MOLECULAR BASIS OF CELLULAR ADHESION

Organizers: Richard Hynes and Erkki Ruoslahti

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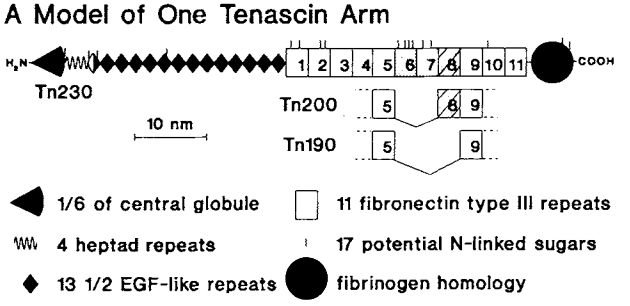
Poster Sessions

Extracellular Matrix Glycoproteins-I

A 001 **STRUCTURE AND POSSIBLE FUNCTIONS OF TENASCIN, AN EXTRACELLULAR MATRIX PROTEIN WITH A DISTINCTIVE TISSUE DISTRIBUTION.**

Ruth Chiquet-Ehrismann and Jürg Spring, Friedrich Miescher Institute, Postfach 2543, CH-4002 Basel, Switzerland.

Tenascin is transiently present in the mesenchyme around epithelia of developing organs and it is reexpressed in the stroma of many epithelial tumors. In vitro its synthesis can be induced by TGF-beta. It is therefore not surprising to note that the tissue distribution of tenascin is very similar to the one reported for TGF-beta. Tenascin is a glycoprotein consisting of six identical disulfide-linked subunits of 190 - 230kD. A structural model of the three splicing variants of the tenascin subunits is shown below. The model was derived from the aminoacid sequence determined from cDNA clones. It is drawn to scale and resembles the structural features of one tenascin arm seen on electron micrographs. Proteolytic fragments of tenascin as well as recombinant parts of tenascin expressed in bacteria were used to identify functional domains. A cell binding site was localized within 104 aminoacids spanning the 10th and the beginning of the 11th fibronectin type III repeat. In contrast, fragments containing the EGF-like repeats showed anti-adhesive activity. The presence of these two contrary signals on the same molecule may be responsible for the apparent contradictory observations reported on the effects of tenascin on cell attachment and migration. Certain cells may primarily be responsive to one or the other site of tenascin and react accordingly.



A 002 FIBRONECTIN ISOFORMS, EXPRESSION AND FUNCTION, Richard O. Hynes, Howard Hughes Medical Institute and Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

The multiple isoforms of fibronectin, which are produced by alternative splicing of the RNA transcript of a single gene, are expressed in a cell- and tissue-specific fashion and are regulated during physiological processes such as wound healing and embryological development. This raises two questions; first, what is the mechanism of regulation of the alternative splicing and, second, what are the different functions of the different spliced forms? We are analyzing the first question by studying the control of RNA splicing *in vivo* by transfection of minigenes and *in vitro* splicing extracts and the second by a variety of methods. Recombinant fibronectins of each of the spliced forms of rat fibronectin have been expressed in lymphoid cells using retroviral vectors. These cells generate homogeneous populations of the different isoforms which can be purified and their functions studied. In this way, we have defined the interaction of a specific integrin receptor, $\alpha_4\beta_1$ with one of the alternatively spliced segments. This interaction mediates adhesive functions in certain cell types. The roles of specific fibronectin isoforms and integrins are also under study *in vivo*, using systems amenable to genetic analysis.

A 003 FIBRONECTIN AND CELL INTERACTIONS Kenneth M. Yamada, Shin-Ichi Aota, and Steven K. Akiyama, Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892 and Howard University Cancer Center, Washington, D.C. 20060. The fibronectin (FN) molecule provides a valuable model system for analyzing cell surface interactions. At least 5 distinct sites in the protein can be used by various adhesion receptor systems for cell attachment and spreading, most of which recognize short peptide sequences. For example, fibroblasts can use the central cell-binding domain with its Arg-Gly-Asp site plus a synergistic site for initial adhesion using the $\alpha_5\beta_1$ integrin; certain other cells can also use specific alternatively spliced regions of FN. The synergistic region of the FN cell-binding domain has been analyzed by site-directed mutagenesis; it is functionally important since its loss leads to >97% loss of adhesive activity. This region also appears to be essential for appropriate integrin receptor binding recognition. A series of overlapped deletions define an important region necessary for function; finer-resolution mapping using deletions designed to retain the type III repeated motif in FN shows a complex series of effects on activity, suggesting the existence of several equivalent sites or conformational requirements. This region appears to differ significantly from other recognition sites in that there is no single, very short peptide sequence that can as yet account for activity.

After initial adhesion, cells often proceed to migrate and/or to elaborate a fibronectin-containing extracellular matrix. Fibronectin matrix assembly on fibroblasts requires β_1 integrins, including $\alpha_5\beta_1$; since the amino-terminal end of FN also appears involved, the "matrix assembly receptor" system appears complex. The mechanisms for recognition of FN during migration appear to differ for different cell types. Human fibroblasts and bladder carcinoma cells do not require the $\alpha_5\beta_1$ receptor for migration on FN, while HT-1080 and colon carcinoma cells do. Although cells can use the central cell-adhesive domain of FN for migration, an unexpected finding is that the sequence requirements for migration can differ substantially from those for initial adhesion. Other FN recognition receptors may be involved, e.g. the $\alpha_3\beta_1$ and vitronectin receptors; in addition, distinct integrins are required for migration on FN, laminin, or collagen. The existence of multiple mechanisms of FN recognition involving specific recognition regions in the molecule and various specific receptors may permit sophisticated regulation of cell-specific adhesion and migration, with the possibility of redundancy for certain key functions.

Extracellular Matrix Glycoproteins-II

A 004 MEROSIN: MOLECULAR STRUCTURE AND ROLE IN DEVELOPMENT, Eva Engvall, Ilmo Leivo, Karin Ehrig and Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Rd., La Jolla, CA 92037.

Basement membranes are morphologically similar in all tissues, but contain tissue-specific and differentiation-specific components that can be detected by immunohistochemistry. One of these tissue-restricted antigens is a protein that we have named merosin [Leivo and Engvall, PNAS 85, 1544 (1988)]. Merosin is present only in basement membranes of trophoblast and mature muscle and nerve. We have isolated and characterized the native merosin molecule from placenta. Electron microscopy after rotary shadowing shows that merosin has a cross-like structure similar to laminin. The molecular weight of merosin, estimated by SDS-PAGE, is about 700 kDa compared to 800 kDa for laminin. The purified merosin contains the B1 and B2 light chains of laminin and the B chains are disulfide cross-linked to a merosin-specific heavy chain. The merosin heavy chain was cloned from a placental λgt11 cDNA library. Sequencing the COOH-terminal one-third of the merosin heavy chain showed that it is 40% homologous with the COOH-terminal portion of the heavy chain of laminin. Immunofluorescence on sections of various tissues with antibodies specific for the heavy chains of merosin or laminin shows that, in the adult, basement membranes contain either laminin or merosin but not both. The differential expression of laminin and merosin in basement membranes suggests different functions dependent on the heavy chains. The cell-binding and neurite-promoting activities of laminin are thought to reside in the COOH-terminal portion of the laminin heavy chain. We have found that merosin also interacts with cells and have begun to analyze the spectrum of cells and receptors recognizing merosin.

A 005 THE STRUCTURE AND FUNCTION OF THROMBOSPONDIN, Jack Lawler, Vascular Research Division, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115. Thrombospondin is a 420,000-dalton glycoprotein that is synthesized and secreted by various cells in culture. Thrombospondin is also secreted by blood platelets at the site of vascular injury. Thrombospondin is similar to the members of the class of adhesive proteins in that it is composed of multiple copies of several structural motifs, it has a heparin-binding domain and it has a RGD sequence. Thrombospondin can support cell attachment through interactions with cell surface proteoglycans and beta-3 integrins. Plasmodium falciparum parasitized erythrocytes also bind to thrombospondin. To further characterize the function of thrombospondin, a mammalian expression system was used to produce normal and variant forms of human thrombospondin in mouse 3T3 cells. Expression of the normal full length cDNA yields a 420,000-dalton trimer which comigrates with platelet thrombospondin during SDS-PAGE in the absence of reducing agents. The expressed protein also binds to heparin and to two monoclonal anti-thrombospondin antibodies. The human specific monoclonal antibody MA-I, also detects a 125,000-dalton single chain polypeptide in the culture media after growth of the expressing cells. Since the epitope for MA-I is very close to the RGD sequence, the 125,000-dalton polypeptide may retain cell binding activity and the relative proportions of the intact molecule and the 125,000-dalton polypeptide may act to modulate the cell's response to thrombospondin. The full length cDNA was also cloned into M13mp8 and used as a template for oligonucleotide-directed mutagenesis. Twenty nucleotides on both sides of the region to be deleted were combined to make the oligonucleotide. Deletion of the type 1 repeats (residues 361-530) and the type 2 repeats (residues 531-673) results in the relative increase in the single chain forms with molecular weights of 91,000 and 94,000, respectively. These results indicate that normal structure is required for the expression and/or stability of native thrombospondin. (This work is supported by NIH grants HL28749 and HL40631).

Integrins-I

A 006 CELL ADHESION FUNCTIONS AND STRUCTURAL ASPECTS OF VLA PROTEINS IN THE INTEGRIN FAMILY, Martin E. Hemler, Mariano J. Elices, Christina Parker, and Yoshikazu Takada, Dana-Farber Cancer Institute, Boston, MA 02115

The VLA proteins are a series of heterodimers composed of unique α chains each associated with the integrin β_1 subunit. The VLA-2 complex ($\alpha^2\beta_1$) which had been well established as a collagen receptor, is now also shown to be a laminin receptor because, 1) anti VLA-2 antibodies blocked adhesion of several cell types to laminin, and 2) the $\alpha^2\beta_1$ complex could be directly isolated from a laminin Sepharose column. However, not all cells utilize VLA-2 as a laminin receptor, since VLA-2 from platelets and fibroblasts appeared to function only as a collagen receptor. No obvious structural differences were found between VLA-2 that bound multiple ligands (collagen, laminin) and VLA-2 which bound only to collagen.

In other experiments, the role of VLA-3 as a fibronectin receptor was firmly established. First, a cell line which selectively expressed VLA-3, but not VLA-5, was shown to bind to fibronectin in a VLA-3 dependent manner, because binding was almost completely inhibited by anti-VLA-3 MAb. Second, VLA-3 from cell extracts in which VLA-5 was either depleted or absent clearly could be bound to fibronectin-Sepharose under physiological salt conditions, and subsequently eluted with RGD-containing peptides.

Also, a cDNA clone for the VLA-4 α^4 subunit was transfected into the K-562 erythroleukemic cell line, thus conferring VLA-4-dependent adhesiveness towards the 40 kDa heparin-binding fragment of fibronectin. Notably, in the transfected cell line, the α subunit of VLA-4 was functional despite being expressed mostly (>95%) in its cleaved (80/70 kDa) form. In further studies of VLA-4, an unusual 180 kDa form of α^4 was identified which appeared in addition to the previously established 150 kDa form. All cells examined so far which express $\alpha^{4/150}$ have also expressed $\alpha^{4/180}$. The $\alpha^{4/180}$ form appears to result from the covalent linkage of $\alpha^{4/150}$ with another, yet to be defined 30 kDa chain.

A 007 ARG-GLY-ASP RECEPTOR SPECIFICITY, Pierschbacher M.D., La Jolla Cancer Research Foundation, La Jolla, CA 92037

Most extracellular matrix proteins carry at least one Arg-Gly-Asp (RGD) sequence and, in many cases, this sequence has been shown to mediate the interaction of the protein with the cell surface. The receptors at the cell surface that recognize and bind the extracellular matrix proteins form a large supergene family and have been given the name integrins. Many of the integrins recognize an RGD sequence in their respective extracellular matrix (ECM) ligand yet they can distinguish one ECM protein from another. Data from our laboratory indicate that these integrins can distinguish the three-dimensional presentation of the RGD sequences in different proteins. The current extent of our knowledge in this area will be presented.

A 008 STRUCTURE OF THE LFA-1 AND ICAM-1 ADHESION SERVOMOTOR, ITS OPERATION BY THE T CELL-RECEPTOR, AND SUBVERSION BY RHINOVIRUS. Staunton,D.S., Dustin,M.L., Erickson,H.P., Hibbs,M.L., and Springer,T.A. The Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.

The integrin LFA-1 on lymphocytes binds to the Ig superfamily member ICAM-1 on other cells in immune and inflammatory cell interactions. Furthermore, the major group of rhinoviruses have subverted ICAM-1 as their receptor. In EM ICAM-1 is a bent rod 19 nm long. Since Ig domains are 4 x 2.5 x 2.5 nm, these dimensions suggest the 5 Ig domains of ICAM-1 are oriented end to end and are unpaired. Mutagenesis studies show LFA-1 and rhinovirus bind to distinct but overlapping sites within the 2 N-terminal Ig domains, with domain 1 most important. The N-terminal domain may be best suited for both types of adhesion because of its accessibility and segmental mobility. LFA-1 does not recognize an RGD or RGD-like sequence in ICAM-1.

Effective interaction between T cells and their targets requires that recognition of specific antigen be coordinated with increased cell-cell adhesion. Antigen-receptor cross-linking increases the adhesiveness of LFA-1, while the adhesiveness of ICAM is unaffected, as shown by binding cells to purified LFA-1 or ICAM-1. Intracellular signals are transmitted from the T cell antigen receptor to the LFA-1 adhesion molecule, as shown by inhibition with dibutryl cAMP and other pharmacologic agents. The high avidity state is maximal after 5-10 min., returning to baseline after 30 min., providing a deadhesion mechanism. Stimulated adhesion is temperature and energy dependent. Antigen recognition can thus drive stable cell-cell adhesion using metabolic energy. This allows antigen recognition to be very sensitive, because the equilibrium between unbound cells and conjugates does not have to be driven solely by the decrease in free energy due to receptor-ligand binding, but rather the antigen receptor can activate cell metabolism-dependent changes in LFA-1 avidity, utilizing ATP to drive the equilibrium toward conjugate formation. This regulatory mechanism and "inside-out" signalling may be of wide importance in cell biology; for example, spatial rather than temporal gradients in adhesion molecule avidity could drive cell migration.

Integrins-II

A 009 RECOGNITION OF DISTINCT ADHESIVE SITES ON FIBRINOGEN BY RELATED INTEGRINS ON PLATELETS AND ENDOTHELIAL CELLS, David A. Cheresh, Jeffrey W. Smith, Shlomo A. Berliner, Vicente Vicente, Thomas Kunicki*, and Zaverio M. Ruggeri, Research Institute of Scripps Clinic, 10666 No. Torrey Pines Road, La Jolla, CA 92037; *Blood Center Southeastern Wisconsin, 1701 W. Wisconsin Ave., Milwaukee, WI 53233

Endothelial cells and activated platelets express integrin-type receptors, termed $\alpha_v\beta_3$ and GPIIb/IIIa, respectively, that are responsible for adhesion to fibrinogen. We have located distinct integrin-directed endothelial cell and platelet attachment sites on immobilized fibrinogen using a combination of synthetic peptides, fibrinogen fragments, and specific anti-peptide monoclonal antibodies. Endothelial cells exclusively recognize an Arg-Gly-Asp-containing site near the C-terminus of the α chain (α residues 572-574), but fail to recognize the Arg-Gly-Asp sequence in the N-terminal region of the same chain (α residues 95-97). In contrast, platelets do not require either Arg-Gly-Asp sequence for binding to intact fibrinogen and are capable of recognizing, in addition to the α 572-574 sequence, a site near the C-terminus of the γ chain (γ residues 400-411). Consistent with these results, purified $\alpha_v\beta_3$ specifically bound to fibrinogen through the Arg-Gly-Asp near the C-terminus of the α chain, whereas the purified platelet integrin GPIIb/IIIa could also recognize the γ chain C-terminal dodeca peptide sequence. These data suggest a molecular mechanism, whereby related integrins on platelets and endothelial cells interact with distinct sites on the fibrinogen molecule during hemostasis and wound healing.

A 010 EXPRESSION OF ß1 INTEGRINS AND THE FUNCTION OF MOUSE AND HUMAN TROPHOBLAST. C. H. Damsky, A. E. Sutherland, C. Librach, L. Moss, G-Y Zhang, P. G. Calarco and S. J. Fisher. Schools of Dentistry and Medicine, University of California San Francisco, San Francisco, CA. 94143-0512. Trophoblast cells and their derivatives mediate the attachment of the mammalian embryo to the uterine wall and its subsequent implantation. In species that establish a hemochorial placenta, including both mouse and human, a subpopulation of trophoblast cells invades the uterine stroma and penetrates basement membranes, behaving much like metastatic tumor cells. This invasive activity is restricted to early stages of gestation. Both adhesion receptors and matrix-degrading proteinases are likely to be relevant to this regulated invasive behavior. In previous functional studies in the mouse, ß1 integrins were found to mediate blastocyst attachment and outgrowth on complex extracellular matrix (ECM) *in vitro* [1], but not to affect earlier stages of preimplantation mouse development [2]. In the present studies, both surface and metabolic labeling of preimplantation embryos, followed by immunoprecipitation with antibodies recognizing the α5 and α6 integrin subunits and the ß1 integrin family as a whole, indicated that none of these could be detected in expanded, unhatched blastocysts. The ß1 and α5 subunits were first detected 24 h post hatching, the time at which blastocysts become attachment competent *in vitro*. RNA phenotyping by the reverse transcription polymerase chain reaction demonstrated that mRNA for these subunits were also not detected prior to the hatched blastocyst stage. Since both α and ß subunits of an integrin heterodimer are required for ligand binding [3], these results suggest that no functional ß1 integrin heterodimers are present in mouse embryos prior to the implantation stage. The onset of expression of other α subunits and their spatial distribution in the post-implantation mouse embryo remain to be determined. In the human system, Fisher, et al.[4] showed that cytotrphoblasts isolated from first trimester placentas degrade complex ECM *in vitro* and express several metalloproteinases that are not expressed by these cells at later stages of gestation. When plated on a Matrigel-coated porous filter, a subpopulation of first trimester cytotrophoblasts, but not later gestation cytotrophoblasts, crosses the filter barrier. This invasion is inhibited by both anti-ß1 monoclonal antibody and by tissue inhibitor of metalloproteinase. Cytotrophoblasts express several ß1 integrins at all stages of gestation. However, the subpopulation of cytotrophoblasts that invades the uterine wall down-regulates expression of α 6 during the first trimester, and the ß1 integrins, but not the ß3 integrin, expressed by first trimester cytotrophoblasts are modified by the addition of polylactosamine chains. The presence of polylactosamines is greatly reduced on late gestations cytotrophoblast ß1 integrins. Taken together, these studies suggest an important role for ß1 integrins both in the initial stages of blastocyst implantation and in the regulation of trophoblast invasive behavior during gestation.

[1] Sutherland, et al. 1988, J.C.B. 106: 1331; [2] Richa, et al. 1985, Dev. Biol. 108: 513; [3] Buck, et al. 1986, J.C.B. 103: 2421; [4] Fisher, et al. 1989, JCB 109: 891.

A 011 ANALYSIS OF A LIGAND BINDING SITE IN CYTOADHESINS, Mark H. Ginsberg, Joseph C. Loftus, Stanley E. D'Souza, Timothy E. O'Toole, Andrew L. Frelinger and Edward F. Plow, Committee on Vascular Biology, Res. Inst. Scripps Clinic, La Jolla, CA 92037
Platelet GPIIb-IIIa and the endothelial vitronectin receptor (VnR) are members of the cytoadhesin (β_3) subfamily of integrins. Ligand binding to these receptors is inhibited by Arg-Gly-Asp (RGD) containing peptides. In addition, peptides derived from the γ chain of fibrinogen (e.g. $\gamma_{402-411}$) also inhibit RGD ligand binding to platelet GPIIb-IIIa. By use of conformation-specific antibodies and flow cytometry, we have analyzed the interaction of 2 RGD peptides (GRGDSP and RGDF) and $\gamma_{402-411}$ to GPIIb-IIIa and VnR. In platelets, the order of potency of these peptides was RGDF > GRGDSP > $\gamma_{402-411}$ which parallels their rank order in inhibition of fibrinogen binding . On endothelial cells' VnR the order was GRGDSP>RGDF>> $\gamma_{402-411}$. On the endothelial VnR, $\gamma_{402-411}$ was approximately 3 orders of magnitude less potent than GRGDSP. To ensure that these differences in peptide specificities were receptor related rather than cell type specific, cell lines expressing recombinant GPIIb-IIIa and VnR were established utilizing the same endothelial β_3 cDNA and different α cDNA's. The recombinant GPIIb-IIIa had similar peptide binding specificities to platelet GPIIb-IIIa and the recombinant VnR was similar to the endothelial receptor. Chemical crosslinking of radiolabelled peptides containing the RGD sequence and γ chain sequence to platelets resulted in prominent labelling of GPIIIa and GPIIb, respectively. The crosslinking sites were identified by mapping with site specific antibodies combined with N-terminal sequence analysis of labelled fragments. The sequences of both crosslinking sites were highly conserved amongst integrins. The GPIIb crosslinking site was within a 21 residue sequence containing a single putative calcium binding site. Patients with a bleeding disorder due to absence of platelet fibrinogen receptor function were identified. GPIIb-IIIa from those individuals lacked RGD and $\gamma_{402-411}$ peptide binding function as assessed by the cytometric assay and affinity chromatography. cDNA's coding for the GPIIb and GPIIIa chain crosslinking sites were amplified from patient platelet mRNA, sequenced, and a point mutation was identified in one of the crosslinking sites. Introduction of this mutation into GPIIb-IIIa lead to loss of RGD binding function. Thus, a ligand recognition site of cytoadhesins may be in proximity to highly conserved regions of α and β chains, and the α chain region probably binds divalent cations . A mutation affecting one of these conserved regions disrupts ligand binding functions of the receptors.

A 012 CHARACTERIZATION OF THE LYMPHOCYTE RECEPTOR FOR AN A NEW ADHESION SEQUENCE, EILDVPST, LOCATED IN THE CS-1 DOMAIN OF PLASMA FIBRONECTIN: FUNCTION IN MEDIATING HETEROTYPIC LYMPHOCYTE ADHESION. Elizabeth A. Wayner, Oncogen Corporation, Seattle, WA, 98104. Using mAb technology we identified a new fibronectin receptor that is identical to the integrin receptor $\alpha4\beta1$. mAbs P4C2 and P3E3 recognize distinct epitopes on the $\alpha4$ subunit and completely inhibit the adhesion of T or B lymphocytes to a 38 kDa tryptic fragment of fibronectin containing Hep 2 and part of IIICS. The minimal ligand in IIICS for $\alpha4\beta1$ is the EILDVPST amino acid sequence located in the carboxy terminal portion of CS-1. The EILDVPST peptide or mAbs to $\alpha4$ completely inhibit lymphocyte adhesion to CS-1, partially inhibit adhesion to intact fibronectin or the 38 kDa fragment, and have no effect on lymphocyte adhesion to an 80 kDa fragment containing the RGDS adhesion sequence. Furthermore, EILDVPST-albumin coated substrates support stable lymphocyte adhesion which can be completely inhibited by mAbs to $\alpha4$. mAb P1D6 to the prototype fibronectin receptor, $\alpha5\beta1$, completely inhibits lymphocyte adhesion to the 80 kDa fragment, also partially inhibits adhesion to intact fibronectin but has no effect on adhesion to the 38 kDa fragment or to CS-1. While investigating the role of $\alpha4\beta1$ and its peptide ligand EILDVPST in lymphocyte function the following observations were made. 1) T or B cell adhesion to monolayers derived from fibroblasts or IL-1 activated endothelial (EC) cells could be completely inhibited by a) the EILDVPST peptide, b) mAbs to $\alpha4$ (P4C2), or c) mAbs to $\beta1$ (P4C10). 2) Lymphocyte adhesion to matrix derived from fibroblasts or EC could also be inhibited by the EILDVPST peptide and by mAbs to $\alpha4$ or $\beta1$. However, lymphocyte-matrix interaction also clearly involved the $\alpha5\beta1$ receptor. 3) Other lymphocyte functions such as homotypic T or B cell aggregation, MLR reactivity, T or NK mediated cytolysis and Con A or LPS activation were not inhibited by the EILDVPST peptide or mAbs to $\alpha4$ or $\beta1$. 4) Alternatively, mAbs to $\beta2$ (P4C9 or 60.3) completely inhibited homotypic aggregation, MLR or mitogen reactivity and cytotoxicity. Anti-$\beta2$ antibodies, however, had little or no effect on lymphocyte adhesion to monolayers or matrix derived from fibroblasts or HUVEs. These data clearly define a dual role for $\alpha4\beta1$ and its EILDVPST peptide ligand in mediating lymphocyte interactions with cells or matrix derived from various tissue domains.

Proteoglycans

A 013 MATRIX AND MORPHOGENESIS: A ROLE FOR SYNDECAN, AN INTEGRAL MEMBRANE PROTEOGLYCAN. Merton Bernfield, Joint Program in Neonatology, Harvard Medical School, Boston, MA 02215.

Syndecan is a cell surface proteoglycan that contains heparan sulfate and chrondroitin sulfate chains attached to a 31 kDa core protein. Based on the cDNA, the syndecan core protein has a cytoplasmic, a hydrophobic transmembrane, and an extracellular domain, which contains 5 potential Ser-Gly GAGylation sites. Both 5' and 3' untranslated regions are highly homologous to identical regions of the human insulin receptor cDNA.

Syndecan behaves as a matrix receptor: It binds mouse mammary epithelial cells to interstitial matrix molecules via its heparan sulfate chains. Binding is to types I, III and V collagens, fibronectin and thrombospondin and is of high affinity (K_d~1nM for type I collagen). It is localized to the basolateral surface of simple epithelial cells both in vivo and in vitro and it associates with the actin cytoskeleton when crosslinked at the cell surface. When cultured cells round up, they rapidly shed the extracellular domain, loosening the cell's association with the substratum. Syndecan also binds basic FGF via its heparan sulfate chains.

Syndecan exhibits a structural polymorphism due to cell-type specific GAGylation: Simple epithelia produce a larger syndecan than stratified epithelia due to increased number and size of heparan sulfate chains. Mesenchymal cells contain 1/100 the amount of cell surface syndecan than epithelial cells and produce a syndecan with even larger size heparan sulfate chains.

Syndecan appears in pre-implantation embryos and is expressed in precursors of the embryo but not the extra-embryonic membranes. Embryonic epithelia transiently induce syndecan in their adjacent mesenchyme and syndecan is often lost with epithelial differentiation. Developing B lymphocytes express syndecan only when and where they associate with interstitial matrix.

Altered syndecan expression correlates with change in cell behavior. In culture, transformed mammary epithelial cells show reduced levels of syndecan mRNA and markedly reduced levels of cell surface syndecan. An SV2-neo construct containing the beta-actin promoter and the syndecan coding region in an antisense configuration has been used to obtain stable, syndecan-deficient epithelial cells. These transfectants have syndecan cDNA in their genome and mRNA corresponding in size and polarity to an antisense syndecan mRNA. Cells showing less than 10% of normal cell surface syndecan are fibroblastic and grow as individual fusiform cells with extensive filopolia that under- and overlap adjacent cells.

These results indicate that syndecan represents a class of membrane proteoglycans that are developmentally regulated and may act during morphogenesis to stabilize the organization of epithelial sheets.

(Supported by HD-06763, CA-28735 and the Lucille P. Markey Charitable Trust).

A 014 STRUCTURE OF THE BASEMENT MEMBRANE PROTEOGLYCAN AND IDENTIFICATION OF ITS CELL SURFACE BINDING PROTEIN. Hassell, J.R., Noonan, D.M., Clement, B., Segui-Real, B., Yamada, Y., Dept. of Ophthalmology, University of Pittsburgh, Pittsburgh, PA 15213, Dept. of Chemical Carcinogenesis, INRC, Genova, Italy, and LDBA NIDR, NIH, Bethesda, MD 20892.

The heparan sulfate containing basement membrane proteoglycan has been extensively characterized using tumor cell lines that produce basement membrane components. This proteoglycan consists of a 400,000 M_r core protein, which contains 5-7 globular domains as visualized by rotary shadowing, with 3-4 heparan sulfate chains attached to a globule at one end of the core. cDNA clones to this proteoglycan have provided the structural characterization of the core. The initial cDNA clones were obtained by screening expression vector libraries with antibodies.[1] We have since obtained additional clones from primer extension libraries to produce over 8 kilobases of continuance sequence. The deduced aminoacid sequence begins with the signal peptide at the N-terminal end of the core and contains 3 major domains showing homology to the lipoprotein binding region of the LDL receptor, to the globules of the short arms of laminin and to N-cam. These results indicate this proteoglycan evolved from exon shuffling.

This proteoglycan plays a role in a number of biological processes including promoting attachment of cells to culture dishes. We have recently identified 3 proteins (M_r=38,000, 36,000 and 26,000) on the surface of cells that binds to the core protein of the proteoglycan.[2] Binding of the core protein to the cell surface proteins was not altered by heparan sulfate, laminin, collagen IV or fibronectin. These cell surface, proteoglycan specific binding proteins were present on a variety of cells including, hepatocytes, EHS cells, MDCK cells, COS cells, melanoma cells and kidney epithelial cells. These observations suggest the proteoglycan has a cell surface binding protein distinct from those for other matrix components.

1) Noonan, D.M., Horigan, E.A., Ledbetter, S.R., Vogeli, G., Sasaki, M., Yamada, Y. and Hassell, J.R. J. Biol. Chem. 263:16379-16387, 1988.
2) Clement, B., Segui-Real, B., Hassell, J.R., Martin, G.R. and Yamada, Y. J. Biol. Chem. 264:12467-12471, 1989.

A 015 ANALYSIS OF PROTEOGLYCANS BY CLONING AND GENE TRANSFER APPROACHES, Erkki Ruoslahti, Cancer Research Center, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, CA 92037. Recent cDNA cloning of proteoglycan core proteins in our laboratory and by others has revealed a number of interesting structural features in these molecules. The core protein of serglycin is to a large extent composed of a serine-glycine repeat that serves as the glycosaminoglycan binding domain. Decorin and biglycan are composed of a 24 amino acid repeat motif found also in many cell surface receptors and in some regulatory proteins active in Drosophila development. The structure of versican, a large proteoglycan we have sequenced quite recently, is particularly interesting[1]. The sequence of the 2389 amino acid core protein has a potential hyaluronic acid binding domain, a glycosaminoglycan attachment domain and a COOH-terminus composed of epidermal growth factor like sequences, a lectin-like sequence, and sequence motif typical of complement regulatory proteins. These same elements are present in the LEC group of lymphocyte homing receptors, while the potential hyaluronic acid binding domain is homologous to another group of homing receptors. These structural features suggest that versican functions in cell recognition. Current gene transfer experiments aim at testing of this hypothesis. Gene transfer with decorin cDNA has shown that Chinese hamster ovary cells expressing high levels of decorin became more quiescent in their growth pattern than the original or control transfected cells[2]. This effect appears to be due to an increased activity of a TGF β-like factor in the culture media of the decorin-transfected cells. Elucidation of the molecular basis of this phenomenon is now in progress.

[1]Zimmerman, D. and Ruoslahti, E., EMBO Journal, in press
[2]Yamaguchi, Y. and Ruoslahti, E., Nature:vol 336, pp.244-246, 1988

Development-I

A 016 **ADHESION AND MOTILITY OF EMBRYONIC AND CANCER CELL,** Thiery, J.P., Duband J.L., Dufour,S., Boyer, B., Tucker, G.C., Valles, A.-M., Gavrilovic, J., Moens, G. and Jouanneau, J. , CNRS-ENS, Laboratoire de Physiopathologie du Développement, 46, rue d'Ulm-8ème Etage, 75230 Paris Cedex 05 FRANCE.
Cell- cell and cell-extracellular matrix adhesion mechanisms play a key role in morphogenesis and in cancer invasion and metastasis. We have analysed in detail the program of expression of several adhesion molecules in epithelial-mesenchymal cell interconversion and in migratory events in embryogenesis.The neural crest, a transient embryonic structure of the neural epithelium undergoes a conversion to a mesenchymal state; these cells subsequently migrate thoughout the embryo to give rise to many derivatives including most of the peripheral nervous system and melanocytes. The pattern of expression and modulations of the cell adhesion molecules (CAMs) and the substrate adhesion molecules (SAMs) correlate with the different morphogenetic steps in the neural crest. During migration crest cells do not express functional CAMs but interact specifically with fibronectins in the extracellular matrix. Several distinct cell binding domains on the fibronectin molecules have been mapped and their relative contribution to adhesion, spreading and motility will be described. A rat bladder carcinoma has been used as a model system to study early events in the dissociation and the acquisition of motility and invasive properties of carcinoma. This epithelial cell line undergoes a conversion to a migratory fibroblast-like state in response to different collagen types but not to fibronectins or laminin. A similar conversion is obtained when acidic FGF is added to the culture medium.This multifunctional growth factor induces a rapid internalization of desmosomes, and a progressive disappearance of cytokeratins which are replaced by vimentin intermediate filaments. Acidic FGF also triggers cell motility. On collagen substrates, the speed of locomotion is enhanced in the presence of acidic FGF and under these conditions the bladder carcinoma cells readily invade 3D collagen gels. Thus this model systems may offer a unique opportunity to evaluate the role of the different adhesion modes and soluble factors in the dispersion of carcinoma cells.

A 017 THE GENETIC ANALYSIS OF DROSOPHILA PS INTEGRINS, Michael Wilcox, Aaron DiAntonio, Maria Leptin and Marcel Wehrli, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom.

The Drosophila PS integrins, PS1 and PS2, have widespread and dynamic distributions during development. Analysis of lethal mutations in the PSβ gene shows that the integrins are required, during embryogenesis, for muscle attachment and for certain cell sheet migrations (1). At such muscle attachments, and in other cell-cell adhesions, the two integrins are found in adjacent positions on either side of the junctions, suggesting that they cooperate in their function. Later, during adult wing development, PS1 and PS2 are again expressed in a complementary way, on the epithelia which give rise to the upper and lower wing surfaces. We have identified two known viable mutations which affect wing morphogenesis, *non-jumper (nj)* and *inflated (if),* as mutations in the β and PS2α subunits, respectively (2). The primary effect of the *nj* mutation is to cause wasting of the thoracic jump muscles (hence its name). However, flies in which the dosage of this allele is reduced (and no wildtype copy is present) show defects also in wing morphogenesis. The two surfaces of the wing fail to connect properly, resulting in 'blistering' and incorrect venation. The *if* mutation leads to a similar failure in wing surface apposition. When the two mutations are combined, the mutant phenotype is greatly enhanced. We are using this double mutant approach to identify putative genes for other components of the integrin system. We find that a number of wing blister mutants from other loci interact with *if* and *nj* to cause greatly exacerbated wing phenotypes, while at least one acts as a phenotypic suppressor.

1. Leptin,M., Bogaert,T., Lehmann,R. and Wilcox,M. (1989) Cell 56 401.
2. Wilcox,M., DiAntonio,A. and Leptin,M. (1989) Development in press.

Development-II

A 018 GENETIC ANALYSIS OF *DROSOPHILA* CELL ADHESION MOLECULES, Corey S.Goodman, Howard Hughes Medical Institute, Division of Genetics, Department of Molecular and Cell Biology, University of California, Berkeley, 94720

We have begun a classical genetic and molecular genetic analysis of cell adhesion molecules to better understand their function during *Drosophila* development, with particular emphasis on their role in the developing nervous system. We have been using two approaches to uncover these molecules in *Drosophila*. First, we have been searching for the *Drosophila* homologues of well known vertebrate cell adhesion molecules. We cloned the genes which encode the three subunits of *Drosophila* laminin, a substrate adhesion molecule shown to be a potent promoter of neurite outgrowth in vertebrate systems. We have isolated a lethal mutation in the gene encoding the A subunit of laminin (*lama*). We also used the PCR method to clone two genes that encode *Drosophila* cadherins (calcium dependent cell adhesion molecule), and have begun a genetic analysis of both (24D and 21D). Both of these cadherins are "E-like", in that both are similarly expressed almost exclusively in the embryonic epidermis and the larval imaginal discs.

Second, beginning with an immunological approach, we identified and subsequently cloned the genes encoding four different surface glycoproteins, which we call fasciclin I, fasciclin II, fasciclin III, and neuroglian. These glycoproteins are dynamically expressed on different overlapping subsets of axon fascicles and glia during embryonic development. Two of these molecules (fasciclin II and neuroglian) are part of the immunoglobulin superfamily and are highly related to a series of vertebrate neural cell adhesion molecules (fasciclin II is highly related to N-CAM and neuroglian to L1). The other two proteins (fasciclin I and III) uncovered by our immunological screen have novel structures. Using transfection, aggregation, and biochemical assays, we have shown that all four proteins are homophilic adhesion molecules, two of which (fasciclin I and fasciclin III) appear to define new classes of neural cell adhesion molecules. We have identified small deletions and/or point mutations in the neuroglian, fasciclin I, and fasciclin III genes and are using genetic analysis to study the function of these molecules in the developing organism.

A 019 **CELL SURFACE AND DIFFUSIBLE MOLECULES THAT GUIDE DEVELOPING AXONS,**
Thomas Jessell*, Andrew Furley*, Marysia Placzek, Marc Tessier-Lavigne* and Jane Dodd, Center for Neurobiology and Behavior, and *Howard Hughes Medical Institute, Columbia University, New York, NY 10032.

Cell surface and diffusible molecules contribute to the patterning of axonal projections in vertebrate embryos. One mechanism of growth cone guidance involves interactions between glycoproteins on the surface of axons and cell surface or matrix molecules in their environment. We have identified a 135 Kd axonal glycoprotein TAG-1, that is expressed transiently on subsets of developing axons and may be involved in the initial stages of axonal growth. To provide further information on the structure and function of TAG-1, cDNA clones encoding this glycoprotein have been isolated. The deduced protein sequence of TAG-1 indicates that it is a member of the immunoglobulin gene family, closely related in structure to other axonal glycoproteins implicated in neuronal recognition, in particular F11, L1 and NCAM. TAG-1 possesses a large extracellular region which contains six immunoglobulin-like domains and four domains that are homologous to type III sequences found in the extracellular matrix protein fibronectin. One of the TAG-1 fibronectin domains contains an Arg-Gly-Asp (RGD) sequence that has been identified in many cell surface and extracellular matrix proteins that interact with integrins. TAG-1 does not possess a transmembrane domain and is anchored to the membrane via a glycosyl phosphatidylinositol linkage. We are now analyzing the function of this axonal glycoprotein.

A group of epithelial cells located at the axial midline of the neural plate, named the floor plate, also contributes to axon guidance in the developing spinal cord by releasing a diffusible chemotropic factor. Evidence for chemotropic guidance has been obtained by co-culturing explants of E11 rat floor plate and dorsal neural tube in a collagen gel matrix. Dorsal spinal cord explants grown in the absence of a floor plate exhibit little or no axon outgrowth, whereas in the presence of a floor plate there is profuse axon outgrowth oriented towards the floor plate. The axons that extend from dorsal explants derive from commissural neurons, since they express TAG-1. The factor released by the floor plate is selective for commissural axons and conversely, the induction of commissural axon outgrowth is highly specific to the floor plate. The action of the floor plate is not mimicked by defined growth factors and may, therefore, reflect the presence of a novel chemotropic molecule. Studies to characterize this molecule are in progress.

A 020 NEURONAL GLYCOPROTEINS THAT REGULATE AXON EXTENSION, Louis F. Reichardt, Blaise Bossy, Deborah Hall, Michael Ignatius, Thomas Large, Karla Neugebauer, Eugene Napolitano, Kevin Tomaselli, Department of Physiology and Howard Hughes Medical Institute, University of California, San Francisco CA 94143-0724. Neurons use a number of distinct glycoproteins that functions as receptors to promote axonal extension on extracellular matrices and other cell types, such as Schwann cells, myotubes and astroglial cells. We have attempted to identify these receptors and their ligands. We have found that neurons use different, but overlapping sets of receptors to interact with different cell types. These include several members of the integrin family of extracellular matrix receptors, at least one member of the calcium-dependent adhesion molecule family (the cadherins), and several calcium-independent adhesion molecules (NCAM, L1). We have characterized the major receptors utilized by neurons to interact with defined extracellular matrix proteins, such as laminin and vitronectin. We have observed considerable heterogeneity in the ability of different neurons to interact with these proteins. We also observed that the function of these receptors is regulated dramatically during development. Novel integrins and cell adhesion molecules have been identified that may account for some additional interactions observed between neurons and their substrates. The identified neuronal receptors and their ligands are regulated in ways that appear capable of explaining major features of growth cone motility in vivo.

Focal Contacts/Cell Junctions (joint)

A 021 CYTOSKELETAL ORGANIZATION AT ADHESIONS TO THE EXTRACELLULAR MATRIX, Keith Burridge, Glen Nuckolls, Carol Otey, Fredrick Pavalko and Christopher Turner, Dept. of Cell Biology and Anatomy, UNC, Chapel Hill, NC 27599.

We are interested in how the actin cytoskeleton is linked to the plasma membrane at sites such as focal contacts where cells adhere to the extracellular matrix. Microinjection of anti-talin antibodies into respreading cells results in the formation of abnormal focal contacts, containing little or no talin. Although these focal contacts reveal an abnormal morphology, they contain vinculin and continue to anchor bundles of actin filaments, arguing that talin is not necessary for the attachment of actin or for the localization of vinculin to these sites. To look for cytoskeletal proteins that bind to integrin, we have used affinity chromatography on synthetic peptides that correspond to the integrin β_1 cytoplasmic domain sequence. One protein that has shown a salt-dependent interaction with these peptide columns is alpha-actinin. Using a solid phase assay with peptide adsorbed to microtiter wells, we have found that ^{125}I-alpha-actinin shows a saturable binding to this peptide, that is competitively displaced by unlabelled alpha-actinin as well as by the free peptide. Scatchard analysis indicates a K_d of 1.6×10^{-8}M for this interaction. Use of shorter peptides in this assay has revealed that the alpha-actinin binding site in this sequence is close to the membrane. We have localized the peptide-binding site on alpha-actinin to a 53 kd fragment that does not bind actin. These results suggest that alpha-actinin may be one direct link between integrins and actin. Supported by NIH grant GM29860.

A 022 ALTERNATIVE MECHANISMS FOR THE MODULATION OF ADHERENS-TYPE JUNCTIONS. Benjamin Geiger, Tova Volberg, Talila Volk and Dorit Ginsberg, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Adherens-type junctions (AJ) are a family of cell contacts which are associated, at their cytoplasmic aspects, with microfilament bundles. The attachment of actin filaments to the junctional membrane occurs through an electron dense plaque which contain vinculin and several additional molecules. It had been shown that cell-matrix and cell-cell AJ display unique mechanical properties, largely contributed by the associated cytoskeleton. The resulting transcellular forces thus generated are, most likely, involved in cellular and tissue morphogenesis. Indeed, localization of specific cell adhesion molecules which partake in AJ formation indicated that the expression of these molecules is tightly regulated and closely correlated to major morphogenetic events in developing embryos. Recent studies have indicated that intercellular interactions in AJ are commonly mediated through A-CAM, an intercellular contact receptor of the cadherin family. Recent results indicate that A-CAM may be modulated by at least 3 alternative mechanisms. The first is a biosynthetic control of A-CAM formation; immunohistochemical staining and in-situ hybridization suggest that the levels of A-CAM closely correspond to the apparent levels of its mRNA. Another process which may affect A-CAM-mediated interactions is proteolysis. Evidence will be presented suggesting that A-CAM may be cleaved by endogenous proteinases and that such processes occur in developing chick embryos. Another mechanism which might be involved in the down regulation of A-CAM mediated interactions was observed in Rous Sarcoma Virus transformed chicken lens cells. When cultured under non-permissive temperature these cells form elaborate intercellular AJ. These junctions rapidly deteriorate following switch to the permissive temperature. This deterioration of the junctions did not involve significant changes in the amount or integrity of surface-associated A-CAM. Moreover, immunoprecipitation with anti-phosphotyrosine antibodies provided preliminary indications that the protein might undergo specific tyrosine phosphorylation in the transformed cells. The possible functional significance of the alternative regulatory mechanisms mentioned above will be discussed.

A 023 SIGNAL-TRANSDUCING PROTEIN KINASES AND THEIR TARGETS, Tony Hunter, Bill Boyle, Rick Lindberg, Dave Middlemas, and John Pines, Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138

Protein phosphorylation is a major mechanism whereby signals are transduced from external stimuli into cellular responses. Many growth factor receptors are ligand-activated protein-tyrosine kinases (PTK), while other PTKs, such as $pp60^{c\text{-}src}$, located on the inside of the plasma membrane may also be involved in signal transduction. We have identified novel PTKs by screening cDNA libraries with oligonucleotide probes to consensus sequences. In this way we have isolated are 2 novel receptor-like PTKs. One of these, *eck*, is predominantly expressed in tissues containing proliferating epithelial cells (skin, lung and intestine). The predicted *eck* protein, has 976 residues, and is closely related to the *eph* and *elk* receptor-like PTKs. Antibodies raised against a TrpE-*eck* fusion protein immunoprecipitate a 125 kDa protein from epithelial cell lines. This protein is phosphorylated on tyrosine in an immune complex kinase assay, indicating that the *eck* protein is a PTK. A second putative PTK, *trk*-B, was isolated from a rat cerebellar library. The predicted *trk*-B protein has 810 amino acids, and it is closely related to but distinct from the *trk* receptor-like PTK. *trk*-B is exclusively expressed in brain, as a series of RNAs ranging from 13 to 0. 8 kb. The smaller RNAs are clearly too short to encode the intact protein. Analysis of additional *trk*-B cDNAs indicates that there are mRNAs which encode a protein truncated just downstream of the transmembrane domain, with a short novel C terminus. We are investigating the distribution of the two types of mRNA in the brain, and trying to identify their protein products, and determine if the full length protein has PTK activity.

Cyclin, is a highly conserved cell cycle-regulated protein that is required in early embryos for entry into mitosis. We have isolated cDNA clones for human cyclin A and cyclin B. The level of mRNA for both cyclins varies during the cell cycle, being highest in G2. For cyclin B, this increase is due to an elevated rate of transcription in G2. Using antibodies against cyclin B we find that the level of cyclin B fluctuates during the cell cycle, being high in G2 and M, and falling rapidly as cells enter anaphase. Immunofluorescence staining shows that cyclin B accumulates in the cytoplasm during G2, enters the nucleus at prophase, and is abruptly destroyed at anaphase. During G2 cyclin B associates with the hyperphosphorylated form of the cell cycle regulatory protein kinase, cdc2, and these complexes have histone H1 kinase activity.

To determine how PKs activated at the cell surface induce nuclear events, we are examining the phosphorylation of nuclear regulatory proteins, which could be targets for a surface-initiated PK cascade. $p48^{v\text{-}myb}$, the AMV oncogene product, and its cellular counterpart, $p75^{c\text{-}myb}$, are phosphorylated at 5 clustered Ser near their C-termini. Among several PKs tested only glycogen synthase kinase 3 (GSK3) phosphorylates these sites in vitro. The transcription factor c-*Jun*, which associates with c-*Fos*, is a phosphoprotein. c-*Jun* has 4 major sites of phosphorylation. Three of these, which show decreased phosphorylation following TPA treatment, are clustered in a region just upstream of the DNA binding domain. These sites are also phosphorylated by GSK3, and we find that GSK3 phosphorylation of bacterially-expressed c-*Jun* protein decreases its ability to bind to a TRE. We propose that c-*Jun* function is negatively regulated by phosphorylation, and that TPA activation of transcription from TRE-dependent genes may in part involve dephosphorylation of c-*Jun*.

Physiology/Pathology-I

A 024 **MOLECULAR DISSECTION OF CARBOHYDRATE-RECOGNITION PROTEINS,** Kurt Drickamer, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032

The mammalian asialoglycoprotein receptor and its avian homolog are receptors which specifically recognize partially deglycosylated serum glycoproteins and mediate their uptake into hepatocytes by endocytosis. We have been attempting to assign specific functions to individual segments of the receptor polypeptides, in order to understand how they are able to perform this function. Signals within the polypeptide direct insertion into the lipid bilayer of the endoplasmic reticulum in an "amino terminus cytoplasmic" orientation, stabilize formation of oligomers in order to achieve the correct cluster of carbohydrate-binding sites, and result in movement into coated pits for endocytosis and return to the cell surface during recycling. In addition, the ligand-binding portion of the receptors are of particular interest because they have led to the identification of modular, calcium-dependent (C-type) carbohydrate-recognition domains which appear in a range of proteins sharing the ability to bind saccharides. We are attempting to define the mechanism of specific carbohydrate binding by a variety of techniques, including three-dimensional structure determination, random cassette mutagenesis, and ligand blotting. Progress in these structure-function studies, and in the structural analysis of other proteins which share the ability to bind saccharides in a calcium-dependent fashion, will be reported. The goal of these studies is to develop a better understanding of the biological role of C-type carbohydrate recognition domains in decoding information found in complex carbohydrates. Such information may be critical for interactions between cells and in the extracellular matrix.

A 025 STRUCTURE-FUNCTION ASPECTS OF THE PERIPHERAL LYMPH NODE HOMING RECEPTOR TYPE LECTIN-CELL ADHESION MOLECULE, Laurence Lasky, Susan Watson, Benjamin Bowen, Christopher Fennie, Donald Dowbenko, Joyce Geoffrey, Mark Singer, and Steven Rosen, Dept. of Cardiovascular Research, Genentech, Inc., 460 Pt. San Bruno Blvd., S.S.F., CA 94080, and Dept of Anatomy, University of California, Sanfrancisco, CA 94143

Lymphocytes traffick to peripheral lymph nodes (PLN) by utilizing a lectin-like adhesion molecule termed the PLN homing receptor. cDNA cloning of the murine and human forms of these molecules has revealed that they are constructed from three different protein motifs which include a type C lectin domain, and egf-like domain, and two copies of a complement regulatory motif. Gene structure analysis has revealed that the different protein motifs are encoded by seperate exons localized on chromosome 1 of the mouse. Analysis of homing receptor reactivity with the adhesion blocking monoclonal antibody, MEL 14, has shown that this antibody recognizes an epitope within the N-terminal 53 amino acids of the lectin domain whose conformation appears to be dependant upon the presence of the adjacent egf-like domain, suggesting that the lectin domain is involved in adhesion to and recognition of the PLN endothelium. Production of chimeric molecules containing either all, or a subset, of these protein domains and the human IgG1 constant region were used in sugar binding, cell blocking, and immunohistochemical experiments. These results showed that these chimeric molecules could be utilized as reagents for the analysis of the roles of each of these domains in PLN homing receptor function.

A 026 ADHESION PROTEINS AND PLASMINOGEN ACTIVATION ON TUMOR CELL SURFACE Antti Vaheri, Ross W. Stephens, Jari Pöllänen, Eeva-Marjatta Salonen and Hannele Tapiovaara, Department of Virology, University of Helsinki, Haartmaninkatu 3, 00290 HELSINKI, Finland

We previously showed that plasminogen interacts with fibronectin and laminin , which led us to introduce the concept of "directional proteolysis". Other work by us and others has shown that several other adhesion proteins also bind plasminogen, supporting the view that they are likely substrates of the plasminogen activation system at the cell surface. Another aspect of directional proteolysis emerged when we found that surface-bound urokinase-type plasminogen activator (u-PA) is localized at focal adhesions of adherent human tumor cells. Adhesion receptors (integrins) are also localized at these sites. In contrast the PA inhibitor PAI-1 is found on the growth substratum, apparently bound to vitronectin (1-3). We have shown that when human tumor cells are grown in serum-containing medium, addition of native plasminogen leads to formation of bound functionally active plasmin on the cell surface. While cell-bound plasmin is protected against inactivation by inhibitors in serum (but not by aprotinin), slow release from cells is accompanied by inactivation (4). Plasmin bound on tumor cells is proteolytically active despite the presence of serum inhibitors and able to activate endogenous bound pro-u-PA to form bound active u-PA. The latter is also protected from serum inactivation (i.e. α_2-macroglobulin), and is able to activate bound native plasminogen to form plasmin. However, cell-bound active u-PA is subject to regulation by the high-affinity specific inhibitors, PAI-1 and PAI-2 (ref. 4).We have recently shown that recombinant PAI-2 may be used to localize active u-PA and to inhibit plasmin generation on tumor cell surface. Our earlier findings indicated a fundamental difference in the way plasminogen activation is initiated on cells derived from solid tumors (adherent cells) compared to initiation on leukemic cells growing in suspension (5). We have now shown that even when grown in serum-containing medium, the former have almost exclusively pro-u-PA bound to their surface, requiring activation by bound plasmin to initiate plasminogen activation. On the contrary, leukemic cells growing in either serum-free or serum-containing medium have a high proportion of active u-PA bound to their surface, which originates from a plasmin-independent pathway.

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A 027 ADHESIVE PROTEINS OF ENDOTHELIAL CELLS AND PLATELETS, Denisa D. Wagner, Roberta Bonfanti, Alessandro Celi, John K. Erban, Barbara C. Furie, Bruce Furie, Gary E. Gilbert, Eric Larsen, New England Medical Center, Tufts University, Boston MA 02111. One of the immediate responses of platelets and endothelial cells to vascular injury is to release their storage granule contents. The α granules of platelets contain several adhesive proteins while the endothelial Weibel-Palade (W-P) bodies store von Willebrand factor only. Both of these granules contain a transmembrane glycoprotein called PADGEM (or GMP140). After granule exocytosis PADGEM becomes exposed on the plasma membrane. PADGEM's primary structure was derived from the nucleotide cDNA sequence (1) and showed remarkable homology with ELAM-1 and MEL-14 adhesion proteins on endothelial cells and lymphocytes respectively. Based upon this structural homology, we have evaluated the possible role of PADGEM in cell adhesion. Activated platelets, but not unstimulated platelets, bind to neutrophils, monocytes, HL60 cells and U937 cells (human monocyte-like cell lines). This interaction is inhibited by anti-PADGEM antibodies, purified PADGEM, purified PADGEM incorporated into liposomes, and EDTA; anti-GPIIb-IIIa, anti-thrombospondin, anti-GPIV, thrombospondin, albumin, and Arg-Gly-Asp-Ser, have no effect. Neutrophils and U937 cells, in contrast to Jurkatt cells (human cell line expressing T cell characteristics) contain saturable PADGEM binding sites. These results indicate that PADGEM mediates adhesion of activated platelets with monocytes and neutrophils. Therefore, PADGEM shares not only structural but also functional homology with ELAM-1 and MEL-14, members of a new family of vascular cell adhesion molecules.

The receptors on endothelial cells involved in signalling the release of W-P bodies remain poorly characterized. Prior studies have demonstrated that the N-terminal sequence of the fibrin β chain is important for secretagogue activity. A synthetic peptide composed of amino acids 15-42 of the Bβ chain of fibrinogen was shown to stimulate release (2). In an attempt to identify a receptor mediating this process endothelial cells were surface labelled, solubilized and applied to a column containing the peptide Bβ 15-42 immobilized on Sepharose. Two iodinated proteins bound and were eluted with Bβ 15-42, but were not eluted with Arg-Gly-Asp-Ser, Bβ 37-56+cysteine, EDTA or an unrelated dodecapeptide. The two eluted proteins were approximately 120,000 and 110,000 in size non-reduced increasing to 130,000 and 120,000 respectively on reduction. The possibility that the smaller protein represents a cleavage product of the larger rather than a separate subunit has not been eliminated. Our results demonstrate that endothelial cells possess surface protein(s) which bind to the N-terminal peptide sequence normally exposed after cleavage of fibrinopeptide B from fibrinogen. It is unlikely that these proteins are known integrin receptors of endothelial cells since their interaction with Bβ 15-42 is not inhibited by EDTA, and they are not recognized by antibodies to the β1 or β3 subunits of integrin. It will be important to determine whether these Bβ 15-42 binding proteins indeed represent the receptor implicated in W-P body release from EC.

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Physiology/Pathology-II

A 028 INTEGRIN MAPPING OF CUTANEOUS WOUNDS, Richard A. F. Clark, Department of Medicine and Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. During cutaneous tissue organization numerous critical interactions occur between cells and the extracellular matrix (ECM). Cell-matrix interactions depend on the presence of ECM receptors. Many ECM receptors are integrin heterodimeric glycoproteins consisting of one α- and β-chain. We have used porcine cutaneous wounds as a paradigm for tissue organization and probed healing wounds with polyclonal antibodies to fibronectin ($\alpha 5\beta 1$) and vitronectin ($\alpha_V\beta 3$) receptors. During reepithelialization the epidermis transits over a provisional matrix containing fibronectin and vitronectin. Migrating epidermal cells expressed both β1 and β3 integrin receptors in a bright linear peripheral pattern. At 10 days when reepithelialization was complete, fibronectin/vitronectin matrices were absent, the basement membrane was reestablished, and β1 and β3 receptor expression was markedly decreased and limited to the basolateral aspect of basal cells, as observed in normal epidermis. Beneath the migrating epidermis in 5 day wounds, granulation tissue had filled 80% of the wound space. Day 5 wound fibroblasts did not express fibronectin nor other β1 integrin receptors, were randomly oriented, and contained no actin bundles. Fibronectin fibrils were assembled on the surfaces of day 5 wound fibroblasts but formed few linkages between cells. Migrating fibroblasts at the periphery of day 5 wounds expressed vitronectin receptors. Day 7 wound fibroblasts expressed fibronectin and vitronectin receptors, contained peripheral cytoplasmic actin bundles consistent with a contractile fibroblast phenotype, and were coaligned across the wound in parallel array with interconnecting fibronectin fibrils. Transmission EM confirmed cell-cell and cell-matrix linkages in day 7 wound fibroblasts. The wounds contracted between 7 and 10 days. Thus the migrating epidermis consistently expressed β1 and β3 integrin receptors and migrating fibroblasts expressed β3 receptors. Fibronectin receptors were expressed by fibroblasts just prior to wound contraction.

A 029 TGFs AS REGULATORS AND MEDIATORS OF CELL ADHESION. Joan Massagué, Jyrki Heino, Joaquin Teixidó, and Atanasio Pandiella. Cell Biology & Genetics Program. Memorial Sloan-Kettering Cancer Center, New York, NY 10021

TGF-ß is a suppressor of cell proliferation and a modulator of cell phenotype. Biochemically, TGF-ß controls the expression of genes implicated in control of cell growth and phenotype as well as the expression of components of the cell adhesion apparatus, In vitro as well as in vivo, integrins, fibronectin, collagens, matrix proteoglycans, and matrix protease inhibitors are in general heavily up-regulated in response to TGF-ß whereas certain collagenases are down-regulated. The net result of these effects is an increased deposition of extracellular matrix and increased cell adhesion. However, TGF-ß action can also switch the pattern of integrins expressed in MG-63 human osteosarcoma cells resulting in loss of the multifunctional $\alpha_3\beta_1$ laminin/collagen/fibronectin receptor and gain of $\alpha_2\beta_1$ and $\alpha_5\beta_1$ collagen and laminin receptors. These changes correlate with loss of cell adhesion to laminin, and suggest that TGF-ß can control the activity of both positive and negative regulators of integrin subunit expression. We are testing the hypothesis that changes in cell adhesion induced by TGF-ß may mediate actions on cell phenotype. For this, we have used L_6E_9 myoblasts as a model system. L_6E_9 myoblasts respond to TGF-ß with strong up regulation of type I collagen, fibronectin, and proteoglycan decorin, and they fail to differentiate into myotubes. Fibrillar collagen matrices block the differentiation of L_6E_9 cells. Recent progress suggests a model in which TGF-ß inhibits myogenic differentation by complementary collagen-dependent and independent pathways that suppress myogenic gene expression.

TGF-α is a secretory mitogen that is generated by cleavage from a integral membrane precursor, proTGF-α. Cleavage of proTGF-α into TGF-α occurs from the cell surface and is an inefficient process in most cell types examined. ProTGF-α accumulated on the cell surface can establish contact with its receptor (the EGF receptor) on an adjacent cell. This interaction can support cell-cell adhesion and lead to mitogenic stimulation. We are exploring various implications of this form of cell-cell communication.

A 030 THE $\alpha_2\beta_1$ INTEGRIN (VLA-2) AND THE PLATELET MEMBRANE GLYCOPROTEIN IIb-IIIa COMPLEX: IMPLICATIONS IN PATHOBIOLOGY, Samuel A. Santoro, William D. Staatz, Mary M. Zutter, Mari A. McGoff and Gregorio A. Sicard, Departments of Pathology, Medicine, and Surgery, Washington University School of Medicine, St. Louis, MO 63110

Studies using intact platelets, inhibitory monoclonal antibodies, and the purified receptor reconstituted into liposomes have revealed that the $\alpha_2\beta_1$ integrin (VLA-2) mediates the Mg^{++}-dependent adhesion of platelets to collagen. The receptor binds specifically to types I, II, III, and IV collagen, but does not bind to other extracellular matrix and adhesive proteins such as fibronectin, vitronectin, laminin, thrombospondin, von Willebrand factor, and fibrinogen. The binding site on collagen recognized by the $\alpha_2\beta_1$ integrin has been localized to the α1(I)-CB3 cyanogen bromide fragment of type I collagen. Immunohistochemical studies of normal human tissues have revealed that the $\alpha_2\beta_1$ receptor has a widespread histologic distribution. The receptor is expressed by fibroblasts, by endothelial cells, and by many epithelia with marked increases in expression associated with sites of epithelial cell proliferation. In contrast to the ductal epithelium of normal breast tissue which expresses high levels of the $\alpha_2\beta_1$ receptor, the expression of $\alpha_2\beta_1$ by the malignant epithelial cells of adenocarcinomas of the breast is markedly reduced. The low levels of expression of $\alpha_2\beta_1$ and other integrin adhesive receptors may contribute to the invasive properties of the tumor cells.

Studies in our laboratory, as well as in other laboratories, have revealed that synthetic peptides containing the arg-gly-asp sequence bind to the platelet membrane glycoprotein IIb-IIIa complex and competitively inhibit the binding of the platelet adhesive proteins fibrinogen, fibronectin and von Willebrand factor to the activation-dependent integrin receptor. The ability of such peptides to effectively inhibit platelet aggregation in vitro raised the possibility that arg-gly-asp-containing peptides could serve as the basis for the development of a novel class of antithrombotic agents. This hypothesis was tested in vivo using a canine model of small diameter synthetic vascular graft thrombosis. Direct determination of platelet deposition, radioisotopic imaging of platelet thrombi, and histologic examination of explanted grafts all indicated that platelet deposition onto the graft surfaces was markedly reduced by infusion above the graft site of a peptide containing the arg-gly-asp sequence. A control peptide in which a glu residue was substituted for the asp was devoid of inhibitory activity.

Adhesive Glycoproteins

A 100 NUCLEIC ACID LEVEL CHARACTERIZATION OF ARTERIAL WALL PROTEOGLYCAN STRUCTURE AND EXPRESSION. Vinod Asundi[1], Kevin L. Dreher[1] and William Wagner[2], [1]Geisinger Clinic 26-17, Weis Center for Research, Danville, PA 17822, [2]Department of Comparative Medicine, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.
Arterial wall proteoglycans (PG) have been shown to effect vascular smooth muscle cell proliferation and postconfluent (multi-layered) growth. Currently, the vast majority of our knowledge regarding arterial PGs has been obtained from histochemical and protein characterization studies. We have extended the characterization of arterial PGs by employing a nucleic acid molecular biology approach designed to determine the structure, function and regulation of PGs expressed by vascular smooth muscle cells (VSMC). Northern blot analyses performed on polyadenylated (polyA$^+$)RNA isolated from arterial medial strips containing quiescent VSMC using a variety of PG cDNA probes have provided new insight into the types and levels of PGs expressed by this tissue. First, there is a species variation with respect to VSMC PG expression. Rat VSMC were found to express transcripts homologous to dermatan sulfate (DS) PGII, DSPGI and chondroitin sulfate (CS) PG. Pigeon VSMC expressed RNA sequences homologous to DSPGII and CSPG whereas rabbit VSMC contained transcripts homologous to DSPGI and CSPG. Secondly, quantitation of Northern blot patterns revealed the level of VSMC PG expression to be DSPGI>DSPGII>CSPG. Finally, no transcripts homologous to the rat cartilage link protein were detected in any of the arterial polyA$^+$RNA samples. This result suggests that the large aggregating CSPG may be organized differently within the arterial matrix when compared to its articular cartilage counterpart. A DSPGII cDNA clone, designated arteriocan II, has been isolated from a cDNA library constructed from polyA$^+$RNA isolated from cultured rat VSMC. Nucleotide sequence homology analysis of arteriocan II has shown it to be approximately 78% homologous to the bovine DSPGII and to the human DSPG known as decorin. Rat arteriocan II RNA levels were enhanced in quiescent VSMC when compared to proliferating VSMC, suggesting that this PG is regulated as a function of VSMC growth state and like decorin it may have antiproliferative properties.

A 101 DIFFERENT CELL SURFACE GLYCOPROTEINS MAY ACT DURING THE SPREADING OF CHICK EMBRYO FIBROBLASTS ON LAMININ AND FIBRONECTIN SUBSTRATA.

M. AUBERY P. CODOGNO R. MOUTSITA and J. BOTTI
U. 180 INSERM 45 Rue des Saints-Pères 75006 PARIS.

Fibroblasts obtained from 8-day old chick embryos (8-day CEF) were shown to adhere to fibronectin (FN) and laminin (LM) substrates respectively. When the lectin concanavalin A (Con A) was added to the adhesion medium the cell attachment was not inhibited either on FN or LM (1). However 8-day CEF spreading was specifically impaired on LM substrate. Futhermore Con A was shown to bind a panel of cell surface glycoproteins. Among them a 72 kDa glycoprotein interacted with LM and was shown to be associated with the cytoskeleton when 8-day CEF were plated on LM substrate whereas this association was not observed on FN substrate. The relationship between this Con A binding protein with 5'-nucleotidase (2) and galactosyltransferase (3) which were previously shown to be involved in cell spreading on LM substrate remains to be determined.

1. CODOGNO P. et al. 1988 J. Cell-Physiol. 136, 463-470
2. CODOGNO P. et al. 1988 Exp. Cell Res. 174, 344-354
3. RUNYAN R.B. et al. 1988 J. Cell Biol. 107, 1863-1871.

A 102 BINDING OF PLASMATIC von WILLEBRAND FACTOR (vWF) TO EXTRACELLULAR MATRICES: ABSENCE OF COMPETITION BY ENDOTHELIAL MATRIX vWF.
Dominique Baruch, Cecile Denis, Laure Coulombel, Corinne Marteaux, Dominique Meyer. INSERM U.143, 94275 Le Kremlin-Bicêtre, France.
vWF is a multimeric glycoprotein mediating platelet adhesion to the subendothelium. vWF circulates in plasma, is stored in platelets and endothelial cells and is also a selective component of the extracellular matrix laid by endothelial cells (E-ECM). Both plasmatic and matrix vWF are required for optimal platelet adhesion to E-ECM at high shear rates. In order to investigate the first step of this process, we assessed the binding of purified 125 I-vWF to the ECM obtained after chemical treatment of confluent human endothelial cells. We found that the binding of vWF to E-ECM is specific and dose-dependent, reaching equilibrium in 14 hours. Optimal binding of vWF to the E-ECM requires pH and ionic strength conditions different from those defined for optimal binding to purified human type I/III collagens. In addition, a monoclonal antibody (MAb B202) to vWF, which specifically blocks vWF binding to purified collagen, only inhibits 30% of its binding to E-ECM. Taken together these findings suggest that vWF binds to an additional ECM constituent different from collagen. We next addressed the question of the role of endogenous E-ECM vWF on the binding of 125 I-vWF to these matrices. To this end we compared the binding of vWF to E-ECM and to a matrix devoid of vWF, obtained by chemical treatment of confluent bone marrow fibroblasts (F-ECM). Interestingly we found that specific binding of 125 I-vWF reached the same value on both F-ECM and E-ECM. In neither case were we able to saturate the matrix with exogenous vWF. However, this could not be accounted for by the amount of endogenous vWF (7ng/cm^2) which represents less than 1% of the total protein content when assessed separately in solubilized E-ECM. This apparent lack of competition between exogenous and endogenous vWF might be explained by differences in their multimeric composition as E-ECM vWF contains a higher number of multimers than plasmatic vWF.

A 103 **A UNIQUE FORM OF FIBRONECTIN RNA IN CARTILAGE, Vickie D. Bennett, Kim M. Pallante, and Sherrill L. Adams, Department of Anatomy and Histology, University of Pennsylvania, School of Dental Medicine, Philadelphia, PA 19104-6003.** Chondrogenesis involves the differentiation of prechondrogenic mesenchymal cells into cartilage-producing chondrocytes. Fibronectin has been observed in prechondrogenic mesenchyme and in mature cartilage. However, the synthesis of several other extracellular matrix (ECM) proteins changes significantly during this developmental process. For example, the cartilage ECM contains type II collagen and chondroitin sulfate proteoglycan that are not present in the ECM of prechondrogenic mesenchymal cells. Thus the structure and function of the fibronectin in cartilage may differ from the fibronectin in prechondrogenic mesenchymal cells. In fact, we have shown using nuclease protection assays for the three alternatively spliced exons of fibronectin (IIIA, IIIB, and V) that chick cartilage contains a unique form of fibronectin RNA. All the fibronectin RNA from prechondrogenic mesenchyme contains exon IIIA and IIIB, whereas all the fibronectin RNA from cartilage contains exon IIIB, but no exon IIIA. In addition, most of the fibronectin RNA in other tissues of mesenchymal origin contains exon IIIA, but little exon IIIB. Thus the presence of exon IIIB in cartilage fibronectin may mediate the interactions of chondrocytes with the unique components of the cartilage ECM. The fibronectin RNA from chondrocytes in culture contain little exon IIIB, even though all of the RNA from intact cartilage contains this exon. Manipulation of the culture conditions to produce chondrocytes that are more adhesive also increases the amount of fibronectin RNA that contains exon IIIA. Thus exon IIIA may play a role in adhesion of chondrocytes to *in vitro* substrates or in cell-cell interactions.

A 104 FIBRONECTIN, HYALURONIC ACID AND A HYALURONIC ACID BINDING PROTEIN CONTRIBUTE TO INCREASED SMOOTH MUSCLE CELL MIGRATION IN THE DUCTUS ARTERIOSUS, N. Boudreau and M. Rabinovitch, Cardiovascular Research Division, The Hospital for Sick Children, Toronto, Canada.

Intimal cushions in the late gestation lamb ductus arteriosus (DA) are characterized by smooth muscle cells migrating into a large subendothelial space. Our previous *in vitro* studies, comparing DA cells with those from the aorta (Ao), have shown, even in early gestation, a tenfold increase in DA endothelial incorporation of hyaluronic acid (HA) into the subendothelial matrix, a twofold increase in smooth muscle fibronectin (FN) synthesis and, in response to endothelial conditioned medium, a twofold increase in chondroitin sulfate. To determine whether these extracellular matrix components may be playing a role in inducing DA smooth muscle migration, we seeded DA or Ao smooth muscle cells onto three dimensional collagen (2.0 mg/ml) gels and assessed migration 2, 5 and 8 days later. After 8 days, significantly greater numbers of DA compared to Ao cells were found invading the gels (23.1%+3.1 vs. 16.2%+2.3, $p<0.05$). Addition of RGDS peptides (5×10^{-4} M) or antibodies against FN significantly decreased migration in the DA cells, but had no effect on migration in the Ao. Addition of endothelial conditioned medium to induce smooth muscle chondroitin sulfate had no effect on DA cell migration. Inclusion of HA in the gel (0.5-1.5 mg), however, further enhanced DA cell migration, being greatest (31.9%+3.1) at a concentration of 1mg/ml. HA was without effect on Ao smooth muscle cell migration. The ability of HA to promote migration in the DA was blocked completely by the addition of antibodies (1:100 dilution) to a cell surface hyaluronic acid binding protein (HABP). As well, addition of anti-HABP to cells on gels containing collagen only, significantly reduced migration in the DA but not the Ao, and immunofluorescent staining revealed a greater concentration of HABP on lamellipodia of DA smooth muscle when compared to Ao. Thus, our studies indicate that both increased FN and HABP contribute to the enhanced migration of DA smooth muscle cells. Together with our previous studies showing a tenfold increase in HA accumulation in the DA endothelial matrix, these results would suggest a mechanism for increased DA smooth muscle migration into the subendothelial matrix observed *in vivo*.

A 105 CHARACTERIZATION OF A TUBULAR BASEMENT MEMBRANE ANTIGEN REACTIVE WITH ANTIBODIES ASSOCIATED WITH TUBULOINTERSTITIAL NEPHRITIS, Ralph J. Butkowski, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455. A kidney tubular basement membrane (TBM) antigen (TIN-antigen) that is bound by antibodies from individuals with anti-TBM antibody-associated tubulointerstitial nephritis was purified and characterized. Two forms, 58KDa and 50KDa, were prepared from rabbit TBM by extraction with 6M guanidine, ion-exchange, gel filtration and reverse phase chromatography. Both forms have the same amino-terminal sequence, indicating that they are structurally related. Their amino acid compositions are enriched in acidic amino acids. The absence of hydroxyproline and hydroxylysine and low levels of glycine indicates that they are non-collagenous. Native TIN-antigen reactivity was solublized by digestion of TBM with collagenase. The native antigen had a gel filtration molecular weight >200,000; which resolved into unbound and tightly bound fractions by ion-exchange chromatography. The unbound fraction contained >200 KDa reactivity by gel filtration re-chromatography. By SDS-PAGE and Western blotting this material contained 58KDa and 50KDa reactive forms and several non-reactive proteins. Tightly bound fractions had a molecular weight of 59,000 by gel-filtration re-chromatography, and consisted of 58Da and 50KDa forms by SDS-PAGE. These results indicate that TIN-antigen either self-associates or is associated with other molecules. This research presents methods to prepare TIN antigen for biochemical studies and investigation of its role in anti-TBM tubulointerstitial nephritis.

A 106 THE ADDITIVE AND SYNERGISTIC ENHANCEMENT BY TGF-β OF PROTEINS INDUCED BY PHORBOL-12-MYRISTATE-13-ACETATE IN HUMAN OSTEOGENIC SARCOMA CELLS. Susan C. Danehower and Kuttikkat A. Chandrabose, Division of Endocrinology, Glaxo Inc., 5 Moore Dr., Research Triangle Park, N.C. 27709.
Human osteogenic sarcoma cells (American Type culture collection, U2OS) were incubated with 10nM phorbol ester for 39 hrs. Cells were pulsed for the last 3 hrs of incubation with [^{35}S] cysteine. The matrix proteins from the medium were adsorbed to gelatin beads, washed, and extracted in SDS containing sample buffer. The extract was analyzed by 4-15% gradient SDS page and autoradiographed. The results show the following: Phorbol treatment results in a) the increased de novo synthesis of fibronectin and an 85 kDa protein, and b) two additional proteins of 110 kDa and 30 kDa are induced in relatively large amounts. When transforming growth factor beta (TGF-β) was present during the last 15 hours of incubation with phorbol, the synthesis of fibronectin and the 85 kDa protein were enhanced additively, and that of the 110 kDa protein was enhanced synergistically. 4α-phorbol, the biologically inactive phorbol analogue, had no effect on the expressions of these proteins. Staurosporin, an inhibitor of the phorbol effects on protein kinase C, abolished the phorbol specific responses of the expressions of the proteins, but had no effects on the TGF-β specific effects. Results suggest overlapping transductional pathways shared by TGF-β and phorbol, which do not involve protein kinase C.

A 107 IDENTIFICATION AND CHARACTERIZATION OF 114/A10, A NOVEL HIGHLY GLYCOSYLATED, INTERNALLY REPETITIVE, PLASMA MEMBRANE PROTEIN EXPRESSED ON EARLY HEMOPOIETIC PROGENITOR CELLS. Graeme J. Dougherty, Robert J. Kay and R. Keith Humphries, Terry Fox Lab., CCABC, Vancouver, V5Z 1L3.
Adhesion between hemopoietic progenitor cells and stromal elements is believed to play a pivotal role in the regulation of hemopoiesis. In order to further define the cell surface molecules which mediate such interactions, we have raised a large panel of monoclonal antibodies against the murine multipotential hemopoietic cell line B6SUtA. One of these antibodies, designated 114/A10, identifies an extensively glycosylated 165kD protein highly expressed on primary hemopoietic progenitor cells and IL-3-dependent cell lines. Full length cDNA clones encoding this molecule were isolated from a plasmid-based expression library. These clones predict a long open reading frame of 573 amino acids having the typical features of a type I integral membrane protein. A particularly striking feature of this sequence is the presence at the amino terminus of a series of 8 highly conserved 27 amino acid serine/threonine rich (55%) tandem repeats with 5 of these 8 repeats being identical at both the amino acid and nucleotide level. This domain has been shown to serve as the site of extensive O-linked glycosylation. The extracellular domain of the 114/A10 antigen also contains a series of 3 epidermal growth factor-like cysteine-rich repeats. Soluble forms of the 114/A10 antigen have been prepared and are presently being used to define the role of this molecule in cellular adhesion.

A 108 THE EXTRACELLULAR MATRIX GLYCOPROTEIN J1/TENASCIN IS AN INHIBITORY SUBSTRATE FOR CENTRAL NERVOUS SYSTEM NEURONS, Andreas Faissner, Department of Neurobiology, University of Heidelberg, 6900 Heidelberg, Germany (FRG).
The functional properties of central nervous system derived J1/Tenascin were investigated in vitro. J1/Tenascin is an extracellular matrix (ECM) constituent consisting of two glycoproteins of 200 kD and 220 kD apparent molecular weight. It is transiently expressed by astrocytes during CNS development and mediates neuron-astrocyte interactions in vitro. Two monoclonal antibodies specific for protein epitopes on J1/Tenascin were used for immunoaffinity purification of the molecule from postnatal mouse brain. The interactions of three defined classes of central nervous system cell populations, embryonic day 14 rat mesencephalic, embryonic day 18 rat hippocampal and postnatal day 6 mouse cerebellar neurons with isolated J1/Tenascin were studied in two in vitro assay systems. When plated onto J1/Tenascin absorbed to bacterial plastic, none of the three neuronal cell types was found to bind to the ECM molecule, whereas they showed differential attachment and process extension on either laminin or, to a lesser extent, fibronectin in the same assay. In a second test system, J1/Tenascin was absorbed to polyornithine (PORN) coated glass coverslips. Subsequently, a pattern was created by removal of the protein from selected areas. All three neuronal cell types avoided J1/Tenascin containing regions but grew equally well on laminin or fibronectin conditioned PORN surfaces as compared to plain PORN. We conclude from these observations that J1/Tenascin defines inhibitory zones for central nervous system neurons during different stages of neural development and could thus contribute to CNS pattern formation.

A 109 GENETIC ANALYSIS OF FIBRONECTIN FUNCTION IN MOUSE

Elizabeth L. George and Richard O. Hynes, Center for Cancer Research and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139

As no known mutations in fibronectin (FN) exist, we are generating FN-deficient mice by gene targeting techniques. Gene targeting entails isolating embryonic stem (ES) cells in which FN gene expression has been disrupted by homologous recombination with a modified FN gene segment. Such modified ES cells are injected into blastocysts and contribute to chimeric embryos. Germ-line chimeric mice will be utilized for two types of analysis. First, they will be examined for defects in the heterozygous and homozygous states. Second, they will be bred with transgenic mice expressing defined alternatively spliced FN variants which are being generated in our laboratory.

At present we have cloned and mapped the mouse FN gene, and determined that high levels of FN mRNA are present in ES cells. Construction and evaluation of two gene targeting vectors is in progress. The first vector disrupts the FN gene in exon 1 with a bacterial neo gene under control of a strong promoter and carrying a polyadenylation site. The second vector takes advantage of the active FN promoter by inserting a promoterless neo gene, also carrying a polyadenylation site, in FN exon 2. Dependence of the neo gene on proximity to the FN promoter may increase the ratio of homologous recombination to random integration events.

A 110 MAMMARY EPITHELIAL CELLS CONTAIN AN INTRGRAL MEMBRANE PROTEIN HOMOLOGOUS TO PLATELET GP IV/CD36,

Dale E. Greenwalt, *Kenneth K. W. Watt, On Yee So and Nilofer Jiwani, Department of Chemistry, San Jose State University, SanJose, CA 95192 and *Department of Protein Chemistry, Cetus Corporation, Emeryville, CA 94608. Platelet GP IV (IIIb) is an integral membrane protein which has been shown to bind thrombospondin and type 1 collagen. CD 36 is a closely related protein which has been shown to bind malaria-infected erythrocytes. We now report the isolation of a protein (PAS IV) from human and bovine mammary epithelial cell membrane which is homologous to GP IV and CD36. Both bovine and human PAS IV were purified from fat-globule membranes isolated from fresh milk by a combination of extraction and phase-partitioning in Triton X-114 and ion-exchange chromatography. Approximately 30 mg of bovine PAS IV can be isolated from one quart of cream. The N-terminal sequences of human and bovine PAS IV contain a 22-residue putative membrane-spanning region identical to sequences reported for GP IV and CD36. Affinity-purified anti-human PAS IV recognized bovine and human PAS IV and human GP IV in western blots. The apparent Mr values for bovine and human PAS IV and GP IV are 78, 80 and. 88 kDa respectively. Endoglycosidase F-catalyzed removal of N-linked oligosaccharides reduced the Mr of the three proteins to approximately 57 kDa. Sequencing of bovine PAS IV proteolytic fragments revealed three peptides homologous to CD36 but which lack the Asn- X -Ser/Thr glycosylation sites present in the CD36 molecule. These data indicate that the difference in Mr between PAS IV and GP IV is largely due to decreased glycosylation of mammary epithelial cell PAS IV. The function(s) of PAS IV is unknown. The demonstration of an epithelial cell homologue of GP IV in conjunction withrecent demonstrations that GP IV and CD36 differ in both their primary structure and ligand binding specificities suggests that these proteins constitute a distinct family of adhesion proteins.

A 111 A HIGH-MOLECULAR-WEIGHT BASEMENT MEMBRANE GLYCOPROTEIN SECRETED BY MELANOMA CELLS FROM ADVANCED LESIONS,

Meenhard Herlyn, The Wistar Institute, Philadelphia, PA 19104.

Melanoma cells secrete a basement membrane glycoprotein of 250,00 dalton (gp250) that was characterized with 5 monoclonal antibodies (MAbs) to 4 different determinants. gp250 was highly secreted by 23 of 25 melanoma, all glioma, and fetal and skin fibroblast cultures tested but was not expressed on cell surfaces. Serum levels of gp250 were significantly elevated in patients with advanced melanomas when compared to normal donors. Tissue sections showed gp250 in basement membranes of normal tissues including skin, vascular system, kidney and placenta. Of malignant tissues, all of 12 metastatic melanomas tested were reactive with MAbs, and the intratumoral stroma showed highest antibody binding. The anti-gp250 MAbs did not bind to purified preparations of collagens type I to IV, fibronectin, or laminin. This monomeric gp250 may be a unique possibly involved in cell-substrate interactions during invasion and metastasis.

A 112 INDUCTION OF FIBRONECTIN AND FIBRONECTIN RECEPTOR BY ONCOSTATIN M, Diane Horn, Peter Gompper, Elizabeth Wayner, and Peter Linsley, Oncogen, 3005 First Ave., Seattle, WA 98121. Alterations in proteins of the extracellular matrix have fundamental effects on a variety of biological processes including metastasis, wound healing, and embryonic development. Oncostatin M is a unique cytokine and tumor inhibitory factor isolated from human histiocytic lymphoma cells and activated T lymphocytes. We have found that oncostatin M causes an increase in both fibronectin and fibronectin receptor levels in A549 human lung carcinoma cells. Total fibronectin synthesis was determined by metabolic labeling followed by immunoprecipitation and by measurements of fibronectin mRNA; fibronectin receptor levels were determined by antibody binding to whole cells followed by flow cytometry. When oncostatin M was combined with TGF-beta, which also caused an increase in the levels of fibronectin and fibronectin receptor in A549 cells, the effect was superadditive for fibronectin at both the protein and RNA levels and less than additive for fibronectin receptor. These observations suggest a role for oncostatin M in the regulation of the extracellular matrix both alone and in combination with other growth factors. Regulation of matrix components may contribute to the tumor inhibitory effects of oncostatin M.

A 113 **Simultaneus Loss of Syndecan Expression and Epithelial Phenotype in S115 Carcinoma Cells Exposed to Steroids.** Markku Jalkanen*, Pirkko Härkönen** and Sirpa Leppä*, Departments of Medical Biochemistry* and Anatomy**, Institute of Biomedicine, University of Turku, Kiinamyllynk. 10, SF-20520 Turku, Finland

Cell-matrix interactions play an important role in the maintenance of cell shape, supposed to be mediated by the anchorage of cellular cytoskeleton to extracellular matrix via matrix receptors. One well characterized extracellular matrix receptor is a cell surface proteoglycan - syndecan, which consists of a matrix binding ectodomain and a membrane embedded domain extending also 34 amino acid residues intracellularly (Saunders, S., M. Jalkanen, S. O'Farrell and M. Bernfield. 1989. J. Cell Biol. 108: 1547-1556). Binding of a matrix ligand to syndecan promotes its association with actin-rich cytoskeleton at the basal site of the the cells, thus participating to the stabilization of epithelial morphology. In this work the expression of syndecan was studied during the hormone-induced change in the phenotype of Shionogi 115 (S115) mouse mammary tumor cells. In the absence of androgen, when S115 cells expressed epithelial morphology, syndecan was localized to the cell borders, organized in long fibers at the edges of the cells and also in small concentrates beneath the cells (adhesion plaques). In the presence of testosterone, when these cells expressed transformed phenotype by becoming fibroblastic in morphology and anchorage-independent in their growth, syndecan expression was reduced and more disorganized. Both the amounts of matrix binding ectodomain of syndecan, when quantitated by radio-immunoassay and by Western-blot, and syndecan mRNA (2.6 kb) declined in hormone-treated S115 cells. We thus propose that the inactivation of syndecan gene and the consequent suppression of syndecan expression is related to the disappearance of epithelial phenotype and, on the other hand, to the appearance of transformed phenotype in hormone-treated S115 cells.

A 114 INTERACTIONS BETWEEN THROMBOSPONDIN AND bFGF ARE INVOLVED IN THE MITOGENESIS OF SWISS 3T3 CELLS. **Kevin Judge, Kristina Wikstrom, and Paul Bornstein. Departments of Medicine and Biochemistry. University of Washington, Seattle, WA 98195**

Thrombospondin (TS), a 420kD extracellular glycoprotein, has been shown to potentiate the mitogenic effect of epidermal growth factor in rat smooth muscle cells. (Majack R, Cook SC, Bornstein P. PNAS 1986;83:9050). In the present study, serum-deprived subconfluent Swiss 3T3 cells were stimulated by TS and bFGF, alone and in combination. The results of a representative experiment are summarized as follows:

Condition	Incorporation of [^{3}H] thymidine % of serum control (+/- SE)
TS, 10μg/ml	11.4 (.01)
bFGF 0.2ng/ml	39.3 (3.0)
bFGF 0.4ng/ml	91.2 (2.1)
TS 10μg/ml + bFGF 0.2ng/ml	81.9 (2.0)

The effect of TS and bFGF in combination was significantly greater ($p<.001$) than the arithmetic sum of the individual effects of TS and bFGF. In addition, significant binding of bFGF to TS was demonstrated by a sequential ELISA method. We conclude that TS is capable of potentiating the mitogenic effect of bFGF in Swiss 3T3 cells, possibly as a consequence of a direct interaction with the growth factor.

A 115 **LAMININ ISOFORMS ARE DEVELOPMENTALLY EXPRESSED DURING C3H10T1/2 MYOGENESIS,** Todd G. Kroll, Barry P. Peters*, and Raymond W. Ruddon. Program in Cellular and Molecular Biology and Department of Pharmacology, University of Michigan School of Medicine, Ann Arbor, MI 48109; *Department of Anatomy, Physiological Sciences, and Radiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606.

We have observed that multiple isoforms of laminin, a basement membrane cell adhesion glycoprotein, are biosynthesized in an *in vitro* model system of mesenchymal muscle development. C3H10T1/2 mouse embryo fibroblasts can be induced to undergo differentiation into myoblasts that have the capacity to terminally differentiate into skeletal myofibers (Taylor and Jones, Cell 17: 771-779, 1979). These events, which resemble the conversion of embryonic mesenchyme into muscle, are initiated in the fibroblasts by the expression of specific genes that regulate muscle differentiation (including myo D1; Tapscott *et al*, Science 242: 405-411, 1988). These genes are activated after brief treatment of the fibroblast cells with 5-aza-2'deoxycytidine (5-azaCdR) or after stable transfection with myo D1. Clonal cultures of undifferentiated C3H10T1/2 cells and of C3H10T1/2 cells that were clonally isolated after they had been activated to differentiate into myoblasts by either 5-azaCdR treatment or myo D1 transfection (isolated and provided by Dr. Peter Jones and co-workers, USC Comprehensive Cancer Center), were compared by laminin immunoprecipitation of radiolabeled cell lysates. The undifferentiated fibroblasts biosynthesized an 850 kDa laminin isoform that was composed of four disulfide-bonded B subunit chains (a B4 isoform containing both the 205 kDa B1 and 200 kDa B2 chains) and that lacked the A subunit chain (400 kDa). The clonal 5-azaCdR-derived myoblasts, prior to and during their terminal differentiation into myofibers, biosynthesized principally a 950 kDa laminin trimer that was composed of the B1, B2, and A subunit chains (an ABB laminin isoform similar to that typically localized to basement membranes). In an identical fashion, clonal myoblasts derived after transfection of the fibroblasts with myo D1 also biosynthesized predominantly 950 kDa laminin ABB. Thus, a switch in the biosynthesis of laminin isoforms from 850 kDa B4 to 950 kDa ABB is correlated with the activation of C3H10T1/2 myogenesis initiated by two different treatments, either 5-azaCdR or myo D1. The biosynthesis of the laminin A subunit and the ABB trimer may be part of the myogenic sequence of gene expression that leads to the muscle phenotype in this system. The increased expression of the ABB laminin trimer early in myogenic differentiation supports the idea that it may play a role in basement membrane formation and muscle morphogenesis. *Supported by USPHS Grant CA-41359.*

A 116 **THE EFFECTS OF LYSOZYME ON ACTIN POLYMERIZATION** *Charles Lee Kuckel,* Peter K. Lambooy# and Patricia N. Farnsworth** * Departments of Ophthalmology and Physiology, University of Medicine and Dentistry, New Jersey Medical School, Newark, New Jersey 07103 # Eli Lilly Corporation, Indianapolis, Indiana 46285

Several methods (fluorescence, high and low shear viscosity, and electron microscopy) have been applied to measure the effects of an extracellular matrix protein, lysozyme, on actin polymerization. Under our conditions, at pH 8.0 and 20°C, lysozyme is predominantly dimeric and its major effect is to inhibit the steady-state polymerization of actin. Those actin filaments formed in the presence of lysozyme are significantly shortened with recurrent amorphous densities along the filament length. In addition, lysozyme produces an immediate increase in the fluorescence of 1-N-pyrene iodoacetamide labelled G-actin. However, at pH 6.4, 37°C, lysozyme is monomeric and actin filament crosslinking is observed *(Griffith and Pollard, J. Biol. Chem., 1982)*. We reasoned that the tripeptide, L-arginyl-glycyl-aspartate [RGD], a sequence capable of mimicking a portion of the receptor sites of extracellular matrix [ECM] proteins *(Singer et al., J. Cell Biol., 1987)*, in hen egg white lysozyme [HEWL] might be important in lysozyme self-association and, therefore, actin-lysozyme interaction. The presence of RGD in the lysozyme-actin polymerizing solutions at pH 8.0, 20°C produced an inhibition of the dimeric lysozyme effects while RGD alone had no effects on actin polymerization. Therefore, RGD most likely binds to a complementary RGD sequence on lysozyme and alters its ability to interact with actin and modify polymerization. *(Supported by NIH grant #565921 [National Eye Institute] and Lions Eye Research Foundation of New Jersey).*

A 117 EXPRESSION OF MEROSIN, A LAMININ-RELATED TISSUE-SPECIFIC BASEMENT MEMBRANE PROTEIN, IN HUMAN SOFT TISSUE TUMORS, Ilmo Leivo[1], Pekka Laurila[1], and Eva Engvall[2], Department of Pathology[1], University of Helsinki, SF-00290 Helsinki, Finland and La Jolla Cancer Research Foundation[2], La Jolla, CA 92037, USA.

Merosin is a novel tissue-specific laminin-related basement membrane protein found in normal basement membranes of trophoblast, striated muscle and Schwann cells. In human placental tissues and tumors of trophoblast origin, merosin was found in cells of the intermediate trophoblast type whereas almost no merosin was found in cytotrophoblast and syncytiotrophoblast cells. In schwannomas, merosin was expressed in areas where the tumor cells are in contact with stromal or vascular tissues. In malignant schwannomas, however, no merosin was seen. Moreover, in other soft tissue tumors including leiomyosarcomas and rhabdomyosarcomas no merosin was found. On the other hand, some biphasic synoviosarcomas showed merosin at the interface of the epithelial and mesenchymal cells. In developing mouse skeletal muscles and nerves, merosin appeared late during the maturation of these tissues. The results indicate that in human tumors merosin is exclusively expressed by specific differentiated cell types such as the intermediate trophoblast cells and schwannoma cells. The protein is not found in immature or poorly differentiated cells. The results also suggest that a contact with mesenchymal cells or matrices may provide an inducing signal for the expression of merosin.

A 118 A-CHAIN DEPENDENCE OF THE ADHESIVE DOMAIN IN THE LAMININ E8 FRAGMENT, Lissitzky J.C. and Martin P.M., Laboratoire de Cancerologie, UA CNRS 1175, Faculte de Medecine Nord, BVD P. Dramard, 13015 Marseille, France. A major laminin-cell interaction site involved in the control on cell movement (adhesion and migration) is located in the long arm of the cross-shaped laminin molecule, a region which corresponds to the laminin elastase fragment E8. The laminin domain responsible for the neurite activity of laminin has also been assigned to this fragment. The laminin long arm consists of the three laminin subunits A, B1, B2, presumably wraped as coiled-coil alpha-helix. The structure-relationship of the E8 related laminin adhesive domain is still unknown. - Due to an expression of the A chain gene lower than the B chains, the endodermal cell line PFHR9 produces equal amounts of heterotrimeric AB1B2 laminin (lam) and B1B2 heterodimers (B1B2). The adhesive properties of these species were analysed by immuno-attachment assay. Lam and B1B2 were separated by gel filtration and immunoselected with Sepharose-6MB beads grafted with a laminin monoclonal antibody (mAb) to the N-terminus of the B1 chain (out of E8) and not interfering in laminin mediated cell adhesion. The material selected by the beads was quantified with a mAb to the laminin B2 chain and was characterized by SDS-PAGE analysis to verify the efficiency of the purification step. - On the beads loaded with lam, the adhesion of the E8 sensitive rat rhabdomyosarcoma cells (RMS), was efficient and specific (it was blocked by polyclonal antibodies to E8). No attachment was obtained with B1B2 or with the B1 chains. Normalization according to the amount of material on the beads indicated that lam was at least 50 times more potent to support the attachment of RMS cells than B1B2.These data indicate a strong A-chain dependence of the adhesive domain in the laminin E8 fragment. This is in contrast to the neurotropic epitope which requires only the B1 and B2 chains chains. The adhesive function of laminin might be under A-chain gene control.

A 119 TUMOUR CELLS WHICH DEVELOP RESISTANCE TO CYTOLYSIS BY TUMOUR NECROSIS FACTOR (TNF) HAVE A DIFFERENT GLYCOFORM OF A 105kD MEMBRANE GLYCOPROTEIN AND LOSE THE CAPACITY TO INVADE AND METASTASISE, M.L.Neale, R.A.Fiera and N.Matthews, Department of Medical Microbiology, University of Wales College of Medicine, Heath Park, Cardiff, CF4.4XN, U.K.
A plastic-adherent variant of human myelomonocytic leukaemia cells (U937A) is highly susceptible to direct TNF cytolysis in vitro. A mutant selected for resistance to TNF cytolysis (U937A/R) is much less motile and more plastic-adherent than the parental line. The U937A and U937A/R cells have different glycoforms of a 105kD cell surface glycoprotein. This protein is also found in the lysozomal membrane and has the physicochemical properties consistent with it being a member of the glycoprotein family containing LAMP-1, lamp A, lep100 and lgp120. In addition, the U937A cells have a 350kD extracellular matrix glycoprotein which is lacking in the U937A/R matrix. In nude mice, U937A cells are highly malignant whereas U937A/R cells form a benign, encapsulated tumour. Therefore, these glycoprotein differences between U937A and U937A/R correlate not only with loss of TNF susceptibility but also with reduced invasiveness and metastasis.

A 120 EXTRACELLULAR MATRIX GLYCOPROTEINS AND PROTEOGLYCANS OF ASTROCYTES REGULATING NEURITE GROWTH OF BRAIN NEURONS, H.W. Müller, H.P. Matthiessen and C. Schmalenbach, Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf,D-4000 Düsseldorf, F.R.G.

Cultured neurons from embryonic rat hippocampus develop bioelectrically active neuritic networks in the presence of neurite growth-stimulating proteins released by cerebral astrocytes from newborn rat. The neurite-promoting activity in serum-free astroglial conditioned medium was shown to bind to polycationic culture substrata. Following FPLC anion exchange chromatography (Mono Q) and gel filtration (Superose 6) of astroglial conditioned medium several active protein fractions could be distinguished (Matthiessen et al., 1989, Glia 2, 177-188). The major peak of neurite-promoting activity contained a modified laminin molecule (lacking the A-chain) which was associated with proteoglycans (heparansulfate and chondroitinsulfate proteoglycan) in a high molecular weight complex. The second peak of activity contained fibronectin, which was simultaneously released with laminin by astroglial cells. Very interesting, in contrast to laminin, fibronectin apparently was not associated with proteoglycans. The characterization and developmental regulation of the appropriate receptors on the responsive brain neurons remains to be investigated.

A 121 ANTITUMOR ACTIVITY OF SURAMIN AND OTHER SULFATED DRUGS WHICH ARE ANALOGS OF SULFATED PROTEOGLYCANS, Charles E. Myers, Renato V. La Rocca and Michael Cooper, Medicine Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Heparan sulfates are known to bind to a range of tumor growth factors and to either stimulate activity of the growth factors or preserve them proteolytic digestion. We have been interested in developing analogs of he-[paran sulfate which have antagnist, rather than agonist action. The first such drug proved to be suramin, a known inhibitor of TGFG, FGF, and PDGF. We have shown that suramin has a broad range of antitumor activity in vitro. In clinical trials, we have noted dramatic responses in patients with cancer of the prostate and a range of lymphomas. Based upon these results, we have looked for other analogs and have found similar activity in other structurally unrelated sulfate drugs. In addition, we have identified a heparan sulfate containing fraction in the urine of patients on suramin which has antitumor activity. These results suggest that there may exist heparan sulfates with a range of growth inhibitory and growth stimulating properties.

A 122 FIBRONECTIN ALTERNATIVE RNA SPLICING: MECHANISMS AND FUNCTIONS

Pamela A. Norton and Richard O. Hynes*, Center for Cancer Research and *Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

We have been pursuing two lines of investigation aimed at: 1) understanding the mechanisms by which fibronectin (FN) alternative splicing events are regulated, and 2) establishing the functional differences between the spliced variants.

1) Transfection of HeLa cells with highly truncated rat FN mini-genes resulted in transcripts from which the EIIIB exon was largely omitted. When pre-mRNAs with similar structures are processed in vitro by HeLa nuclear extract, EIIIB exon skipping is also observed. Increasing the magnesium concentration of the in vitro reaction results in an alternative processing event: removal of the intron upstream of EIIIB. We have been characterizing this latter, conditional splicing event to determine which regions of the pre-mRNA are important. In addition, proteins which bind to the FN pre-mRNA in a magnesium-sensitive fashion are under investigation.

2) The biological consequences of FN alternative splicing remain largely obscure, particularly with regard to EIIIA and EIIIB, as no specific functions have been attributed to these segments. To study the role of these two segments in vivo, we are generating transgenic mice expressing defined splice variants. cDNAs encoding specific forms have been placed under the control of the FN promoter. It is hoped that expression of inappropriate spliced forms will exert dominant effects despite the presence of the endogenous, correctly spliced FNs.

A 123 GLUTACTIN, A NEW ACIDIC BASEMENT MEMBRANE GLYCOPROTEIN OF *DROSOPHILA*

Pamela F. Olson, Robert E. Nelson, Katherine Garrison, Liselotte I. Fessler, and John H. Fessler, Molecular Biology Institute and Biology Department, UCLA, Los Angeles, CA 90024-1570

The extracellular matrix that is immediately adjacent to cells influences their differentiation and function. A new glycoprotein component of this pericellular matrix in *Drosophila* is described here. Glutactin was isolated from Kc cell culture medium and shown by immunofluorescence microscopy to occur in the basement membranes of *Drosophila* embryos, particularly to the fibroblast-like hemocytes, the segmentally invaginated envelope of the central nervous system, the dorsal median cell processes and the muscle apodemes. Glutactin is a single polypeptide which migrates in SDS-PAGE with an apparent MW of 155 kD. The cDNA sequence encoding glutactin was confirmed by partial amino acid sequencing. The glutactin gene is located at chromosome locus 29D. The 1023 residues of glutactin are separated into 2 domains by a stretch of 13 contiguous threonine residues. The amino domain is strongly similar to several serine esterases, but lacks the catalytically active serine residue. The carboxyl domain has an excess of 52 acidic amino acids, and 44% of its residues are either glutamine or glutamic acid. Glutactin preferentially binds Ca^{++} in the presence of excess Mg^{++} and several of its tyrosines are O-sulfated. The molecular characterization and developmental profile of glutactin will be presented.

A 124 INTERACTION BETWEEN GLYCOPROTEIN IB-IX COMPLEX AND VON WILLEBRAND FACTOR: A CENTRAL EVENT IN PLATELET ADHESION AND AGGREGATION, Zaverio M. Ruggeri, Department of Molecular and Experimental Medicine, and Committee on Vascular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037. The binding of von Willebrand factor (vWF) to the platelet receptor glycoprotein (GP) Ib-IX complex is a key event in platelet adhesion and aggregation, thus participating in hemostasis and thrombogenesis. The studies reported here have been aimed at localizing the relevant functional regions in both vWF and GP Ibα that mediate their interaction. The GP Ib-binding site of vWF involves two discontinuous, disulfide-linked segments of sequence located between residues Cys^{474}-Pro^{488} and Leu^{694}-Pro^{708} of the mature subunit. One of these two sequences is in close proximity to a putative heparin-binding site that might be involved in mediating vWF binding to proteoglycans of the subendothelium. Moreover, a collagen-binding site is located in the same proteolytic fragment of vWF that contains the heparin- and GP Ib-binding sites of the molecule. The spatial proximity of these distinct sites suggests the possibility of functional modulation. In GP Ibα, the vWF-binding domain is located in the 45 kDa extracytoplasmic amino terminal domain. A segment of sequence comprising residues Ser^{251}-Tyr^{279} of the mature α-chain has been identified as being involved in vWF binding. In all cases, appropriate synthetic peptides and/or monoclonal antibodies to epitopes comprising the functional sites identified above have been shown to inhibit vWF binding to GP Ib-IX. Effective inhibition has also been obtained with recombinant vWF fragments containing the sequences indicated above. Knowledge of the molecular anatomy supporting vWF interaction with GP Ib-IX may indicate strategies for anti-thrombotic intervention.

A 125 Lp(a) LIPOPROTEIN HAS SERINE PROTEINASE ACTIVITY AND BINDS TO FIBRONECTIN
Eeva-Marjatta Salonen, Department of Virology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland

We have demonstrated that plasminogen and its activator bind to immobilized fibronectin (1). Recently it was reported that apolipoprotein (a), which is associated with apoB-100 (the main protein component of LDL), is highly homologous with plasminogen (2). This led us to study whether Lp(a) and fibronectin interact. We found that apo(a) binds to immobilized fibronectin and cleaves it (3). The binding was localized to the C-terminal heparin-binding domain of fibronectin. The cleavage pattern, as visualized by SDS-PAGE and immunoblotting was was distinct from that obtained upon proteolysis of fibronectin by plasmin or kallikrein. Experiments with inhibitors and synthetic peptide substrates indicated that the proteolytic activity was of serine proteinase-type and, unlike plasmin, preferred Arg rather than Lys at the scissile bond. The observed interaction between Lp(a) and fibronectin leading to its degradation, is of interest since plasma concentration of Lp(a) is correlated with the risk of heart disease and in view of the observation of Lp(a) (4) and fibronectin in association of smooth muscle cells (5) in early atherosclerotic lesions.

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A 126 FIBRONECTIN FIBRILLOGENESIS REQUIRES MULTIPLE DOMAINS,
Jean E. Schwarzbauer, Department of Biology, Princeton University, Princeton, NJ 08544.

The fibroblast extracellular matrix (ECM) is, in large part, structured by a fibronectin (FN) fibrillar network. Within this network, the FN dimer interacts with cells and with other ECM components such as proteoglycans, collagen, and other FN molecules. To follow the assembly of a FN matrix, rat FN cDNAs encoding normal and mutant polypeptides are being expressed in transformed mouse fibroblasts. ECM incorporation of the recombinant rat FN polypeptides is assayed in two ways: presence in the deoxycholate-insoluble matrix fraction of the cell layer and detection of fibrils by indirect immunofluorescence with a rat-specific monoclonal antibody. An internal 75kD deletion of the first seven type III repeats produces a 175kD FN polypeptide that is fully competent for fibril formation. However, this FN polypeptide must be in the form of a disulfide-bonded dimer and the dimer subunits must contain the amino-terminal 50kD for efficient fibril formation. Polypeptides lacking either the C-terminal interchain disulfide bonds or the N-terminal domain are released into the culture medium. Deletion of the RGDS cell binding peptide from type III-10 has no detectable effect on the extent of incorporation into the ECM. Other functional domains and splice variants of FN are currently under study to determine their roles in this process.

A 127 RESPONSE OF FIBROBLAST AND MACROPHAGE FIBRONECTIN MRNA TO HYPEROXIA. Robert A. Sinkin, Michael LoMonaco, Department of Pediatrics (Neonatology), University of Rochester, Rochester, NY 14642. Fibronectin (FN) is involved in diverse biological phenomena including mediation of cell matrix interaction through its ability to bind to cells, collagen, and other connective tissue components and to attract cells by virtue of chemotactic properties. Continuous exposure of the lung to high concentrations of oxygen produces a well recognized pathology including fibroproliferation in which the normal alveolar walls are progressively thickened by a fibrotic process characterized by an increase in fibroblasts and collagenous extracellular matrix secreted by these cells. The effects of hyperoxia (95% O_2) on rabbit alveolar macrophages from adult animals and on a rabbit fetal lung fibroblast cell line (R9ab) were investigated with respect to FN mRNA expression. R9ab were cultured in macrophage conditioned medium (CM) with and without serum. RNA was extracted from the cultured cells and subjected to dot blot analysis by hybridization with a cloned rabbit FN cDNA probe [isolated from a rabbit liver library, ~2.2 Kb (unpublished data)]. Preliminary data reveals that oxygen exposure increases R9ab FN mRNA expression in the absence of serum but decreases expression when serum is in the culture medium. R9ab cultured in CM +/- serum demonstrate differential FN mRNA expression depending on the environment in which the macrophages and R9ab were cultured. Ongoing experiments to determine mechanisms of action for oxygen and its effect on FN expression are proceeding.

A 128 SECONDARY STRUCTURE PREDICTIONS FOR THE 65-67 KD LAMININ RECEPTOR, Jean R.Starkey, Terry H. Landowski and Sam L. Helgerson, Departments of Microbiology and Chemistry, Montana State University, Bozeman MT 59717.

Although the complete amino acid sequence of the 65-67 Kd laminin receptor is known (Yow et al., PNAS, 85:6394, 1988), little information is available on the functional domains of this protein. Using the Stroud membrane protein secondary structure prediction program (Finer-Moore, J. and Stroud, R.M., PNAS 81:155, 1984), we found the receptor to have 30% alpha helix, 22% beta sheet, 19% beta turn and 29% random coil. No simple putative transmembrane hydrophobic domain was found and no homologies were found with proteins having beta sheet transmembrane regions by the EMBO "Alimat" program which looks for structure:function rather than strictly sequence homologies (Argos, P., J. Mol. Biol. 193:385-396). The most likely transmembrane region was determined to be an amphipathic helical region from residue 49-70. To allow for shielding of complementary charged side chains in the transmembrane region, the receptor may be present in the membrane as a dimer or higher oligomer. This correlates with our observation of the tendency of the receptor to self-aggregate at high concentrations in physiological saline. Within the cytoplasmic region of the receptor, we have identified a sequence which demonstrates a high degree of homology with the ATP-binding site of members of the protein kinase family, followed closely by a serine, which is a potential phosphorylation site. As the laminin binding site is known to be in the carboxy terminal half of the protein (Wewer, U., et alk, PNAS 83:7137, 1986), we predict the ligand binding region to be a highly charged alpha helix (residues 208-228) flanked on one side by a motif homologous with C' regulatory protein sequences in ELAM-1. CTR Grant #2405.

A 129 RECOMBINANT PROTEIN FRAGMENTS AND SYNTHETIC PEPTIDES FROM THE "EGF-LIKE" DOMAIN OF LAMININ WITH CELL ADHESION ACTIVITY. David G. Streeter, Dorris L Taylor, Jerome B. Lazar, Shin-ichiro Abe*, Paul H. Johnson Molecular Biology Dept. SRI International, Menlo Park CA. 94025. Bioscience Research Laboratory, Nippon Mining Co., Ltd., Saitama, Japan.

Laminin is a major glycoprotein component of basement membrane that has been shown to function in the attachment and invasion of capillary basement membrane by certain metastatic tumor cells. Various peptide sequences have been identified in laminin that may account for this activity. Two of these peptides are found in a repeating sequence on the B1 chain of laminin which bears homology to epidermal growth factor (EGF) and EGF-related proteins. We have synthesized the entire 40 amino acid sequence of this region of laminin by recombinant DNA methods and have prepared various synthetic peptides from the C-terminal half of this sequence. The recombinant protein fragment promotes attachment of B16 mouse melanoma cells. Synthetic peptides of 20-25 amino acids comprising the C-terminal half of this fragment, containing the reported YIGSR cell binding sequence, are similarly active. However, the C-terminal 10 amino acid sequence of this fragment, also containing YIGSR, is much less active in cell attachment. Cell attachment activity remains low when this 10 amino acid sequence is extended further on the C-terminal side by 15-20 amino acids. These findings indicate that the EGF-like region of the laminin B1 chain does indeed contain cell attachment activity, but that determinants other than YIGSR contribute significantly to this activity.

A 130 THE ROLE OF A HYALURONAN BINDING PROTEIN (HABP) IN p21-ras STIMULATED FIBROBLAST LOCOMOTION, Eva A. Turley, Carol Clary, Karen Vandeligt and Lea Austen, Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 4N1.
A 10T1/2 cell line transfected with an EJ ras gene linked to an MT-1 promotor, was used to study cell locomotion. Induction of the ras gene with zinc resulted in a 2-3-fold increase in random locomotion and conferred sensitivity to hyaluronan: The addition of picogram amounts of this glycosaminoglycan promoted locomotion by a further 3 fold. This effect was specific in that heparin and chondroitin sulfate had no effect. Sensitivity of cells to hyaluronan correlated with increased expression of a hyaluronan binding protein (HABP). Further, several monoclonal antibodies to HABP inhibited the hyaluronan-stimulated locomotion. Possible roles of hyaluronan/HABP in transformation-mediated increases in cell motility are presented. This work was supported by the National Cancer Institute of Canada and Alberta Cancer Board.

Integrins

A 200 CHANGES IN KERATINOCYTE-EXTRACELLULAR MATRIX INTERACTIONS DURING TERMINAL DIFFERENTIATION: THE ROLE OF FIBRONECTIN RECEPTORS, Josephine C. Adams and Fiona M. Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London, WC2A 3PX, England.
In the epidermis proliferation takes place in the basal layer and cells undergo terminal differentiation as they move upwards towards the tissue surface. Keratinocytes leave the basal layer because their ability to adhere to a range of extracellular matrix proteins is reduced at an early stage in the differentiation process. We have begun to examine the molecular basis for the reduction in adhesion to fibronectin. Cultured keratinocytes adhere to fibronectin via the peptide RGDS and do not recognise cell binding sites within the IIICS region of the molecule. Adhesion to fibronectin is blocked by an antiserum raised against the $\alpha_5\beta_1$ integrin. Using this antiserum, we have measured the level of fibronectin receptor on the surface of keratinocytes by immunoprecipitation at different times during suspension- or TPA-induced terminal differentiation. In each case loss of adhesion precedes a decrease in the level of the receptor on the cell surface by several hours. However, loss of adhesiveness does coincide with a marked reduction in the ability of detergent-extracted receptors to bind fibronectin. We are currently investigating the mechanisms that regulate receptor affinity in keratinocytes.

A 201 MOLECULAR CHARACTERIZATION OF A NOVEL INTEGRIN RECEPTOR FROM CHICK EMBRYO FIBROBLASTS, Blaise Bossy and Louis F. Reichardt, UCSF and Howard Hughes Medical Institute, U426, San Francisco, CA 94143-0724. Two groups of cDNA clones have been purified from a chick E10 cDNA library using as a probe a cDNA encoding the α subunit of the human vitronectin (VN) receptor. The first set of overlapping cDNAs appears to define the avian homologue of the human α_{VN} subunit. The coding sequence defined by these cDNAs is ca 80% identical to the sequence of the human subunit. The second group of cDNAs encode an apparently novel chick α subunit lacking an I domain, whose amino acid sequence does not exhibit high homology to any described integrin α subunit (42% identity to the human α_{VN}; 39% to the human α_{FN}; less to any other published subunit sequence). Antibodies to two synthetic peptides derived from the sequence of this α subunit immunoprecipitate an integrin heterodimer containing the chick β_1 subunit in association with a putative α subunit with a Mr of 155 kD in non-reducing SDS-PAGE that is shifted to 130 kD by reduction. Immunodepletions and peptide mapping experiments demonstrate that this α subunit is not the 150 kD chick integrin α subunit immunoprecipitated with a serum specific for the 19 C-terminus residues of the human α_5 subunit. Functional assays using the immunopurified integrin heterodimer are being performed to determine the specificity of this receptor.

A 202 ROLE OF INTEGRINS $\alpha 2\beta 1$ AND $\alpha 3\beta 1$ IN CELL-CELL AND CELL-SUBSTRATE ADHESION OF HUMAN EPIDERMAL CELLS, W. G. Carter, E. A. Wayner, T. S. Bouchard and P. Kaur. Hutchinson Cancer Ctr. Seattle, WA 98104. We have examined cultures of neonatal human foreskin keratinocytes (HFKs) in order to determine the ligands and functions of integrins $\alpha 2\beta 1$, and $\alpha 3\beta 1$ in normal epidermal stratification and adhesion: (i) $\alpha 2\beta 1$, and an unidentified $\beta 1$-containing integrin, contribute to initial HFK adhesion to exogenous laminin. (ii) $\alpha 3\beta 1$ interacts with a secreted laminin/type IV collagen-containing complex, in preference to exogenous laminin or type IV collagen. Anti-$\alpha 3\beta 1$, detached prolonged cultures of HFKs from culture plates or from partially purified HFK extracellular matrix indicating that interaction of $\alpha 3\beta 1$ with the secreted BMZ-like complex was responsible for HFK adhesion in long term culture. (iii), Ca^{+2}-induced aggregation of HFKs resulted in relocation of $\alpha 2\beta 1$ and $\alpha 3\beta 1$ from FAs to cell-cell contacts. A dual function for $\alpha 3\beta 1$ in cell-cell as well as cell substratum adhesion was indicated by inhibition of HFK intercellular adhesion by anti-$\alpha 3\beta 1$ MAb P1B5 and anti-$\beta 1$ MAb P4C10. (iv) It is suggested that interaction of $\alpha 3\beta 1$ with a secreted, BMZ-like complex in cultured HFKs, duplicates the role of $\alpha 3\beta 1$ in basal cell adhesion to the BMZ in skin. Further, relocation of $\alpha 2\beta 1$ and $\alpha 3\beta 1$ to cell-cell contacts may result in detachment of cells from the BMZ and increased cell-cell adhesion in the suprabasal cells contributing to stratification of the skin.

A 203 EXPRESSION OF FIBRONECTIN AND VITRONECTIN RECEPTORS IN WOUND FIBROBLASTS, Richard A.F. Clark, James Gailit, Michael D. Pierschbacher, Erkki Ruoslahti, Departments of Medicine and Pediatrics, National Jewish Center, Denver, CO, 80206; LaJolla Cancer Research Foundation, LaJolla, CA, 92037. Mesenchymal organization of many tissues requires fibroblast migration and extracellular matrix (ECM) assembly. Appropriate ECM receptor expression is sine que non for these processes. The $\beta 1$ and $\beta 3$ families of integrin membrane glycoproteins are two important classes of ECM receptors. The fibronectin receptor ($\alpha 5/\beta 1$) and vitronectin receptor ($\alpha v/\beta 3$) are members of these families. In this study we have used porcine cutaneous wound repair as a paradigm of mesenchymal organization in which to correlate fibronectin and vitronectin receptor expression with fibroblast function. Polyclonal antibodies to fibronectin or vitronectin receptors isolated from human placenta or antibodies to synthetic $\alpha 5$ peptides were used for immunofluoresence probing of wound tissue. A fibroblast-rich granulation tissue filled approximately 80% of the wound space by day 5. Vitronectin, but not fibronectin, receptors were present on 5 day wound fibroblasts that were aligned along the wound margins and presumably migrating inward. Fibroblasts in the center of 5 day wounds were randomly oriented and expressed neither vitronectin nor fibronectin receptors. Day 7 fibroblasts were coaligned with ECM across the wound, contained cytoplasmic f-actin bundles, expressed fibronectin and vitronectin receptors, and formed cell-matrix linkages. Subsequently the wound contracted. These findings demonstrate that vitronectin but not fibronectin receptors were present during fibroblast migration into wounds, and that both receptor types were expressed coordinately with f-actin bundles and cell-matrix links just prior to wound contraction.

A 204 INTEGRIN RECEPTORS ON AORTIC SMOOTH MUSCLE CELLS MEDIATE ADHESION TO FIBRONECTIN, LAMININ, AND COLLAGEN, Ronald I. Clyman and Randall Kramer, Cardiovascular Research Institute and Departments of Anatomy and Stomatology, University of California, San Francisco, CA 94143. Extracellular matrix (ECM) receptors on vascular smooth muscle cells help in anchoring the cells during contraction and in promoting cellular migration after vessel injury. Rat aortic smooth muscle cells attach to surfaces coated with fibronectin, laminin, and collagen types I and IV. Members of the β_1 family of integrin receptors appear to mediate attachment to these ECM components. Antibodies to the β_1-subunit demonstrated the presence of integrin complexes in focal adhesion plaques. Ligand affinity chromatography isolated a series of receptor complexes that were recognized by antisera to β_1 integrin receptors. Each of the receptors appeared to be a heterodimer in which one of several α-subunits shared a common 120 kD (nonreduced) β_1-subunit protein. There was a 185 kD (nonreduced, 200 kD reduced) α-subunit that was promiscuous and bound to collagen types I and IV as well as to laminin; the 185 kD α-subunit appeared to bind to collagen more efficiently than to laminin. In addition, there was a 150 kD (nonreduced, 160 kD reduced) α-subunit that bound exclusively to collagen type I and a 150 kD (nonreduced) α_5-subunit that bound exclusively to fibronectin. Two additional α-subunits (125 kD and 56 kD, nonreduced) were associated with the β_1-subunit and bound only to laminin. Consistent with the affinity chromatography data, antibodies to the β_1-subunit blocked (> 80%) cell adhesion to the different substrates. On the other hand, a monoclonal antibody (3A3) against the 185 kD α-subunit blocked (> 90%) adhesion to type IV collagen, only partially inhibited (17%) adhesion to type I collagen and laminin, and had no effect on adhesion to fibronectin. Thus, smooth muscle cell express multiple integrin receptors that appear to mediate cell interactions with the ECM.

A 205 INTEGRIN VLA-5 MEDIATES ADHESION OF HUMAN ERYTHROBLASTIC CELLS TO FIBRONECTIN (Fn). Laure Coulombel, Marie-Hélène Gaugler, Mario Rosemblatt, Christine Leroy. Laboratoire d'hématologie, Hopital Bicêtre, 94270 Kremlin-Bicêtre, France.

Our purpose is to delineate the role of cell-matrix interactions in the bone marrow during human hematopoietic differentiation. We have recently shown (Blood,1988,71,329) that attachment to Fn is a selective property of erythroblastic progenitors BFU-E and CFU-E. We now provide evidence that Fn-adhesion is modulated during terminal erythroid differentiation and is mediated by VLA-5. Fn-adhesion was sequentially assessed in CFU-E and their immediate progeny preproerythroblasts, highly purified from fresh human marrow and cultured 7-10 days up to the reticulocyte stage. CFU-E attached to Fn in the highest proportion, 50-70%, as compared to only 25% for preproerythroblasts. With further differentiation, adhesion declined steadily and was totally lost at the time of enucleation. A similar loss of Fn-adhesion was observed during differentiation of human erythroleukemic KU 812 cells. Adhesion to Fn of CFU-E, preproerythroblasts and KU 812 cells was inhibited by a polyclonal antibody (Ab) against the $\alpha 5\ \beta 1$ Fn-receptor, and also by a mAb against the $\alpha 5$ subunit. Presence of VLA-5 on both normal immature erythroblasts and erythroleukemic KU 812 cells was further confirmed by immunoprecipitation. An additional band, which behaves as $\alpha 4$ on gels, was identified in KU 812 cells. Abs against both Fn and the FnR also blocked the spreading of KU 812 cells. However, interestingly, the concentrations required to inhibit spreading were much lower than those required to block adhesion, which suggests that each property might depend on different domains of the Fn molecule binding to different receptors. Based on these findings, we conclude that VLA-5 is differentially expressed during normal human erythropoiesis. A challenging question will be to investigate if VLA-5 binding to Fn will trigger events associated with erythroid differentiation.

A 206 CHARACTERIZATION OF AN 82 000 DALTON COMPONENT OF ADHESION PLAQUES Aaron W. Crawford and Mary C. Beckerle, Department of Biology, University of Utah, Salt Lake City, UT 84112

An 82 kD component of adhesion plaques was recently identified through use of a nonimmune rabbit serum. We have purified the 82 kD protein from low ionic strength extracts of avian smooth muscle by ammonium sulfate fractionation followed by chromatography on DEAE-cellulose, hydroxylapatite, and HPLC gel filtration. Purified 82 kD protein was used to generate polyclonal antisera in mice. These antisera specifically recognize the 82 kD protein originally identified by the nonimmune serum. We have characterized a number of the properties of the 82 kD protein. It is a relatively insoluble protein at neutral pH, having an isoelectric point of 6.9. By calibrated gel filtration chromatography we have determined that the protein has a Stokes radius of 5.6 nm. We do not believe that the 82 kD protein is a transmembrane or extracellular component of adhesion plaques since 1) it fractionates with the aqueous phase in Triton X-114 phase separation experiments, 2) detergent does not facilitate extraction of the 82 kD protein from smooth muscle, 3) detection of the 82 kD protein by indirect immunofluorescence requires detergent permeabilization indicating that all antigenic determinants, at least, are intracellular, and 4) it does not bind Con A. The subcellular distribution of the 82 kD protein is particularly intriguing. It is localized at adhesion plaques as well as along actin filament bundles near where they terminate at sites of substratum adhesion.

A 207 DIFFERENT ROLE OF $a_E\beta_4$ INTEGRIN IN MEDIATING HUMAN KERATINOCYTE ADHESION IN ACTIVELY GROWING COLONIES OR QUIESCENT RECONSTITUTED EPIDERMIS, Michele De Luca*, Pier Carlo Marchisio°, Sergio Bondanza*, Richard N. Tamura^, Ranieri Cancedda* and Vito Quaranta^, *IST, Ist. Naz. per la Ricerca sul Cancro, 16132 Genova, Italy, °Dip. di Scienze Biomediche e Oncologia, Univ. di Torino, 10126 Torino, Italy, ^Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Indirect immunofluorescence and laser scan confocal microscopy of *in vitro* reconstituted human epidermis showed that the major integrins expressed by keratinocytes are $a_E\beta_4$ localized at the basement membrane-contacting surface of basal cells and $\alpha_2\beta_1/\alpha_3\beta_1$ absent from the basal surface and found on the lateral surface of basal and spinous cells. Accordingly, cell adhesion inhibition assays with anti-β1 or anti-β4 antibodies showed that 80-90% inhibition was obtained only with anti-β4 sera on keratinocytes plated on purified laminin or matrigel. Poor inhibition of attachment was obtained when cells were plated on fibronectin, vitronectin ot type I collagen. Beta $_4$ and β_1 integrins were identically distributed in actively growing keratinocytes. However, cell adhesion inhibition assays performed on growing cells showed no inhibition of attachment with either antibodies. Inhibition with anti-β_4 (20-35%) appears when colonies attain confluence and was complete (about 90%) 2 days after reaching confluence. Then, although $a_E\beta_4$ is localized at the basal surface both in growing and stationary keratinocytes its role in functionally supporting adhesion to the basement membrane takes place only wgen cells are organized in a reconstituted epidermis. The two integrins belonging to the β_1 subfamily are not apparently involved in the cell-substratum but rather in cell-cell adhesion of human keratinocytes.

A 208 DIFFERENTIAL REGULATION OF EXPRESSION OF SPECIFIC INTEGRIN RECEPTORS BY TRANSFORMING GROWTH FACTOR β AND NERVE GROWTH FACTOR ON A NEUROBLASTOMA CELLS, Shoukat Dedhar, Virginia Gray and Christopher Haqq, Department of Advanced Therapeutics, Cancer Control Agency of B.C., Vancouver, Canada. We have recently isolated variant neuroblastoma cell lines based on their resistance to detachment from culture by a synthetic peptide containing the cell attachment-promoting sequence Arg-Gly-Asp. Peptide-resistant SK-N-SH cells specifically overproduced the integrin VLA-1 (α1/β1) whereas similarly treated SK-N-MC cells overproduced VLA-5 (α5/β1) [Dedhar et al, J. Biol. Chem., 264, 4832-4836 (1989)].The VLA-5 overproducing SK-N-MC variant cells expressed 10-fold higher TGF-β1 mRNA levels than their parental counterparts, whereas, TGF-β1 mRNA levels were similar in the parental and variant SK-N-SH cells. On the other hand the VLA-1 overproducing SK-N-SH cell variants expressed higher levels of nerve growth factor (NGF). To determine whether TGF-β1 and NGF regulate the levels of expression of different VLA-integrin α subunits in neuroblastoma cells, cell surface expression of integrins on TGF-β1 and NGF-treated and untreated SK-N-SH cells was determined by immunoprecipitation of ^{125}I surface labelled cells. Whereas the primary effect of TGF-β1 was to upregulate the integrin subunits α2-6 and β1 the level of α1 subunit was not altered within the same time period. On the other hand NGF specifically and rapidly upregulated the expression of α1/β1 (VLA-1). The NGF treated SK-N-SH cells attached more strongly to type IV collagen but less strongly to fibronectin acquired a neuronal morphology, and expressed higher amounts of neurofilament p170 mRNA levels. These properties are similar to those expressed by the VLA-1 overproducing, Arg-Gly-Asp selected SK-N-SH variant cells. TGF-β treated cells, however attached more strongly to fibronectin and did not express a neuronal phenotype. Thus by regulating different integrin α subunits the two growth and differentiation factors, TGF-β1 and NGF, may differentially modulate those aspects of the cell phenotype that are mediated by cell-extracellular matrix interactions.

A 209 **In Vitro and Ex Vivo Characterization of a Monoclonal Antibody (AP-2) to Platelet Glycoprotein IIb/IIIa in Rabbits,** T Deisher, J Cohen, C Muir, M Moyle, M Napier, S Bunting. **Department of Pharmacology, Genentech, Inc., South San Francisco, CA 94080**

Platelet glycoprotein IIb/IIIa is essential for mediating platelet aggregation. Both monoclonal and polyclonal antibodies (Ab) for this receptor are available. However, none of the previously available Abs has been reported to inhibit rabbit platelet aggregation, making it necessary to use larger animals in models for the study of these Abs. We have evaluated the ability of the f(ab')2 fragment of the monoclonal AP-2 Ab to inhibit aggregation in rabbit and human platelets.

IN VITRO INHIBITION OF AGGREGATION

	ADP: Max	IC50	Collagen : Max	IC50
HUMANn=6	100%	15nM	98%	15nM
RABBITn=7	75%	28nM	68%	2 4 n M

In vivo administration of 1.0 to 5.0 mg/kg f(ab')2 iv reduced measured ex vivo rabbit platelet aggregation by only 50%. Additional ex vivo f(ab')2 at 10ug/ml did not enhance the inhibition seen with in vivo administration of the f(ab')2. However, following in vivo administration of the AP-2 f(ab')2 to rabbits, 80% of all IIb/IIIa receptor sites were found to be occupied, which, according to other reports, should fully inhibit platelet aggregation. Competitive dissociation studies revealed that in rabbit platelets, unlike human platelets, the AP-2 readily dissociates from the receptor. Conclusion: 1) The rapid dissociation kinetics of the AP-2 from rabbit platelets may partially explain the inability of AP-2 to competitively block platelet aggregation in vitro and ex vivo, 2) additionally, other platelet structural components may play a role in mediating platelet aggregation in rabbits.

A 210 MODULATION OF VITRONECTIN RECEPTOR BINDING BY MEMBRANE LIPID COMPOSITION, E.Dejana,A.Zanetti,P.Neyroz and G. Conforti,Mario Negri Institute for Pharmacological Research,20157 Milano,Istituto di Chimica Biologica,Universita' di Parma,Parma,Italy.We found that vitronectin(vn)-receptor binding to different matrix proteins is influenced by the surrounding lipid composition of the membrane. Human placenta affinity purified vn-receptor was inserted into liposomes of different composition: i) phosphatidylcholine (PC); ii) PC+ phosphatidylethanolamine (PE); iii) PC+PE+phosphatidylserine (PS)+ phosphatidyl inositol (PI)+cholesterol (chol). The amount of purified material incorporated into the three lipid vesicle preparations did not differ. By electron microscopy analysis, the homogeneity and size of the three liposome preparations were also comparable. In contrast their binding capacity to a series of substrata differed widely. Vn-receptor inserted into PC-liposomes bound only vn, while when it was inserted into PC+PE and PC+PE+PS+PI+chol liposomes it also attached to vonWillebrand factor (vWf) and fibronectin (fn). Vn-receptor had higher binding capacity for substrata when it was inserted into PC+PE+PS+PI+chol than PC+PE liposomes. Antibodies to vn-receptor blocked vn-receptor liposome binding to vn, vWf and fn. The intrinsic emission fluorescence spectrum of the vn-receptor reconstituted in PC+PE+PS+PI+chol liposomes was blue shifted in respect to PC liposomes, thus suggesting a conformational change of the receptor in the membranes. The nature of the membrane lipid composition surrounding the receptor could thus influence its binding affinity, possibly by changing its conformation and/or its exposure.

A 211 PHOSPHORYLATION OF PLATELET GPIIIa, Ellen Freed[1], Edward F. Plow[2], Mark H. Ginsberg[2] and Tony Hunter[1], [1]The Salk Institute, San Diego, CA 92138; [2]Scripps Clinic and Research Foundation, La Jolla, CA 92037

We and others have described the phosphorylation of the fibrinogen receptor (GPIIb-IIIa) β subunit (GPIIIa) upon treatment of platelets with PMA and thrombin. GPIIIa is phosphorylated on threonine and to a smaller extent on serine. Phosphorylation of both increases upon treatment. Only trace amounts of phosphotyrosine are present under any treatment condition. GPIIb is not detectably phosphorylated. Upon 2-D tryptic phosphopeptide mapping, we originally observed only one phosphopeptide. Under new conditions, however, two distinct phosphopeptides are resolved. Both are constitutively phosphorylated and become more highly phosphorylated upon treatment. In in vitro kinase assays, purified protein kinase C incubated with immunoprecipitated GPIIIa preferentially phosphorylates one of these sites while purified calmodulin(CAM)-dependent protein kinase II preferentially phosphorylates the other. Three other kinases we have tested give no detectable phosphorylation of GPIIIa in vitro. By the use of synthetic peptides corresponding to GPIIIa cytoplasmic domain sequences we have determined which peptide contains the site of phosphorylation by CAM-kinase II, and are working on a similar analysis for the protein kinase C site. We are also conducting kinase assays with purified myosin light chain kinase, a CAM-dependent kinase known to be present in platelets and stimulated during platelet activation. Experiments to address the functional implications of GPIIIa phosphorylation are also in progress.

A 212 OVEREXPRESSION OF INTEGRIN $\alpha_5\beta_1$: EFFECT ON THE BEHAVIOR OF A TRANSFORMED CHO LINE, Filippo G. Giancotti and Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 N. Torrey Pines Rd., La Jolla, CA 92037. In order to investigate the function of the $\alpha_5\beta_1$ integrin and its role in transformation, we have introduced this fibronectin receptor in a transformed CHO cell line by gene transfer. Amplification of the cDNA sequences encoding both subunits allowed isolation of clonal cell lines overexpressing this fibronectin receptor. Proper biosynthesis and function of the transfected receptor was assessed by a number of criteria. The $\alpha_5\beta_1$ overexpressor cells were found to deposit a richer fibronectin pericellular matrix. The effect observed was specific since the assembly in the matrix of other components was not modified. The overexpressor cells migrated less in an in vitro "wound" assay. Examination of the growth properties of the overexpressors revealed characteristic modifications. They were found to grow slower and to a lower saturation density, as compared to control clones. Moreover, while control cells grew efficiently in soft agar, the overexpressor cells gave only a limited number of colonies. The results demonstrate that $\alpha_5\beta_1$ integrin is involved in matrix assembly, as well as in the control of cell migration and proliferation. The change we have observed in the behavior of transfected cells indicate that modifications of the $\alpha_5\beta_1$ fibronectin receptor may be crucial for the acquisition of altered adhesive phenotype and for the anchorage independent cell growth of transformed cells.

A 213 ORGANIZATION OF DIFFERENT ACTIN ISOFORMS BY INTEGRIN β_1 IN ARTERIAL SMOOTH MUSCLE CELLS CULTURED ON FIBRONECTIN

Ulf Hedin, Bradford A. Bottger, Staffan Johansson, and Johan Thyberg
Department of Medical Cell Biology, Karolinska Institutet, S-104 01 Stockholm, Sweden and Department of Medical and Physiological Chemistry, Uppsala University, S-751 23 Uppsala, Sweden

If smooth muscle cells are enzymatically isolated from the aortic media of adult rats and seeded in primary culture on a fibronectin substrate, a rapid cell spreading and organization of actin filaments takes place and subsequently the smooth muscle phenotype shift from a contractile to a synthetic state. Previous results have revealed that the organization of actin into bundles of stress fibres and the shift in phenotype of the smooth muscle cells is mediated by an interaction between the RGD sequence of fibronectin and a fibronectin receptor at the cell surface. In this study, the organization of different actin isoforms by integrins in smooth muscle cells was studied in more detail using indirect immunofluorescence microscopy with antibodies against integrin β_1, rhodamine phalloidin and a monoclonal antibody against smooth muscle specific α-actin. In the initial formation of a linkage complex between extracellular fibronectin, β_1-containing integrins and actin filaments, smooth muscle α-actin was absent whereas actin filament bundles of other isoforms where localized to these sites. Moreover, as the spreading of the cells proceeded, most α-actin disappeared from the cytoplasm. The findings demonstrate that β_1-containing integrins in smooth muscle cells early in primary culture primarily promote the organization of stress fibre actin but lacks the capacity to interact with 'contractile' α-actin. It is suggested that integrins can specifically interact with different actin isoforms and that such linkages may participate in the control of cellular phenotype.

A 214 IDENTIFICATION AND CHARACTERIZATION OF THE CHICK FIBRONECTIN RECEPTOR AS A HETERODIMERIC COMPONENT OF THE AVIAN INTEGRIN

Urs Hofer, Josef Syfrig and Ruth Chiquet-Ehrismann, Friedrich Miescher-Institut, Postfach 2543, CH-4002 Basel, Switzerland

The chick fibronectin receptor was isolated from chick embryo fibroblasts by affinity chromatography to a cell binding fragment of chick fibronectin and was eluted with the synthetic peptide GRGDSP or with EDTA. The binding to the affinity column was greatly enhanced by the use of 1 mM $MnCl_2$. The purified receptor was a heterodimeric complex like the characterized mammalian integrins and showed an RGD-dependent binding to fibronectin coated wells after incorporation into liposomes. Monoclonal antibodies (mabs) were raised against the two subunits of the fibronectin receptor. Immunoprecipitations with β-chain specific mabs gave the same pattern of multiple bands as with the avian integrin-specific mab JG22. In contrast α-chain specific mabs precipitated only the dimeric fibronectin receptor. In immunoblots anti β-chain mabs recognized the chick gizzard integrin β_1-chain. Anti α-chain mabs did not crossreact with the α-chain of gizzard integrin and in a reverse experiment antisera against gizzard integrin recognized only the β-chain of the fibronectin receptor. These results indicate that we have purified the chick homologue of the mammalian $\alpha_5\beta_1$ integrin.

A 215 OSTEOCLAST VITRONECTIN RECEPTOR : IMPLICATIONS FOR THE REGULATION OF BONE RESORPTION.

Michael Horton, I.C.R.F. Haemopoiesis Group, Dept. of Haematology, St. Bartholomew's Hosp.,London,U.K.

Osteoclasts (OC) are bone resorbing cells which display a unique repertoire of integrin receptors when compared to other cells of haemopoietic origin. Of note is their high level expression of Vitronectin Receptor, CD51 (VNR). Lower levels of VNR are observed in vivo in a restricted range of human tissues including placenta, kidney, endothelium and smooth muscle; in contrast, most cultured adherent cell lines express low levels of VNR. The function of this receptor is unclear though it is known to recognise a wide range of extracellular matrix proteins through their RGD cell binding sequence. We have begun to explore the role of VNR in skeletal tissue. Previously we have raised mabs., 13C2 and 23C6, to distinct epitopes of OC VNR and demonstrated that 13C2 but not 23C6 blocks bone resorption in vitro in an analogous manner to the calcitropic hormone calcitonin. This observation suggests a function for VNR in skeletal homeostasis. The preservation of a number of VNR antibody defined epitopes on OCs across a wide range of species also supports this contention. The normal ligand for VNR in bone is as yet unknown - various data suggest that the RGD-containing non-collagenous bone phosphoproteins, osteopontin and BSPII, may interact with VNR. We are currently examining the nature of the OC VNR ligand by analysing the range of proteins prebound to OCs in vivo prior to cell isolation and in situ by immunocytochemical techniques. Candidate proteins, and derived cell binding peptides, are also being examined for their effect upon bone resorption in vitro.

A 216 A *CANDIDA ALBICANS* PROTEIN SHARES STRUCTURAL AND FUNCTIONAL PROPERTIES WITH MAMMALIAN INTEGRINS. M. K. Hostetter, K. S. Gustafson, G. M. Vercellotti, and K. E. Kendrick, Departments of Pediatrics and Internal Medicine, University of Minnesota, Minneapolis, MN 55455, and Department of Microbiology, Ohio State University, Columbus, OH 43210.

The pathogenic yeast *Candida albicans* expresses a receptor for C3 fragment iC3b which is recognized by Mab to α-chain epitopes of Mac-1, a member of the leukocyte adhesion glycoprotein family. Antibodies recognizing p150,95 also bind to *C. albicans*. Western blotting of yeast cytosolic extracts under non-reducing conditions reveals a single band of M_r 165±15 kD with either anti-p150,95 or anti-Mo1.

Using anti-p150,95 to screen a cDNA library of *C. albicans* in lambda gt11, we have isolated three immunoreactive clones containing *EcoRI* fragments of 1.1, 3.7, and 3.8 kbp, the latter two of which overlap. These recombinant phage direct the synthesis of fusion proteins that also react specifically with anti-Mo1. Alignment of the deduced amino acid sequence from the 3.8 kbp fragment discloses similarities with the L-domains of Mac-1 and p150,95 as well as with regions more proximal to the carboxyl terminus of both mammalian proteins. Like its mammalian counterparts, the yeast protein also mediates the binding of iC3b and the adhesion of *C. albicans* to human umbilical vein endothelium. Binding of [^{3}H] iC3b is specific, saturable, and reversible in the presence of a 100-fold excess of unlabeled iC3b. The affinity constant (K_a=2.45x10^6 L/M) is virtually identical to that described for the binding of iC3b to Mac-1/CR3 on neutrophils. Increased expression of the iC3b receptor on the yeast, induced by growth in glucose, correlates with increased adhesion of the yeast to human umbilical vein endothelium. Proteolytic removal of the yeast protein decreases adhesion. Glucose-enhanced adhesion of *C. albicans* is inhibited by the anti-Mo1 Mabs 44 and 17, which inhibit adhesion-dependent functions in the neutrophil. Based on antigenic, structural, and functional homologies with members of the β_2 integrins, we suggest that the iC3b receptor on *Candida albicans* may be an evolutionary precursor of the leukocyte adhesion glycoproteins.

A 217 MAMMALIAN CELL ATTACHMENT TO SURFACE PROTEINS OF *BORDETELLA PERTUSSIS*,
James G. Kenimer, Elizabeth Leininger and Michael J. Brennan, Food and Drug Administration, Center for Biologics Evaluation and Research, Bethesda, MD 20892
The bacterial surface proteins pertactin (PN) and filamentous hemagglutinin (FHA) are putative cell adhesins for *Bordetella pertussis*, a human pathogen which causes whooping cough. Amino acid sequences deduced from DNA sequence analyses indicate that both PN and FHA contain the cell attachment sequence Arginine-Glycine-Aspartic Acid (RGD). We have used synthetic peptides to examine the role of the RGD sequence in the attachment of Chinese Hamster Ovary (CHO) cells to purified preparations of *B. pertussis* PN and FHA. A 14 amino acid peptide (P1), corresponding to one of the two RGD containing sequences of PN, inhibited the attachment of CHO cells to PN. A control peptide, which was identical to peptide P1 except that the aspartic acid of the RGD sequence was changed to glutamic acid, did not affect binding. These results suggest that CHO cell attachment to PN is mediated through an RGD-containing cell binding site which may interact with an integrin receptor. Conversely, CHO cell attachment to FHA was not inhibited by a 14 amino acid peptide which corresponded to the RGD-containing sequence of FHA. FHA may interact with CHO cells via a lectin-binding site since variant CHO cells (15B cells), which possess altered carbohydrate surface structures, attach better to FHA. Therefore, PN and FHA may utilize different mechanisms to mediate mammalian cell attachment.

A 218 STUDIES ON THE CONTRIBUTION OF THE CLEAVAGE OF THE α_5 SUBUNIT TO $\alpha_5\beta_1$ FIBRONECTIN RECEPTOR FUNCTION, Charlotte J.R. Kennedy and John A. McDonald, Washington University School of Medicine, St.Louis, MO 63110.

VLA integrins share a β_1 subunit, but each has an unique α subunit and different ligand specificity. The α_5 subunit of the $\alpha_5\beta_1$ fibronectin receptor (FNR) contains an unique region surrounding the proposed proteolytic cleavage site at dibasic residues. Other α subunits, including α_3, α_4, α_V and α_{IIb}, have unique regions surrounding their cleavage sites. Because differences in primary structure and/or proteolytic cleavage may alter ligand binding avidity (e.g., Yoshimasa et al., Science 1988 249:784-787) or specificity, we have investigated the ability of FNRs containing cleaved or uncleaved α_5 subunits to bind fibronectin (FN). Metabolically labeled IMR-90 fibroblasts or K562 erythroleukemia cells were extracted in octylthioglucoside with 1 mM $MnCl_2$ or 1 mM $CaCl_2$/0.5 mM $MgCl_2$, applied to ligand affinity columns, and FNR was eluted with 10 mM EDTA and immunoprecipitated with an α_5-specific antipeptide antibody. In the presence of Mn^{2+}, 100% of FNRs with cleaved α_5 subunits and 50% of FNRs with uncleaved α_5 subunits bound to ligand. In the presence of Ca^{2+}/Mg^{2+}, less than 10% of FNRs bound ligand; of these, 75% contained cleaved α_5 subunits. Thus, cleavage increases the ability of FNR to bind FN in the presence of Ca^{2+}/Mg^{2+}, and Mn^{2+} enhances binding of both cleaved and uncleaved FNR. Currently we are expressing the α_5 subunit (cDNA from Drs. F. Giancotti and E. Ruoslahti) and plan to mutate the proteolytic cleavage site to determine its role in FNR function.

A 219 FIBROBLASTOID-TYPE CELL GROWTH OF A HUMAN ERYTHROLEUKEMIC (HEL) CELL VARIANT MEDIATED BY UPREGULATED EXPRESSION OF THE VITRONECTIN RECEPTOR. Nelly KIEFFER and David R. PHILLIPS, COR Therapeutics Inc., South San Francisco, CA 94080.
HEL cells are nonadherent erythroleukemic cells that grow in suspension and express cell surface antigens of the erythroid and megakaryocytic lineage (glycophorin; GP IIb-IIIa). In order to identify adhesion receptors responsible for variations in adhesion phenotype, adherent cells from the HEL cell line were selected from cultures grown in medium supplemented with fetal calf serum (FCS) and analyzed for integrin expression. One HEL variant (HEL-AD1) was isolated which expressed glycophorin but displayed morphologic characteristics of fibroblasts. HEL-AD1 cells attached and spread on fibrinogen (Fb) and vitronectin (Vn), whereas HEL parental cells only attached to Fb. Attachment and spreading of HEL-AD1 cells was inhibited by the synthetic peptide RGDS but not by RGES, suggesting that the receptor responsible for the fibroblastoid phenotype is a member of the integrin family. FACS analysis and immunoprecipitation experiments revealed surface-expression of both proteins of the β3 subfamily (the vitronectin receptor and GPIIb-IIIa) in HEL-AD1 cells, in contrast to HEL parental cells which only express GPIIb-IIIa. Similarly, Northern blot analysis identified mRNA for vitronectin receptor α chain, GP IIb and the common β3 chain in HEL-AD1 cells. The attachment and spreading of HEL-AD1 cells to Fb and Vn could be specifically inhibited by MoAb A2A9 (anti-GPIIb-IIIa) and MoAb LM609 (anti-vitronectin receptor) respectively. These results provide evidence that fibroblastoid-type cell growth of HEL-AD1 cells in FCS-supplemented medium is caused by upregulation of the vitronectin receptor. In current experiments, DNA constructs encoding integrins of the β3 subfamily are being transfected into nonadherent hematopoietic cells in order to determine whether expression of these integrins is solely responsible for this adhesion phenotype.

A 220 $\alpha_2\beta_1$ INTEGRINS FROM DIFFERENT CELL TYPES SHOW DIFFERENT BINDING SPECIFICITIES, Daniel Kirchhofer, Lucia R. Languino, Erkki Ruoslahti and Michael D. Pierschbacher
La Jolla Cancer Research Foundation, Cancer Research Center, 10901 North Torrey Pines Rd., La Jolla, CA 92037.
Purified $\alpha_2\beta_1$ integrin from human platelets was compared in its function and immunoreactivity to $\alpha_2\beta_1$ from endothelial cells. Both $\alpha_2\beta_1$ integrins appear to be principal receptors for collagen type I, and when analyzed by a panel of monoclonal and polyclonal α_2-specific antibodies, the receptors were found to be indistinguishable. However, functional analysis using re-chromatography of purified receptors on laminin and collagen-Sepharose showed that endothelial $\alpha_2\beta_1$ was able to bind to laminin, whereas its counterpart from platelets did not. Moreover, a receptor binding assay indicated that, in contrast to platelets, endothelial cells might also use $\alpha_2\beta_1$ to bind to fibronectin. These results suggest that the $\alpha_2\beta_1$ binding specificity may be modulated by cell-type specific factors.

A 221 REGULATION OF FIBRONECTIN RECEPTOR (FN-R; VLA-5) GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC), F. R. Kohn, M. E. Grigg and H.-G. Klingemann, Terry Fox Laboratory, British Columbia Cancer Research Centre and University of British Columbia, Vancouver, British Columbia, V5Z 1L3, Canada.

Expression of FN-R α5 and β1 subunit mRNAs in purified human PBMC subpopulations, before and after culture with various proliferation/differentiation signals, were determined using Northern analysis (Telios cDNA probes). Resting T cells (>98% CD2+), isolated from PBMC by E-rosetting, expressed detectable levels of α5 and β1 mRNAs. Message levels of both subunits increased following culture for 3 days with concanavalin A and phorbol myristate acetate (PMA). Such cells were "activated" as shown by expression of interleukin-2 receptor mRNA and an increased proliferative response (^{3}H-thymidine incorporation). Resting B cells (>99% surface IgM+), isolated from PBMC using flow cytometry, did not express α5 mRNA, but did express detectable levels of β1 mRNA. Resting monocytes (>85% nonspecific esterase+), isolated from PBMC using percoll density centrifugation, expressed substantial levels of α5 and β1 mRNAs. Message levels of both subunits were decreased following short-term (4-6 hr) culture of monocytes with γ-IFN or lipopolysaccharide; levels did not change following culture with α-IFN, GM-CSF, or CSF-1. Monocytes cultured for 6-7 days in Teflon beakers and human AB serum ("macrophages" by morphological and histochemical criteria) also expressed substantial levels of α5 and β1 mRNAs. Message levels were increased following additional culture of such cells for 2 days in medium containing PMA. Culture with PMA also increased α5 and β1 mRNA levels in the U937 monocytoid cell line. These conditions induced morphologic changes of U937 cells to a "macrophage-like" phenotype. Our results indicate that various proliferation/differentiation signals can regulate FN-R (VLA-5) gene expression in human T cells and monocytes/macrophages.

A 222 VERIFICATION THAT A GPIIIa POLYMORPHISM IS RESPONSIBLE FOR THE PI^{A1} AND PI^{A2} ALLOANTIGENS BY HETEROLOGOUS EXPRESSION OF THE PLATELET GLYCOPROTEIN IIIa, Michael Kolodziej, Amy Goldberger, Mortimer Poncz, Peter Newman, and Joel S. Bennett, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and The Blood Center of Southeastern Wisconsin, Milwaukee, WI 53201
The platelet integrin subunit glycoprotein IIIa (GPIIIa) forms a calcium-dependent heterodimer with glycoprotein IIb that contains binding sites for fibrinogen, von Willebrand factor, and fibronectin on activated platelets. GPIIIa is a single chain protein containing a large number of intrachain disulfide bonds. It also contains the PI^{A1}/PI^{A2} alloantigens that are often involved in the syndromes of neonatal thrombocytopenia and post-transfusion purpura. PI^{A1}/PI^{A2} are associated with a leucine/proline polymorphism at amino acid 33 of GPIIIa. To determine if this polymorphism is responsible for the PI^{A1}/PI^{A2} epitopes, we replaced the codon for leucine 33 of a GPIIIa cDNA with the codon for proline. Mutant and non-mutant cDNA were then inserted into the plasmid pMT2ADA and introduced into COS-1 cells. Newly synthesized GPIIIa was labeled with ^{35}S-methionine and immunoprecipitated with either PI^{A1} or PI^{A2} antisera. GPIIIa synthesized by cells containing pMT2ADA-$IIIa^{Leu}$ was immunoprecipitated by SSA6, a murine anti-GPIIIa monoclonal antibody and by PI^{A1}, but not PI^{A2}, antisera. In contrast, the GPIIIa synthesized by cells containing pMT2ADA-$IIIa^{Pro}$ was immunoprecipitated by SSA6 and PI^{A2} antisera, but was not recognized by the PI^{A1} antisera. These studies indicate that GPIIIa expressed by COS-1 cells is sufficiently processed to express the PI^{A1}/PI^{A2} alloantigens. Furthermore, they demonstrate the that the leucine/proline polymorphism at GPIIIa residue 33 is both necessary and sufficient to direct the expression of this clinically important alloantigen system.

A 223 NOVEL INTEGRIN COMPLEX IS A LAMININ RECEPTOR, Randall H. Kramer, Mai P. Vu, Yao- Fen Cheng, Dan Ramos, and Ronald L. Clyman. Departments of Anatomy, Stomatology, and Pediatrics, and the Cardiovascular Research Institute, University of California, San Francisco, CA 94143. We examined whether human melanoma cells express integrin-related receptors that mediate their adhesion to laminin. An unusual β_1 heterodimer was identified on various human and mouse melanoma cell lines (Kramer et al., J. Biol. Chem. in press, 1989). Chromatography of detergent extracts of ^{125}I-surface-labeled cells on laminin-Sepharose columns recovered two major laminin-binding proteins (100 and 130 kDa, reduced) that bound with moderate affinity to the columns and were eluted with EDTA. Laminin-binding by the complex was independent of Arg-Gly-Asp (RGD)- or Tyr-Ile-Gly-Ser-Arg (YIGSR)-like sequences. This complex was not recovered from columns of fibronectin- or collagen type I or IV-Sepharose. Both proteins were specifically immunoprecipitated from column fractions with monoclonal and polyclonal antibodies to the β_1 integrin subunit, and could be covalently linked with DSP cross-linker indicating that they form a noncovalent heterodimer complex. The α-like subunit is composed of a 30 kDa light chain that is joined by a disulfide bond to the 100 kDa heavy chain. The 100 kDa α-like subunit was electrophoretically distinct from the other known α subunits, and was not immunoprecipitated with monoclonal antibodies to α_1-α_6 and α_v subunits. Preliminary evidence suggests that this receptor complex is not heavily expressed in cultured normal human melanocytes but was detected in $\approx$ 75% of the melanoma cell lines tested. Most other cell lines did not appear to express this complex (fibroblasts, epidermal cells, endothelial cells) except for smooth muscle cells which expressed variable amounts of the receptor. The results indicate that human melanoma cells express a novel laminin-specific β_1 integrin complex which may mediate some of the cells' interactions with this ligand.

A 224 ENDOTHELIAL CELLS USE $\alpha_2\beta_1$ INTEGRIN AS A LAMININ RECEPTOR, **Lucia R. Languino, Kurt R. Gehlsen, Elizabeth Wayner*, Eva Engvall and Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 N. Torrey Pines Rd., La Jolla, CA 92037 and * Fred Hutchinson Cancer Research Center, Seattle, WA 98104.** **Human umbilical vein endothelial cells attach and spread on laminin coated substrates. Affinity chromatography was used to identify the attachment receptor. Fractionation of extracts from surface iodinated endothelial cells on human laminin-Sepharose yielded a heterodimeric complex whose subunits showed MW of 160/120 kD and 160/140 kD under nonreducing and reducing conditions, respectively. The purified receptor bound to laminin and slightly less to fibronectin and type IV collagen in a radioreceptor assay. Monoclonal and polyclonal antibodies directed against the α_2 and β_1 subunits immunoprecipitated the receptor. Cytofluorometric analysis and immunoprecipitation showed that the α_2 subunit is an abundant integrin α subunit in the endothelial cells and that the α subunits associated with laminin binding in other types of cells are expressed in these cells only at low levels. Moreover, an anti-α_2 monoclonal antibody inhibited the attachment of the endothelial cells to human laminin. Our results show that the endothelial cells bind laminin through an integrin composed of α_2 and β_1. Since the $\alpha_2\beta_1$ integrin in other types of cells as platelets binds only to collagens, this finding reveals cell-type specific functional heterogeneity of the $\alpha_2\beta_1$ integrin. The molecular basis of this heterogeneity is being studied.**

A 225 AN AVIAN INTEGRIN BINDS TO FIBRILLAR COLLAGEN WITH HIGH EFFICIENCY, Charles D. Little and A. Jeannette Potts, Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, VA 22908.
Native collagen molecules attached to agarose beads were used as an affinity matrix to isolate embryonic avian integrins. Immunoprecipitation with CSAT (a gift of C. Buck, Wistar Inst.) and rabbit antibody 363 (a gift of R. Hynes and co-workers, MIT) identified two integrins in the β_1 subfamily. Chromatographs developed with gradients of 0-1.0 M NaCl under non-denaturing conditions, yielded a 155/110 kDa integrin complex that eluted early in the gradient and a 135/110 kDa integrins which eluted later. In contrast, if insoluble fibrillar collagen (not attached to agarose beads) is used as an affinity matrix, the 155/110 kDa integrin complex remains bound at higher ionic strength than the 135/110 complex. Moreover, the initial binding of the 135/110 complex at physiological ionic strength is weak, whereas the 155/110 complex binds efficiently. Thus, under conditions which approximate the physiological state, (0.15 M NaCl, pH 7.4, fibrillar collagen) one complex binds weakly, and has an α-subunit similar in molecular weight to the promiscuous avian integrin $\alpha^3\beta_1$; while the second complex, has a larger α-subunit, and binds with relatively high-affinity.

Burridge, Dept. of Cell Biology and Anatomy, UNC, Chapel Hill, NC 27599. We have identified an interaction between α-actinin and the cytoplasmic domain of the β-subunit of the chicken fibronectin receptor (β_1 integrin). A synthetic peptide corresponding to the 47 amino acid cytoplasmic domain of β_1 integrin was HPLC-purified and coupled to sepharose for use as an affinity chromatography matrix. Triton X-100 extracts of chicken embryo fibroblasts were applied to this column and bound proteins eluted with high salt. Several major bands were eluted from the β-peptide column; one protein of 100 kDa was identified by immunoblotting as α-actinin. ^{125}I-α-actinin bound to β-peptide adsorbed to microtiter plates with a K_D of 1.5×10^{-8}M. Additionally, smooth muscle integrin was used in solid phase assays and also bound ^{125}I-α-actinin, but with a lower affinity ($K_D = 10^{-6}$M). α-actinin fragments of 27 kDa (actin-binding domain) and 53 kDa (dimeric rod segment) were generated by cleavage with thermolysin and separated by FPLC. The 53 kDa, but not the 27 kDa, fragment inhibited α-actinin binding to the β-peptide. Using 4 shorter peptides of the β-chain cytoplasmic domain, we have localized the binding site for α-actinin on the β-peptide to the N-terminal 13 amino acids. (Supported by NIH grant GM-29860 to KB)

A 227 STRUCTURE AND EXPRESSION OF THE VLA-4 GENE, Glenn D. Rosen and Douglas C. Dean, Washington University School of Medicine, St. Louis, MO 63110. Very late anitgen-4 (VLA-4) is a receptor in the integrin family which is found predominantly on lymphocytes. It has been implicated as a lymphocyte "homing" receptor as well as a fibronectin receptor. A primer extension cDNA library was constructed to obtain the full lenght VLA-4 sequence. VLA-4 mRNA contains ~500 nucleotides of 5'- untranslated region which agrees with values obtained with primer extension assays. Southern blot hybridization to genomic DNA revealed the presence of a single band with 5 different enzymes suggesting that a single gene encodes VLA-4. Northern blot of poly A+ RNA from MOLT-4 cells revealed multiple species of 7 kb, 4 kb and 2 kb suggesting that different forms of VLA-4 may be present in the cell. We are currently isolating genomic clones to analyze the promoter region for possible transcriptional regulatory sequences.

A 228 COLLAGEN RECEPTORS FROM PRIMARY RAT HEPATOCYTES AND FIBROBLASTS. **Rubin,K.[1], Gullberg,D.[1], Tingström,A.[1], Terracio,L.[2] and Borg,T.K.[3]** [1]Dept. of Medical and Physiological Chemistry, Biomedical Center, S-751 23 Uppsala, Sweden, and Depts. of [2]Anatomy and [3]Pathology, School of Medicine, University of South Carolina, SC 292 08.

We have recently reported on the isolation and partial characterization of RGD-independent collagen receptors belonging to the β_1-subfamily of integrin matrix receptors [Gullberg, et al., J. Biol. Chem. 264:12686-12694, 1989]. We have investigated the role of such integrin collagen receptors in fibroblast mediated collagen lattice contraction. A novel assay that allowed for the quantification of collagen gel contraction was developed. Under conditions favoring a rapid collagen gel contraction, both fetal calf serum and 'platelet derived growth factor' (PDGF), but not transforming growth factor-ß (TGF-ß) stimulated the kinetics of the contraction process, both when investigated with primary rat heart fibroblasts and diploid human dermal fibroblasts. Anti-rat-β_1-integrin antibodies, but not anti-fibronectin antibodies inhibited the collagen lattice contraction. The presence of synthetic GRGDTP- and GRGESP-peptides did not affect the contraction process. Increasing concentrations of PDGF decreased the sensitivity for anti-β_1-integrin IgG, suggesting that PDGF may regulate integrin activity.

Two different integrin collagen receptors could be isolated from surface labeled primary rat fibroblasts by affinity chromatography on collagen type I immobilized to Sepharose. These β_1-integrin collagen receptors did not possess any affinity for fibronectin-Sepharose. The M_r's of the two respective α-chains were similar to those reported for the human VLA-1 and VLA-2 α-chains. Of the β_1-integrin complexes with affinity for collagen, primary rat hepatocytes mainly expressed the high molecular weight species.

A 229 AN RGD-PEPTIDE ANALOG WITH POTENT ANTITHROMBOTIC ACTIVITY IN VIVO, J. Samanen[1], F. Ali[1], T. Romoff[1], R. Calvo[1], P. Koster[2], J. Vasko[2], M. Strohsacker[2], J. Stadel[2], A. Nichols[2], [1]Peptide Chemistry Department, [2]Pharmacology Department, Smith Kline and French Laboratories, King of Prussia, PA 19406-0939. In the canine coronary thrombus model, thrombus inhibition was previously demonstrated by intracoronary infusion of >100 μM Ac-RGDS-NH_2 at 0.1 ml/min (R. Shebuski et al. Thromb. Haem. 61, 183-8, 1988). Structure - activity studies [in vitro antiaggregatory activity, dog platelet rich plasma (PRP)/ADP] led to the cyclic disulfide SK&F 106760 [Ac-Cys-(N^{α}Me)Arg-Gly-Asp-Pen-NH_2] with improved plasma stability (100% activity after 3 hr), affinity (K_i 7.6 nM purif. human receptor) and potency (IC_{50} 0.26 ± 0.08 μM dog PRP/ADP, 0.48 ± 0.10 μM human PRP/ADP, 0.26 ± 0.02 μM dog PRP/collagen, 0.49 ± 0.09 μM dog PRP/U-46619). SK&F 106760 displays dose dependent thrombus inhibition in the canine coronary thrombus model by bolus intravenous administration: Time to thrombus formation was 5 ± 1, 28 ± 2, and 118 ± 27 min with 0.1, 0.3, and 1.0 mg/kg iv bolus SK&F 106760 respectively, 4 ± 1 min with 1 ml/kg 0.9% saline iv bolus (n=4). No effects on blood pressure, heart rate or platelet count were observed.

A 230 MOLECULAR MECHANISMS OF CANDIDA ALBICANS ADHERENCE, R. Sawyer, A. Richardson, M. Horst, R. Garner and J. Hudson, Mercer University School of Medicine, Macon, GA 31207.

The molecular basis for the adherence of Candida albicans was studied in the isolated perfused mouse liver model (Infect. Immun. 14,1349, 1976). When 10^6 C. albicans were infused into ICR mouse livers approximately 68±5% (X=SEM) were recovered from the liver and 21±3% in the effluent for a total recovery of 81±4%. This suggests that 19±4% of the infused yeasts were eliminated within the liver and a total of 86±3% were trapped (% in liver + % killed) by the liver. In contrast, the ability of hepatic tissue to trap yeasts was significantly reduced (P=0.01, White rank order) by pre-treating yeasts with KCl, urea or dithiothreitol but not with beta-mercaptoethanol, Triton X100, SDS, or overnight digestion with papain. Yeasts were extracted with Triton X100 plus 9.5M urea. Perfusion of C. albicans in the presence of 2% crude extract, reduced the ability of hepatic tissue to trap yeasts. Hepatic trapping was also reduced when perfusions were carried out in the presence of 100 mg/L GP, a mannoprotein extract, or 100 mg/L extracted mannan. Perfusion in the presence of mannan from Sacchromyces cerevisiae failed to inhibit trapping of C. albicans. SDS-PAGE analysis of crude TX100-urea extracts of C. albicans suggest that GP containing structures on the surface of C. albicans, mannoproteins or mannans, mediate adherence of yeasts within deep tissues of the host. (Supported by grants 16121-50 from the Medical Center of Central Georgia, and HL 30619 from NHLBI.)

A 231 BIOCHEMICAL AND FUNCTIONAL EVIDENCE THAT HLA CLASS I ANTIGEN MODULATES THE FUNCTION OF AN INTEGRIN. Shaw, A. R. E., Seehafer, J. G., Masellis-Smith, A., Slupsky, J. R. Department of Medicine, University of Alberta, and Cross Cancer Institute, Edmonton, ALTA T6G 2J7

MHC antigens are suspected to play a role in the regulation of cellular adhesion, but biochemical evidence has been lacking. We report here the novel finding that HLA class I antigens are in close physical association with an integrin on the surface of the "resting" platelet, and that anti-HLA class I monoclonal antibodies (mAb) modulate integrin function. Following radio-iodination, and cross-linking of platelet surface proteins with the reversible heterobifunctional cross-linking agent dithiobis (succinimidyl propionate) (DSP) anti-HLA class I monoclonal antibodies (mAb) co-precipitated proteins of 127 kDa, and 110 kDa from Nonidet P-40 (NP40) solubilized resting platelets which were identified as sub-units of the platelet fibrinogen receptor, and β_3 integrin GPIIb/IIIa. The specificity of the association was demonstrated by the absence of other coprecipitating major platelet proteins, by the failure of mAb to the abundant cell surface antigen CD9 to coprecipitate GPIIb, or GPIIIa from cross-linked resting platelets, and by the inability of the anti-GPIIb/IIIa mAb P2, or HUPI-m1a to reciprocally co-precipiate HLA class I. Although anti-HLA class I antigen mAb do not activate gel-filtered platelets in the presence of fibrinogen, suggesting that they cannot initiate platelet activating signals both anti-β2 microglobulin, and anti-HLA class I mAb trigger aggregation on addition of subthreshold doses of epinephrine, or ADP indicating that the mAb may amplify signal induced by the fibrinogen receptor. Since fibrinogen binding is an obligate step in platelet activation these results provide evidence that HLA class I antigen is physically associated with a major platelet integrin, and modulates its activity.

A 232 BIOSYNTHESIS OF AN INTEGRIN-LIKE COMPONENT OF THE 13762 MAMMARY TUMOR CELL SURFACE SIALOMUCIN COMPLEX, Z. Sheng, S. Hull and K.L. Carraway, Departments of Cell Biology & Anatomy and Biochemistry & Molecular Biology, University of Miami School of Medicine, Miami, FL 33101

Cell surfaces of metastatic 13762 ascites rat mammary adenocarcinoma cells are covered with a sialomucin complex, which has been proposed to protect the cells from immune destruction. This complex is composed of a high Mr (approx. 600 kDa) sialomucin (ASGP-1) and a 120 kDa, integral membrane glycoprotein (ASGP-2) whose N-terminal sequence is similar to integrin α chains. Biosynthesis of ASGP-2 was investigated by pulse-chase labeling followed by immunoprecipitation after dissociation of complex in sodium dodecyl sulfate. After a 10 min pulse label the only immunoprecipitated components observed by gel electrophoresis corresponded to bands at apparent Mr values of about 400 (major band, pSMC-1) and 330 (minor band, pSMC-2) kDa. Chase analyses demonstrated disappearance of both species and the appearance of 120 kDa ASGP-2. The kinetics were consistent with a precursor-product relationship between pSMC-1, but not pSMC-2, and ASGP-2. Immunoblot and lectin blot analyses indicated that both pSMC-1 and ASGP-2 from immunoprecipitates bind anti-ASGP-2 and Concanavalin A, but pSMC-2 binds neither. Both pSMC-1 and ASGP-2, but not pSMC-2, can be labeled with mannose; the mannose was removed from 30 min pulse-labeled immunoprecipitates by incubation with endo-β-N-acetylglucosaminidase H. One dimensional peptide maps of pSMC-1 and 120 kDa ASGP-2 showed several corresponding bands. From these results we propose that ASGP-2 is synthesized as a high Mr precursor (pSMC-1) which is cleaved to its 120 kDa form early in the transit pathway from the endoplasmic reticulum to the cell surface.

A 233 IMMUNOELECTRON MICROSCOPIC CO-LOCALIZATION OF CD11b CYTOPLASMIC DOMAIN AND TALIN ANTIGENS ON THE EXTERNAL SURFACE OF POLYMORPHONUCLEAR LEUKOCYTE SPECIFIC GRANULES (ADHESOMES). I.I. Singer,* S.Scott,* D.W. Kawka,* D.M. Kazazis,* S.D. Wright,+ and K.W.T. Burridge,# MSDRL, Merck & Co., Inc., Rahway, NJ 07065*; Rockefeller Univ., New York, NY 10021+; Univ. North Carolina, Chapel Hill, NC 27599#. It was previously demonstrated that antigens of the extracellular domains of CD11b/CD18 complex, alpha$_5$ fibronectin receptor, alpha$_v$ vitronectin receptor, and 67 kD laminin receptor were co-distributed within the specific granules of human polymorphonuclear leukocytes (PMNs) and monocytes (MO); these granules were termed adhesomes. Here, we show using double-label immunoelectron microscopy that antibodies to the cytoplasmic domain of CD11b and against human platelet talin are co-localized on the external surfaces of PMN adhesomes, and in patches beneath the plasma membrane. These observations suggest that talin might tether adhesomes to actin via their transmembrane integrin molecules. The actin cytoskeleton might thus modulate the up-regulation of integrins as well as their lateral mobility and distribution upon the PMN surface.

A 234 INTEGRIN ($\alpha_v\beta_3$) - LIGAND INTERACTION: IDENTIFICATION OF THE RGD BINDING DOMAIN OF THE VITRONECTIN RECEPTOR. Jeffrey W. Smith and David A. Cheresh. Scripps Clinic. La Jolla, CA 92037. We have employed photoaffinity cross-linking to examine RGD recognition by the vitronectin receptor (VNR). A photoaffinity derivative of an RGD peptide was constructed and used to photolabel regions of VNR proximal to the ligand binding domain. These regions were subsequently identified by peptide mapping and amino acid sequence analysis. The majority of affinity label (80%) was associated with a region extending from beta subunit residues 61 - 203. The remainder of the affinity label was split between two sites on the alpha subunit. Both sites (residues 139 - 224 and 225 - 349) are proximal to the putative divalent cation binding sites, an observation indicating that the RGD and divalent ion binding sites are spatially and functionally linked. As an extension of these studies, we have identified a 17 residue peptide from the affinity labeled region of the beta subunit which inhibits the interaction between matrix proteins and VNR in a solid-phase ligand binding assay. This peptide also inhibits photoaffinity labeling of VNR by RGD peptides, indicating that it may correspond to the RGD binding site. Collectively, our results suggest that residues on the beta subunit of VNR contribute directly to RGD binding, and that regions on the alpha subunit proximal to this site are required for proper orientation of an "active" ligand binding site.

A 235 THE INTEGRIN $\alpha^6\beta^1$, BUT NOT $\alpha^6\beta^4$ FUNCTIONS AS A SPECIFIC RECEPTOR FOR THE E8 FRAGMENT OF LAMININ, Arnoud Sonnenberg[¶], Caroline Damsky[T], Monique Aumailley[π] and Rupert Timpl[π] [¶]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, [T]University of California, San Franscico, USA and [π]Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany. Three proteolytic fragments of laminin (E3, E8 and P1) support adhesion of a variety of cell types. Cell adhesion to E8 was blocked completely by Mab GoH3, directed against the integrin α^6 subunit and by Mab AIIB2, directed against the common β^1 subunit. The integrin α^6 antibody did not inhibit cell adhesion to E3 or P1. Furthermore, the antibody only blocked adhesion to laminin of cells that exclusively adhered to E8. Finally, expression of $\alpha^6\beta^1$ was closely correlated with the ability of cells to bind to E8. These results demonstrate that $\alpha^6\beta^1$ functions as a specific receptor for the E8 fragment of laminin. Many cells express instead of or in addition to $\alpha^6\beta^1$, the recently described integrin $\alpha^6\beta^4$. Although the ligand of $\alpha^6\beta^4$ was not identified, it must be different from that of $\alpha^6\beta^1$, because (1) cells that express $\alpha^6\beta^4$, but not $\alpha^6\beta^1$ do not adhere to E8 and (2) the integrin β^1 antibodies could completely block the attachment of cells to E8. Further experiments indicated that Arg-Gly-Asp (RGD) dependent β^1 and β^3 (= vitronectin receptor) integrins are involved in cell adhesion to P1. Biochemical analysis of the $\alpha^6\beta^1$ and $\alpha^6\beta^4$ complexes revealed several interesting features (1) all cells that express the $\alpha^6\beta^4$ complex express in additon to this complex free β^4, (2) no evidence was obtained for novel α subunits associated with β^4, (3) variability in size of α^6 from different cell types was shown to be largely due to differences in modifications of N-linked glycans. Additional heterogeneity may be caused by differential proteolytic cleavage of the α^6 precursor.

A 236 A NOVEL RAT LIVER GLYCOPROTEIN MEDIATING HEPATOCYTE ADHESION ON FIBRONECTIN. S.C. Stamatoglou, R.C. Hughes and S. Johansson*, National Institute for Medical Research, Mill Hill, LONDON NW7 1AA, U.K. *Biomedical Center, University of Uppsala, Sweden.

We identified a bile canalicular membrane glycoprotein (CGp110) in rat liver that mediates adhesion of hepatocytes on fibronectin. CGp110 has a molecular weight of 110K and an isoelectric point of 4.2. Antibodies against CGp110 reverse the spreading of hepatocytes on fibronectin substrata, but have no effect on cells spread on type IV collagen or laminin. CGp110 and integrin fibronectin receptor from rat hepatocytes migrate to different molecular weight positions in SDS gels and, using specific antisera, no immunological cross-reactivity could be demonstrated between these two proteins. In immunofluorescence and immunogold labelling experiments at the light and electron microscope level CGp110 was found to be confined to the canalicular membrane, whereas integrin was observed on all three faces of the hepatocyte, i.e. sinusoidal, lateral and bile canalicular. In double-label experiments, co-localization of the two proteins was indicated on the bile canalicular surface. Fibronectin was found on all surface domains. The distribution of the two proteins in cultured hepatocytes was studied by laser confocal microscopy: CGp110 and integrin co-localize along the periphery of the basal cell surface at adhesion sites where actin fibres terminate.

A 237 INTEGRINS OF THE BETA 1 FAMILY REDISTRIBUTE AT THE LEADING EDGE IN MIGRATING CORNEAL EPITHELIA, Mary Ann Stepp and Ilene Gipson, Cornea Unit, Eye Research Institute of Retina Foundation, Boston, MA 02114.

We demonstrate an altered localization of integrins of the beta 1 family at the tip of the leading edge of epithelia migrating over basement membrane in a *in vitro* rat corneal organ culture model. The integrins at the tip of the leading edge are found only at the sites of interaction of basal cells with the underlying basement membrane. Distal to the leading edge, integrins localize under and between the basal and supra-basal cells, a pattern identical to that found in stationary, non-migratory epithelia. To study integrin expression in migrating epithelia further, we employed subunit specific antisera for the beta 1 family of integrins. The major beta 1 integrin found in corneal epithelias is alpha 3 beta 1, the receptor which interacts with laminin, fibronectin, and collagens. We could detect no alpha 4 or alpha 5 in control or migrating epithelia and are currently assessing whether or not alpha 1 and alpha 2 are found. A monoclonal antisera which recognizes the alpha 6 chain in mouse (GoH3; Sonnenberg et al JBC 263:14030-14038, 1988) also localized to basal and supra-basal cells and underlying the tip of the leading edge in mouse cornea. We have yet to determine if this alpha 6 is associated with beta 1 or with beta 4 as reported for other types of epithelia. Analysis of the beta 3 family of integrins identified the alpha V beta 4 vitronectin receptor as the primary member; this integrin also localized to basal and supra-basal cell surfaces. In addition to extending our studies to include the beta 4 integrin family, we have begun quantitating beta 1 and beta 3 integrins to determine if their expression changes during migration. In summary, several integrin types are synthesized by rat corneal epithelium. These integrins are found at locations of cell:cell and cell:substrate interactions; upon onset of migration, localization of beta 1 family integrins at the leading edge is restricted to areas in contact with basement membrane.

A 238 Expression of extracellular matrix receptors in the periimplantation mouse embryo. A.E. Sutherland, R. Sanderson, M. Bernfield, P.G. Calarco and C.H. Damsky. University of California, San Francisco, CA, and Stanford University School of Medicine, Stanford, CA.

Normal embryonic development requires a broad range of temporally and spatially regulated adhesive interactions between cells and their extracellular matrices (ECM). Such interactions play an essential role in embryonic cell migration, invasion and differentiation. Implantation of the mouse embryo is an example of a complex process that involves extensive cell-ECM interactions as well as dynamic interaction between embryonic and uterine cells. We have studied the pattern of expression and regulation of several cellular receptors for ECM in periimplantation stage embryos in order to determine the role that each might play in invasion of trophoblast cells (TB) and the differentiation of embryonic and extraembryonic tissues. The receptors that we have examined are the cell-surface proteoglycan, syndecan, and the integrin receptors for fibronectin ($\alpha5\beta1$) and laminin ($\alpha6\beta1$). We have found that syndecan is first expressed in the mouse embryo at the 4-cell stage, and that the pattern of its expression changes dramatically after blastocyst hatching and differentiation of endoderm and secondary TB giant cells. At the time of implantation, syndecan is localized almost exclusively at the interface of the primitive ectoderm and primitive endoderm. In the ectoplacental cone, syndecan is expressed in the differentiating TB giant cells but not in the diploid core cells. The deglycosylated syndecan protein from hatched blastocysts and embryo outgrowths has an M_r of approximately 75 kD, which is larger than that isolated from mature tissues. While the size of the unmodified core protein does not change with development, the size of the intact proteoglycan does. In hatched blastocysts and embryo outgrowths, the size of the intact proteoglycan is extremely heterodisperse, ranging from 90 kD to 400 kD. The size of the intact proteoglycan isolated from EPC explants is also heterodisperse but has a much more restricted size range of approximately 200-260 kD. In contrast, $\beta1$ integrins are not expressed until after the embryo has hatched and developed attachment competence. They are expressed on all parts of the embryo, including the external surface of trophoblast cells. Antibodies to $\beta1$ integrins inhibit TB-ECM interactions in-vitro, suggesting that integrins mediate these interactions during implantation. Thus separate classes of ECM receptors show distinct spatial and temporal regulation indicative of different roles in cell-cell and cell-ECM interactions during early embryogenesis. (Supported by NIH grants HD06763, HD06703, HD22593 and the Arthritis Foundation)

A 239 CHARACTERIZATION OF CYTOADHESIN INTEGRINS ON HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS (HDMEC), RA Swerlick, E Brown*, E Garcia-Gonzalez, and TJ Lawley, Emory University, Atlanta, GA 30322, *Washington University, St. Louis, MO. Cytoadhesins, such as the vitronectin receptor (VnR) and the IIb/IIIa complex of platelets are integrins that play an important role in cell-matrix interactions, usually via recognition of the amino acid sequence arginine-glycine-aspartic acid (RGD). Since microvascular endothelial cell interactions with RGD containing proteins are important, we determined whether human dermal microvascular endothelial cells expressed cytoadhesin integrins using flow cytometric analysis and also examined their functional role using a cell-matrix adhesion assay. Polyclonal rabbit anti-human VnR antibodies, monoclonal antibody (Mab) 3F12, which recognizes the VnR alpha chain, and Mab 7G2, which recognizes the VnR beta chain, all stained human endothelial cells derived from umbilical vein (HUVEC) and human dermis (HDMEC). Immunoprecipitation with these antibodies demonstrated a complex consistent with the VnR. Mab B6H12, which identifies an integrin on neutrophils distinct from the VnR, also stained both HDMEC and HUVEC. Staining with B6H12 was consistently brighter on small-vessel HDMEC than staining with 3F12, while the opposite was observed on large-vessel HUVEC. B6H12 and 3F12 blocked the adherence of HDMEC to vitronectin-coated plastic, but 7G2 had no effect. Mab 2E1.3, which identifies the VnR by ELISA but does not stain cells by FACS, also profoundly blocked adherence of HDMEC to vitronectin. None of the antibodies affected adherence to fibronectin. This data suggests that multiple integrins may be present on endothelial cells, that their expression may be different on large- vs. small-vessel endothelium, and that these integrins may play an important role in endothelial cell-matrix interactions.

A 240 HIGH LEVEL FIBRONECTIN RECEPTOR EXPRESSION IN K562: ALTERED CELL MORPHOLOGY, ADHESION AND LOSS OF SOFT AGAR GROWTH. B. E. Symington, M.D. Fred Hutchinson Cancer Research Center, M421, Seattle, WA. 98104.

Selection for tight adhesion by K562 to human plasma fibronectin (FN) results in isolation of a stable fibroblastoid variant of the K562 erythroleukemic cell line (called FA-K562, for Fibronectin Adherent). Prominent changes in cellular morphology, cytoskeletal organization, and fibronectin receptor (FNR) expression, as well as changes in growth in soft agar and in conventional culture accompanied tight adhesion to FN. FA-K562 expresses four-fold more fibronectin receptor (FNR) and CD36 (by immunoprecipitation and flow cytometry) than parental K562. FA-K562, unlike parental K562, displays an organized cytoskeleton, complete with filamentous vimentin, when cultured on FN-coated plastic. FA-K562 proliferate more slowly than K562 and grow poorly in soft agar in comparison to K562 (5% vs 75% colony formation). Binding to the cell-binding domain of FN is required to elicit the morphologic changes, cytoskeletal organization, and filamentous vimentin expression as glycine-arginine-glycine-aspartate-serine (GRGDS) peptide inhibits these responses. In contrast, increased FNR expression, slow proliferation, and inefficient growth in soft agar were independent of immobilized FN. High-resolution two-dimensional gel electrophoresis analysis of biosynthetically labelled proteins failed to detect any qualitative differences in over 2000 spots identified in each cell line. Identical patterns are observed by DNA restriction fragment analysis of the two cell lines, confirming that the two cell lines are related. Adaptive induction of receptor expression does not occur, as growth of K562 on immobilized collagen or laminin did not result in adhesion of cells or selection of variant cells. FA-K562 demonstrates that immobilized ligand can be used to select stable variants expressing high levels of receptor for the specific ligand. FA-K562 should prove useful for studying control of FNR expression and the influence of increased FNR expression on cell morphology and behaviour.

A 241 NGF INDUCES INCREASED EXPRESSION OF A LAMININ-BINDING INTEGRIN (180/135kDa) INVOLVED IN NEURITE OUTGROWTH IN PC12 CELLS, Guido Tarone & , Isabella Gavazzi*, Rupert Timpl*, Monique Aumailley*, Marzia Abbadini*, Filippo Giancotti*, Lorenzo Silengo*, PierCarlo Marchisio* and Paola Rossino*, *Dipartimento di Scienze Biomediche and *Dipartimento di Genetica Biologia e Chimica Medica, Universita' di Torino, Italy and *Max-Planck-Institut für Biochemie, FRG.
Rat pheochromocytoma PC12 cells exposed to nerve growth factor differentiate as sympathetic neurons and extend neurites on laminin and to a much lesser extent on fibronectin. Analysis of laminin fragments indicated that neurite outgrowth occur mainly on fragment P1 corresponding to the center of the cross, and only poorly on fragment E8, a long arm structure, that is active with other neuronal cells. Integrin antibodies prevented adhesion and neurite sprouting of these cells on laminin, fragment P1 and fibronectin. By affinity chromatography we isolated an integrin binding to laminin and consisting of two subunits with molecular masses of 180kDa and 135kDa. The latter is recognized by an antiserum to integrin β_1 subunit. The bound laminin receptor could be displaced by EDTA, but not by Arg-Gly-Asp or Tyr-Ile-Gly-Ser-Arg peptides. Affinity chromatography on laminin fragments showed that the 180/135kDa receptor binds to fragment P1. The expression at the cell surface of the 180kDa α subunit of the laminin receptor was increased tenfold after NGF treatment. The effect of NGF is specific since the amount of a 150kDa fibronectin-binding integrin α subunit remained unchanged. Moreover, the increased expression of the 180/135kDa receptor at the cell surface corresponded to a selective increase in cell adhesion to laminin and to fragment P1. The 180/135kDa complex is, thus, an integrin receptor for laminin whose expression and binding specificity correlates with the capacity of NGF-induced PC12 cells to extend neurites on laminin.

A 242 EXPRESSION OF INTEGRINS IN HUMAN TUMORS, Virtanen I., Laitinen L., Korhonen M. K. and Ylänne J. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, 00170 Helsinki, Finland and (LL) the Jorvi Hospital, Espoo, Finland
The expression of integrins in human tumors was studied by using monoclonal antibodies against the $beta_1$, $beta_3$ and various alpha subunits. Generally, three different types of reactivity were observed in various primary carcinomas (including breast, gastrointestinal tract, thyroid gland, kidney and lung). (i) In some of them, the tumor cells showed a polarized distribution of $beta_1$ integrins including the $alpha_2$ and $alpha_6$ subunits but almost all of them lacked the $alpha_5$ integrin immunoreactivity. However, the $alpha_5$ integrin subunit was prominently expressed in the stromal cells. (ii) Within some primary tumors, a remarkable heterogeneity was seen in the expression of both $beta_1$ and $alpha_6$ subunits. (iii) Some primary tumors lacked $beta_1$ integrins. In contrast to the three types of reactivity in primary carcinomas, the metastatic tumors expressed a generalized cell surface reactivity in all cells. All the carcinomas studied lacked $beta_3$ integrins.

A 243 THE INTEGRIN β1 SUBUNIT FROM THE YEAST, CANDIDA ALBICANS. Theodore C. White and Nina Agabian, Dept. of Pharm. Chem., U. of California, San Francisco, San Francisco, CA. 94143 Recently, E.E. Marcantonio and R.O. Hynes [(1988) J. Cell Biol. 106, 1765-1772] have shown that antibodies to a peptide from the cytoplasmic domain of the integrin β1 subunit from chicken cross react with a membrane associated protein from Candida albicans, a dimorphic parasitic fungus. This cross reactivity was not found with membrane proteins from Saccharomyces cerevisiae. The presence of integrins in Candida may be related to its ability to attach to and invade host tissues. We are using the polymerase chain reaction and conserved regions from vertebrate integrin β1 subunits to identify and clone the C. albicans gene. Candida has two cell types, a budding yeast form and a hyphal form associated with invasion. The integrin β1 subunit should only be necessary and therefore only expressed in the invasive hyphal form. Inactivation of the β1 subunit gene may limit the invasive nature of the hyphal forms, establishing a correlation between integrins and Candida adhesion and invasion. The expression of the integrin β1 subunit will also be monitored in clinical Candida isolates from normal and immunosuppressed patients with varying clinical symptoms of candidiasis, to determine if adhesion involving integrins correlates with the clinical manifestations of the disease.

A 244 A GLYCOSYLATION VARIANT OF THE MOUSE FIBRONECTIN RECEPTOR WITH REDUCED BINDING AFFINITY TO FIBRONECTIN, Tien-wen Tao and Orhan Oz, Division of Nuclear Medicine, Stanford University School of Medicine, Stanford, CA 94305

The acquisition of resistance to wheat-germ-agglutinin (WGA) by a WGA-resistant (WGA-R) mutant selected from B16 mouse melanoma cells is accompanied by a dramatic reduction in its malignant potential. The structure of its complex N-linked oligosaccharides is altered, including decreased sialic acid and increased fucose residues with the expression of the Le^x antigenic structure (Galß1 →4[Fucα1→3]GlcNac), determinant of the stage-specific-embryonic-antigen (SSEA). The altered carbohydrate structure is present in a number of membrane glycoproteins including the ß1 integrins, which mediate cellular adhesive interactions with such extracellular matrix proteins as fibronectin and laminin. Associated with the glycosylation change is a decrease in the adhesion and spreading of the WGA-R cells on the ligands. To test the hypothesis that the alteration in the carbohydrate structure of the receptor affects the functional expression of the receptor, the fibronectin receptor (FNR) was studied. Cell-binding-domain of fibronectin immobilized on Sepharose was used to compare the binding affinity and the structure of the mature FNR prepared from the detergent extracts of ^{125}I-surface-labeled cells. Results showed: (1) The FNR bound to CBD-Sepharose and eluted with GRGDS or EDTA contained two α subunits (α5 and α3?) and one ß subunit (ß1). All subunits of the variant FNR derived from the mutant cells were smaller than those of the wild-type FNR derived from the parental cells due to the glycosylation difference. (2) The binding affinity of the variant FNR to the ligand was lower as demonstrated by the lower concentration of the GRGDS needed to elute the bound receptor from the ligand. (3) The α5 subunit of the variant FNR bound to the ligand was greatly reduced, and thus could be responsible for the reduced binding affinity of the receptor. The decreased affinity of the receptor for the ligand could be causally related to the reduced adhesive interaction of the cells with fibronectin.

A 245 DIFFERENT LOCALIZATION OF $BETA_1$, $BETA_3$ AND $ALPHA_{IIb}$ SUBUNITS OF THE INTEGRIN FAMILY AT FOCAL ADHESIONS IN TPA-EXPOCED SPREADING HUMAN ERYTHROLEUKEMIA (HEL) CELLS DEPENDING ON CULTURE SUBSTRATES AND ON CULTURE TIMES, Ylänne, J. and Virtanen I. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, SF-00170 Helsinki, Finland

Human erythroleukemia (HEL) cells, normally grown in supension, can be induced with a phorbol ester to spread rapidly (in 10-15 min) on fibronectin or on vitronectin. In the absense of attachment promoting proteins similar spreading was seen first after 1 day. After the cells were cultured for 15-30 minutes on vitronectin or on fibronectin, integrin $beta_3$, $beta_1$ $alpha_5$ and $alpha_{IIb}$ subunits were all localized at focal adhesions, as revealed by double labeling immunofluorescense microscopy with specific monoclonal antibodies and talin antiserum. After prolonged culture on adhesive substrata the $alpha_{IIb}$ subunit disappeared from the focal adhesions. In the cells cultured on vitronectin but not in those on fibronectin also the $beta_1$ and $alpha_5$ subunits disappeared from the focal adhesion. The $beta_3$ subunit remained associated with talin-containing focal adhesions. The $beta_3$ subunit was even observed in the focal adhesions formed in the cells on glass substratum afted 1 day of culture. These results suggest a combined function of different integrins in the same focal adhesion structures.

A 246 POSSIBLE TARGETS FOR THE ATTACHMENT AND ENTRY OF MAMMALIAN CELLS BY *YERSINIA ENTEROCOLITICA*, Vincent B. Young, Stanley Falkow, and Gary K. Schoolnik, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

Yersinia enterocolitica is a facultative intracellular pathogen that causes a broad range of food- and waterborne gastrointestinal syndromes. *Y. enterocolitica* traverses the intestinal epithelium and can be found in the underlying lymphoid tissues of infected patients. The virulence of pathogenic *Y. enterocolitica* has been correlated with their capacity to invade cultured mammalian cells. This capacity is encoded by a single chromosomal gene called *inv* which codes for a protein termed "invasin." The interaction of invasin with eukaryotic cells has been studied using *inv* in an *E. coli* expression system. Cloned *inv*, when introduced into a non-invasive *E. coli* K-12 strain, enables this strain to penetrate cultured eukaryotic cells. Invasin is localized on the bacterial surface, within the outer membrane of strains harboring *inv*; bacterial membranes containing invasin bind cultured mammalian cells. Moreover, latex microspheres coated with invasin-containing membranes attach to eukaryotic cells *in vitro* and are apparently ingested. Antibodies against the β_1 subunit of the integrin superfamily competitively inhibit binding of eukaryotic cells by invasin-containing membranes and the invasion of the same cells by *Y. enterocolitica* and *E. coli* carrying the cloned *inv* gene. However, the synthetic peptide RGDS has no effect on binding or invasion and invasin itself does not contain the sequence RGD. Thus the interaction between invasin and β_1 may represent a novel interaction between an integrin and a non-RGD ligand.

Development and Differentiation

A 300 A NEW MONOCLONAL ANTIBODY, 1AA3, AFFECTS GENERALIZED MOTILITY IN A2058 MELANOMA CELLS, Marie E. Beckner, Mary L. Stracke, Cynthia Fischler, Reuben P. Siraganian, Elliott Schiffmann, and Lance A. Liotta, Tumor Invasion and Metastasis Section, Laboratory of Pathology, National Cancer Institute and Clinical Immunology Section, National Institute of Dental Research, National Institutes of Health, Bethesda,MD 20892.

Hybridomas were produced by injecting mice with whole cells from the highly motile, human melanoma A2058 cell line and were screened for antibody mediated effects on motility. One clone, 1AA3, produced antibodies, subclass IgG1, with a narrow range of strong motility inhibition and a wide range of weak stimulation. The antibody was found to affect motility stimulated by matrix components (Type IV collagen, laminin, and fibronectin) and soluble factors (autocrine motility factor and insulin-like growth factor-I). By Western blotting a single protein band migrating with a MW of 90-105 kD (unreduced) was identified. In comparison, anti-integrin antibodies (gift of K. Yamada) specific for the B1 subunit produced a different pattern of biological activity and identified a protein of higher MW on immunoblots. Thus 1AA3 antibodies react with a protein involved in the generation of motility that is potentially distinct from integrins.

A 301 MONOCLONAL ANTIBODIES TO CD9 RECOGNIZE AN EXTRACELLULAR EPITOPE OF HUMAN KERATIN IN INTEREPITHELIAL JUNCTIONS. Marie C. Béné, Jianqing Tang, Gilbert C. Faure. Laboratoire d'Immunologie. Faculté de Médecine de Nancy. 54500 Vandoeuvre, France.

The human leukocyte-associated antigen CD9 is found on platelets, basophils, eosinophils and expressed transiently in the lymphocyte lineage differentiation. We report on the expression of the epitope recognized by antibodies of this cluster of differentiation in molecules present in interepithelial spaces.

Human cutaneous epidermis, glandular epithelia, mucosal epithelia from lung, digestive and urinary tract were labelled in intercellular spaces by a monoclonal antibody against the CD9 molecule. Proteins extracted from the membrane fraction of epidermal cells, subjected to SDS-PAGE and western blot, allowed the detection of various bands according to the origin of the sample (fresh, frozen or cultured skin), a 43 KD molecule being constantly evidenced. Similar bands were obtained using an antikeratin antibody. Cross-immunoprecipitations were performed with similar epidermis extracts using either CD9 or different antikeratin antibodies and the same 43 KD band was detected. These results suggest the participation of the CD9 epitope in cell junctions between epithelial cells.

A 302 CHANGES IN ADHESION, MOTILITY AND INVASION OF RETINAL CELLS TRANSFORMED BY ROUS SARCOMA VIRUS. Susann Brady-Kalnay, David Soll+ and Robert Brackenbury, Dept. of Anatomy and Cell Biology, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267 and +Department of Biology, Univ. of Iowa, Iowa City, IA 54242

To investigate how changes in cell-cell adhesion affect cell movement we studied chicken retinal neuroepithelial cells transformed by Rous sarcoma virus (RSV). RSV reduces adhesion and causes alterations in cell movement. To describe and quantitate these alterations, we analyzed video recordings of cultured cells using the computer-assisted Dynamic Morphology System. Control cells showed very low levels of movement that increased slightly after transformation due to changes in cell shape. The behavior of control and RSV-transformed retinal glial cells was also examined. The control glial cells were highly motile and showed a slight decrease in movement after transformation. To determine whether the control neuroepithelial cells were capable of rapid movement but were restrained by cell-cell adhesions, we examined the motility of cells plated at low density or in the presence of adhesion-blocking antibodies. In both cases, the movement of the isolated cells remained low, implying that the reduction in adhesion and increase in cell shape changes are independently induced by RSV transformation. To analyze how these changes in adhesion and movement affect invasive behavior, we compared the ability of the control and RSV-transformed cells to invade the chorioallantoic membrane of chicken embryos. Control neuroepithelial cells were not invasive. RSV-transformed neuroepithelial cells were invasive, although they showed only low levels of cell motility. Similarly, RSV-transformed glial cells were invasive while control glial cells, although highly motile, were not invasive. The combined results suggest that the degree of cell movement does not directly correlate with the ability to invade.

A 303 INDUCTION OF ALBUMIN GENE TRANSCRIPTION IN HEPATOCYTES BY EXTRACELLULAR MATRIX PROTEINS, Joan M. Caron, Department of Medicine, University of California, San Francisco, CA 94143.
Expression of liver-specific proteins in primary cultures of rat hepatocytes is dependent on the culture substrata: albumin production is maintained in hepatocytes plated on a basement membrane-like matrix derived from the EHS mouse sarcoma (EHS gel), but decays rapidly in cells plated on a thin layer of type I collagen (TIC). Maintenance of albumin production in cells on EHS gel is due primarily to increased stability of albumin mRNA: transcriptional activity of the albumin gene decayed rapidly in cells plated on TIC or EHS gel. To determine if EHS proteins can affect transcriptional activity, I examined induction, rather than maintenance, of albumin expression. Cells were plated onto TIC. After 3 days in culture, when albumin gene transcription was not detectable, dilute concentrations of EHS (dilute EHS) were added to the cultures. This addition caused a marked increase in transcriptional activity of the albumin gene; albumin mRNA levels and albumin secretion increased in parallel. By immunofluorescence microscopy with anti-albumin antibody, the population of hepatocytes responded homogeneously to dilute EHS. The effect of dilute EHS was selective in that cytochrome P450, another liver-specific protein with labile activity in cultured cells, exhibited little response. Of the three major components of EHS, purified laminin was as effective as the complete matrix at inducing increases in albumin mRNA levels and albumin secretion; type IV collagen and heparan sulfate proteoglycan were ineffective. Experiments are in progress to identify regions of the laminin molecule involved in induction of albumin expression.

A 304 EVIDENCE FOR MULTIPLE N-CADHERIN MESSENGER RNAs IN BRAIN AND HEART OF THE NEWBORN CHICKEN. Anne-Marie Dalseg, Anna-Maria Andersson and Elisabeth Bock, Research Center of Medical Biotechnology, Copenhagen University, Protein Laboratory, 34 Sigurdsgade, DK-2200 Copenhagen N, Denmark. The neural cadherin (N-cadherin) is a calcium-dependent cell-cell adhesion molecule, which presumabely plays an important part during embryonic development. It has been studied in mouse and chicken, and is probably identical to the molecules NcalCAM and ACAM. The nucleotide sequence of a chicken N-cadherin cDNA has been reported, and Northern blotting, using a cDNA probe of about 350 bp covering the putative NH_2-terminus, displayed a single mRNA band of ca. 4.3 kb. We report the demonstration of at least three N-cadherin mRNAs by Northern blotting using two oligonucleotide probes derived from the published cDNA sequence. The mRNA pattern in heart and brain of newly hatched chicken was identical using a 34-mer probe derived from the extracellular EC2 region, showing the main mRNA of 4.7 kb and a weaker band of 3.3 kb. When using a 37-mer probe covering the putative NH_2-terminus, we saw the same picture in brain as with the 34-mer probe, but in heart there was a band of 3.8 kb instead of the 3.3 kb band. Western blotting revealed weak bands smaller than the 135 kD band, which so far has been regarded as degradation products. The possibility exists, that some of these bands are primary translation products of the less abundant mRNA forms observed.

A 305 ISOLATION AND CHARACTERIZATION OF INTEGRINS AND FIBRONECTIN ISOFORMS EXPRESSED IN EARLY XENOPUS EMBRYOS, Douglas W. DeSimone*, Richard O. Hynes+▲, Pamela A. Norton+ and David Ransom*, *Dept. of Anatomy and Cell Biology, University of Virginia 22908, +Center for Cancer Research, and ▲Howard Hughes Medical Institute at MIT, 02139. The onset of gastrulation in Xenopus laevis embryos is accompanied by the expression of integrins and alternatively spliced forms of fibronectin (FN). Several components of this developmentally activated cell-matrix adhesion system display differences with regard to the regulation of their expression at the mRNA and protein levels. We have isolated cDNA clones encoding Xenopus FN. These cDNAs have been used to determine the pattern of alternative splicing in early embryos and adult tissues. Antisense oligodeoxynucleotide probes can also be used to eliminate specific splice variants of FN present as maternal transcripts in Xenopus oocytes and early embryos. Additional variability is noted in the expression of integrin $\alpha\beta_1$ heterodimers during development. Some of these integrins (e.g. α_3 β_1) are localized to the presumptive ectoderm and mesoderm just prior to gastrulation. The α subunit heterogeneity described for Xenopus suggests that integrins may play an important role in regulating cell-adhesion and morphogenesis in early vertebrate embryos.

A 309 LAMININ-BINDING PROTEIN AND EMBRYOGENESIS IN A SEA URCHIN (*S. PURPURATUS*), Richard L. Hawkins, Nancy A. Torkelson and Merrill B. Hille, Department of Zoology, NJ-15, University of Washington, Seattle, WA 98195.

Laminin is an extracellular matrix glycoprotein known to affect mammalian cell adhesion and developmental processes and also has been reported to exist in the basal lamina of the sea urchin embryo (Wessel *et al. Dev. Bio.* 103:23, 1989). We therefore sought to isolate a membrane-associated laminin binding protein from the sea urchin, *Strongylocentrotus purpuratus*, by affinity chromatography using a published cell attachment sequence pentapeptide (Tyr-Ile-Gly-Ser-Arg, YIGSR) (Graf *et al. Cell* 48:989, 1987). We also examined the developmental effect of exogenous YIGSR added to cultured *S. purpuratus* embryos. The detergent extract from membranes of mid-blastula to gastrula stage embryos was bound in the presence of divalent cations to an affinity matrix to which YIGSR-amide had been covalently coupled. Subsequent elution by cation chelation yielded a major band at M_r 200K on reducing SDS-PAGE. Transfer to nitrocellulose and incubation with 125I-laminin also showed a M_r 200K band by autoradiography. Addition of YIGSR-amide (25-100 μg/ml) to cultured embryos disrupted archenteron elongation by either exogastrulation or failure of the archenteron to complete elongation across the blastocoel. Skeletal spicule formation was unaffected. This effect of YIGSR was only seen when added between the 8-cell to hatched blastula stages. Earlier or later additions of YIGSR were without effect on development. Rabbit antisera have been produced to the M_r 200K protein and studies are in progress to determine the developmental expression and localization of this apparent laminin-binding protein.

A 310 REGULATION OF A VASCULAR CADHERIN-LIKE CELL ADHESION MOLECULE IN ENDOTHELIUM BY BASIC FGF , Ronald. L. Heimark and J. D. Coffifn, Dept. of Pathology, University of Washington, Seattle, WA 98195

Since the vasculature must undergo normal developmental growth and remodeling it is reasonable that coordinated interactions between cells are important. To characterize intercellualr adhesions in endothelium a hybridoma clone was isolated producing a monoclonal antibody, Ec6C10, which inhibits Ca^{2+}-dependent adhesion of cultured aortic endothelial cells. There was no inhibition of Ca^{2+}-independent adhesion of endothelial cells or Ca^{2+}-dependent adhesion of smooth muscle cells. MAb Ec6C10 recognizes a protein with an apparent molecular weight of 135,000 in endothelium, but was not found in uvomorulin containing cells. As the cells reach confluence the immunofluorescent staining pattern is concentrated at sites of cell-cell contact. This patttern resembles that of monolayers after silver staining of cell borders. Immunochemical staining in vivo shows that only endothelium is stained and that the staining is localized to the region of intercellular junctions. This organization at intercellualr regions seen in the adult does not appear to be present in the 12 day rat embryo using a polyclonal antibody. The properties of this protein are like that of the cadherins of epithelial cells and we suggest it be, Vascular-Cadherin. Sequence analysis of the has shown that there are regions of homology with the other identified cadherins. Addition of the angiogenic factors, basic FGF or acidic FGF with heparin to confluent endothelial cells induces a time dependent decrease of V-cadherin as shown by quantative Western blotting. Replication is stimulated 20 hours after basic FGF addition and saturation density is increased 2-3 fold. These obsevations are consistent with early responses in angiogenesis showing a loss of junctional contact.

A 311 REGULATION OF MYOGENIN GENE EXPRESSION AND MYOGENESIS BY TRANSFORMING GROWTH FACTOR-β AND EXTRACELLULAR MATRIX COLLAGEN, Jyrki Heino and Joan Massaguè, Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Transforming growth factor-β1 (TGF-β1) inhibits the differentation of rat L6E9 myoblasts. This effect of TGF-β1 is concimitant with a rapid elevation of type I collagen expression. Myoblasts plated in dishes precoated with fibrillar collagen do not differentiate. To determine the role of up-regulated collagen synthesis in the anti-myogenic action of TGF-β1, we have examined the effect of TGF-β1 and collagen on myogenic gene expression. Expression of myogenin, a critical regulatory gene in myogenic differentiation, increases 4 hours after L6E9 cells are exposed to differentiation medium and reaches a maximal level 36-48 hours later. In the presence of TGF-β1 myogenin mRNA level remains at basal level. Precoating cell culture dishes with fibrillar collagen completely inhibits biochemical and morphological myogenic differentiation without preventing myogenin mRNA expression. These results suggest that TGF-β1 inhibits myogenic differentiation by at least two pathways, one that involves down-regulation of myogenin expression and another pathway that involves up-regulated production of collagen-rich matrix that inhibits differentiation independently of myogenin expression.

A 312 STRUCTURE AND DISTRIBUTION OF TRANSMEMBRANE FORMS OF THE HUMAN NEURAL CELL ADHESION MOLECULE, John J. Hemperly, Jane K. DeGuglielmo and Robert A. Reid, Becton Dickinson Research Center, P.O. Box 12016, R.T.P., NC 27709

Cell-cell adhesion is vital in both the establishment and maintenance of tissue form and function. At least one molecule mediating such adhesion, the neural cell adhesion molecule (N-CAM), has been well characterized in a number of vertebrate species. N-CAM consists of multiple polypeptide variants generated by alternate splicing of RNA transcribed from a single gene. We have recently analyzed several cDNA clones encoding human N-CAM from a neuroblastoma cDNA library. One of these clones contains the complete amino acid coding region of a transmembrane form of the molecule and another encodes a neuronal form of the molecule not reported previously in humans. Other clones encode additional N-CAM variants which arise by utilization of some of the same RNA splice sites seen in mice, rats and chickens. These clones are being used in Northern blot analyses to catagorize the occurrence of these variant sequences in human tissues and established cell lines.

A 313 MODULATION OF EXTRACELLULAR MATRIX PROTEINS OCCURS DURING ENDOTHELIAL "SPROUT" CELL FORMATION AND SUBSEQUENT IN VITRO BLOOD VESSEL ORGANIZATION. Pamela S. Howard, Jeanne C. Myers and Edward J. Macarak. Connective Tissue Research Institute and Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

"Sprouting" vascular endothelial cells were used as an in vitro model to determine if morphologic changes result in a modulation of extracellular matrix macromolecules. In vitro, "sprouting" endothelial cells formed spontaneously in cultures of cloned left ventricular endothelium, and eventually formed tubular, capillary-like structures. To determine if the cultures of "sprouting" endothelial cells were characterized by any changes in extracellular matrix macromolecules, we analyzed their major biosynthetic products. The cultures were metabolically labeled with 3H-proline and the extracellular matrix proteins were analyzed by SDS-PAGE. Additional collagenase and chymotrypsin assays were performed to determine the collagenous and non-collagenous nature of the proteins. Immunofluorescent staining of the cultures in the cobblestone, sprouting and tube-like capillary stages also revealed a switch in the expression of collagen types I, III and IV. These studies demonstrate that the progression from cobblestone to sprouting cultures, and subsequent capillary organization involves major alterations in extracellular matrix protein expression, emphasizing the importance of these macromolecules in the in vitro angiogenic process. Supported by NIH grants AM20553, HL34005 and HI41882.

A 314 REGULATION OF EXPRESSION OF THE GENES ENCODING CYTOSKELETAL PROTEINS VINCULIN, TALIN AND ALPHA-ACTININ: EFFECTS OF SERUM GROWTH FACTORS, Paul Jackson and David R. Critchley, Department of Biochemistry, University of Leicester, Leicester, United Kingdom. LE1 7RH.

A number of recent observations have indicated that expression of genes encoding cytoskeleton -extracellular matrix proteins may be intimately associated with the primary responses of cells to factors which affect cellular proliferation. For example, fibronectin, integrin, tropomyosin and actin mRNA expression is co-ordinately induced within 30 minutes of serum stimulation of fibroblasts (Ryseck et al, Exp. Cell Res. **180**, 537, 1989).

We are investigating the possibility that expression of genes encoding other cytoskeleton proteins such as vinculin, talin and alpha-actinin, may also be induced as an early response to serum growth factors. It has already been shown that after exposure of quiescent cells to platelet-derived growth factor (PDGF), there is a rapid (less than 10 minutes) and reversible loss of vinculin from focal contacts. Studies with inhibitors of transcription and translation suggest that the return of vinculin to focal contacts (by 60 minutes) requires de novo transcription and protein synthesis (Herman et al., J. Cell Physiol., **126**, 115, 1986). Little is known of the effect of PDGF on expression of the genes encoding alpha-actinin or talin.

We have recently isolated cDNAs encoding vinculin (Price et al. Biochem. J., **245**, 595, 1987), alpha-actinin (Baron et al. J. Biol. Chem. **262**, 27623, 1987) and talin (Critchley et al. unpublished data). As part of an investigation to identify factors which regulate expression of these cytoskeletal proteins and their possible relevance in the cellular responses to growth factors, we have used these cDNAs to examine the changes in expression of vinculin, talin and alpha-actinin mRNAs after serum growth factor stimulation of fibroblast cell growth.

A 315 HEMATOPOIETIC PROGENITOR CELL ADHESION TO IRRADIATED BONE MARROW STROMA *IN VITRO*.
William Janssen, Kimberly Johnson, Carlos Lee, and Srisurang Wathana. University of Florida, Division of Pediatric Hematology/Oncology, Gainesville, FL; and Bone Marrow Transplant Program, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL.
In the bone marrow, specific spatial arrangements exist between hematopoietic progenitor cells (HPC) and their cellular microenvironment. Whether stromal adhesion is unique to the most primitive HPC, or a stochastic process involving all HPC, is uncertain. In these studies, re-attachment of murine HPC to in vitro marrow stromal elements was measured. Long term bone marrow cultures (LTBMC), in which both stroma and HPC may be functionally maintained, were established and then irradiated (10Gy) to eliminate existing HPC. 10^6 marrow cells were then seeded into these LTBMC. Following periods of 1 to 20 hours, the stroma in the seeded LTBMC were vigorously washed to remove all cells not tightly adhering, and nonadherent cells were recovered. Recovered cells were counted, and assayed for the presence of the most primitive, spleen colony forming, HPC (CFU-S) and their close, but differentiated progeny which form granulocte and macrophage colonies in tissue culture (CFU-GM). Total non-adhering cells and non-adhering CFU-GM declined <20% after one hour, and then were invariant over the remaining 19 hours of incubation. Conversely, nonadherent CFU-S declined by one half after two hours, and remained reduced at subsequent samplings. Verification of CFU-S attachment to stroma was made by demonstrating restored hematopoietic function. These data support a hypothesis of specific adhesion between primitive HPC and the marrow stroma, which adhesive properties are lost with differentiation of the cells.

A 316 PURIFICATION AND CHARACTERIZATION OF SOLUBLE NEURAL CELL ADHESION MOLECULE FROM RAT BRAIN, Lisbeth Krog, Marianne Olsen, Niamh Moran and Elisabeth Bock, Research Center for medical Biotechnology, The Protein Laboratory, Univ. of Copenhagen, 2200 N, Denmark.
Soluble Neural Cell Adhesion Molecule (NCAM) was purified from rat brains by affinity chromatography on an anti-NCAM antibody-column. Purified soluble NCAM appeared as two major bands of Mr 115,000-120,000 and approximately Mr 60,000. Occasionally higher bands were seen. Iodinated soluble NCAM was submitted to ultracentrifugation. The preparation was placed on top of a 1.21 g/ml salt suspension and centrifuged for 20 hours at 200,000 g. Soluble NCAM was found in the 1.21 g/ml salt suspension indicating that this isoform did not contain lipid. In a solid-phase assay the binding constant (K_B) for the homophilic binding between soluble NCAM and membrane NCAM from newborn rats was found to be 3×10^{-7} M and in a cell-to-substrate adhesion assay where membrane NCAM and soluble NCAM was immobilized on glass, using BSA/gelatine as background, an NCAM expressing glioma cell line (BT4C) bound to the same extent to soluble NCAM as to membrane NCAM. This adhesion could be blocked by anti-NCAM Fab' fragments. This indicates that soluble NCAM contains the homophilic NCAM binding domain and has a possible functional role in cell adhesion events.

A 317 CHARACTERIZATION AND PURIFICATION OF AN EMBRYONIC CHICKEN BOWEL SPECIFIC HNK-1 ANTIGEN: THE PUTATIVE TARGET FOR NEURAL CREST CELLS IN THE DEVELOPING GUT. Th.M. Luider, J.H.C. Meijers, M.J.H. Peters-van der Sanden, S. van Gaalen, A.W.M. van der Kamp, D. Tibboel, and J.C. Molenaar, Depts. of Cell Biology and Pediatric Surgery, Erasmus University, P.O. Box 1738, 3000DR Rotterdam, the Netherlands
Monoclonal antibody HNK-1 binds a family of morphoregulatory molecules essential for the normal development of the chicken embryo (e.g. N-CAM, Ng-CAM, MAG, cytotactin, integrin). We investigated the spatio-temporal distribution of proteins carrying the HNK-1 epitope during organogenesis of the chicken embryo by Western blot technique. We found a general pattern of expression of HNK-1 proteins (21, 25 major band, 31, 81 kD), but in addition we found tissue and stage specific HNK-1 proteins. In the aneuronal gut we found two tissue specific HNK-1 positive proteins 42 and 44 kD, which disappeared after the arrival of neural crest cells in the gut. Histochemical staining of tissue sections of aneuronal bowel with the monoclonal antibody HNK-1 showed a band of mesenchymal cells. This characteristic staining disappeared on the arrival of neural crest cells. After the arrival of neural crest cells in the gut, the developing plexuses (plexus of Auerbach and plexus of Meissner) became HNK-1 positive. Preliminary results will be presented of the purification of these bowel specific proteins from aneuronal chicken bowel and transplants of aneuronal chicken bowel cultured on the chorioallantoic membrane. The purification is performed by a combination of affinity chromatography and HPLC gel filtration techniques.

A 318 **INHIBITION OF TURNOVER OF DESMOSOMAL GLYCOPROTEINS IN CULTURED EPITHELIAL CELLS,** Anthony I. Magee and Paris Ataliotis, Laboratory of Eukaryotic Molecular Genetics, National Institute for Medical Research, The Ridgeway, London NW7 1AA, U.K.

Desmosomes are the majpr stress-bearing intercellular junctions of epithelial cells. They are composed of a variety of proteins including two membrane-spanning glycoproteins, DGl (M_r 150kDa) and DGII/III (M_r 120/100 kDa). Formation of desmosomes *in vitro* is dependent on extracellular calcium. We have shown that DGI and DGII/III both have half lives of about 20 h when MDCK cells are cultured in standard calcium medium (SCM; 1.8 mM Ca^{2+}). In low calcium medium (LCM; <0.1 mM) there is a marked increase in the turnover of these proteins with half lives decreasing to 2-3 h. Associated with this increased turnover is the failure of cells to form desmosomes. The absence of junction formation may result from an increase in the turnover of its constituent proteins or turnover may increase because desmosomes do not assemble. We have used protease inhibitors and perturbation of intracellular traffic with low temperature and lysosomotropic agents, to prevent turnover in order to address the mechanism and site of this phenomenon.

A 319 **IDENTIFICATION OF A *DROSOPHILA* GENE WHICH ENCODES A PROTEIN WITH EXTENSIVE HOMOLOGY TO VERTEBRATE CADHERINS.**
Paul A. Mahoney,[1] Patricia Onofrechuk,[2] Peter J. Bryant,[2] and Corey S. Goodman[1],[1]HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, [2]Developmental Biology Center, University of California, Irvine.

Cadherins, or calcium-dependent cell adhesion molecules, constitute a highly conserved family of membrane-bound glycoproteins which have been isolated from a wide variety of vertebrate species. We have attempted to determine whether homologous molecules exist with similar functions in invertebrates, particularly in *Drosophila melanogaster*, where the process of cell adhesion can be studied using genetic as well as molecular methods. By using primers in the polymerase chain reaction (PCR) which are based on conserved regions of the vertebrate sequences, we have amplified and cloned a region of fly DNA which is highly homologous to vertebrate cadherins. The predicted protein product of a 5kb cDNA (not full-length) which we subsequently isolated contains over 13 repeated domains which are homologous to the 5 extracellular domains found in vertebrate cadherins. Thus, while the *Drosophila* domains are homologous to the vertebrate extracellular domains, the size of the predicted protein is much larger. In addition, we present evidence which suggests that this fly cadherin is encoded by the *Drosophila fat* locus, a gene which causes tumor-like overgrowths when mutated.

A 320 PHENOTYPIC CHANGES IN ENDOTHELIAL CELL MORPHOLOGY ARE ASSOCIATED WITH DIFFERENTIAL MATRIX GENE EXPRESSION. Jeanne C. Myers, Pamela S. Howard, Stephen F. Gorfien and Edward J. Macarak. Connective Tissue Research Institute and Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104.
Endothelial cell adhesion, proliferation and migration can be regulated by various extracellular matrix macromolecules. In vivo and in vitro, the endothelium normally consists of a contiguous monolayer of quiescent, polygonal-shaped cells. However, under certain growth conditions, endothelial cells exhibiting the cobblestone morphology are replaced by an interconnecting network of elongated cells termed "sprouts." Since it is well-established that changes in biosynthesis and cell shape are elicited by the extracellular matrix, we characterized the expression of collagen, fibronectin and thrombospondin genes in normal and "sprouting" bovine endothelium in vitro. Our results revealed that the sprout cells exhibited a pattern more closely resembling a non-epithelial cell type including differences in the synthesis of type I collagen mRNAs and fibronectin ED-containing transcripts. Similar endothelial morphologic changes occur during blood vessel development and response to injury, suggesting that the associated alterations in matrix gene expression are integral components of the initial events in these processes. (Supported by NIH grants: AM20553, HL34005 and HL41882).

A 321 CHARACTERIZATION OF SOLUBLE NEURAL CELL ADHESION MOLECULE FROM RAT BRAIN, CEREBROSPINAL FLUID AND PLASMA, Marianne Olsen, Lisbeth Krog, Anne-Marie Dalseg and Elisabeth Bock, Research Center for medical Biotechnology, The Protein Laboratory, Univ. of Copenhagen, 2200 N, Denmark.

Soluble Neural Cell Adhesion Molecule (NCAM) from supernatant made from homogenized rat brains appeared as a highly glycosylated major smear of Mr 250,000-350,000 and three bands of approximately Mr 200,000, Mr 140,000 and Mr 115,000-120,000 in Western blotting. Occasionally bands of lower molecular weight were seen. The same pattern was seen in cerebrospinal fluid from rat. In immunopurified rat plasma however only bands of Mr 115,000-120,000 and Mr 100,000 were seen. To investigate the glycosylation of soluble NCAM the supernatant was treated with endoglycosidase N resulting in the disappearence of the 250,000-350,000 band, indicating that this band is highly polysialylated. During development from newborn to adult the Mr 250,000-350,000 band disappears while the 115,000-120,000 band increases in intensity. The Mr 115,000-120,000 band carried the same N-terminal sequence as membrane NCAM. The monoclonal antibody P61, which recognizes a cytoplasmic domain common to membrane spanning NCAM isoforms of Mr 200,000 and Mr 140,000, does not recognize any of the soluble forms of NCAM.

A 322 SEQUENCE AND DOMAIN STRUCTURE OF TALIN. D J G Rees, S E Ades[1], V Ohanian[2], D R Critchley[2] and R O Hynes[1], Center for Cancer Research and [1]Howard Hughes Medical Institute, MIT, Cambridge, MA 02139, [2]Department of Biochemistry, Leicester University, Leicester, United Kingdom.

The cytoskeletal protein talin is found in the focal contacts of adherent cells, in the cytoskeleton of platelets and in the cell-cell contact zone of antigen dependent T cell-target cell conjugates. In each location it colocalises with members of the integrin family of membrane glycoproteins. It is also a substrate for the calcium-dependent protease, calpain II. We have undertaken the molecular cloning of mouse and chicken talin cDNA in order to determine the structure and study the function of talin at a molecular level.

Sequence analysis of cDNA clones for mouse talin shows that the protein is 270 kD in size and encoded by an 8.3 kb mRNA. The N-terminus of talin is found to be homologous with the N-terminal domains of erythrocyte band 4.1 and the microvillar core-associated protein ezrin. The C-terminal 220 kD domain shows no relationship with any known protein, but 20% of its residues are alanine and it probably forms an extended α-helical structure. We have cleaved purified chicken talin with the protease calpain II, and determined the N-terminal amino acid sequences of the two fragments so obtained. The smaller, 50 kD, fragment derives from the N-terminus of the intact protein, while the larger, 220 kD, fragment is C-terminal. The calpain II cleavage site is located immediately C-terminal to the band 4.1/ ezrin related domain.

We are currently investigating the structure-function relationships of talin using β-galactosidase/talin fusion proteins to raise domain-specific antibodies and to study protein-protein interactions with vinculin and integrins.

A 323 SQUAMOUS EPITHELIAL CELL DIFFERENTIATION IS ASSOCIATED WITH REDUCED EXTRACELLULAR MATRIX PRODUCTION AND REDUCED CELL-SUBSTRATE ADHESION, Bruce Riser and J. Varani, Department of Pathology, Univ. of Michigan,Ann Arbor, MI 48109. Human epidermal keratinocytes and human squamous epithelial cells derived from a tumor of the oral cavity were grown in culture in a low-Ca^{2+} (0.15 mM) containing medium (MCDB-153) supplemented with epidermal growth factor (EGF). The cells were Then exposed to an elevation in the external Ca^{2+} concentration (1.4 mM), treated with interferon-γ (IFN-γ) or subjected to removal of the EGF from the culture medium. Each of these treatments inhibited keratinocyte proliferation, decreased cell-substrate adhesion, induced morphological changes consistent with differentiation and increased cornified envelope formation. These changes were accompanied by a reduction in thrombospondin production. The reduction in thrombospondin production may be directly related to altered adhesion since monoclonal or polyclonal antibodies to thrombospondin inhibited epithelial cell attachment and spreading. The malignant squamous epithelial cells were similar to the keratinocytes in their response to IFN-γ and to elevation of the external Ca^{2+} concentration. In contrast, the malignant cells showed no response to removal of EGF from the culture medium. These data suggest that the malignant squamous epithelial cells do not differ from their normal counterpart cells in capacity to undergo differentiation but rather, differ in their requirements for exogenous growth-promoting factors. These data suggest further that inhibition of proliferation and onset of differentiated features are associated with reduced production of extracellular matrix components, particularly thrombospondin.

A 324 ADHESION AND GROWTH OF HUMAN OVARIAN CARCINOMA CELLS ON EXTRACELLULAR MATRIX, D.M. Skrincosky, R.J. Bernacki. Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, NY 14263.

The interaction between human ovarian carcinoma (HOC) cells and reconstituted extracellular matrix was studied to assess the role of adhesion in cell proliferation. The adhesion of HOC cells (line A121) to a bovine corneal endothelial cell extracellular matrix (ECM), a basement membrane matri-gel extracted from the EHS murine tumor, and tissue culture plastic (TCP) was compared. A121 cells adhered to both ECM and matri-gel at a faster rate than to TCP. Although the rates on both types of matrix were similar, ECM promoted earlier and more extensive cell spreading. While the growth of A121 cells on all three substrates in serum-containing media was similar, in serum-free media, cells grew only on ECM. A121 cells suspended in media containing methylcellulose or in spinner flasks grew at a much slower rate than normal, adherent monolayer cultures. In suspension, cells always formed clusters and aggregates. Implanted as an i.p. xenograph in nude mice, A121 cells formed distinct solid tumor nodules attached throughout the peritoneum. Free-floating tumor cells and ascites fluid were not observed. This suggests that A121 cells need to adhere in order to grow and under selected conditions cell-substrate interactions can be replaced by cell-cell interactions for cell growth.

A 325 GROWTH FACTOR-INDUCED EXTRACELLULAR MATRIX PRODUCTION AND GROWTH CONTROL IN HUMAN COLON CARCINOMA CELLS, James Varani, Lucia Schuger and Subhas Chakrabarty, Dept. of Pathology, Univ. of Mich., Ann Arbor, MI and Dept. of Pharmacol., Baylor College of Medicine, Houston, TX. A series of human colon adenocarcinoma cell lines were examined in vitro for production of extracellular matrix components including fibronectin (FN), laminin (LN) and thrombospondin (TSP). Production of all three matrix components was low as compared to normal colonic mucosal epithelium or various squamous carcinoma cell lines derived from tumors of the head and neck. Treatment of one of the colon carcinoma lines (Moser) with transforming growth factor-β (TGF-β) stimulated matrix production, increased cell-substrate adhesion and reduced proliferation. A variant cell line which was resistant to the growth inhibiting effects of TGF-β bound TGF-β with the same characteristics as the parent cells but did not respond with increased matrix production. Treatment of the cells with epidermal growth factor (EGF) also stimulated matrix production and cell-substrate adhesion. Moser cells were transfected with DNA containing human EGF-coding sequences. Several clones of the EGF gene-transfected cells were found to produce increased amounts of FN, LN and TSP as compared to the untransfected parental Moser cells. These cells were also more adherent to the substrate than the parent cells. These findings suggest that the phenotypic characteristics of human colonic adenocarcinoma cells may be a reflection of their low level of extracellular matrix production. Matrix production and the phenotypic properties that depend on the production of extracellular matrix components may be modulated by addition of exogenous growth-regulating peptides or by altering the endogenous production of these factors.

A 326 CLONING OF DESMOSOMAL GLYCOPROTEIN I WITH AFFINITY PURIFIED ANTI-DG1 ANTISERA, Grant N. Wheeler, Paris Ataliotis, Claire L. Thomas, Roger S. Buxton and Anthony I. Magee, Laboratory of Eukaryotic Molecular Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

One of the major cell-cell adhesion structures in epithelial tissues is the desmosome junction. The biochemical composition of desmosomes is well documented, with a number of proteins and glycoproteins ranging from 250kd down to 75kd. Desmosomal glycoprotein 1 (DG1) is a transmembrane glycoprotein of 150kd. Immunochemical studies have shown cross reactivity between DG1 of various species and tissues. Anti DG1 sera raised against gel purified DG1 from bovine muzzle desmosome preparations was affinity purified and used to screen a human keratinocyte λgt11 cDNA library. Possible clones were verified using anti-DG1 monoclonal antibodies and comparison with amino acid sequence obtained in this laboratory. Features of DG1 will be discussed in relation to its possible role as a cell adhesion molecule.

A 327 THE ROLE OF FIBRONECTIN IN MESODERM CELL MIGRATION DURING XENOPUS GASTRULATION, Rudolf Winklbauer, Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, Federal Republic of Germany.
In the Xenopus embryo, mesodermal cells migrate on a substrate cell layer which is covered by a sparse network of fibronectin (FN) fibrils. It is shown that these migratory mesoderm cells attach to their in vivo substrate independently of FN, but cell spreading and the formation of stable cytoplasmic protrusions requires interaction with FN. The motile behavior of mesoderm cells on their in vivo substrate is correlated with these FN-dependent differences in cell morphology. Cells migrate persistently, on straight pathways, when interacting with FN, but persistence is lost, and cells perform a random walk on the substrate cell layer, when cell-FN interaction is blocked by an inhibitory RGD-containing peptide. It is proposed that in this system, FN is not a simple substrate molecule which allows cells to attach and exert traction, but rather controls the protrusive activity of cells. By promoting the formation of stable lamelliform protrusions, the sparse network of FN fibrils enables intrinsically motile cells to migrate efficiently on the surface of a substrate cell layer.

A 328 CARBOHYDRATE RECOGNITION IN AXONAL PATHFINDING / SYNAPSE FORMATION INVOLVES A FAMILY OF LEECH 130 KD PROTEINS. Birgit Zipser, Mary Lynn Bajt, Robert N. Cole, Robert J. Morell, Irmgard Thorey, Karl Zipser. Department of Physiology, Michigan State University, East Lansing, MI 48824
A family of 130 kD integral membrane glycoprotein is distinguished via their carbohydrate epitopes by MAbs. One member of this family (recognized by MAb Lan3-2) is expressed by the entire sensory afferent system; three others (MAbs Laz2-369, Laz6-212 and Laz7-79) are expressed by unique sensory subsets. Two lines of evidence suggest that carbohydrate recognition plays a role in central pathfinding/synapse formation: (1) Immunocytochemical staining of serial sections determined that the sensory neuron processes distinguishable by their particular carbohydrate epitopes occupy distinctly different regions within the central synaptic neuropile. (2) Segregation of sensory afferents into their discrete domains within the synaptic neuropile is exquisitely sensitive to antibody perturbation with Lan3-2 binding to a mannosidic epitope (threshold: 6 nM of Fab). Preliminary results suggest that the 130 kD leech glycoproteins are related to the L3, L4, L5 epitope family characterized by M. Schachner's group (Bajt, Schmitz, unpubl.).

Leukocyte Adhesion and Metastasis

A 400 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE INTEGRIN VLA-4. John L. Bednarczyk, James N. Wygant and Bradley W. McIntyre, Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, Tx. 77030
The monoclonal antibody L25 immunoprecipitates the integrin VLA-4. Double determinant binding assays show that mAb L25 binds to the α_4 subunit. The structure of VLA-4 is unusual for an integrin in that four non-covalently associated polypeptides of M_r 150,000 (α_4), 130,000 (β_1), 85,000 and 75,000 are immunoprecipitated from CHAPS lysates of cell surface radiolabeled lymphocytes. The p85 polypeptide is a fragment of α_4 that contains the L25 epitope. Unlike the other integrins, which are receptors for extracellular matrix proteins, the ligand for VLA-4 is unkown. However, the binding of mAb L25 to VLA-4 induces the homotypic aggregation of human lymphocytes and lymphoblastoid cell lines. Aggregation induced by mAb L25 is not mediated by the adhesion proteins LFA-1, ICAM-1, LFA-3, CD2, CD8 or CD4. MAb L25-induced aggregation requires energy, an intact cytoskeleton and the divalent cation Mg^{++}. IL-2 dependent CTLs do not aggregate upon mAb L25 binding and VLA-4 immunoprecipitated from these cells consists of only the M_r 130,000 (β_1), 85,000 and 75,000 polypeptides. These studies indicate that the ability of mAb L25 to induce homotypic adhesion is dependent on the expression of the intact α_4 polypeptide.

A 401 IDENTIFICATION OF THE PERIPHERAL LYMPH NODE VASCULAR ADDRESSIN AND COMPARISON WITH THE MUCOSAL VASCULAR ADDRESSIN, Ellen L. Berg, Philip R. Streeter and Eugene C. Butcher, Department of Pathology, Stanford University, Stanford, CA 94305. Lymphocytes continuously recirculate, exiting the blood by binding selectively to high endothelial venules (HEV) in lymphoid tissues and sites of inflammation. The entrance of lymphocytes into peripheral lymph nodes and mucosal lymphoid tissues is controlled by organ-specific lymphocyte-HEV interactions, mediated by tissue-specific lymphocyte homing receptors and the corresponding vascular addressins. In the mouse, the peripheral node and mucosal vascular addressins (PNAd and MAd), defined by monoclonal antibodies MECA-79 and MECA-367, are molecularly distinct species as are the peripheral node and mucosal homing receptors, the MEL-14 antigen and LPAM-1. MAd is a single chain glycoprotein of 58-66 kDa and contains sialic acid but no N-glycanase-sensitive carbohydrate. MAd binds to wheat germ agglutinin but not to lentil lectin. In addition, MAd migrates more slowly in SDS-PAGE gels under non-reducing conditions (54-62 kDa) suggesting the presence of disulfide linked bonds. In contrast, PNAd (defined by MAb MECA-79) is a determinant that appears to exist on several glycoprotein species (60, 70, 92, 110, 130, and 150 kDa). Some, but not all of these species contain N-glycanase sensitive carbohydrate and bind to lentil lectin, while all of the species contain sialic acid. These data suggest that lymphocyte-HEV interactions involve molecules from distinct gene families and with distinct evolutionary histories, but which serve homologous functions.

A 402 IMMUNO-ELECTRONMICROSCOPY OF CYTOTOXIC GRANULES IN T LYMPHOCYTES LEADS TO A NOVEL MODEL FOR LETHAL HIT DELIVERY, *Jannie Borst[1], Hans J. Geuze[2] and Peter J. Peters[2], [1]Division of Immunology, The Netherlands Cancer Institute, Amsterdam, 1066 CX, & [2]Laboratory of Cell Biology, Medical School, University of Utrecht, The Netherlands.*

Human cytotoxic T lymphocyte (CTL) granules contain an electron-dense core and small membrane vesicles. By immuno-electronmicroscopy we have localized membrane molecules relevant for CTL-target cell (TC) interaction on the membranes of the dense core and small vesicles within the granule. Moreover, perforin, the component implicated in the lethal hit, as well as serine esterases are localized within these granule substructures. These observations necessitate a reversion of the current model for lethal hit delivery. There may be a dual role for T cell membrane molecules. First, the TCR/CD3 complex and accessory molecules ensure antigen recognition, followed by tight adhesions between CTL and TC, which allow the formation of an intercellular clefts. The cytotoxic mediators are exocytosed into the clefts between CTL and target cell not in soluble form, but are membrane enveloped. On these membranes the molecules relevant for TC recognition and adhesion are again present, which may ensure unidirectional delivery of the lethal compounds to the TC.

Abstract Withdrawn

A 404 THE ROLE OF ICAM-1 IN ANTIGEN PRESENTATION, Anne-Marie Buckle and Nancy Hogg, Macrophage Laboratory, ICRF, London WC2A 3PX, UK.
The leucocyte integrin LFA-1 plays an important role during the initial interactions between monocytes and T cells in antigen presentation (Dougherty and Hogg EJI 17 943 1987). Antibodies to intercellular adhesion molecule-1 (ICAM-1), an established ligand for LFA-1, are able to effectively block antigen presentation, suggesting that this is the molecule with which LFA-1 is interacting. We have found that ICAM-1 is readily expressed on the surface of monocytes and is weakly expressed on the memory T cell population, and thus would be available as a ligand for LFA-1 on both monocyte and T cell surfaces during the memory response to recall antigen. We have now characterised an anti-ICAM-1 antibody and prepared $F(ab')_2$ and Fab' fragments for use in antibody blocking studies. Initial studies of the kinetics of ICAM-1 involvement in this interaction have shown that it mirrors the early role in events that has previously been demonstrated for LFA-1. Blocking studies are now in progress to establish the relative contributions of monocyte and T cell derived ICAM-1 in this interaction.

A 405 EXPRESSION AND FUNCTIONAL ANALYSIS OF MURINE ICAM-1. C. Carpenito, K.J. Horley, and F. Takei, Terry Fox Laboratory, B.C. Cancer Research Centre; Departments of Medical Genetics, Microbiology, and Pathology, University of British Columbia, Vancouver, B.C., Canada.
The rat monoclonal antibody YN1/1.7 detects a murine lymphocyte surface antigen, MALA-2. We have reported the isolation of a putative 3.1 kb cDNA (K3-1.1) of MALA-2 which shows significant homology to the human ICAM-1 gene. Purified MALA-2 was crosslinked to a solid support and found to bind Con A blasts that express LFA-1. This binding was blocked by the YN1/1.7 antibody or the anti-murine LFA-1 antibody. These results coupled with the sequence homology strongly suggest that MALA-2 is the murine homologue of ICAM-1. However, expression of the K3-1.1 insert could not be detected on the surface of transfected COS cells, even when the 5' end (which has 10 potential translation start sites) was deleted. A second clone, K4-1.1, has been isolated that is virtually identical to the K3-1.1 clone except for the 5' end. Expresson is detected on COS cells transfected with the K4-1.1 insert. Northern blots probed the K4-1.1 insert detected transcripts of 2.5 kb and 3.1 kb. When the blot was probed with the 5' end of K3-1.1 insert, only the 3.1 kb transcript was detected suggesting that the two transcripts, represented by K3-1.1 and K4-1.1, are probably the result of alternate splicing. By deleting the transmembrane and cytoplasmic regions of the K4-1.1 insert, a secreted form of ICAM-1 can be generated. The modified protein was purified from the supernatant of transfected COS cells and is presently being used in binding studies and examining its role in cell-cell interactions.

A 406 EXTRACELLULAR MATRIX REGULATION OF ORGAN SPECIFIC METASTASIS. RF Cerra*, SD Nathanson* and BF Sloane+. *Department of Surgery, Henry Ford Hospital, Detroit, MI 48202, and +Department of Pharmacology, Wayne State University, Detroit, MI 48201.
The extracellular matrix is a complex regulatory structure which may control, in part, the organ specific pattern of metastasis demonstrated by many primary tumors. We have previously shown that 4M guanidine extracts of extracellular matrix material, prepared by high salt extraction and DNAase/RNAase digestion, induce differential migration of organ specific tumor cells. Cells which preferentially colonize liver (B16-L4b and M5076) or lung (B16-F10 and B16-BL6) migrate towards extracts derived from liver or lung, respectively. These preparations contain only trace amounts of contaminating cellular material, laminin, fibronectin and collagen IV. Since it is likely that these organ specific chemoattractants regulate a key step in the metastatic cascade we have attempted to both characterize these organ specific chemoattractants and to elucidate a possible mechanism by which these molecules are released from the extracellular matrix. The guanidine extracts from liver and lung were extensively dialyzed and subjected to gel filtration chromatography. The liver extract resolved into 4 peaks of activity of Mr >250,000, ~245,000, ~120,000, and ~30,000 with the Mr~120,000 species inducing a 5-fold greater migration of liver colonizing cells than lung colonizing cells. The lung extract resolved into 5 peaks of activity of Mr>250,000, ~148,000, ~120,000, ~71,000 and ~30,000 with the Mr~71,000 and ~30,000 species inducing 6- and 10-fold, respectively, greater migration of lung colonizing cells than liver colonizing cells. To investigate a mechanism of release of these molecules from the extracellular matrix we digested liver and lung matrix with cathepsin B, a protease secreted from tumor cells which correlates with metastatic potential. The solubilized material contained a high amount of organ specific chemotactic activity. The liver colonizing cell lines exhibited 5-fold greater chemotaxis toward the liver digest than toward the lung digest. Conversely, the lung colonizing cells demonstrated a 4-fold increase in migration toward the lung digest when compared to liver digest. These results suggest that organ specific chemotactic factors are present in the extracellular matrix and may be released as a result of the proteolytic digestion of the matrix by an invading tumor cell.

A 407 TUMOR PROGRESSION IN VIVO: INCREASED SBA LECTIN BINDING AND LIVER METASTASIS, Donna A. Chow and Martin R. Reese, Department of Immunology and Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada, R3M OP9.
Tumors which manage to grow out from threshold SC inocula of L5178Y-F9 and SL2-5 murine T-cell lymphomas in syngeneic DBA/2 mice exhibited a unified natural defense-resistant phenotype including a higher tumor frequency upon SC inoculation. They also produce more liver foci upon IV injection. Analysis of lectin binding using FITC-labelled Con-A, WGA, PNA and SBA showed that the most consistent change, 3 of 4 lines, was an increase in N-acetyl-galactosamine (galNAc)-specific SBA binding. A strong direct correlation was demonstrated between the log of liver metastasis formation and SBA binding. The in vivo grown variants also bound to hepatocytes 2-4 times as well as the parental lines and a strong correlation was seen between hepatocyte rosette formation and specific SBA binding. Therefore there was also a strong correlation between the log of liver foci formation and hepatocyte rosetting by the tumors. Tumors treated with neuraminidase bound hepatocytes better, consistent with the idea that tumor/hepatocyte binding was mediated by galactose/galNAc specific hepatocyte receptors. In addition, galNAc or galactose added to the binding reaction inhibited rosette formation better than glucose. However, preincubation of the tumor cells with the monosaccharide inhibited rosette formation well for galNac and moderatly for galactose, while no inhibition was seen with similar preincubation of hepatocytes. The relationship between the increased expression of the SBA-binding ligand on the in vivo grown tumor cells and the galNAc inhibitable tumor receptor involved in tumor/hepatocyte binding is being investigated as a possible dominant feature in the in vivo generated increase in the liver specific metastatic capability of murine lymphomas. Supported by NCI of Canada and MHRC.

A 408 IDENTIFICATION OF TWO THYMIC EPITHELIAL GLYCOPROTEINS SPECIFICALLY BOUND BY THYMOCYTES. Clément Couture and Edouard F. Potworowski, Institut Armand-Frappier, P.O. Box 100, Laval, Qc, Canada, H7N 4Z3.
Intrathymic differentiation of T cells depends to a large extend on the formation of complexes between thymocytes and various stromal cells. In order to elucidate the molecular nature and role of adhesion molecules involved in such lympho-stromal complexes, we have developped an *in vitro* system in which a thymic medullary epithelial cell line (E-5) forms complexes both with a discrete subset of CD4+CD8+ thymocytes and with cells of a thymic lymphoma line (Ti-6). We show that Ti-6 cells and normal thymocytes adhere to E-5 cells using a common or closely related receptor, thought to recognize the same E-5 surface ligand. In fact, both Ti-6 cells and thymocytes bind specifically a 23 kDa and a 45 kDa membrane glycoprotein of E-5, thus validating Ti-6 cells as a faithful model of molecular interactions between thymocytes and medullary epithelial cells. The electrophoretic mobility of gp23 indicated the presence of intrachain disulfide bonds. These data were corroborated by the immunoprecipitation of gp23 and gp45 by a set of monoclonal antibodies able to inhibit thymocyte adherence to E-5 cells. We therefore postulate that E-5 cells adhere both to thymocytes and to Ti-6 cells through a ligand generated by the non-covalent association of gp23 and gp45, and that this novel adhesion molecule, composed of two subunits, could be part of the integrin family of adhesion molecules. Supported by the Medical Research Council of Canada and the Cancer Research Society.

A 409 REGULATED EXPRESSION OF MG^{2+} BINDING EPITOPE ON LEUKOCYTE INTEGRIN α SUBUNITS, Ian Dransfield and Nancy Hogg, Imperial Cancer Research Fund, London, WC2A 3PX, UK.
The leukocyte integrins LFA-1, CR3 and p150,95 are a family of heterodimeric receptors that mediate divalent cation-dependent cellular adhesion reactions. We have identified a novel antibody-defined epitope present on all three α subunits (Dransfield and Hogg, in press) representing a common structural motif. Antibody recogntion is eliminated in the absence of divalent cations and restored by addition of Mg^{2+} but not Ca^{2+}, thus epitope expression reflects the Mg^{2+} occupancy of these molecules. Preliminary studies have revealed that the ligand binding site is remote from the site recognised by mAb 24 and that inhibition of antigen presentation function by this antibody (Dougherty et al. Eur. J. Immunol. **18** 2067) occurs after LFA-1/ICAM-1 ligation has taken place although the mechanism has yet to be defined. Expression of this epitope on intact cells requires metabolic energy suggesting that control of Mg^{2+} binding to the extracellular domains from within the cell represents one potential mechanism for regulation of leukocyte integrin function.

A 410 ANTI-CD18 ANTIBODY R15.7 ATTENUATES NEUTROPHIL LOCALIZATION IN ISCHEMIC MYOCARDIUM, W.J. Dreyer, L.H. Michael, C.W. Smith, R.D. Rossen, R. Rothlein, M.L. Entman and D.C. Anderson, Baylor College of Medicine, Houston, TX 77030; Boehringer Ingelheim Pharm Inc, Ridgefield, CT 06877. Neutrophil localization (NL) has been associated with injury during ischemia/reperfusion of myocardial tissue. We previously reported that chemotactic factors generated in ischemic canine myocardium elicit CD18-dependent neutrophil adherence reactions (*Circ Res*, in press). An anti-CD18 Mab, R15.7 (IgG1) inhibits selected canine neutrophil functions in vitro (e.g. homotypic aggregation, attachment to endothelium and myoctyes, H_2O_2 release). In this study we evaluated the possibility that systemically administered R15.7 could attenuate NL into reperfused ischemic canine myocardium. Six control and 5 Mab-treated animals (1 mg/kg IV 3 hrs prior to ischemia) underwent 1 hr of circumflex coronary artery occlusion followed by 1 hr of reperfusion. Regional myocardial blood flow was measured by radioactive microsphere technique. 99mTc-labeled autologous neutrophils (4-6 X 10^7/animal) were given by left atrial injection 15 min into reperfusion and allowed to circulate 45 min prior to removal and sectioning of hearts. In control animals, significant NL occurred in endocardial and mid-myocardial segments in which occlusion flow was reduced to <50% the maximum flow to nonischemic segments. The extent of NL was inversely related to blood flow (endocardium: R=-.82, p<0.001, mid-myocardium: R=-.67, p<0.001). NL did not occur in epicardial segments, regardless of flow. In R15.7-treated animals vs. control animals, R15.7 had no effect on NL in segments with flow <10% maximum flow. In segments with 11-30% and 31-50% maximum flow, NL (expressed as fold increase over background in nonischemic segments) was significantly decreased in Mab treated animals:

	% Max Flow	Control	R15.7	p Value
endocardium	11-30%	8.4 ± 1.1	2.6 ± 0.7	0.001
	31-50%	6.0 ± 1.3	2.2 ± 1.5	0.02
mid-myocardium	11-30%	6.5 ± 1.1	1.5 ± 0.4	0.001
	31-50%	4.5 ± 1.2	1.6 ± 0.5	0.06

We conclude that NL in regions of reduced myocardial blood flow is in part CD18-dependent. We speculate that therapy with anti-CD18 Mab may salvage reversibly damaged ischemic myocardial tissue.

A 411 METABOLIC COUPLING BETWEEN METASTATIC TUMOR CELLS AND VASCULAR ENDOTHELIUM. Marwan El-Sabban, Susi Sturzenegger and Bendicht U. Pauli, Department of Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853.
Metastases are tumor colonies that develop in a non-random fashion in distant, often multiple organ sites by dissemination of cancer cells from a primary malignancy. Metastatic colonization appears to be initiated by the attachment of blood-borne tumor cells to organ-specific adhesion molecules expressed on the surfaces of microvascular endothelial cells. Using timed fluorescence activated cell sorting and digital video imaging techniques, we show here that tumor cells previously labeled with the fluorescent dye BCECF begin to transfer dye to endothelium shortly after organ-specific adhesion is established. Dye transfer proceeds from endothelial cells that are in immediate contact with tumor cells to neighboring endothelial cells without dye leakage into the media. It is inferred from these experiments that metabolic coupling proceeds along heterotypic, membrane-junctional channels (gap junctions) and facilitates extravasation of tumor cells, possibly, by initiating endothelial cell retraction and capillary recanalization around the arrested tumor cell. Supported by NIH grant CA47668.

A 412 EXPRESSION OF ADHESION MOLECULES ON MURINE BRAIN MICROVASCULAR CELLS, Zsuzsa Fabry, Mari Waldschmidt and Michael N. Hart, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242
The expression of adhesion molecules on murine brain endothelial (En) and smooth muscle (SM) cells and their role in interactions with lymphocytes were studied. Brain En and SM cells expressed class II antigens. This expression was enhanced by IFN-γ. The Ia^+ En and SM cells were able to present antigens to different T cell clones and hybridomas. This interaction could be inhibited by anti Ia monoclonal antibodies. Intercellular adhesion molecule-1 (ICAM-1) expression was detected using flow cytometry analysis with biotinylated anti ICAM-1 antibody (γN1/1.7.4) kindly provided by Dr. Fumio Takei. According to our results, about 20-25% of the non-activated BALB/c brain En cells expressed ICAM-1 molecules. However, we could not detect any ICAM-1 expression on BALB/c brain SM cells. Interestingly, En cells isolated from SJL/J mouse strain showed much lower, if any, ICAM-1 expression. Activation of En cells by different cytokines (IFN-γ, IL-α, TNF-α) increased the ICAM-1 expression in a time and dose dependent manner, in contrast to TGF-β, which did not have any effect. The role of ICAM-1 on brain En cell-T cell interaction has been tested in adhesion, antigen presentation, and T cell activation assays using the anti ICAM-1 monoclonal antibody, anti LFA-1 and EDTA. The expression of adhesion molecules on brain vascular cells may participate in initiation of local immune response in the central nervous system.

A 413 DIFFERENTIAL EXPRESSION OF LFA1 ON CD4+ AND CD8+ CELLS IN AIDS AND AUTO-IMMUNE DISEASES IN MAN, Gilbert C.Faure, Jianqing Tang, Bernard Gobert, Corinne Amiel, Marie C.Béné. Laboratoire d'Immunologie. Faculté de Médecine de Nancy, 54500 Vandoeuvre les Nancy, France.
LFA1 is an adhesion molecule of the integrin family, involved in cell-cell interactions of immune recognition, and normally expressed on 100% of peripheral blood nucleated cells. Cytometric analyses confirm that more than 90% of peripheral blood lymphocytes (PBL) are labelled by anti-LFA1 antibodies in control subjects and often show a gaussian shape of the fluorescence peak. In previous studies, we have demonstrated an alteration of LFA1 expresssion on PBL from AIDS patients, and patients with such auto-immune diseases as Graves'disease or rheumatoid arthritis. This abnormal expression of LFA1 is characterized by a significant percentage of LFA1-negative cells, and also by a bimodal fluorescence peak in flow cytometry. We used computer-assisted analyses of cytometric data to investigate the expression of LFA1 on CD4+ and CD8+ PBL from patients of these three groups of diseases. CD4 or CD8 depleted suspensions of PBL were prepared using monoclonal antibody-coated metallic beads to confirm modelization data. These experiments demonstrated that LFA1 expression is high on CD8+ cells and low on CD4+ cells. This differential expression might have to deal with cytotoxicity in AIDS and with attempts of immunoregulating overactive CD4+ cells in auto-immunity.

A 414 TRIGGERING OF CD2 OR CD3 PROMOTES LYMPHOCYTE ADHESION BY ACTIVATION OF LFA-1. *Carl G. Figdor, Pauline Weder, Elly van de Wiel-van Kemenade, Theo W. Kuijpers[1] and Yvette van Kooyk.* Division of Immunology, The Netherlands Cancer Institute, 1066CX, Amsterdam, [1]Central Laboratory of the Netherlands Red Cross Blood transfusion Center, Amsterdam.

LFA-1 acts as a general leukocyte adhesion molecule mediating a variety of cell-cell interactions through binding to its ligand ICAM-1. Despite the fact that resting lymphocytes express both LFA-1 and ICAM-1 they do not spontaneously adhere. This indicates that an additional signal is required to induce LFA-1 mediated adhesion. A unique anti-LFA-1 antibody, NKI-L16, can activate the LFA-1 molecule by inducing a conformational change resulting in LFA-1 dependent cell adhesion. Furthermore, triggering of CD2 or CD3 molecules by monoclonal antibodies leads to activation of LFA-1, possibly by signals transduced via PKC. LFA-1 becomes persistently activated upon CD2 induction, whereas triggering of CD3 transiently activates LFA-1. These findings suggest that CD2 and CD3 can differentially regulate the affinity of LFA-1 for its ligand, via PKC dependent mechanisms resulting in a conformational change of the LFA-1 molecule. Furthermore, we demonstrate that this enhanced lymphosyte adhesion is caused by activation of the LFA-1 moleculeand not of its ligands.
From these data it is concluded that LFA-1 can be expressed on the cell membrane in an inactive and an active state in which Ca^{2+}/Mg^{2+} cations play an important role.

A 415 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF E2, A 32 kDa MOLECULE INVOLVED IN T-CELL ADHESION PROCESSES. Catherine Gelin, Didier Zoccola, Brigitte Raynal, and Alain Bernard, Laboratoire d'Immunologie des Tumeurs de l'Enfant, Institut Gustave Roussy, 94805 Villejuif Cedex, France.
E2 is a 32 kDa glycoprotein, which in addition to CD2, is involved in the process of rosette formation. A 1.11 kb cDNA was isolated from a λgt11 expression library. The primary structure deduced from the nucleotide sequence of its gene, comprises 185 amino acids and is devoid of N-linked glycosylation sites. The E2 protein is rich in proline residues and displays an organization typical of an integral membrane protein. Moreover, nucleotide sequencing revealed that E2 is the MIC2 gene product. Four epitopes are actually defined on E2 and are differentially involved in T-cell rosette formation. Moreover, the expression of those epitopes varies on different cell types, subdividing T cells into subpopulations with distinct functional repertoires. Finally, results obtained with E2 and CD2 transfected cells, permit us to analyze the involvement of E2, besides CD2, in T-cell rosette formation.

A 416 HUMAN NK CELLS EXPRESS ON CELL SURFACE AN INTEGRIN STRUCTURE WHICH MEDIATE THEIR ADHESION TO FIBRONECTIN, Gismondi A., Morrone S., Tarone G.£, Piccoli M., Santoni G.*, Frati L. and Santoni A.Department of Experimental Medicine, University of Rome "La Sapienza"; * Institute of Forensic Sciences, University of Macerata; £ University of Torino, Italy.
A large body of evidence has demonstrated that NK cells express the leukocytes members (LFA-1, Mac-1, p150-95) of integrins, a superfamily of dimeric receptors which share the ability of recognizing RGD sequence on adhesive proteins. These receptors, have been found to play a major role in the cytotoxic function of NK cells. In this study we have examined whether NK cells express another set of integrins mainly consisting of extracellular matrix receptors and initially identified at surface of stimulated T cells (VLA). Highly purified human LGL were stained with a monoclonal antibody recognizing the B1 subunit of VLA antigens (A1A5) and analyzed by flow cytometry. 90% CD16+ LGL were found to be positive while resting T lymphocytes did not react with antibody. We then functionally analyzed whether human LGL could adhere to fibronectin (25 ug/ml) coated on a solid substrate. 20-30% of CD 16+ LGL adhered to this glycoprotein. The adhesion was specifically blocked by a goat anti-FNr antiserum, arisen against affinity purified human placenta FNr, which reacts with human LGL when tested by FACS analysis and by a RGD-containing peptide. Moreover, A1A5 and anti-Fnr antibodies were able to immunoprecipitate from human LGL two bands migrating at 150 and 130 Kd. Taken together our data suggest that human NK cells express on cell surface a FNr-like molecule. The possible involvement of this molecule on NK cell functions is under investigation. Supported by: M.P.I. 40%

A 417 CHARACTERIZATION OF A cDNA ENCODING A MAJOR SURFACE PROTEIN OF VASCULAR ENDOTHELIAL CELLS. Anne Gougos and Michelle Letarte, The University of Toronto and The Hospital for Sick Children, Toronto, Ontario M5G 1X8.
The 44G4 glycoprotein is expressed constitutively at high levels on human endothelial cells of arteries, veins and capillaries. The antigen, originally localized with monoclonal antibody 44G4 generated against a pre-B leukemic cell line, is also expressed at lower levels on a small percentage of normal bone marrow cells. The 44G4 antigen is a dimer composed of subunits of apparent Mr 95,000; glycosidase digestion of 44G4 reveals the presence of both N- and O-linked oligosaccharide side chains on a polypeptide of Mr 60,000. A polyclonal antibody suitable for screening a cDNA expression library was generated against reduced and alkylated placental 44G4, purified by adsorption to pure placental antigen, and shown to react specifically with the reduced antigen of leukemic, endothelial and placental extracts. Eleven positive clones were isolated from an umbilical vein endothelial cell lambda-gt11 cDNA library (provided by J.E. Sadler, Howard Hughes Medical Center, St. Louis, MO), including a polyadenylated 2.6 kb cDNA. Comparison of this cDNA sequence to Genebank sequences indicates that 44G4 is a previously undefined molecule. An open reading frame of 1930 bp is present and contains the N-terminal sequence of placental 44G4, proving the identity of the isolate. The deduced amino acid sequence predicts a cysteine-rich polypeptide containing three potential N-linked glycosylation sites and a region of O-linked glycosylation rich in clusters of serine and threonine residues located proximal to the putative membrane-spanning domain. Northern hybridization reveals the presence of a major mRNA species of 3.4 kb which correlates with the cell surface expression of 44G4. The possible role of this newly identified major surface component of endothelium in interactions with lymphocytes, neutrophils and/or monocytes will be investigated.

A 418 INHIBITION OF CANINE PERIPHERAL NEUTROPHIL ADHERENCE BY URICASE, Dale F. Gruber, Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145
Xanthine oxidase and uricase both occupy penultimate positions in different pathways (or mechanisms) of purine nucleotide degradation. Besides their in vivo ability to degrade purine nucleotides, xanthine oxidase and uricase have both demonstrated some similarities in vitro to modulate neutrophil function(s). Both xanthine oxidase and uricase induce the cytosolic H_2O_2 production of isolated peripheral canine neutrophils. Although qualitatively similar in the induction of H_2O_2, xanthine oxidase and uricase are qualitatively divergent in other biological effects. Xanthine oxidase, and/or it's degradation products, have demonstrated significant chemotactic activity to neutrophils whereas uricase, at the levels examined, has not demonstrated any similar effects. It is functionally imperative to the role of the neutrophil in nonspecific resistance that it has the capacity to adhere prior to it's initiation of migratory function(s). The experimental effects in the presence of xanthine oxidase or uricase on the neutrophil's in vitro ability to adhere to plastic and glass surfaces was examined on lysis-isolated peripheral canine neutrophils. It was observed that uricase significantly reduced the ability of neutrophils to adhere to either type of solid substrate. The effects of uricase on neutrophil adherence were significant and linear throughout the concentration range examined (0.0075 to 0.03 U/ml, final concentration), were not ameliorated over time, and were not reversible upon washing. Xanthine oxidase at final concentrations between 0.125 and 0.5 U/ml did not significantly alter neutrophil adherence properties.

A 419 ENHANCEMENT OF MONOCYTE ADHERENCE TO MONOLAYERS OF BOVINE AORTIC ENDOTHELIAL CELLS BY NEURAMINIDASE TREATMENT. David G. Hassall and Ann-Marie Gladwin, Biochemical Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, Kent, U.K.
Human peripheral blood monocytes (PBMs) were exposed to 5U/ml neuraminidase for periods of up to 1 hour at 37°C in the presence of an endothelial cell monolayer. The cells were then washed to remove non-adherent cells and enzyme prior to the assessment of monocyte adherence by measurement of myeloperoxidase. Under these conditions, there was a time-dependent increase in the adherence of monocytes to endothelial cells. Pre-treatment of endothelial cells with neuraminidase did not enhance adherence, suggesting that exposure of new sites for adherence was likely to be occurring on PBMs. Labelling of the cell surface of PBMs with antibodies recognising the integrin subunits CD11a, CD11b, and CD11c following treatment with neuraminidase, demonstrated no increase in surface binding, indicating that enhancement of adherence was not mediated by an increased expression of these adherence molecules. We conclude that treatment of monocytes with neuraminidase exposes new sites for adherence separate from the CD11/CD18 integrin family.

A 420 CELLULAR RECEPTOR FOR UROKINASE-TYPE PLASMINOGEN ACTIVATOR. G. Høyer-Hansen, N. Behrendt, E. Rønne, V. Ellis, P. Kristensen, V. Kielberg, A.L. Roldan, M.V. Cubellis, F. Blasi and K. Danø. Finsen Laboratory, Rigshospitalet, DK-2100 Copenhagen Ø., and Institute of Microbiology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K., Denmark.

A specific cellular receptor (u-PAR) for human urokinase-type plasminogen activator (u-PA) was initially detected by cell-binding experiments. u-PAR can be visualized by cross-linking to radiolabeled amino terminal fragment of urokinase (ATF) or to DFP-inhibited u-PA followed by SDS-PAGE and subsequent autoradiography.

The human u-PAR has been purified, by affinity chromatography, from detergent extracts of PMA-stimulated U937 cells. The protein is heavily glycosylated, which is the reason for a marked molecular weight heterogeniety. The amino acid composition revealed a high content of cysteins. The N-terminal amino acid sequence of the purified human u-PAR was neither identical nor homologous to any known protein sequence.

With the aid of an oligonucleotide synthesized on the basis of the amino terminal sequence of the protein, a 1.4 kb cDNA clone coding for the entire human u-PAR has been isolated. The cDNA codes for a protein of 313 amino acids, preceded by a 21-residue signal peptide.

Cell-binding experiments using Lewis lung cells and radiolabeled mouse u-PA indicate that a cellular receptor for mouse u-PA is present on Lewis lung cells.

Several findings indicate that u-PAR plays a crucial role in focalizing pericellular proteolysis.

A 421 HUMAN LYMPHOCYTE HOMING RECEPTOR (CD44) INTERACTS WITH THE HEPARIN BINDING DOMAIN OF FIBRONECTIN, Sirpa Jalkanen and Markku Jalkanen, Departments of Medical Microbiology and Medical Biochemistry, University of Turku, SF-20520 Turku, Finland
Lymphocyte-high endothelial venule (HEV) cell interaction is an essential element of the immune system as it controlls lymphocyte recirculation between blood and lymphoid organs in the body. This interaction involves an 85-95 kDa class of lymphocyte surface glycoprotein(s), gp90Hermes (CD44). A subset of Hermes-defined homing receptor molecules is modified by covalent linkage to chondroitin sulfate. In this work we show that chondroitinase treatment of lymphocytes, or chondroitin sulfate treatment of HEV does not significantly inhibit lymphocyte binding to HEV suggesting that chondroitin sulfate is not involved in endothelial cell recognition of lymphocytes. However, affinity purified Hermes-antigen was observed to bind the heparin binding domain of fibronectin, and heparin, heparan sulfate and chondroitin sulfate were inhibitory for the binding. These findings suggest that lymphocytes use gp90Hermes molecules not only for binding to HEV at sites of lymphocyte entry to lymphoid organs, but also within the lymphatic tissue where this molecule - especially the subset modified by chondroitin sulfate - is used for interaction with matrix molecules such as fibronectin.

A 422 EXPRESSION OF ADHESION MOLECULES ON NORMAL, ACTIVATED B LYMPHOCYTES AND MALIGNANT PRE-PLASMA CELLS. Gitte S. Jensen, Rupesh Chawla, Eva A. Turley and Linda M. Pilarski, Dept. of Immunology, University of Alberta, Edmonton, Canada T6G 2H7

We have demonstrated expression of certain adhesion molecules on normal B lymphocytes after Pokeweed Mitogen-stimulation. Normal, predominantly resting, B cells do not express CD11b (LFA-1 family) or CD9 (platelet aggregation molecule), and express low densities of 4B4 (CD29, VLA-beta chain), but do express the Hyaluronate binding protein (HABP) which complexes with Hyaluronan (HA). We found a high expression of CD11b and CD9 after 3 days of culture, and of 4B4 at day 7 of culture. The expression of HABP went down over time. The cells were negative for LeuM3 (CD14) and LeuM1 (CD15) at all times. We hypothesize that the increase in expression of adhesion molecules coupled with a decrease of HABP/HA detachment complex on activated B cells reflect the changing needs for cell interactions and locomotion during stimulation and differentiation of the cells, and their capacity to home to potential sites of infection in vivo.

When analyzing malignant pre-plasma cells from peripheral blood of patients with multiple myeloma we found that these cells do express high amounts of CD11b, CD9, and 4B4, corresponding to normal, activated peripheral blood B cells. However, the malignant cells express high amounts of LeuM3 and LeuM1 as well. The expression of adhesion molecules on these abnormal B lineage cells might reflect their stage of activation as well as a capacity to metastasize/home to the bone marrow, thereby playing a crucial role in the biology of the disease.

A 423 CARCINOEMBRYONIC ANTIGEN (CEA) MAY BE A CELL ADHESION MOLECULE INVOLVED IN HUMAN COLORECTAL CARCINOMA (CRC) METASTASES. J.M. Jessup, R. Hostetter, C.A. Toth, and P. Thomas. UT M.D. Anderson Cancer Center, Houston, TX and Harvard Med. School, Boston MA.
CEA is a glycoprotein that is an important tumor marker for CRC but whose function is not defined. Since CEA is a member of the immunoglobulin supergene family, it may function in extracellular recognition and adhesion. These functions were assessed in experimental metastasis assays in athymic nude mice and in cell adhesion assays. To test whether CEA directly promoted metastasis, purified CEA was injected intravenously (iv) into groups of mice that 30 min later were injected intrasplenically (is) with CRC cells. CEA serum levels were 400 ng/ml at this time and CEA was present on murine Kupffer cell and hepatocyte membranes. When CRC cells are injected (is), they enter the circulation and may implant in the liver to form experimental metastases. Four CRC were tested: mHC 1410, a highly metastatic line; HC 2998, a nonmetastatic CRC; and KM-12c and MIP 101, 2 weakly metastatic CRCs. CEA pretreatment enhanced experimental metastasis by KM-12c and MIP 101 but not mHC 1410 or HC 2998. Asialo CEA and bovine serum albumin (BSA) did not enhance experimental metastasis. The implantation of 125I IdUdr labelled KM-12c and mHC 1410 cells was enhanced by CEA pretreatment while that of HC 2998 was not (MIP 101 was not tested). The CRC lines whose metastatic potential was enhanced by CEA bound to CEA immobilized on a solid phase. CRC cell lines did not bind to alphal-acid glycoprotein or BSA attached to a solid phase. CEA did not make a nonmetastatic CRC metastatic in nude mice or a metastatic CRC more aggressive, but did enhance the metastatic potential of 2 CRC that were weakly metastatic. Thus, CEA may promote metastasis by facilitating the binding of CRC to liver cells.

A 424 MECHANISMS OF CR3 ACTIVATION IN THE FORMATION OF STABLE AGGREGATES OF NEUTROPHILS. Taco Kuypers, Leo Koenderman, Arthur Verhoeven and Dirk Roos. Centr. Lab.of the Neth. Red Cross Blood Transf. Serv., and Lab. Exp. Clin. Immunol. Univ. of Amsterdam, Amsterdam, The Netherlands
Human neutrophils aggregate after stimulation with various stimuli. This process depends primarily on the presence of complement receptor type 3 (CR3), which is not present on neutrophils from Leukocyte Adhesion Defiency (LAD) patients. Neutrophils, separately stained red with hydroethidine (HE) and green with sulfofluorescein (SFDA), were mixed and the number of double-coloured aggregates was subsequently measured by FACS analysis. Formation of aggregates required continuous triggering, as indicated by the rapid disaggregation observed after removal of the stimulus PdBu (an activator of protein kinase C) or after depletion of intracellular ATP by the addition of glycolysis inhibitors. Several data indicate that neutrophil activation is needed to convert CR3 to an active state able to recognise its putative counterstructure on the opposing cell. Interestingly, staurosporine, completely inhibiting intracellular protein phosphorylation in response to PMA, only slightly affected the PMA-induced aggregation. In contrast, the aggregation induced by the diacylglycerol analog OAG was completely inhibited. Thus, activation of CR3 leading to neutrophil aggregation can be induced in a PKC-dependent as well as in a PKC-independent way. The role of PKC in homotypic neutrophil aggregation in response to other stimuli and in other adhesive responses will be discussed.

A 425 IDENTIFICATION OF PROTEINS INVOLVED IN INVASIVENESS OF T-CELL LYMPHOMAS, Geertje La Rivière, Cor A. Schipper, Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; Lotte Kuhn, Ivan Lefkovits, Basel Institute for Immunology, Basel, Switzerland.

Fusion of normal T-lymphocytes with non-invasive, non-metastatic BW5147 T-lymphoma cells resulted in hybrids, most of which were highly invasive in both hepatocyte and fibroblast monolayers in vitro and highly metastatic in syngeneic mice in vivo. In addition, non-invasive hybrids were obtained which were tumorigenic subcutaneously, but did not metastasize after intravenous injection. This suggests that for T-cell lymphomas, invasive potential is a prerequisite for colonization of tissues from the blood stream.

To identify proteins involved in invasion we analyzed a panel of 5 highly invasive and 4 non-invasive T-cell hybridomas, as well as the BW5147 lymphoma, by two-dimensional gel-electrophoresis after metabolic labeling with ^{35}S-methionine or ^{3}H-leucine. Among 925 spots detected, we found only two major differences between invasive and non-invasive cells. One of these proteins was specifically expressed on highly invasive hybridomas, had an apparent M_r of $\pm$ 130 kD, a pI of $\pm$ 5.3 and was detected on the leucine but hardly on the methionine gels. This protein might be involved in the invasion process itself. In contrast, the other protein was found exclusively in the non-invasive hybridomas and the BW5147 cells. The apparent M_r was 15 kD and the pI $\pm$ 7.6, and fractionation experiments suggested it to be cytoplasmic. This protein might serve as an invasion suppressor. To study the function of this protein we are presently purifying it to obtain amino acid sequence data and to clone the corresponding gene.

A 426 EXPRESSION OF CD44 IN MALIGNANT CELLS, Michelle Letarte, Sonia Vera, Adonna Greaves and Elizabeth J.Quackenbush, Department of Immunology, Hospital for Sick Children, and University of Toronto, Toronto, Canada, M5G 1X8.

The expression of the p85 glycoprotein (CD44) in human brain and leukemic cells has been the focus of our laboratory for several years. The recent demonstration of the identity of Hermes, a molecule implicated in the adhesion of lymphocytes to endothelium, and of CD44 proteoglycan, raises interesting questions as to the role of this molecule on fibrous astrocytes in normal brain and in malignant cells of diverse origin. The expression of CD44-specific mRNA transcripts was analysed in several melanoma, leukemia and astrocytoma cell lines using PBL32-cDNA clone (provided by E. Butcher, Stanford University, CA). The same three mRNA species of 5.0-2.3- and 1.7-kb were seen in all CD44-positive lines tested. Negative cell types included Nalm-6 leukemia line, Y79 retinoblastoma line and normal endothelial cells derived from umbilical cord vein. A correlation between the amount of surface antigen and the level of mRNA was observed. Blasts of immature pre-B ALL and myeloid phenotypes, obtained from patients with leukemia, expressed very high levels of CD44 as did the astrocytoma lines. CD44 molecules were purified from leukemic cells, treated with trypsin and CNBr and fractionated by HPLC. Three peptides could be aligned with the amino acid sequence deduced from the cDNA (residues 49-54, 59-66 and 309-323). The isolation of peptide 309-323 confirms the existence of a processed glycoprotein bearing the 72 amino acid long cytoplasmic tail. Two distinct forms of CD44 (Mr 85,000 and 77,000) can be seen in leukemic cells and may represent molecules cross-linked differentially to intracellular signal transduction and/or cytoskeletal elements. CD44 is expressed in large amounts on malignant cells and could play a role in the adhesion to matrix proteins and/or other cells within a tumor, in tumor invasiveness and in metastatic potential.

A 427 MONOCLONAL ANTIBODIES DIRECTED AGAINST CD9 INDUCES HOMOTYPIC CELL-CELL INTERACTION OF A HUMAN B CELL LINE BY A NOVEL MECHANISM A. Masellis-Smith, J. Seehafer, J.R. Slupsky, and A.R.E. Shaw. Department of Medicine, Cross Cancer Institute, University of Alberta, Edmonton, Canada

Monoclonal antibodies (mAb) directed to cluster designation antigen-9 (CD9) trigger a variety of biological function of which homotypic platelet aggregation is the best characterized. We have investigated a panel of mAbs (50H.19, ALB_6 and BA-2) directed against the CD9 antigen for their ability to induce homotypic cell-cell interactions of cells other than platelets. All anti-CD9 mAbs tested induced homotypic interaction of NALM-6 and HOON, but not of the CD9 negative B cell line Raji. MAb directed against β_2 microglobulin and CD24 failed to mediate aggregation, suggesting that the homotypic interaction induced is specific for mAb activation of CD9. Such cell adhesion has been ascribed to lymphocyte function-associated antigen-1(LFA-1). However, as both Nalm-6 and Hoon are negative for β2 integrins, this implicates CD9 as a novel LFA-1 independent mechanism of cell adhesion. In contrast to LFA-1 mediated adhesion, the aggregation mediated by anti-CD9 mAbs was found to be dependant upon Ca^{2+}. Mg^{2+} was not sufficient for induction of aggregation, but did enhance Ca^{2+} mediated adhesion. The induction of cell-cell interaction also required an intact cytoskeleton, as cytochalasin B, a microfilament inhibitor, abrogated the induction of aggregation, did not proceed at 4C and was not inhibited by RDG containing peptides. These results suggest at novel mechanism of cell adhesion which appears to be mediated by receptors other than LFA-1 or RGD dependent receptors.

A 428 EXPRESSION OF IL-8 mRNA IN HUMAN MONOCYTES, ENDOTHELIAL CELLS, AND DERMAL FIBROBLASTS IS REGULATED BY A DISTINCT SET OF CYTOKINES/MEDIATORS. V. Mielke, T. Ibs, M. Sticherling, J.-M. Schröder, W. Sterry, E. Christophers., Dept. of Dermatology, University of Kiel, FRG. Recently, a neutrophil-activating peptide, Interleukin 8 (IL-8) produced by LPS stimulated human peripheral blood monocytes was biochemically purified and functionally characterized by different investigators. Work conducted in our lab showed that IL-8 as well as variants of this peptide are produced by a variety of cells and that lesional psoriatic scales contain large amounts of IL-8. Functionally, IL-8 is a potent chemoattractant for PMNL and for T-lymphocytes. Additionally, IL-8 induces the binding of PMNL to endothelial cells, via a mechanism that involves altered expression of the leukocyte CD11/CD18 glycoproteins. In this study we analysed the inducibility (IL-1, TNFα, LPS, etc.) and subcellular distribution of IL-8 mRNA in relation to the 28s rRNA in monocytes, dermal fibroblasts and endothelial cells using non-radioactive in situ hybridization techniques. All three cell types express IL-8 mRNA upon stimulation with IL-1 and TNF; whereas LPS is only a stimulus for endothelial cells and monocytes. Especially in dermal fibroblasts IL-8 mRNA has a distinct subcellular distribution pattern and is found adjacent to the nucleus and with small spots in the nucleus, whereas the the corresponding protein (Il-8, detected by a monoclonal antibody 46E5) was only found in the cytoplasm.

A 429 NEURAL CELL ADHESION MOLECULE ASSOCIATED WITH POOR PROGNOSIS IN LUNG CANCER, Kitty Moolenaar, Robby Kibbelaar, Nico van Zandwijk, Wolter Mooi, and Rob Michalides. Dept. of Tumor Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.
Monoclonal antibody, Mab 123C3, generated against small cell lung carcinoma (SCLC) stains also 20 % of non-SCLCs. The follow-up data show a poor postoperative clinical course of the group of 123C3-positive non-SCLC. The 123C3 antigen is, therefore, a prognostic marker for lung cancer. Mab 123C3 appears directed against the neural cell adhesion molecule, NCAM, which is a cell surface glycoprotein involved in intercellular adhesion. NCAM occurs in various isoforms with different biological functions, as result of alternative RNA splicing and/or post-translational modifications.
One such a modification, the alpha 2,8 polysialylation, occurs as a characteristic modification of an embryonal form of NCAM, and results in a reduced adhesion. This alpha 2,8 polysialic acid form of NCAM is present on SCLC. In immunoprecipitates, this appears as a diffusely stained band of 200-250 kDa, which, after neuraminidase treatment, resolves into two bands of 145 and 185 kDa. These proteins represent the transmembrane isoforms of NCAM. The 4.3 and 2.9 kb mRNAs of NCAM in the SCLC cell line H69 suggest also the presence of non-transmembrane isoforms of NCAM. At present we are studying whether splice variants of NCAM which are characteristic for SCLC do exist.

A 430 *DIFFERENTIAL EFFECTS OF THE PROTEIN KINASE INHIBITORS H7 AND STAUROSPORINE ON PHORBOL ESTER AND CYTOKINE STIMULATED ICAM-1 EXPRESSION IN ENDOTHELIAL CELLS.* C. Myers, S. Desai, L.G. Letts and R. W. Wallace, Department of Pharmacology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877.

ICAM-1, an adhesion glycoprotein expressed on endothelial cells (ENDO), plays an important role in leukocyte-ENDO interactions, an early and necessary event in the inflammatory process. ICAM-1 is induced by cytokines such as IL1β and TNFα, but little is known concerning the intracellular regulatory mechanisms which trigger ICAM-1 expression. Using an ELISA assay, we found that the phorbol ester PMA, a protein kinase C agonist, increased the level of ICAM-1 more than ten-fold (IC50 of 2±1 ng/ml, n=3); PMA-induced changes in another cell surface antigen, 14E5, ranged from 1.2 to 1.6 fold. Neutralizing antibodies to IL1β and TNFα failed to block the induction of ICAM-1 by PMA; thus, ICAM-1 expression in response to PMA is not secondary to PMA-induction of IL1β and/or TNFα. Pretreatment with H7 blocked PMA-induced ICAM-1 expression (IC_{50}=15 μM, n=2) as did pretreatment with staurosporine (IC_{50} between 50 and 100 nM, n=2); >90% inhibition was achieved with 50 uM H7 and 300 nM staurosporine. H7 also inhibited IL1β- and TNFα-induced ICAM-1 expression (IC50 of 6±1 μM and 7±3 μM, n=3; >97% inhibition at 50 uM); however, staurosporine did not inhibit IL1β- and TNFα-induced ICAM-1 expression. H7 and staurosporine were slightly toxic at the highest concentrations tested; H7 caused morphological changes (cell rounding) at 50 uM. Our results suggest that PMA-induced ICAM-1 expression may be triggered through activation of protein kinase C, but that ICAM-1 induction by IL1β and TNFα may involve other regulatory pathways.

A 431 THE USE OF THYMIC EPITHELIAL CELL LINES TO STUDY THYMOCYTE/EPITHELIUM INTERACTION. Ph. Naquet, H. Lepesant, H.T. Hé and M. Pierres. Centre d'Immunologie INSERM/CNRS Marseille-Luminy, Case 906, Cedex 9, 13288 Marseille, France.
The goal of our study was to use murine thymic epithelial (MTE) cell lines as a model system to study adhesion between thymocytes and epithelial cells. With a quantitative adhesion assay described by Mc Clay, we found that thymocytes efficiently interacted with MTE lines. Both LFA-1, CD8 and CD4 molecules were shown to play a role as adhesion molecules. The involvement of CD4 and CD8 was dependent upon the induction of MHC antigens by interferon; as a result $CD4^+CD8^+$ thymocytes and less so immature thymocytes were interacting the most efficiently with these cell lines. Polymorphic regions of MHC molecules did not significantly alter the level of adhesion. We then focused our interest on the identification of other adhesion molecules on thymocytes and epithelium. A panel of thymocyte- or epithelial cell-specific monoclonal antibodies were tested for their ability to interfere with cell adhesion. Histologic analysis on thymus sections allowed us to establish the tissue distribution of various markers in vivo. We will present our initial results suggesting the implication of other T or epithelial cell markers in cell interaction. Similarly, when epithelial cells were treated with phospholipase C, the interaction between thymocytes and cell lines was diminished, supporting the involvement of glypiated molecules. We have shown that these lines could serve as efficient antigen-presenting cells for T cell hybridomas but not T cell clones. We are currently testing the possibility that these lines might lack accessory molecules for cell activation.

A 432 MACROPHAGE RECOGNITION OF TUMORIGENIC MURINE ERYTHROLEUKEMIA CELLS: CELL SURFACE STUDIES. Charles C. Pak and Isaiah J. Fidler, Department of Cell Biology, UT M.D. Anderson Cancer Center, Houston, TX 77030. Tumoricidal macrophages recognize and lyse tumor cells yet leave non-tumorigenic cells unharmed. The mechanisms used by macrophages for this selective recognition are unknown. Murine erythroleukemia cells (MELC) are tumorigenic erythroid precursor cells that can be chemically induced to differentiate to a non-tumorigenic non-dividing cell (DIFC). We have studied the interaction between tumoricidal macrophages and MELC in order to better understand the initial macrophage recognition of tumor cells. Macrophages activated to a tumoricidal state recognize and bind MELC but not DIFC. Removal of sialic acid residues by neuraminidase from tumoricidal macrophages or MELC does not affect binding by activated macrophages, suggesting that the binding is not a simple ionic interaction nor are the terminal sialic acids involved in the binding at either the target or effector cell surface. The binding does involve proteins and/or glycoproteins since protease treatment of the macrophages or MELC significantly reduces the binding. Divalent cations are required for the macrophage-MELC interaction. MELC at various stages of their cell cycle were bound equally well by macrophages, showing that recognition is independent of cell cycle. These observations provide a closer understanding of the mechanisms involved in the tumoricidal macrophage-tumor cell interaction.

A 433 MATRIX REGULATION OF ENDOTHELIAL CELL ADHESION MOLECULES IN ORGAN PREFERENCE OF TUMOR METASTASIS. Bendicht U. Pauli, Department of Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853.
Many cancers display characteristic organ colonization patterns that do not fit simple, anatomical-mechanical trapping theories of tumor cell dissemination. Organ preference of metastasis appears to be mediated partly by the selective attachment of tumor cells to organotypic adhesion molecules on the surfaces of microvascular endothelium. These molecules are lost rapidly when endothelial cells are cultured on plastic surfaces, but are recovered when cells are grown on extracellular matrix components (ECMC) of the metastasized organ. Using monolayers of endothelial cells that are grown on ECMC from various organs, we show that tumor cells which metastasize to a given organ, have a significantly higher binding affinity for endothelial cells grown on ECMC of the preferred metastasized organ, than they have for endothelial cells grown on ECMC of any other organ not colonized by tumor cells. Various adhesion inhibition experiments and enzyme studies indicate that endothelial adhesion of lung-metastatic tumor cells appears to be mediated by lectin-like molecules, whereas that of liver-metastatic tumor cells may be mediated by a galactosyltransferase. Supported by NIH grant CA47668.

A 437 THE MIGRATION OF LARGE GRANULAR LYMPHOCYTES, Kristina Somersalo and Eero Saksela, Department of Pathology, University of Helsinki, Haartmanink. 3, 0290 Helsinki SF.

In immune defence against tumor cells and virus-infected cells the interaction of lymphocytes with the extracellular matrix plays an important role. We have examined the migration of large granular lymphocytes (LGL) through 3μm pore sized Nuclepore filters and the effect of the matrix proteins on migration. A strong enrichment of $CD16^+$ cells with LGL morphology, but reduction of $CD3^+$ cells was found among migrating cells after 1, 2, 4 or 8 hours incubation in a Boyden invasion chamber. After 24 hour incubation also agranular lymphocytes, mainly $CD3^+$ T lymphocytes, had started to migrate. About 3/4 of LGL was capable of migrating during 24 hours. With fibronectin coated filters significantly more ($p<0.05$) LGL migrated through filters when compared to untreated filters. Also laminin coating slightly facilitated migration. When filters were coated with both laminin and fibronectin, rather than with either protein alone, more LGL migrated through the filters. Collagen I coating prevented LGL migration, whereas collagen IV had no effect. The RGD sequence binding site had no role in LGL migration through untreated filters, but when filters were coated with fibronectin or laminin, or with both fibronectin and laminin, RGD prevented migration to the level of untreated filters. Both original and migrated cells had high cytotoxicity towards LGL sensitive target cells (K562). Our results show that unstimulated LGL are capable of rapid migration through porous membranes and cell surface fibronectin and probably laminin receptors are utilized in this process.

A 438 EXPRESSION OF CD44 CONFERS A NEW ADHESIVE PHENOTYPE ON TRANSFECTED CELLS. Tom St. John, Joey Meyer, Rejean Idzerda and W. Michael Gallatin, Division of Basic Sciences, Fred Hutchinson Cancer Research Center 1124 Columbia Street, Seattle, WA 98104.

Normal function of the immune system requires that adhesive contacts be formed between many disparate cell types. For example, lymphocytes interact with specialized endothelium in lymphoid tissues as part of a process which targets functionally distinct subsets to appropriate microenvironments. One cell surface glycoprotein, CD44, has been linked to this process. The broad tissue distribution of CD44 on cells of nonhemopoietic origin would argue for its involvement as an adhesion molecule in other tissue systems as well. Accordingly, we have found that this glycoprotein can promote cell-cell adhesion in another cellular context, namely aggregation of fibroblasts. The function of the CD44 glycoprotein as an adhesion molecule was directly tested by transformation of a CD44 cDNA into mouse fibroblasts. This cDNA was expressed as a heavily modified cell surface protein reactive with several monoclonal antibodies recognizing glycoproteins now identified as CD44. Independent transfectants exhibited a new self-adhesive phenotype, forming large aggregates in when placed in suspension. In variants derived from a clone of cells, aggregation competence segregated with expression of the transfected gene. This CD44-mediated adhesion was blocked specifically by monoclonal antibodies binding one immunologically defined region of CD44. Nontransfected L cells did not self-aggregate but were capable of adhering to the transfectants indicating that at least one ligand for this adhesion molecule is expressed by mouse fibroblasts.

A 439 UPREGULATION OF ADHESION MOLECULES IN INFLAMMATORY SKIN DISEASES, W. Sterry, I. Kellner, G. Sterry, U. Konter, Dept. of Dermatology, University of Kiel, F.R.G.

Cellular adhesion is a major prerequisite for leukocyte infiltration in inflammatory dermatoses and mediated by various cell surface molecules. In relation to the expression pattern in normal human skin we investigated the expression pattern of adhesion molekules (β1-integrins, β2-integrins, ICAM-1, LFA-3 and CD2) in a variety of inflammatory skin disorders (n=49). Compared with normal human skin, keratinocytes in diseased skin showed upregulated expression of ICAM-1, VLA-3 (multifunctional receptor), VLA-5 (fibronectin receptor) and VLA-6 (CD49f; laminin receptor), which correlated well with the extent of the intraepidermal infiltrate. Almost every infiltrate-cell expressed LFA-1 (CD11a/CD18), LFA-3 (CD58; ligand of CD2) and CD2 (ligand of LFA-3). The percentage of VLA-4 and 5 positive cells in the subepidermal infiltrate was about 60-70%. Remarkably, VLA-2 (CD49b; a collagen-receptor expressed in long term T cell cultures) was not observed in our biopsies. In lichen planus, the subepidermal infiltrate showed a high percentage of VLA-1, 3 and 6 positive cells, whereas no or only moderate expression of these molecules was found in psoriasis, parapsoriasis en plaques and contact dermatitis. Interestingly, the intraepidermal lymphocytes in psoriasis and pityriasis lichenoides were VLA-1 positive, while the subepidermal infiltrate was VLA-1 negative. A variable portion of epidermal Langerhans cells showed neoexpression of ICAM-1, VLA-4 and 5, especially in psoriasis and pityriasis lichenoides.

A 440 COLLAGEN RECEPTORS AND THE CONTROL OF T-LYMPHOCYTE MOTILITY

Karl-Gösta Sundqvist, Ignacio Arencibia and D. Hauzenberger. Dept. of Clinical Immunology, Karolinska Institute, Huddinge, Sweden

T lymphocytes attached to two-dimensional collagen substrata both in the presence and absence of serum but did not adhere or adhered poorly to substrata coated by fibronectin, laminin, IgG and albumin. In contrast, T-blasts (MLC-induced) adhered to fibronectin-coated substratum. During contact with substratum-bound collagen for a 24 hour period 47±15% of the freshly purified lymphocytes from separate individuals developed motile behaviour whereas 16±4% of the cells became motile on fibronectin. Gelatin (denatured collagen) also mediated attachment of lymphocytes to surfaces but only at comparatively high concentrations (40 mg/ml). Collagen and gelatin in solution also caused agglutination and motility of the vast majority of fresh T lymphocytes whereas fibronectin and other proteins, when presented in soluble form, did not. Cell agglutination was maximal at moderate (10 or 20 mg/ml) and cell motility at "low" gelatin concentrations (1 to 10 mg/ml). High gelatin concentrations (20 and 40 mg/ml) did not induce motile behaviour. Cytochalasin B (CB) augmented the proportion adherent cells on gelatin coated substrate. Taken together these results indicate that circulating lymphocytes have a collagen/gelatin binding plasma membrane component. Crosslinking of this component is a likely explanation for the selective inducing effect of gelatin and collagen on lymphocyte motility. The lymphocyte plasma membrane contains collagen binding components with a molecular weight of >200 kd, 130 kd, 90 kd, 55 and 45 kd. The components with a molecular weight of >200 kd, 130 and 55 kd also react with with anti-fibronectin antibodies. Thus, interactions with the extracellular matrix may control lymphocyte locomotor capacity.

A 441 **A NOVEL MULTIGENE FAMILY ENCODING MOUSE CELL SURFACE PROTEINS WITH LECTIN-LIKE DOMAINS**, Fumio Takei, Simon Wong, Colm Kelleher, Po-Ying Chan and Dixie Mager, Terry Fox Laboratory, BC Cancer Research Center and the Departments of Pathology and Medical Genetics, University of British Columbia, Vancouver, B.C., Canada

YE1/48 is a murine cell surface glycoprotein consisting of two disulfide- linked 45-50 kd chains. It is expressed at high levels on transformed T lymphocytes and pre-B cells and at very low levels on normal lymphocytes. We have recently isolated a cDNA encoding YE1/48. The deduced amino acid sequence of YE1/48 indicates that it is a type II transmembrane protein. It contains a lectin-like domain that has significant homologies with lectin domains of several cell surface proteins including the rat asialoglycoprotein receptor, the human low affinity IgE receptor, the murine lymphocyte homing receptor Mel-14, the human endothelial leucocyte adhesion molecule ELAM-1, and the human platelet granule membrane protein GMP-140. At the nucleotide level, YE1/48 has no homology with any of the above proteins. Using genomic Southern blot analyses, we have shown that the region of the YE1/48 cDNA encoding the putative lectin domain hybridizes with multiple restriction fragments whereas other regions give the pattern of a single copy gene. Therefore, YE1/48 seems to belong to a multigene family with similar lectin-like domains. We have now isolated genomic clones that encode YE1/48 as well as two other genes homologous to YE1/48. The YE1/48-related genes have open reading frames and appear to encode functional proteins, suggesting that they are members of a multigene family that encodes cell surface proteins containing lectin-like domains. These proteins may function as cell adhesion molecules.

A 442 LAK CELL ADHERANCE TO TUMOR CELL GLYCOPROTEINS: BLOCKING BY ANTI CD-18 MONOCLONAL ANTIBODIES. Joseph A. Tami, Philip D. Hall, David J. Friedman, and David H. Boldt. The Department of Medicine, Division of Hematology, University of Texas Health Science Center at San Antonio, San Antonio, TX. 78284-7880.

Lymphokine activated killer (LAK) cells, used in conjunction with interleukin 2 (IL-2), are currently being tested as a form of adoptive immunotherapy in the treatment of cancer. We have developed a unique immunobinding assay which allows one to examine the binding of intact radiolabeled LAK cells to specific tumor cell glycoproteins. Tumor whole cell lysates or membrane preparations (Daudi and K562 cells) are seperated on 3-17% gradient SDS-polyacrylamide gels and electrophoretically transferred to polyvinylidene difluoride (PVDF, Immobilon-P) membranes. The tumor glycoproteins are incubated with chromium labeled human LAK or NK cells and the strips then autoradiographed overnight. Several major and minor protein bands are consistently labeled. LAK and NK cells appear to recognize similar molecular weight bands. Pre-treatment of the tumor cells with trypsin eliminated LAK cell adherance. The pre-treatment of the LAK cells with monoclonal antibodies to CD-18 (LFA Beta chain, gp 90) inhibits binding as well. Many, if not all, of the previously observed bands disappear, indicating that the beta chain of LFA plays an important role in the adherance of LAK cells to their tumor targets.

A 443 A NOVEL T CELL ADHESION MOLECULE THAT IS INVOLVED IN IMMATURE T CELL PROLIFERATION, toshimitsu Uede, Yoshihiro Torimoto, Satoshi Nagoya and Kokichi Kikuchi, Department of Pathology, Sapporo Medical College, S-1, W-17, Sapporo, 060, Japan. A monoclonal antibody recognizing a novel rat T cell antigen was generated and was designated as 8H3 antibody. During thymic ontogeny, 8H3 antigen is one of the earliest T cell specific molecule. Early fetal double negative thymocytes express 8H3 antigen which lack the expression of T3/T cell receptor (TCR). Crosslinking of 8H3 antigen on double negative thymocytes and adult thymocytes as wekk as splenic T cells by 8H3 antibody and second antibody initiated an increase in the concentration of cytoplasmic free Ca^{2+} and cell proliferation was observed in the presence of exogenous IL-2. Interestingly, crosslinking of 8H3 antigen on double negative thymocytes induced cell proliferation in the abscence of IL-2. The ligand for the 8H3 antigen was searched. 8H3 antibody immunoprecipitated 180,000, 120,000 and 90,000 dalton components from rat thymocytes and T cells. Rat thymocytes specifically bound to fibronectin, but not to laminin, vitronectin, and collagen. The binding of rat thymocytes to immobilized synthetic peptide, Gly-Arg-Gly-Asp-Ser-Pro-Cys-BSA was inhibited by 8H3 antibody as well as Gly-Arg-Gly-Ser-Pro-Cys, but not by Gly-Arg-Ara-Asp-Pro-Lys. In order to clarify the relationship between 8H3 antigen and Integrin, polyclonal rabbit antiserum against 8H3 antigen was prepared. Antiserum against beta-1 of VLA immunoprecipitated alph and beta subunit of VLA as well as unknown 70,000 components from U937 cells. Preliminary data indicated that polyclonal 8H3 antibody recognize the same 70,000 component from U937 cells. These data indicate that 8H3 antibody recognize a receptor molecule which may belong to Integrin family.

A 444 Plasma membrane ruffling: a correlate of invasive capacity? Nicolas van Larebeke, Marc Bracke and Marc Mareel. Laboratory of Experimental Cancerology, University of Gent, B9000 Gent, Belgium. We developed a simple method for quantifying plasma membrane ruffling using automatic image analysis. We studied three pairs of cell lines. The human mammary cell line MCF-7/6, the ras transformed dog kidney cell line ras-MDCK and the ras transformed mouse mammary gland cell line NM9pneoT24E12, all of which were invasive in precultured embryonal chicken heart fragments, showed more intense ruffling than the respective related or parental MCF-7/AZ, MDCK(AZ) and NM9 cell lines, none of which were invasive. Retinoic acid 10^{-6}M induced the capacity to invade in MCF-7/AZ cells, and also increased ruffling in these cells. In MCF-7/6 cells, on the contrary, retinoic acid 10^{-6}M inhibited both invasive capacity and ruffling activity. Dexamethasone (dexa)10^{-6}M and the commercial serum surrogate Ultroser G both inhibited as well invasive capacity as ruffling in MCF-7/6 cells. Triiodothyronine (T3)10^{-5}M, ß-oestradiol (E_2)10^{-6}M and dexa 10^{-6}M did not induce invasive capacity in MCF-7/AZ cells, and did not affect ruffling activity or lowered (E_2 and dexa) it. E_2 and T3 did not inhibit invasion by MCF-7/6 cells, and did not affect (T3) ruffling activity or increased (E_2) it. So, agents that alter plasma membrane ruffling also manipulate the invasive phenotype.

A 445 ADHESION OF NORMAL & NEOPLASTIC LYMPHOID CELLS TO FIBROBLASTS. K.J. Chapman & A.S. Jack, Department of Pathology, University of Leeds, Leeds, LS2 9JT. Neoplastic lymphoid cells displace normal lymphocytes from lymphoid organs in vivo. To study whether this is a function of differential adhesion to connective tissues we studied the binding of the T-cell lymphoma lines Hut78 (ICRF) and J6 and normal peripheral blood lymphocytes to normal fibroblasts in vitro.

J6 cells adhered much more avidly than either Hut78 or peripheral lymphocytes, which were not significantly different. This result was surprising in view of the almost complete lack of expression of LFA-1 by J6. Furthermore the addition of anti-LFA-1 to Hut78 and PBL had only a marginal effect on adhesion. Hut78 differed from J6 and PBL in having a very low level of CD2 expression. When anti-CD2 was added to J6 and PBL adhesion was significantly increased; no effect was seen with Hut78. This CD2 stimulated enhancement was completely abrogated by anti-LFA-1.

Thus, although this simple assay did not show a clear difference in adhesion between neoplastic and normal lymphoid cells it does illustrate how complex interactions between cell adhesion molecules could be perturbed by altered expression of one component in a neoplastic cell.