYTE DERIVED MACROPHAGES,
Martha K. Cathcart, Diane W. Morel and Guy M. Chisolm III, Cleveland Clinic Foundation and Case Western Reserve University, Cleveland, OH 44106

We have shown that human neutrophils and adherent populations of peripheral blood monocytes (PBM) can be stimulated to oxidize LDL and make it cytotoxic to growing fibroblasts. Both the oxidation and cytotoxicity can be prevented by butylated hydroxytoluene, a general free radical scavenger, glutathione or vitamin E. Our current results indicate a significant linear correlation between LDL oxidation (as measured by thiobarbituric acid reactivity) and LDL toxicity to fibroblasts ($p<0.01$). SOD (75 u/ml) and CAT (200 u/ml) inhibit the PBM ($2x10^6$ cells/ml) mediated oxidation of LDL at lower than normal plasma concentration (500 μg cholesterol/ml); boiled SOD and CAT are ineffective. Identically prepared PBM populations exhibit donor variation. For example, adherent PBM do not always require addition of activators to oxidize LDL. Our results with various activators reveal that a lymphocyte supernatant is more effective than opsonized latex or phorbol myristate acetate (PMA), and that PMA is more effective than N-formyl-methionyl-leucyl-phenylalanine in promoting oxidation of LDL by PBM. Our results are consistent with oxidation of LDL involving PBM-derived H_2O_2 and/or superoxide anion. That the oxidation of LDL by PBM may involve metal ions is suggested by our finding that certain chelating agents inhibit the oxidation. The proximity of high concentrations of interstitial LDL to lipid laden macrophages in early arterial fatty streak lesions leads to speculations that oxidized LDL may be produced *in vivo* and damage adjacent cells.

0382 SURFACE RECEPTOR MODULATION OF BOVINE MAMMARY NEUTROPHILS DURING INFLAMMATION, Peter J. Didier and John A. Shadduck, University of Illinois, Department of Veterinary Pathobiology, Urbana, Illinois 61801

Functional activity of neutrophils in extravascular sites can be substantially different from the activity of neutrophils in circulation. We compare the expression of Complement and Fc receptors over time on neutrophils isolated from the bovine mammary gland after inoculation with oyster glycogen. Our results demonstrate decreased expression of the Complement receptor over time relative to Fc receptor expression on milk and blood derived neutrophils.

0383 MACROPHAGE PRODUCTION OF HSF AND IL-1 HAVE DIFFERENT INDUCING PATHWAYS, Gerald M. Fuller, Sang-Uk Nham and B. Mickeal Woloski, Department of Human Biological Chemistry and Genetics, The University of Texas Health Science Center, Galveston, TX 77550.

One of the hallmarks of acute inflammation is a significant alteration in the synthesis of plasma proteins made by hepatocytes. We know that the major regulator molecule controlling this hepatic response is a 24-27kd protein produced by monocytes/macrophages. We call this protein the hepatocyte stimulating factor (HSF). It is also known that macrophages secrete interleukin-1, a protein that controls fever, thymocyte and fibroblast proliferation and muscle proteolysis. We have examined some of the signals which cause the production of these two monokines in an attempt to determine if there are separate inducing pathways. We show that both of these monokines can be produced by monocytes, peritoneal exudate macrophages, and leukemic cell lines which can acquire macrophage-like features ($P388D_1$, HL-60 and U937). The plasminolytic product of fibrin(ogen), fragment D, is a potent inducer of HSF but not IL-1. Bacterial lipopolysaccharide stimulates IL-1 synthesis but is an ineffective inducer of HSF production in $P388D_1$ cells. Both monokines are produced in the leukemic cells when treated with phorbol myristate acetate, however, the IL-1 production is more pronounced. Conditions which lead to a "superinduction" of IL-1 (exposure of cells to PMA and cycloheximide followed by actinomycin D and sodium butyrate) completely inhibits HSF production. These data suggest that the two macrophage products HSF and IL-1 which regulate events distal to the inflammatory lesion respond to different inducing signals.

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0384 *PSEUDOMONAS AERUGINOSA* EXOTOXIN-A ABROGATION OF MURINE SPLENIC MONONUCLEAR TRANSFORMATION, Dale F. Gruber and Thomas A. Davis, Armed Forces Radiobiology Research Intitute, Bethesda, MD 20814-5145.

Pseudomonas aeruginosa a normally commensal enteric organism, becomes pathogenic following immunoparalysis of the host. We report that one of its' extracellular products, exotoxin-A can enhance immunosuppression by abrogating splenic mononuclear transformation capabilities. Cells cultured in the presence of exotoxin for 72 hours demonstrated significant changes in lymphocyte transformation characteristics. Based on ^{3}H thymidine uptake the order of sensitivity to exotoxin-A was determined to be Con-A >PHA>LPS. Increasing concentrations of exotoxin resulted in proportionately greater inhibition of thymidine uptake kinetics. In four repetitive experiments mononuclear cells were preincubated with exotoxin for 1 hour and washed three times. Maximal concentrations of toxin (2000 ng/ml) decreased Con-A response levels to $25 \pm 7\%$ of controls. PHA respondant cells decreased to $89 \pm 4\%$ of controls while LPS respondant cells demonstrated little, if any, inhibition. Thymidine uptake kinetics were not significantly effected by increasing either the temporal availability of exotoxin from 1-3 hours or by increasing the number of washings from 1-3X. Cytotoxicity, measured by trypan blue exclusion, was evident (40%) at 48 hours and later only in populations cocultured in the presence of CON-A. The data indicate that the CON-A respondant population of cell mediated immunity is sensitive to the effects of exotoxin-A.

0385 STIMULUS-RESPONSE COUPLING AND REGULATION IN THE FC RECEPTOR MEDIATED MACROPHAGE RESPIRATORY BURST, Thomas A. Hamilton, Paul A. Johnston and Dolph O. Adams, Duke University Medical Center, Durham, N.C. 27710

The interaction between immune complexes and Fc receptors ultimately leads to activation of the NADPH oxidase complex which is responsible for generation of O_2^-. However, there is little known in molecular terms of how the signal is transduced. We have examined macrophages treated with polyclonal immune complexes for alterations in protein phosphorylation and observed a stimulus dependent phosphorylation of at least 6 different macrophage proteins (73,67,53,37,31, and 25 kD) which exhibits a dose and time dependency consistent with a role in signal transduction. Furthermore, the phosphorylation pattern induced in response to immune complexes is similar to that induced in response to phorbol diesters. These results suggest that the Ca^{++}, phospholipid dependent kinase activity recently identified as the site of action of phorbol diesters, may be involved. Furthermore we have observed that long term treatment of macrophages with bacterial lipopolysaccharide (LPS) leads to a dramatic alteration of immune complex mediated oxidative metabolism while that stimulated in response to phorbol esters remains unaltered. Whether the change results in enhancement or diminution of the respiratory burst depends upon the stage of functional development of the macrophage population in use. The ability of such macrophages to bind and/or ingest radiolabeled immune complexes is unaltered or increased by such treatment. Furthermore the immune complex mediated protein phosphorylation is also altered following LPS exposure. Thus we conclude that regulation of respiratory burst capacity in mononuclear phagocytes can be regulated, at least, in part, at the level of stimulus-response coupling.

0386 UPTAKE BY MOUSE PERITONEAL MACROPHAGES OF LARGE CHOLESTEROL ESTER-RICH PARTICLES ISOLATED FROM HUMAN ATHEROSCLEROTIC LESIONS , Henry F. Hoff and Beverly A. Clevidence, The Cleveland Clinic Foundation, Cleveland, OH 44106.

We have previously shown that a lipoprotein fraction (LP) consisting of large particles and representing most of the extracellular lipid in human atherosclerotic lesions, is recognized by a high affinity binding site on mouse peritoneal macrophages (MPM) resulting in unregulated uptake, stimulation of cholesterol esterification (CEst) and massive accumulation of cholesteryl esters (Arteriosclerosis 1: 196-210, 1981). To better characterize the interaction between LP and MPM, we isolated LP from supernatants of plaque homogenates by first removing a cream layer via high speed centrifugation and then gel filtering (A-15m) the infranatant. LP (void volume fraction) possessed a cholesterol-to-protein ratio of 2.5, and stimulated CEst in MPM in a dose-dependent fashion with saturation kinetics. LP isolated from plaques by extracting tissue minces for 24 hr, i.e. avoiding homogenization, gave the same ratio and CEst, suggesting that homogenization had not created a new fraction with enhanced CEst. Incubation of MPM with LP for up to 16 hr resulted in a time-dependent increase in number of extracellular vacuoles containing LP and believed to be secondary lysosomes. However, typical cholesteryl oleate-rich lipid droplets were rarely present, even though CEst was high. When subject to SDS PAGE, LP isolated from both homogenates and leached fractions demonstrated several bands, some of which were identified immunochemically as albumin (strong band) as well as apoB, apoE, and fibronectin (weak bands). Ongoing studies seek to determine whether any of these proteins are responsible for the binding to MPM by testing whether antibodies to them inhibit stimulation of CEst.

0387 ENDOTHELIAL CELL INTERACTIONS IN THE CONTROL OF GRANULOPOIESIS, Elaine McCall and Grover Bagby Jr., Oregon Health Sciences University, Portland, OR 97201

Granulocytes and monocytes are formed from progenitor cells present in the bone marrow. _In vitro_ growth of these cells depends upon the presence of a family of glycoproteins known as colony stimulating activity (CSA). We have recently described a monocyte product (monocyte-derived recruiting activity, MRA) which stimulates endothelial cells (EC) to produce CSA. We asked if the increase of CSA in inflammation might be related to increased MRA production by stimulated inflammatory cells. We now describe the response of stimulated and resting monocytes, and compare this with granulocyte responses. Blood monocytes or granulocytes (10^2-10^6/ml) were incubated 1h-3d in RPMI 1640 containing 10% lactoferrin depleted fetal calf serum, and dilutions of the supernatants were incubated a further 3d with EC cultures. The EC conditioned media were tested for CSA against low density bone marrow cells depleted of T-lymphocytes and macrophages. GM colonies were counted at 7d. Neither monocyte, granulocyte nor EC supernatants contained significant levels of CSA; detectable CSA therefore indicated the presence of recruiting factor in monocyte or granulocyte supernatants. MRA production was detected from 10^3-10^6 resting monocytes after 24h, and rose to a peak at 3d, while stimulating activity was detected only with 10^6 granulocytes, at 2d. With LPS (50ug) added to monocytes, MRA was detected at 8h, and rose to 3-10 times maximum resting levels by 24h. Stimulation of granulocytes with 10^{-6}M f-MLP did not increase production of stimulatory activity, nor did granulocyte lysates (from 4.10^4 cells) stimulate CSA production from EC. These results suggest that both in the steady state and in granulopoietic stress, monocyte products are important regulators of EC CSA production, and thus of granulopoiesis.

0388 REDUCED CYTOLYSIS BY ACTIVATED MACROPHAGES OF P815 TUMOR CELLS TREATED WITH THE OLIGOSACCHARIDE PROCESSING INHIBITOR DEOXYNOJIRIMYCIN. Arthur M. Mercurio and Phillips W. Robbins. M.I.T., Cambridge, MA. Tumoricidally activated macrophages recognize a structure(s) common to the surfaces of most types of transformed cells but not present on the surfaces of normal cells. The molecular nature of this recognition structure(s) is not known. We are examining the hypothesis that specific carbohydrates expressed on the tumor cell surface as a result of oncogenic transformation are involved in this recognition. Deoxynojirimycin (dNM) is an inhibitor of the glucosidases which are essential for the processing of the asn-linked precursor $Glc_3Man_9GlcNAc_2$ to complex oligosaccharides. In the present experiments, P815 cells were incubated in normal medium or in medium containing dNM for 24 hours, and the susceptibility of these cells to lysis by γ-interferon activated mouse macrophages was then assessed by an 18 hour ^{51}Cr-release assay. The dNM had no effect on cell viability, rate of growth or morphology. Compared to P815 cells incubated in normal medium, a 29% inhibition of cytolysis was observed for cells incubated in 1mM dNM, a 48% inhibition for cells incubated in 2mM dNM, and a 71% inhibition for cells incubated in 3mM dNM. Biochemically, P815 cells incubated in dNM accumulated $Glc_1Man_9GlcNAc_2$ indicating a disruption of processing. Labeling studies with neuraminidase/galactose oxidase/NaB^3H_4 indicate that dNM reduces considerably the amount of complex oligosaccharides on the cell surface. These data suggest that complex-type oligosaccharides on the tumor cell surface are involved in the lytic interaction between activated macrophages and tumor cells. Oligosaccharides may be involved in this interaction either directly or indirectly, e.g., by affecting protein conformation.

0389 MODULATION BY LOW DENSITY LIPOPROTEIN (LDL) OF ENDOTOXIN-INDUCED ENDOTHELIAL CELL (EC) TOXICITY, Diane W. Morel, Paul E. DiCorleto, and Guy M. Chisolm, Cleveland Clinic Foundation and Case Western Reserve University, Cleveland, OH 44106.

The mechanisms responsible for EC damage by endotoxin (lipopolysaccharide, LPS) are not known. We and others have found that even very high concentrations of LPS (in our system, up to 1000 μg/ml of LPS from S. typhosa in serum-free medium) did not cause cytotoxicity (cell detachment and morphological changes in 24 hr) to confluent human umbilical vein EC (HUVEC), whereas lower concentrations of LPS (10 to 100 μg/ml) were very cytotoxic to bovine aortic EC (BAEC). Addition of LDL (500 μg cholesterol/ml) virtually prevented the cytotoxic effects of LPS (even at 50 μg/ml) on BAEC. In contrast, LDL added to HUVEC with higher concentrations of LPS (500 and 1000 μg/ml) resulted in marked cytotoxicity, an effect that was absent with either LPS or LDL alone. Butylated hydroxytoluene (BHT, 20 μM), a general free radical scavenger, did not significantly alter the toxic effect of LPS on BAEC or its inhibition by LDL, but BHT inhibited or even prevented the toxic effect of LPS plus LDL on HUVEC. Further evidence that an oxidation is involved in the synergistic effect of LPS plus LDL on HUVEC is our finding that lipid peroxidation products (measured as thiobarbituric acid reacting substances) are increased in HUVEC supernatants containing LPS and LDL unless BHT is present. This oxidation is consistent with our previous finding that HUVEC but not BAEC can oxidize LDL lipids. We speculate that LDL prevents LPS-induced cytotoxicity to BAEC by binding to the LPS and interfering with the interaction of LPS with BAEC. Oxidation of the LPS-LDL complex by HUVEC may result in the formation of an LPS-derived toxic product or increased uptake of the toxic complex.

0390 The Human Lymphotoxin Gene: Structure and Homology with Tumor Necrosis Factor, Glenn E. Nedwin, Diane Pennica, Julie A. Jarrett, Douglas Smith, Patrick W. Gray, and David V. Goeddel, Genentech, Inc., 460 Pt. San Bruno Blvd., S.S.F., CA 94080.

Human lymphotoxin (LT) and tumor necrosis factor (TNF) are cytokines produced by mitogen activated peripheral blood leukocytes. These molecules have similar and/or identical cytostatic and cytolytic activities on transformed cells both in vitro and in vivo. We have recently isolated and sequenced cloned cDNAs for both human LT and TNF, proving that they are distinct molecules. We have also determined the structure of a unique single copy gene for human LT. The gene was isolated from a genomic DNA library using a single 120 bp ^{32}P-labelled synthetic DNA probe whose sequence was based on the first 60 amino acids of human LT. The sequence of a 3 kb region of genomic DNA containing the entire LT gene indicates that the gene contains three introns. The site of initiation of transcription was determined by S1 mapping. By introducing two gaps the LT sequence can be aligned with the TNF sequence so that distinct homologies are apparent. Comparison of the nucleotide sequence of the coding regions of LT and TNF shows that they are 46 percent homologous. At the amino acid level LT and TNF are 28 percent homologous, and many of the remaining differences between the two proteins result from conservative amino acid changes. Two particularly conserved regions are found at amino acids 35-66 and 110-133 (TNF numbering) where 50 percent of the residues are identical. The hydrophobic carboxy-terminal of the two molecules are also significantly conserved. These conserved regions may be crucial for the shared cytotoxic activities of LT and TNF.

0391 GLYCOPROTEIN PROFILES OF MACROPHAGES AT DIFFERENT STAGES OF ACTIVATION REVEALED BY THE BINDING OF LECTINS AFTER SDS-PAGE. Garth L. Nicolson, Tatsuro Irimura, Susan M. North, and Carolyn P. Cooke, Dept. of Tumor Biology, The University of Texas-M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Glycoconjugate changes occur upon myeloid cell differentiation; however, little is known about glycoconjugate differences among macrophages of different activation stages. We have estimated the classes of cellular glycoprotein carbohydrate chains after SDS-PAGE separation of the glycoproteins by lectin binding in combination with *in situ* chemical modifications (Irimura, T. and Nicolson, G.L. Carbohyd. Res. 115:209-220, 1983; Carbohyd. Res. 120:187-195, 1983; Cancer Res. 44:791-798, 1984), and we have applied this technique to rat peritoneal macrophages. Resident macrophages, thioglycolate-elicited macrophages, thioglycolate-elicited macrophages stimulated with LPS and BCG-activated macrophages were washed and lysed with 0.25M sucrose 10mM Tris-HCl buffer pH 7.3 containing 0.5% NP-40 and 10 µM PMSF and immediately mixed with electrophoresis sample buffer. After SDS-PAGE the samples were transblotted to nitrocellulose membranes and stained with ^{125}I-labeled lectins before or after sialic acid removal. Although LPS stimulation *in vitro* did not change glycoprotein profiles, macrophages at different stages revealed remarkably different glycoprotein profiles. For example, $M_r \sim 180{,}000$ and $\sim 160{,}000$ glycoproteins revealed by *Lens culinaris* hemagglutinin and *Ricinus communis* agglutinin after desialyzation were prominent only with resident macrophages, whereas the same lectins revealed $M_r \sim 41{,}000$ and $\sim 31{,}000$ components of thioglycolate-elicited macrophages. Wheat germ agglutinin revealed a $M_r \sim 87{,}000$ component of BCG-activated macrophages. $M_r \sim 35{,}000$ and 33,000 components stained with peanut agglutinin after removal of sialic acid or with pokeweed mitogen were seen in thioglycolate-elicited or BCG-activated macrophages.

0392 MODULATION OF HUMAN VASCULAR ENDOTHELIUM BY IMMUNE INTERFERON, Jordan S. Pober, Alan H. Stolpen, Eva C. Guinan, Walter Fiers and Tucker Collins, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 and State University of Ghent, Belgium

Recombinant immune interferon (IFN-γ) increases expression of class I (HLA-A,B) and induces *de novo* appearance of class II (HLA-DR, DC and SB) major histocompatibility complex surface antigens on cultured human endothelial cells by regulating mRNA levels. Recombinant fibroblast interferon (IFN-β) increases mRNA and surface expression of class I but does not induce class II mRNA or surface antigen. IFN-γ, but not IFN-β, causes HEC to elongate and to lose contact inhibition of migration (i.e. the cells extensively overlap at confluence) without loss of growth control (i.e. the cells remain mitotically quiescent at confluence and are actually growth inhibited at subconfluence). IFN-γ treated HEC at confluence rearrange their actin filaments from dense peripheral bands into longitudinal arrays of stress fibers. IFN-γ treated HEC also lose their underlying fibronectin meshwork. These studies show that cultured vascular endothelium is a highly responsive target of IFN-γ action, and the endothelial modulations observed *in vitro* in response to purified lymphokine may underlie some of the vascular changes that accompany cell-mediated immunity.

0393 GENETIC CONTROL OF MACROPHAGE INFLAMMATORY RESPONSE TO INFECTION AND MALIGNANCY, Emil Skamene, Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4

Intraspecies differences in the expression of various facets of the macrophage response to infection are invariably the result of a particular genetic make-up of the host. Appropriate breeding experiments can be designed for the study of the mode of inheritance and of the chromosomal location of the "macrophage genes." Phenotypic expression of such genetic control can formally be proven by analysis of the linkage of the effective or defective macrophage responses to their postulated cellular and molecular mechanisms in segregating progeny and in recombinant, mutant and congenic inbred strains. By division of the course of macrophage response to infection into discrete phases, the genetic control of multiple steps of this complex phenomenon becomes apparent and can be analyzed. Results of experiments described in this presentation will demonstrate the genetic linkage of resistance to intracellular parasites *in vivo* with macrophage microbicidal activity *in vitro*, linkage of resistance to *Listeria monocytogenes* with the macrophage inflammatory response and the genetic linkage of macrophage activation for tumoricidal and schistosomulicidal activities *in vitro*.

0394 LOW DENSITY LIPOPROTEIN (LDL) INHIBITS PLATELET SECRETORY AND MEMBRANOLYTIC RESPONSES TO MONOSODIUM URATE CRYSTALS (MSUC). R. Terkeltaub, D. Smeltzer, L. Curtiss, M. Ginsberg, Scripps Clinic and Research Foundation, La Jolla, CA.

LDL is the major plasma inhibitor of neutrophil responses to MSUC *in vitro*. We studied platelets to determine if similar effects occur with other urate-responsive cells. Plasma inhibition of MSUC-induced platelet ^{3}H-serotonin (5HT) release was dose-dependent and virtually complete at a 1:4 dilution. Plasma depleted of lipoproteins (LPDP) by KBr density gradient ultracentrifugation effected minimal inhibition. Reconstitution of LPDP with LDL, but not high denisty lipoprotein, restored the inhibitory activity of plasma. LPDP was also less inhibitory than plasma for MSUC-induced membrane lysis (^{51}Cr release). LDL (225 g/ml) abrogated MSUC-induced 5HT release from cytochalasin B-treated platelets under conditions where lysis was minimal. LDL was thus shown to inhibit the platelet secretory response to MSUC. LDL (22.5-450 g/ml) did not inhibit platelet 5HT secretion in response to 0.5 mg/ml collagen, heat aggregated IgG (5 mg/ml) and thrombin (1 U/ml). At these LDL concentrations, responses to MSUC and silica (4 mg/ml) were profoundly inhibited. We conclude that LDL inhibits both platelet secretory and membranolytic responses to MSUC *in vitro* and that this inhibitory activity is not due to effects on platelet responsiveness to all stimuli. Lipoproteins may thus regulate cellular interactions with negatively charged particulates and surfaces.

0395 REGULATION OF EXPRESSION OF CSF RECEPTORS AND OTHER FUNCTIONAL ANTIGENS ON HUMAN NEUTROPHILS, Mathew A. Vadas, Nicos A. Nicola and Angel F. Lopez. The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

G-CSF (a pure murine colony stimulating factor that preferentially stimulates the maturation of neutrophils from mouse and human progenitors) and monoclonal antibodies WEM-G1 and 11 (recognizing granulocyte specific antigens of Mr 110000 and 95000 respectively) stimulate human neutrophil function. G-CSF enhances antibody dependent cytotoxicity (ADCC) of tumor targets by neutrophils but not eosinophils, WEM-G1 enhances ADCC by neutrophils and eosinophils and WEM-G11 ($F(ab')_2$ stimulates both ADCC and phagocytosis of Ig coated targets. Radio-iodinated G-CSF shows specific binding to human neutrophils that is inhibited by cold G-CSF and human CSF-β but not by GM-CSF or CSF-α. Incubation of human neutrophils with f-met-leu-phe (FMLP) at 37°C but not at 4°C resulted in rapid time and dose dependent loss of G-CSF binding (by 80% at 20') and simultaneous enhancement (by 330%) of the binding of WEM-G1 and 11. The capacity of the MAb's to stimulate neutrophil function was also increased by FMLP. In contrast to WEM-G's the binding of a MAb against β_2-microglobulin was unaltered. The changes observed were due to modulation of membrane structures rather than changes in affinity. Cytochalasin B caused a further dose and FMLP dependent enhancement of binding of WEM-G11 but not of WEM-G1 and a prolongation of the effect of FMLP or G-CSF receptors. These studies show an independent modulation of human neutrophil surface structures involved in function.

0396 METHIONINE, BY SCAVENGING PMN-DERIVED OXIDANTS, PRESERVES ANTI-ELASTASE ACTIVITY OF α1 ANTITRYPSIN (AT) AND CONCOMITANTLY PROTECTS HUMAN ENDOTHELIUM: RAMIFICATIONS FOR SMOKER'S ATHEROSCLEROSIS. G Vercellotti, D Stroncek, , P Huh, HS Jacob. U of Minn, Mpls.

Emphysema and atherosclerosis result from smoking. PMN are thought doubly responsible for lung dissolution: they release tissue-destructive elastase and generate reactive O_2 species that oxidize a methionine (MET) group in AT rendering it, in turn, impotent as an anti-elastase. We hypothesized an analogous scenario: namely, PMN elastase might mediate vascular damage and underlie smoker's atherosclerosis. Our strategy used ^{51}Cr-labeled human endothelial cells (EC) exposed to intact PMN or to enucleate "neutroplasts" (NP); the latter are elastase-free cytoplasmic blebs derived from PMN. When activated, both PMN and NP generate similar amounts of toxic O_2 species; yet NP cause insignificant EC damage, measured as ^{51}Cr "lift- off" from anchoring matrix (PMN=14.8 ± 2.6% vs NP=1.2 ± .4; $p < .001$). Adding pure elastase (10^{-6}M) back to NP reconstitutes EC lift-off (7 ± .2%). Surprisingly, pure AT, a potent anti-elastase, does not protect EC from PMN assault, suggesting PMN oxidants inactivate it. Indeed, added physiologic levels of MET, a scavenger of one such oxidant, chloramine ($ClNH_2$), completely protects AT from inactivation by reagent $ClNH_2$ or that generated by suitably-treated NP; concomitantly it prevents PMN-provoked EC lift-off ($p < .01$). We conclude that stimulated PMN injure vascular tissue by combining elastase secretion with oxidative inactivation of the anti-elastase, AT. We suggest that smoke-stimulated PMN may engender atherosclerosis by this mechanism and that methionine by preserving anti-elastase activity in serum may modulate this deleterious combination.

0397 Ca^{++} REDISTRIBUTION BY CHEMOATTRACTANTS IN HUMAN PMNs MAY UTILIZE A NOVEL N PROTEIN,
M Verghese and R Snyderman, Howard Hughes Med Inst, Duke Univ, Durham, NC 27710
The chemoattractant receptor(CTX-R) for fMet-Leu-Phe(FMLP) has high(~1nM) and low affinity (~20nM) states which are regulated by GTP. Although this suggests coupling to nucleotide regulatory proteins(N), adenylate cyclase is not coupled to CTX-R. PMNs appear to use intracellular $[Ca^{++}]_i$ as a second messenger for chemoattractant signals and we found that exposure of PMNs to FMLP, Con A or WGA caused immediate rises in $[Ca^{++}]_i$ (Quin 2 measurements) of 3 to 4 times resting levels(140$\pm$35nM). Quin 2 responses occurred at 0.5nM FMLP and plateaued at 10nM FMLP, showing similar sensitivity as chemotaxis. To determine whether N protein coupled to CTX-R could regulate $[Ca^{++}]_i$ instead of cAMP-mediated transduction processes in PMNs, we studied the effects of islet activating protein(IAP) which specifically inactivates N_i but not N_s. Treatment of human PMNs with IAP caused nearly 100% inhibition of Quin 2 monitored $[Ca^{++}]_i$ rises and chemotaxis towards FMLP. Inhibition was irreversible and dose and time dependent. In contrast, changes in $[Ca^{++}]_i$ induced by Con A or WGA were not affected by IAP. Similarly, IAP inhibited FMLP induced O_2^- production and lysosomal enzyme release by ~70% and ~50%, respectively, but equivalent responses to PMA, Con A or WGA were inhibited by only 15%. Thus, N proteins apparently regulate the affinity of the CTX-R and its ability to redistribute $[Ca^{++}]_i$, thereby affecting functional responses. Since the release of inositol-1,4,5 trisphosphate from phosphatidylinositol 4,5 bisphosphate(PIP_2) is thought to result in enhanced $[Ca^{++}]_i$, we suggest that CTX-R-N-GTP activates a specific phosphodiesterase responsible for the hydrolysis of PIP_2.

0398 PHENOTYPIC CHARACTERIZATION OF γ INTERFERON-INDUCED HUMAN MONOCYTE POLYKARYONS (MP).
J.B. Weinberg, M.A. Misukonis and M.M. Hobbs, VA and Duke, Durham, NC 27705
We have previously demonstrated that highly purified recombinant human γ interferon (IFN-γ) causes normal human peripheral blood monocytes to fuse and form MP. These MP formed over a 36 to 72 hour period in cultures with 10% autologous, unheated serum. The MP were 28 to 1000 microns in diameter and contained 2 to 150 nuclei/MP with a fusion index of 40 to 70%. The peak effects were seen at doses of 20 to 100 units/ml (0.1 to 0.5nM). The IFN-γ effect was abolished by treatment at 56°C for 4 hours, pH 2 for 3 hours, or with mouse monoclonal anti IFN-γ antibody. As determined by autoradiography, the MP did not incorporate tritiated thymidine into their nuclei. The MP contained nonspecific esterase and tartrate-resistant acid phosphatase. Various preparations of recombinant and natural α and β interferons did not cause the MP formation. Populations of IFN-γ-treated monocytes had increased levels of acid phosphatase, plasminogen activator, and H_2O_2 production in response to PMA. However, when assessed on an individual cell basis, the MP reduced little or no NBT, while the uninuclear monocytes reduced large amounts. The MP phagocytized latex spheres normally, but there was diminished phagocytosis of antibody-coated sheep erythrocytes. The uninuclear monocytes contained antigens recognized by the monoclonal antibodies LeuM3 (antimonocyte), 9E1 (anti HL-60), lysozyme, HLA-DR and TE5 (antithymic macrophage). The MP had normal lysozyme and normal or increased HLA-DR, but there was no or very little LeuM3, 9E1, and TE5 in these MP. Thus, IFN-γ induces MP formation by fusion of blood monocytes, and the MP are phenotypically different than the monocytes.

Lymphocytes

0400 THE MHC AND PLASMINOGEN ACTIVATOR: FUNCTIONAL RELATIONSHIPS. Paul Gladstone, Mark Leahy, Genetic Systems Corp. Seattle.
We are studying possible relationships between HLA antigens and serine proteases, principally plasminogen activator (P.A.). HLA-DR antigens are involved in antigen presentation; proteases are presumably involved in antigen processing. Interferon (IFN) inducible expression of HLA class II antigens (HLA-DR) and P.A. are strongly correlated in tissue distribution (macrophages, endothelial cells, etc.). Beyond this correlation does either of the two components regulate the other? In an Ia-inducible mouse macrophage line, Wehi-3, a specific inhibitor of P.A., e-ACA (60-120 mM), in the presence of γ-IFN causes a 5-10X increase in membrane Ia (I-A^d and I-E^d) and a two-fold increase in class I over that seen with γIFN alone. Cell associated P.A activity, measured in a chromagenic assay, was inhibited by eACA; maximal inhibition was seen at concentrations giving maximal Ia induction. The inhibitor, TLCK, neither stimulated Ia nor inhibited cellular P.A. Apparently, there is a mechanism for downregulation of Ia induction which involves a protease(s). Is this control reciprocal, i.e., does Ia have an influence on the protease activity? P.A. activity was measured in blood mononuclear cells free of serum. When these cells are incubated with anti-HLA-DR monomorphic monoclonals for 16-18 hours, cell-associated PA activity is inhibited by 80-90% in some individuals. Other individuals show 40-50% inhibition and a few less than 5% inhibition. An individual's "Ia-dependent P.A. activity", based on limited repeat tests, is reproducible. We hypothesize that DR acts as an intercellular transducer of a signal for P.A. expression and that some DR-antibodies block this signal. Elastase doesn't seem to be under this type of control.

0401 FUNCTIONAL AND IMMUNOCHEMICAL CHARACTERIZATION OF MONOCLONAL ANTI-INTERLEUKIN-2 ANTIBODIES, John A. Hare, Linfield, College, Rich Jones and Denis R. Buger, VA Medical Center, Portland, OR 97207.
The production of monoclonal anti-interleukin 2 (IL-2) antibodies will be useful in development of assays for IL-2, in purifying IL-2 from natural souces, and in defining the interaction of IL-2 with its receptor on T cells. An antibody to the active site of the receptor for IL-2 was used as the source of immunogen and the anti-(anti-receptor) antibodies which were produced by the use of standard hybridoma techniques were screened for anti-IL-2 activity. Ultimately, protein A purified antibodies from one such hybridoma (H3) were shown to inhibit the functional activity of IL-2 in an assay with an IL-2 dependent cell line. The inhibition was IL-2 specific and not due to an indirect effect of the antibody on the cells. An enzyme immunoassay was developed with H3 and it was able to detect recombinant human IL-2. The results indicate that the approach used may be useful in producing antibodies to other growth factors and lymphokines which are difficult to obtain in sufficient quantities and purity to be directly used as immunogens.

0402 THE ANTIGEN-PRESENTING ROLE OF ENDOTHELIAL CELLS IN VIVO, B. Standage, C. Wagner, R. M. Vetto, D. Burger, VA Medical Center, Portland, Oregon 97207.
In order to ask questions regarding the role of EC in immunological responses in vivo, we have developed a model system for transferring cell-mediated immune (CMI) responses in rats. Spleen cells from immunized rats are cultured with antigen for 48 hours in vitro and then transferred into recipient animals. The recipients are then tested for a CMI response using ear induration and histology as a measure of delayed type hypersensitivity (DTH). Using this system we have been able to transfer CMI to KLH and tuberculin with as few as 10^7 cultured spleen cells. By constructing radiation-induced, bone marrow-reconstituted chimeras, we can produce potential recipients with bone marrow-derived cells which are compatible to donor lymphocytes but disparate to the endothelium of the recipient. Therefore, the transferred immune T cells are only histocompatible with the bone marrow-derived cells (e.g. MΦ and dendritic cells) and incompatible with the non-bone marrow derived cells in the recipient (e.g. the EC). Since EC presentation of antigen has been shown to be MHC restricted in vitro, the EC in the recipient rat can not present antigen to the histoincompatible transferred cells. Therefore, if EC presentation of antigen is a critical step in the development of a DTH response, the F_1 to P chimera recipients should not develop positive responses to the test antigens. Using this in vivo system in conjunction with in vitro experiments, we are attempting to elucidate the antigen presenting role of EC in immunological responses.

0403 PRODUCTION OF T CELL DIFFERENTIATION FACTOR (TCDF) AND CYTOTOXIC CELL DIFFERNTIATION FACTOR (CCDF) IN SYNGENEIC LYMPHOCYTE CULTURES. C.C. Ting, N-N Loh, and M.E. Hargrove. National Cancer Institute, NIH. Bethesda, MD. 20205
TCDF is required to induce the antigen- or mitogen-activated cytotoxic T cell (CTL) precursors to be differentiated into mature CTL. Previously we demonstrated that CCDF is required for the differentiation of the lymphokine-activated cytotoxic precursors into a unique class of cytotoxic effectors, the lymphokine-induced cytotoxic cells (LICC). In the present study we found that both TCDF and CCDF were produced in a syngeneic lymphocyte culture containing normal mouse spleen cells, peritoneal cells and indomethacin. There was no requirement of antigenic or mitogenic stimulation for their production. CCDF was produced by the macrophages but was distinct from interleukin 1; it was present at peak levels in day 1-2 culture supernatants. TCDF was produced by T cells, was first detected in day 3 culture supernatants, peaked on day 5, and then declined. CCDF syngerized with interleukin 2 (IL2) to generate LICC which selectively killed the lymphoid or solid tumor targets of different etiological origins and of different H-2 haplotypes. The LICC did not kill lymphoblasts even in the presence of lectin. On the other hand, TCDF synergized with IL2 to generate antigen- or mitogen-activated CTL. The antigen-induced CTL only killed targets with the appropriate antigenic specificity whereas mitogen-induced CTL killed a variety of targets, including lymphoblasts, in the presence of lectin. Our results indicate that two lymphokines, TCDF and CCDF, with distinct biological activities are produced in a syngeneic lymphocyte culture.

0404 **A FUNCTIONAL SUBSET OF HUMAN T HELPER CELLS DEFINED BY A MONOCLONAL ANTIBODY TO THE IL-2 RECEPTOR,** **Paul Yoshihara, Denis Burger, VA Medical Center, Portland, OR 97201.**

Human T helper cells (T_H OKT-4+ cells) are heterogeneous with respect to biological function. We have prepared monoclonal antibodies to subsets of cells in the T_H family and attempted to relate phenotypic expression of surface markers to functional properties of the subsets. Mice were immunized with cloned, human T_H lymphoma cells and after fusion hybrids were screened for production of antibodies against the T_H subset from which the lymphoma was derived. A variety of screening patterns was observed including one defining a minor poulation of normal T_H cells. This hybrid culture was cloned (designated T_H5.2) and the resulting monoclonal antibody characterized as a cytotoxic IgM isotype recognizing 3-5% of normal blood mononuclear cells (about 20% of blood T_H cells). Addition of T_H5.2 to lymphocyte cultures produced significant augmentation of mitogen and antigen-induced proliferation particularly when the stimulant concentration was suboptimal. Depletion of T_H5.2+ cells by sorting or cytotoxicity eliminated antigen responsiveness as well as suppressing (65-80%) mitogen-induced proliferation. This monoclonal antibody also blocked the ability of IL-2 dependent cell lines (HT-2) to respond to IL-2. Taken together the data suggests that this antibody recognized the IL-2 receptor.

0405 INTERACTION OF IMMUNE CELLS WITH TARGET CELL MEMBRANES: IS THERE A COMMON MECHANISM OF KILLING? John Ding-E Young, Carl Nathan and Zanvil A. Cohn, Rockefeller University, New York, N.Y. 10021

We have been investigating the role of pore-forming proteins in mediating cell -mediated cytotoxicity. We have evidence that eosinophils and cytotoxic T lymphocy contain cytolytic proteins localized in cytoplasmic compartments which induce pore- -formation in model lipid membranes and cause hemolysis. Pore-formation was examin planar lipid bilayers and the ionic current associated with the pores directly assa The pores formed by these cytolytic proteins are large, aqueous, ion non-selective insensitive to electrical field. The insertion of pores by granule proteins derive cytotoxic T cell clones was dependent on calcium in the bathing medium and was most effective at 37 °C. Pore-formation could be triggered by treatment of cell suspens with A23187. The stimulated cell supernatant contained high concentrations of pore forming activity. For eosinophils, the protein has been identified as the eosinoph cationic protein, previously described by Per Venge and Inge Olsson.

Similar pore-forming proteins have also been identified in cytopathic strains *Entamoeba histolytica*, the causative agent of amebiasis. This protein has now been purified to homogeneity and shown to induce hemolysis and pore-formation in planar ayers.

The pores formed by these lytic proteins resemble closely to the ones formed b terminal attack complex of human complement. With Eckhard R. Podack, we are studyi comparative electrical properties of all these membrane-active proteins. It is pos that working models such as this one, involving similar effector mechanisms, are ap cable to a number of other cytotoxic cell types.

Effector Functions of Leukocytes and Platelets

0406 RECOGNITION AND DESTRUCTION OF TUMOR CELLS BY ACTIVATED MACROPHAGES: REGULATION AT THE MOLECULAR LEVEL, Dolph O. Adams and Thomas A. Hamilton, Department of Pathology, Duke University, Durham, NC 27710

The ability of macrophages (MØ) to kill tumor cells is an acquired function strictly regulated by two signals: interferon-γ (IFNγ) and the lipid A moiety of endotoxin (LPS). The two must be added in that precise sequence, and treatment with IFNγ reduces the amount of LPS that must be subsequently added. Kill is a two-step process: 1) target recognition and capture (binding); and 2) secretion of toxic substances including a cytologic proteinase (CP). IFNγ induces competence for binding and prepares the MØ for release of CP, but LPS is required to trigger release of the enzyme and induce cytolyis. To probe the transductional events that follow cellular perception of these two signals and that lead to the above functional consequences, we have analyzed protein phosphorylation and synthesis. IFNγ enhances the enzymatic activity of protein kinase C (PKc). Several lines of evidence suggest that this is due to covalent modification of the enzyme, resulting in a higher turnover number. LPS stimulates a specific pattern of protein phosphorylation (proteins of M_r 103,75,67,60,57,45,37,31,28,20 kD) similar to that induced by PMA, a known stimulant of PKc. Phosphorylation induced by LPS is enhanced in IFNγ-treated MØ. In LPS resistant C3H/HeJ mice, LPS does not induce lytic function or phosphorylation, though PMA readily triggers phosphorylation via PKc. PMA (10^{-7}M) plus the calcium ionophore A23187 (10^{-6}M) induce phosphorylation similar to that induced by IFNγ plus LPS and mimic the preparation for cytolysis induced by IFNγ, though LPS is still required to initiate actual kill of tumor cells. LPS further stimulates a distinctive pattern of new protein synthesis (i.e., new proteins of M_r 85,80,75,65,57,38,kD); cycloheximide blocks the effects of LPS but not of IFNγ on induction of lytic competence. Treatment with IFNγ lowers the LPS threshold by ~ 100 fold. We interpret these data that IFNγ enhances the "potential" activity of PKC, while LPS triggers phosphorylation of new proteins via PKc and a burst of new protein synthesis as well. These changes, in cooperation with altered intracellular levels of Ca^{++}, thus appear to be cardinal events in the intracellular signal transduction of stimulation by IFNγ and LPS that lead to macrophage activation.

1. Johnson, W.J., Somers, S.D. and Adams, D.O. Activation of Macrophages for Tumor Cytotoxicity: In, Contemporary Topics in Immunobiology. D.O. Adams and M.G. Hanna (eds), Plenum Press, New York, 14: 127-146, 1984.
2. Adams, D.O. and T.A. Hamilton: The Cell Biology of Macrophage Activation. Ann. Review of Immunol., Vol. 2, pp. 283-318, 1984.

0407 NK MEDIATED CELL LYSIS: A DIRECTED SECRETORY EVENT OF CYTOLYTIC TUBULAR STRUCTURES. Gunther Dennert, Department of Microbiology, Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033.

Events that lead to target cell lysis by cloned natural killer (NK) cells (1) have been studied with light microscopy and electron microscopy. NK cells and targets collide and bind randomly. After the initial binding the Golgi apparatus (GA) and the microtubule organizing center (MTOC) in the NK cell reorient towards the target binding site, an event that takes place about 30 min., requires recognition of specific cell surface structures on the target and the presence of Ca^{+2} (2,3). Reorientation of the GA/MTOC complex only takes place in the effector cell and is not observed in a situation in which the killer cell lost its cytolytic activity. This and the finding that non lysable targets do not induce reorientation in the effector cell (3) suggests that reorientation is an important and integral part of the events leading to target lysis. The integrity of the microtubules appears to be important for GA/MTOC reorientation, because depolymerization of the microtubules by nocodazole inhibits it (2). Interestingly, one of the conditions that inhibit reorientation, i.e., treatment of cells with nocodazole does not completely inhibit cytolysis in particular when the lectin Con A is added to NK target mixtures. This suggest that reorientation is not an obligatory event in target cell lysis. It is therefore proposed that reorientation serves to direct the secretion of cytolytic components rather than enables it. Support for this comes from the observation that the dense granules tend to collect in the area of target cell binding. The recognition of target structures by the NK cell leads to secretion of vesicles that originate from the granules (4). The vesicles carry P_1 and P_2 perhaps in a preassembled way and either fuse with the target membrane or transfer P_1 and P_2 by an as yet unknown mechanism (4,5). Since vesicle mediated cytolysis appears to be nonspecific the effector cell must have a protective mechanism to avoid autolysis. Future experiments will have to elucidate what these protective mechanisms may be and whether the vesicles carry yet other lymphocytotoxic substances besides P_1 and P_2.

1. G. Dennert. Cloned lines of natural killer cells. Nature (Lond), 287:47 (1980).
2. A. Kupfer, G. Dennert, and S.J. Singer. Polarization of the Golgi apparatus and the microtubule organizing center within cloned natural killer cells bound to their targets. Proc. Natl. Acad. Sci. USA 80:7224 (1983).
3. A. Kupfer and G. Dennert. Reorientation of the microtubule organizing center and the Golgi appartus in cloned cytotoxic lymphocytes triggered by binding to lysable target cells. J. Immunol., (in press).
4. E.R. Podack and G. Dennert. Cell-mediated cytolysis: assembly of two types of tubules with putative cytolytic function by cloned natural killer cells. Nature (Lond) 302:442 (1983).
5. G. Dennert and E.R. Podack. Cytolysis by H-2 specific T killer cells. Assembly of tubular complexes on target membranes. J. Exp. Med. 157:1483 (1983).

0408 ROLE OF THE MOUSE MACROPHAGE Fc RECEPTOR AS A LIGAND-DEPENDENT ION CHANNEL, John Ding-E Young and Zanvil A. Cohn, Rockefeller University, New York, N.Y. 10021

The mouse macrophage FcRγ2b/γ1 has previously been purified by means of the monoclonal antibody 2.4G2. Our data indicate that FcR functions as a ligand-dependent ion channel. (a) Using [^{3}H]tetraphenylphosphonium$^+$ ([^{3}H]Ph_4P^+) uptake by the J774 macrophage cell line as a probe for membrane potential changes, we found a biphasic change in membrane potential following treatment with immunocomplexes, monoclonal antibody 2.4G2 and 2.4G2 Fab-Sephadex particles. An immediate depolarization, whose magnitude and duration were dose and Na^+-dependent, was followed by prolonged hyperpolarization. (b) [^{3}H]Ph_4P^+ uptake experiments were done with plasma membrane vesicles prepared from J774 cells by nitrogen cavitation, which showed binding of FcR led to monovalent cation flow through the membrane. (c) Purified FcR was reconstituted into phospholipid vesicles by octylglucoside dialysis. These vesicles showed an ion flow response following binding of specific ligands comparable to the plasma membrane vesicles. (d) The purified FcR was incorporated into planar lipid bilayers and the conductance of the membrane monitored. Addition of specific ligands resulted in a transient conductance increase across the bilayer, which occurred in discrete steps. Single channel fluctuations were resolved in 1 M KCl. The mean conductance was 60 pS and average lifetime 250 ms (in the first 5 min after exposure to ligands). The channel conductance showed ohmic behavior and was equally permeable to Na^+ and K^+ but relatively impermeable to Ca^{2+}.

However, direct measurements of intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) showed a rise in $[Ca^{2+}]_i$ to micromolar levels following binding of the FcR by immunocomplexes. An optimal range of $[Ca^{2+}]_i$ was found to be required for phagocytosis, below and above which inhibition of ingestion was observed. We suggest the involvement of localized cytosolic ion (Na^+, Ca^{2+}) gradients in generating the signals necessary for phagocytosis and secretion.