INTEGRINS: CELL ADHESION AND TRANSMEMBRANE COMMUNICATION IN DEVELOPMENT AND DISEASE

Organizers: Robert Pytela and Vito Quaranta

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Integrins: Cell Adhesion and Transmembrane Communication in Development and Disease

Integrins: Expression and Ligand Binding

X 001 STRUCTURAL BASIS OF THE DISTINCT CELL BIOLOGICAL RESPONSE PROMOTED BY INTEGRINS $\alpha v\beta 3$ AND $\alpha v\beta 5$, David I. Leavesley[1], Greg Ferguson[1], Elizabeth Wayner[2], and David A. Cheresh[1], [1]The Scripps Research Institute, La Jolla, CA, [2]University of Minnesota, Minneapolis, MN.

The integrin αv subunit has the unique property of associating with multiple β subunits. Among these, $\beta 3$ and $\beta 5$, when associated with αv, promote cell adhesion to vitronectin. The amino acid sequence of $\beta 3$ and $\beta 5$ are approximately 60% identical overall, yet their cytoplasmic tails are structurally distinct, suggesting that $\alpha v\beta 3$ and $\alpha v\beta 5$ promote distinct biological responsiveness to a vitronectin matrix. To this end, we have found that $\alpha v\beta 3$ can promote a vitronectin mediated cellular adhesion event leading to spreading and migration, while cells attach to vitronectin via $\alpha v\beta 5$ attach, yet fail, to spread or migrate. Expression of chimeric $\beta 3/\beta 5$ integrins suggest that the variation in cytoplasmic tails accounts for the distinct biological properties promoted by $\alpha v\beta 3$ and $\alpha v\beta 5$ during cell adhesion to vitronectin.

X 002 LIGAND-INTEGRIN INTERACTIONS IN CELL ADHESION AND MIGRATION, Kenneth M. Yamada, Shin-ichi Aota, Steven K. Akiyama, and Susan E. LaFlamme, Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892.

The specificity of integrin heterodimer binding to ligands appears to dictate which integrin(s) mediate cell adhesion and migration on a specific substrate or adhesive protein such as fibronectin, collagen fibrils, or laminin. Although short peptide adhesive-recognition sequences in these molecules are crucial for such binding, other polypeptide information also appears to be essential for the specificity and full affinity of these interactions. Studies with the Arg-Gly-Asp and Leu-Asp-Val minimal peptide sequences from fibronectin (and apparently other sequences from type I collagen), suggest that cell adhesion and migration depend on both these minimal core sequences and on other polypeptide information that contributes synergistically to function. For example, fibronectin appears to utilize distant "A" and "B" regions in addition to the RGD site according to deletion mutagenesis analyses. Monoclonal antibodies against the "B" region can block cell adhesion, migration, and organization of a fibronectin fibrillar matrix dependent on the $\alpha_5\beta_1$ integrin. If a non-inhibitory monoclonal antibody that binds between the "B" and RGD sites is adsorbed to a substrate, it can bind and present truncated fibronectin molecules in fully active form to cells; these truncations otherwise display artifactually low activity if tested by standard substrate-adsorption methods. An alternative approach to test for synergy regions will be to test chimeras between two different proteins, both of which contain an RGD sequence. The functional consequences of ligand-integrin interactions can also be dissected by substituting substrate-adsorbed monoclonal antibodies for the ligand of a particular integrin. Anti-β_1 monoclonal or polyclonal antibodies can mimic the functions of the central cell-binding domain of fibronectin and of the CS1 alternatively spliced domain in mediating cell attachment, spreading, and promotion of intracellular actin microfilament bundle organization. Other studies show, however, that antibodies with lower avidities are needed to mimic most efficiently the role of fibronectin in cell migration. A final approach to dissecting ligand-integrin interactions involves the use of chimeras between the intracellular domains of integrins and an extracellular reporter molecule. The β_1 intracellular domain was found to be capable of regulating receptor localization to sites enriched in occupied fibronectin receptors (focal contacts and fibrillar streaks). Since occupancy of either fibronectin or vitronectin receptors with a soluble ligand can also produce a similar redistribution of receptors, a free β_1 intracellular domain may mimic the intracellular face of an occupied receptor. The various approaches described here should help elucidate integrin function in terms of straightforward ligand-receptor binding interactions coupled with intracellular regulatory contributions.

Cell Matrix Interactions in Development (Joint)

X 003 SYNDECAN, THE PROTOTYPE OF A FAMILY OF INTEGRAL MEMBRANE PROTEOGLYCANS, ACTS AS A HIGHLY REGULATED "RECEPTOR PARTNER", Merton Bernfield, Harvard Medical School, Boston, MA 02115

All adherent vertebrate cells have heparan sulfate, a heparin-like glycosaminoglycan (GAG), at their surfaces. These GAG can bind a wide variety of components in a cell's microenvironment, including extracellular matrix, growth factors, degradative enzymes and proteinase inhibitors. These components change during embryonic development and tumor invasion and can influence the adhesion, shape, growth, and differentiation of cells. Indeed, adding heparin to model systems developing in culture modifies their morphogenesis. Therefore, we hypothesized that changes in the amount and type of cell surface heparan sulfate could control the influence of these components during morphogenesis and tumorigenesis. A major source of cell surface heparan sulfate is an integral membrane heparan sulfate-containing proteoglycan, syndecan (from the Greek, *syndein*, to bind together), that is the prototype of a proteoglycan gene family. The expression of syndecan in embryos and neoplastically transformed cells is highly regulated. Syndecan appears soon after fertilization and localizes to the cells that will form the embryo. It is lost from the anterior mesenchyme following gastrulation and its subsequent expression follows morphogenetic rather than histologic patterns and is dictated by epithelial-mesenchymal interactions. It also shows cell-specific expression: syndecan is predominantly at the cell surface on epithelia, where it has smaller heparan sulfate chains, but is predominantly intracellular in mesenchymal cells, where its heparan sulfate chains are larger. Indeed, these heparan sulfate chains from epithelial and mesenchymal cells differ in the number and size of N-sulfated, iduronic acid-rich domains. Syndecan on epithelia associates with the actin cytoskeleton via its cytoplasmic domain and inducing syndecan deficiency in cultured epithelia alters their shape, ability to migrate within matrix and response to growth factors. Syndecan expression is reduced upon neoplastic transformation. Thus, because syndecan may interact with a variety of extracellular effectors, we propose that syndecan is a "receptor partner", acting in combination with more highly specific receptors, which mediates the actions of matrix components and growth factors on cells. Thus, change in expression of the syndecan family of proteoglycans at distinct times or sites may regulate the effects of the microenvironment on cells.

Bernfield M and Sanderson RD. Syndecan, a developmentally regulated cell surface proteoglycan that binds extracellular matrix and growth factors. Phil Trans R Soc Lond Ser B. 1990; 327:171-186.

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Integrins: Cell Adhesion and Transmembrane Communication in Development and Disease

X 004 GENETIC ANALYSES OF CELL ADHESION IN FLIES AND MICE, Richard O. Hynes,[1,2] Elizabeth L. George[1,2], Elisabeth N. Georges,[2] Yevgenya Grinblat,[3] Fotis Kafatos,[3] Stephenie Paine-Saunders,[1,2] Helen B. Rayburn[3], Joy T. Yang,[1,2] Gene Yee[2] and Susan Zusman,[1,2] [1]Howard Hughes Medical Institute and [2]Center for Cancer Research, Department of Biology, M.I.T., Cambridge, MA and [3]Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA.

Cell-matrix adhesion plays crucial roles in embryonic development, in normal physiological processes such as hemostasis and wound healing and in pathological processes such as thrombosis, inflammation and cancer. The molecules involved in these processes have been studied extensively *in vitro* but the functions *in vivo* have been much less analyzed.

In order to analyze the functions of the adhesive extracellular matrix protein, fibronectin, and of cell surface integrin adhesion receptors, we are using genetic methods to investigate their roles in intact organisms, namely flies and mice.

Fibronectin is being analyzed in mice using techniques of homologous recombination in embryonic stem (ES) cells to derive strains of mice which contain defective fibronectin genes. The fibronectin transcript is alternatively spliced to produce multiple different fibronectin isotypes. These alternatively spliced forms are expressed in tissue and developmentally specific fashion but their differences in function are not yet understood. We have generated both null mutants of fibronectin and alleles altered in their pattern of alternative splicing. The results so far demonstrate that FN-null is a recessive early embryonic lethal mutation. Mice heterozygous for fibronectin are viable but express reduced levels of the protein in their blood. They will be examined for more subtle defects in functions thought to involve fibronectin. The mutations altered in pattern of splicing will provide information on the functions of the alternatively spliced segments of fibronectin. Work in progress to generate transgenic mice expressing different alternatively spliced forms of fibronectin should provide complementary information. We are also analyzing the functions of certain integrin subunits which participate in fibronectin receptor function by similar approaches in mice.

We have been unable to detect fibronectin in *Drosophila* but integrins are present. Using preexisting mutations in the gene encoding the β subunit of the position-specific (PS) integrins we have been analyzing the functions of this family of integrins in the development of *Drosophila* embryos, muscles, wings and eyes. Using various genetic combinations and somatic clones, we have shown the involvement of these receptors in all four situations. Using P-element transposons, we have reintroduced wild type and mutant integrin subunits under the control of either their own promoter or a heat-inducible promoter. This allows definition of the times of requirement for integrins in the various processes and the requirement for specific structural elements within the protein.

We have also detected alternative splicing in the extracellular domain of the PS integrin β subunit and again using P-element rescue, have shown that some systems can use either spliced form but that proper embryonic development requires both forms. Further work should reveal the significance of the alternative splice for the structure and functions of this family of integrins.

Many processes involving cell adhesion and movement and axonal outgrowth proceed almost normally in flies in the complete absence of these integrins, suggesting that alternative adhesion molecules can suffice. Accordingly we have used PCR to search for other integrins in *Drosophila* and have discovered a novel β subunit which shows a highly restricted pattern of expression in the midgut of the developing embryo. The gene for this subunit has been mapped and small deficiencies obtained. These deficiencies should allow isolation of mutants in the gene encoding this novel β subunit.

Regulatory Pathways Involving Integrins

X 005 THE STRUCTURAL BASIS FOR LIGAND BINDING TO INTEGRINS, Edward Plow, Randy Piotrowicz, Lynn Deng, Stanley D'Souza, Jeffrey Smith and Dario Altieri, The Scripps Research Institute, La Jolla.

Analysis of the structural basis for ligand recognition by integrins has been greatly facilitated by delineation of small peptide ligands and development of quantitative assays to assess their interaction with receptor-bearing cells. Two peptide ligands for $\alpha_{IIb}\beta_3$, the RGD and γ chain, bound specifically and saturably to platelets, and chemical crosslinking permitted identification of regions which contribute to the ligand binding function of this integrin. β_3 109-172 and α_{IIb} 294-314, the RGD and γ chain crosslinking site, respectively, are envisioned as contact sites for macromolecular ligands bound to $\alpha_{IIb}\beta_3$. The second metal binding motif of α_{IIb} is contained within the γ chain crosslinking site. Cobolt oxidation ablates the ligand binding functions of $\alpha_{IIb}\beta_3$ and selectively alters this site within the receptor, suggesting a close relation between bound divalent cation, bound ligand and α_{IIb} 294-314.

As an initial step to identify ligand interactive regions within other integrins, the binding of peptide ligands to representative β_1 and β_2 integrins have been characterized. A radiolabeled peptide from the IIICS region of fibronectin bound to $\alpha_4\beta_1$-bearing cells in a saturable manner but not to cells lacking this integrin. The interaction was temperature- and time-dependent and was inhibited by structurally related peptides and by an $\alpha_4\beta_1$ monoclonal. The K_d of the interaction was 1.4 X 10^6 M^{-1}, and binding was divalent ion dependent. In other studies, a peptide ligand for $\alpha_M\beta_2$ has been identified. The D_{30} fragment of fibrinogen previously has been shown to bind to $\alpha_M\beta_2$. Proteolytic fragmentation and antibody inhibition data implicated the midregion of the γ chain of fibrinogen in mediating this interaction. A thirteen amino acid peptide from this region inhibited $\alpha_M\beta_2$-dependent fibrinogen binding and cell adhesion. Specific and saturable binding of this peptide to $\alpha_M\beta_2$-bearing cells and to the purified receptor was demonstratable. This interaction was divalent ion dependent and inhibited by certain antibodies to $\alpha_M\beta_2$. Thus, specific peptide ligands for $\alpha_4\beta_1$ and $\alpha_M\beta_2$ have been identified, their interaction with their respective integrin receptors is being quantitatively assessed, and the mechanisms and sites of their interaction with their integrin receptors can now be resolved.

X 006 RELATIONSHIPS BETWEEN PLATELET GP IIb-IIIa AND CELL SIGNALING, Sanford J. Shattil[1], Lawrence F. Brass[1], Michael Cunningham[1], Charles Abrams[1], Peter Sims[2], Therese Wiedmer[2], Beatrice Haimovich[3], Karen Cichowski[3], and Joan S. Brugge[3], [1]Departments of Medicine and Pathology and Laboratory Medicine, University of Pennsylvania, [2]Oklahoma Medical Research Foundation, Oklahoma, City, OK, and [3]Howard Hughes Medical Institute and Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

GP IIb-IIIa ($\alpha_{IIb}\beta_3$) is a platelet-specific integrin which undergoes a change during platelet activation that allows it to bind RGD-containing adhesive ligands, including fibrinogen. We have used the monoclonal antibody, PAC1, to examine the structural basis of fibrinogen receptor expression and the intracellular signals that initiate it. Three observations indicate that PAC1 recognizes the fibrinogen receptor: 1) PAC1 binds only to the activated form of IIb-IIIa; 2) a peptide derived from a hypervariable loop of PAC1 competes with fibrinogen for binding to IIb-IIIa; 3) anti-idiotypic antibodies raised against PAC1 bind to platelet recognition sites in the Aα and γ chains of fibrinogen. Fluoresecence resonance energy transfer studies using fluorophore-labeled antibodies directed against IIb and IIIa have shown that expression of the PAC1 epitope on activated platelets is due to a conformational change in the extracellular portions of IIb-IIIa. In order to study the regulation of this process, platelets were permeabilized with either saponin or the complement proteins, C5b-9, and potential mediators were incorporated into the cell. The results obtained indicate that for some agonists, such as thrombin, activation of IIb-IIIa is a response to the G protein-mediated stimulation of phospholipase C, leading to phosphoinositide hydrolysis, diacylglycerol formation and protein kinase C activation. However, agonists such as ADP and epinephrine cause little, if any, phosphoinositide hydrolysis and may work independently of protein kinase C. Preliminary evidence suggests that one or more of the Src-related tyrosine kinases present in platelets may be involved in IIb-IIIa activation. All agonists studied stimulated the tyrosine phosphorylation of platelet proteins, including a 120 kDa GTP-ase activating protein for $p21^{ras}$. In addition, relatively selective tyrosine kinase inhibitors, such as the tyrphostins, prevented agonist-induced IIb-IIIa activation. Collectively, these results suggest that fibrinogen receptor expression involves several different protein kinases, including those which phosphorylate tyrosine as well as serine and threonine residues. Since platelet activation is not accompanied by the stoichiometric phosphorylation of either IIb or IIIa, we propose an alternative model in which fibrinogen receptor expression is regulated by interaction of IIb-IIIa with an unidentified phosphorylated signaling molecule. This type of interaction may also regulate platelet responses that occur after fibrinogen binding. For example, additional protein substrates were phosphorylated on tyrosine residues after platelet aggregation or after the spreading of platelets on fibrinogen-coated surfaces. Thus, the IIb-IIIa complex appears to be both a target for and a modulator of platelet signaling reactions that converge to induce the phosphorylation of platelet proteins. Identification of specific cytoplasmic, cytoskeletal and membrane phosphoproteins that physically interact with GP IIb-IIIa may help to clarify how the adhesive functions of this integrin are regulated.

Integrins: Cell Adhesion and Transmembrane Communication in Development and Disease

Cytoskeleton-Plasma Membrane Interactions

X 007 α6β4 INTEGRINS: THEIR ROLE IN THE ASSEMBLY OF THE HEMIDESMOSOME (HD) AND IN SIGNAL TRANSDUCTION. J.C.R. Jones, D.H. Klatte, S.B. Hopkinson, M.A. Kurpakus, M. Langhofer, V. Quaranta* and K.S. Riddelle, CMS Biology, Northwestern University Medical School, Chicago, IL and *Department of Immunology, Scripps Research Institute, La Jolla, CA.

In epithelial cells the HD is the structural link between the extracellular matrix and the keratin filament network. Recently, it has been shown that the HD is enriched in α6β4 integrins. We will describe studies which indicate that α6β4 integrins play roles in both signal transduction and in the nucleation of HD assembly.

Although many epithelial cells express α6β4 integrins in vitro, such cells assemble HDs only in rare instances. One cell line which readily forms HDs in vitro is the rat bladder line 804G. We wondered whether this unusual ability of 804G cells is related to the matrix that these cell secrete. To study this, we have placed epithelial cells on matrix produced by the 804G cells and analysed the influence of this matrix not only on the location of α6β4 integrins but also on components of the HD plaque. In certain human epidermal-derived cell lines maintained on uncoated substrates, α6β4 integrins localize along the cell substratum in streaks and spots towards the periphery of the cell. In contrast, HD plaque components are diffusely located in the cytoplasm. Remarkably, when such cells are maintained on 804G matrix, both α6β4 integrins and the HD plaque components show a Swiss cheese type punctate distribution along the substratum attached surface of each cell. This is the type of distribution that is characteristic of 804G cells. We speculate that the matrix influences the location (possibly the solubility) of HD plaque components via α6β4 integrins. In other words, the α6β4 integrin complex is capable of transducing signals originating in the 804G matrix which impact on cytosolic elements of the HD. We will detail the characterization of three glycosylated polypeptides of 125, 140 and 165kD that are the major components of the matrix secreted by the 804G cells and that presumably play a role in this phenomenon.

We have analysed HD assembly using an in vitro tissue explant model of wound healing. In this model, α6β4 integrins first appear along the entire surface of epithelial cells which have migrated over wound sites and which are in contact with the connective tissue (CT). These integrins appear before plaque components of the HD but at the same time as laminin and a 125kD component of anchoring filaments (CAF). The latter have been proposed to "anchor" the HD to the basal lamina, the interface between the epithelium and the CT. Polarization of α6β4 integrins to the surface of the epithelial cell in contact with the wound bed occurs at about the same time that the cells become positive for HD plaque components. We speculate that this change in localization of α6β4 to the basal surface of epithelial cells is induced by deposition of matrix elements such as laminin and CAF. Furthermore, the matrix-integrin complex is likely to be a nucleating center for the assembly of the HD. This would be consistent with the observations described above which indicate that 804G cell matrix via α6β4 influences HD plaque component distribution in epidermal cells in vitro. Antibodies directed against CAF and the extracellular domains of both α6 and β4 integrins inhibit HD assembly in the wound model and induce disruption of already formed HDs. Furthermore, these same antibodies appear to inhibit expression or deposition of collagen type VII by epithelial cells which have populated wound sites. This provides further support for a role of α6β4 in signal transduction. Supported by NIH and ACS.

Cancer and Metastasis

X 008 THE LAMININ-BINDING $\alpha_7\beta_1$ INTEGRIN: REDUCED LEVELS CORRELATE WITH INCREASED METASTATIC POTENTIAL IN MALIGNANT MELANOMA. Randy Kramer, Nahid Waleh, Mai P. Vu, Yao-Fen Cheng, and Daniel M. Ramos. University of California, San Francisco, CA 94143.

Malignant melanoma develops through a series of stepwise alterations that convert normal melanocytes to benign lesions to in situ melanoma, and finally to metastatic melanoma. This process of tumor progression evolves with the expression of melanoma-associated antigens and the acquisition of enhanced adhesion to laminin substrates. We have identified a novel laminin binding receptor, $\alpha_7\beta_1$, that is present on human and mouse melanoma cells (J. Biol. Chem. 264:15642, 1989; Cell Regulation 2:1233, 1991). In normal tissues, the $\alpha_7\beta_1$ complex is expressed at high levels in cardiac and skeletal muscle. This receptor binds with high affinity and selectively to part of the long arm of laminin (the E8 region) but not to the P1 fragment containing the cross-region. The α_7 subunit is unusual since it co-migrates with the β_1 subunit in non-reducing SDS-gels. The apparent lower mass of α_7 appears to be a result of under glycosylation as compared to other α subunits, such as α_6. The expression of the $\alpha_7\beta_1$ was examined in normal and malignant melanocytes. Whereas the $\alpha_7\beta_1$ complex was commonly expressed in melanoma cells, this integrin was not detected in normal melanocytes, suggesting that α_7 expression may be associated with malignant transformation. A cDNA clone for the α_7 subunit was used to probe for its expression in a series of mouse K1735 melanoma cell lines with differing metastatic potential. Surprisingly, the α_7 mRNA and corresponding protein levels was highest in cells of low metastatic potential (K1735 clones 10, 16, 19, and 23). In contrast, the level of mRNA for α_7 was several-fold lower or non-detectable in cell lines that were highly metastatic (clones 2 and 26 and the M2 and M4 cell lines). The expression pattern of the laminin-binding $\alpha_1\beta_1$ and $\alpha_6\beta_1$ integrins was opposite from that of $\alpha_7\beta_1$, in that these integrins were significantly elevated in the highly metastatic cell lines (e.g., M2) and reduced in the non-metastatic cell lines (e.g., clone 23). The results indicate that the acquisition of metastatic potential is associated with loss of α_7 expression and a coordinate increase in α_1 and α_6 expression. It is significant that the metastatic K1735 cell lines but not the non-metastatic cell lines are highly migratory on laminin substrates. The mechanism by which these shifts in integrin expression profiles influence metastatic potential may be related to how the cell interacts with laminin. This suggests that the reduction of expression of $\alpha_7\beta_1$ is associated with or causes highly metastatic behavior and may act as a suppressor protein for metastasis. (Supported by NIH grants DE00242, CA33834, and CA51884).

X 009 CHARACTERIZATION OF INTEGRIN EXPRESSION IN HUMAN CANCER CELL LINES AND REGULATION BY CYTOKINES, Marc A. Shuman, Robert Pytela, and Eric Small, University of California, San Francisco, California.

We have studied the expression of the vitronectin receptor (VNR) and beta2 integrins in cancer cell lines with the goal of determining their role in tumor growth and metastasis. Evidence for alphav mRNA in every type of human tumor cell line studied was found on Northern blotting. Thus HEL (leukemia), A431 (epidermoid carcinoma), U937 (lymphoma), PC-3 (prostate cancer) cells all had the normal size 7.5 kB band on Northern blotting using a 2.9 kB alphav cDNA probe. Alphav mRNA was also identified in A431 cells using PCR homology subcloning. Primers were derived from highly conserved alpha integrin sequences and designed to anneal with nucleotides encoding sequences that flank a 300-nucleotide region beginning 200 amino acids from the amino terminus in many of the alpha integrins. Purified PCR fragments were sequenced confirming the presence of alphav. Northern blotting using a human 2.0 kB beta3 cDNA probe revealed a 6.0 kB band consistent with beta3 mRNA in HEL, PC3 and A431 cells. Thus, it is likely that the alphav/beta3 VNR is expressed in all three cancer cell lines.

We also examined the effect of gamma-interferon (IFN), a cytokine with anti-tumor effect, on expression of of the alphav/beta3 VNR on A431 cells. Cells were grown in the presence of 300 units/ml of IFN then mRNA was isolated. Both alphav and beta3 mRNA was reduced on Northern blotting with no change in the control tubulin mRNA. Thus, this agent has the potential to inhibit VNR expression and presumably its interaction with matrix adhesion proteins. This could significantly effect tumor growth.

An unexpected finding was the detection of beta2 mRNA in two non-leukocyte tumor cell lines, PC3 and A431 on Northern blotting. A 300 base pair cDNA fragment confirmed to be beta2 by sequencing was used as a probe. A 3.2 kB band identical in migration with mRNA from normal leukocytes was observed under low stringency conditions (0.1 x SSC, 55° C). Under more stringent conditions (0.1 x SSC, 65° C), only the band seen with PC3 cells persisted. It is unclear whether the loss of hybridization in the A431 cells is due to less mRNA compared to PC3 cells or the presence of a beta2-like mRNA in the A431 cells with significant but not complete homology. Previously, beta2 has only been seen in leukocytes. PCR homology probing of A431 has not revealed an alpha subunit usually associated with beta2. Northern blot analysis with an alphaM cDNA fragment failed to detect mRNA in A431 or PC3 cells. Metabolic labelling with ^{35}S-methionine and immunoprecipitation with a rabbit polyclonal antibody raised against a 15 amino acid peptide in the C-terminus of the beta2 cytoplasmic domain revealed the presence of beta2 in PC3 cells. No co-precipitating alpha subunit was identified. Surface labelling with Na^{125}I and immunoprecipitation failed to reveal beta2. Thus it appears that beta2 in PC3 cells is not paired with a corresponding alpha subunit and is not transported to the cell surface.

X 010 PLASMINOGEN ACTIVATION AT THE TUMOR CELL - MATRIX INTERFACE,
Antti Vaheri, Hannele Tapiovaara, Jozef Bizik, Vappu Sirén, Heli Myöhänen, Tuuli Reisberg, Athina Lymboussakis, Miina Palolahti and Ross W. Stephens. Department of Virology, University of Helsinki, SF-00290 Helsinki, Finland.

Plasminogen activation is a cascade-like process regulated at a number of levels (1,2). We have shown that plasminogen interacts with adhesion proteins, such as fibronectin and laminin, supporting the view that they are likely substrates of the plasminogen activation system at the cell surface. Thus there is direct evidence that adhesion proteins of the pericellular matrix can provide a molecular link between integrins and plasminogen. We found that urokinase-type plasminogen activator (uPA) is localized at focal adhesions, cell-cell contact sites and tips of microspikes of adherent cells. These findings led us to introduce the concept of "directional plasminogen activation", thought to operate in matrix invasion. We showed that cell-bound plasminogen is directly activated on the surface of adherent tumor cells by uPA and that, while cell-bound, plasmin is protected from inactivation by plasma inhibitors, i.e. a_2-antiplasmin and a_2-macroglobulin (a_2M), but not from aprotinin (4). We found that cell-bound uPA is subject to regulation by the high-affinity inhibitors, PAI-1 and PAI-2. Recombinant PAI-2 was used to localize and inhibit u-PA on tumor cell surface (5). More recently, in collaboration with the laboratory of K. Danø, we have shown (Myöhänen *et al.*, in preparation) that also the uPA receptor (uPAR) is located in focal adhesions. Since uPAR is known to have a lipid anchor and to lack a cytoplasmic tail, we are studying which other interactions, such as those with integrins or their ligands, might drive the uPA-uPAR complexes to the cellular contact sites.We have now identified a peptide sequence in the kringle domain of uPA that interacts with heparin (and thus possibly with heparan sulfate proteoglycans) and have demonstrated, in collaboration with R. Timpl, that high molecular weight uPA binds to defined domains in the laminin/nidogen complex (Stephens *et al.*, submitted). The above pattern withuPA, seen in many types of adherent tumor cells, is not the only possibility. On the surface of melanoma cells plasmin is generated by tissue-type PA, a process unaffected by the a_2M these cells secrete (5). We have shown that leukemic cells growing in suspension (unlike adherent tumor cells) convert pro-u-PA to active u-PA which is on the cell surface. Our recent experiments show that in fact leukemic cells carry active plasmin continuously in their cell surface compartment, protected from the high molecular weight serum/plasma inhibitors (Tapiovaara *et al.* submitted) and that a_2M restricts plasminogen activation to the surface of leukemic cells where u-PA is protected from inactivation by a_2M (6).

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Induction Phenomena-I (Joint)

X 011 REGULATORY FACTORS IN ADIPOCYTE DEVELOPMENT, Bruce M. Spiegelman, Reed A. Graves, Peter Tontonoz, and Lisa Choy, Dana-Farber Cancer Institute, Division of Cellular and Molecular Biology and Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, MA

We have been studying the regulation of adipocyte gene expression during cell differentiation and tissue development. The intracellular factors that activate adipocyte-specific gene regulation are not well understood. Toward this end, we have recently discovered an adipocyte-specific enhancer, and are performing a molecular dissection to identify key transcription factors binding to this DNA. This enhancer, from the adipocyte P2 gene, functions in cultured cells and transgenic animals. It binds several nuclear proteins including a member of the NF-1 family and mutational analysis indicates that binding at 5 separate sites are necessary for full activity. One factor, termed ARF6, appears to be developmentally regulated and may be the switch that activates this enhancer only in fat cells. The ARF6 factor itself is observed only in nuclear extracts from adipocytes. Multimers of the ARF6 binding site are sufficient to activate gene expression from a minimal promoter in adipose cells. C/EBP, a transcription factor that is induced during adipocyte differentiation, does not bind to this enhancer and an expression vector for C/EBP cannot transactivate this enhancer in preadipocytes. Current experiments address the general importance of the ARF6 factor in the activation program of differentiation in adipocytes. We have also been examining adipocytes for production of extracellular factors that may influence systemic energy balance. Our recent data indicates that adipocytes make and secrete several complement factors including factor D/adipsin. They also activate part of the alternative pathway of complement and generate several complement peptides with known biological effects. The role of this pathway in systemic energy metabolism is being investigated.

X 012 GROWTH FACTOR-MEDIATED INDUCTIVE SIGNALLING DURING *C. elegans* DEVELOPMENT, Paul W. Sternberg, Russell Hill, Phoebe Tzou, Jing Liu, Helen Chamberlin, Gregg Jongeward, Junho Lee, Linda Huang, and Raffi Aroian, Howard Hughes Medical Institute, Division of Biology 156-29, California Institute of Technology, Pasadena, CA.

An intercellular signalling pathway consisting of a growth factor, transmembrane receptor tyrosine kinase and a ras protein acts in several defined inductive events during nematode development. During vulval induction in the hermaphrodite, a single cell in the gonad, the anchor cell, induces three of six multipotent vulval precursor cells [VPCs] to undergo three rounds of mitosis and generate vulval cells. The three uninduced VPCs undergo a single mitosis and generate nonspecialized epidermis. Among genes necesary for this induction are *lin-3*, *let-23* and *let-60*. Defects in any of these three genes leads to a failure of vulval induction. Transgenic nematodes carrying high copy *lin-3* or *let-60* transgenes have excessive vulval differentiation. Using these dominant transgenes, we have ordered the action of these three genes: *lin-3* acts via *let-23*, which acts via *let-60*. *let-60* encodes a ras protein. *let-23* encodes a *C. elegans* homolog of the human EGF receptor and related receptor tyrosine kinases. *lin-3* encodes a putative growth factor precursor with the architecture of TGFα. Predicted *lin-3* proteins have an N-terminal leader sequence, a single EGF growth factor repeat, a membrane-spanning domain. Two alternatively spliced forms of *lin-3* differ in the presence of 15 amino acids between the EGF repeat and the transmembrane domain. *lin-3* is expressed in the anchor cell at the time of vulval induction. We propose that *lin-3* encodes the signal that induces vulval development. During male spicule development, we have shown that the F and U cells use this *lin-3-let-23-let-60* pathway to induce particular neurectoblasts fates including Bα and Bγ, which generate particular subsets of spicule cells. Ablation of F and U has the same result as mutation of *lin-3*, *let-23* or *let-60*: defects in the Bα and Bγ cell lineages. We are examining the negative regulation of this pathway by examining mutations that result in excessive vulval differentiation. Some of these mutations are in the *let-23* gene, others are in unlinked loci, *lin-15*, *unc-101*, and *rok-1*.

X 013 ADHESION AND MOTILITY OF EMBRYONIC AND CANCER CELLS. Jean Paul Thiery, Jean Claude Boucaut, Brigitte Boyer, Florence Broders, Thierry Darribere, Annie Delouvée, Sylvie Dufour, Jeanne Marie Girault, Jacqueline Jouanneau, Victor Koteliansky, Giovanni Levi, Ginette Moens, Jean Pierre Saint-Jeannet, Pierre Savagner, Lionel Simonneau and Ana Maria Valles. Laboratoire de Physiopathologie du Développement, CNRS-Ecole Normale Superieure 46, rue d'Ulm, 75230 Paris Cedex 05 FRANCE.

We have analysed in detail the program of expression of several adhesion molecules during morphogenesis focusing primarily on epithelial-mesenchymal cell interconversions and on migratory events. 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. The role of cell adhesion is also investigated in amphibian embryos particularly during gastrulation.

A rat bladder carcinoma has been used as a model system to study the conversion of an epithelial to a migratory fibroblast-like state. This morphological transformation is triggered by collagens but not by fibronectins or laminin. A similar conversion is induced by acidic Fibroblast Growth Factor (aFGF) in subconfluent cultures while this multifunctional growth factor acts as a mitogen on high density cultures. In low density cultures, aFGF and several other growth factors acting through tyrosine kinase receptors induce a rapid internalization of desmosomes, a major adhesive structure of epithelia. The newly formed fibroblasts progressively lose their cytokeratins which are replaced by vimentin intermediate filaments. The transformation is fully reversible upon removal of the growth factor. Acidic FGF also triggers cell motility and production of gelatinases. On collagen substrates, the speed of locomotion is enhanced in the presence of aFGF and under these conditions the bladder carcinoma cells readily invade 3D collagen gels. The bladder carcinoma line can also become fibroblastic after transfection with an expression vector coding for aFGF, most likely through an autocrine mechanism. Thus, this model system may offer a unique opportunity to evaluate the role of the different adhesion modes and signalling factors in morphogenetic processes.

Inflammation and Immunity

X 014 THE DISTINCTIVE FUNCTIONS OF SELECTINS, INTEGRINS, AND IG FAMILY MOLECULES IN REGULATION OF LEUKOCYTE INTERACTION WITH ENDOTHELIUM, Timothy A. Springer, The Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.

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

Differentiation and Development

X 015 HOW DOES EXTRACELLULAR MATRIX REGULATE GENE EXPRESSION?: VIA INTEGRINS AND TRANSCRIPTIONALLY, Mina J. Bissell, Lawrence Berkeley Laboratory, Berkeley, 94720

Along with the spectacular advances in our understanding of the structure of genes and their regulatory sequences, the pains-taking work of developmental and cellular biologists has determined that the microenvironment in which a cell finds itself decisively and specifically regulates the expression of tissue-specific genes. In the last decade, my laboratory has used two versatile model systems to define the important regulatory elements of such microenvironments: 1)the interaction of the extracellular matrix (ECM) with the mouse mammary epithelial cells in culture and 2)the interaction of Rous sarcoma virus with the embryonic limb *in ovo*.

While I will concentrate on the first system, the conclusions from both systems are that the microenvironment is dominant in allowing the expression of both normal and malignant phenotypes, and that extracellular matrix and the three dimensional structure of the tissues are crucial determinants of such regulations.

Our evidence indicates that the basement membrane in general, and laminin in particular, regulate the expression of β-casein gene, that the regulation is via interaction with integrins, that such regulation is transcriptional and that there is a unique ECM and prolactin response element (an enhancer) in the 5' region of the β-casein gene that requires both ECM and hormones for induction of gene expression. I will present a working model for how such elements may play a role in expression of tissue-specific genes in general.

Furthermore, the ECM selectively suppresses the expression of a number of other genes including growth factors and the ECM molecules themselves. I will discuss the importance of ECM *in vivo* and the possible relevance of these findings to malignancy and metastasis.

These studies were made possible by funding from the Office of Health and Environmental Research of the Department of Energy and by a gift for research from the Monsanto Company.

X 016 TENASCIN VARIANTS AND THEIR LIGANDS

Ruth Chiquet-Ehrismann and Urs Hofer, Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland

Tenascin is a large extracellular matrix protein. In the chicken, three tenascin variants have been characterized that are generated by alternative splicing of 3 of its 11 fibronectin type III repeats. Recently, we identified a 12th fibronectin type III repeat in the chicken tenascin gene, located between the previously identified repeats 7 and 8.

These tenascin variants show distinct distribution patterns in cell cultures and tissues and they bind differentially to fibronectin. Using the purified proteins in various types of solid phase binding assays, only the smallest tenascin variant bound strongly to fibronectin.

These biochemical data were paralleled by the observation that in chick embryo fibroblast cultures only the smallest form of tenascin could be detected in the fibronectin-rich extracellular matrix laid down by the cells.

In contrast to the differential binding to fibronectin, all tenascin variants bind equally well to heparin. Furthermore all tenascin variants have identical lectin activity and agglutinate erythrocytes.

This hemagglutination can be inhibited most efficiently by gangliosides containing two consecutive sialic acids, such as i.e. GD2 and GD3. We thus postulate that gangliosides, or maybe polysialic acid containing membrane proteins could be cellular ligands of tenascin.

Since GD2 and GD3 have been shown to be necessary for fibronectin receptor function, we propose that tenascin interferes with fibronectin-mediated cell adhesion by binding to the receptor-associated gangliosides.

X 017 MIGRATORY CELLS FROM DIFFERENT REGIONS OF THE EARLY MOUSE EMBRYO UTILIZE DISTINCT ADHESION STRATEGIES TO INTERACT WITH FIBRONECTIN AND LAMININ.

Ann Sutherland, Carol Burdsall, Roger Pedersen, Patricia Calarco and Caroline H. Damsky. University of California San Francisco, San Francisco, CA 94143-0512.

Cell-extracellular matrix (ECM) interactions mediated by integrins are critical for morphogenesis and may also play a regulatory role in differentiation. The onset of expression of individual integrin subunit proteins, as detected by immunoprecipitation and immunofluorescence is staggered. The α6, αV, β1 and β3 subunits are detected as early as the 4 cell stage, α5 at the hatched blastocyst stage and α1 and α3 following blastocyst attachment. Three migratory populations of the early mouse embryo are: the primary trophoblast, which establishes the initial contact with the uterine wall and ultimately forms the chorionic placenta; parietal endoderm, which differentiates from the primitive endoderm, migrates over the inner surface of the trophoblast and ultimately forms the partietal yolk sac; and early mesoderm, which is derived from the embryonic ectoderm and migrates through the primitive streak during gastrulation to form embryonic mesoderm. The attachment and migratory activity of each of these cell populations was studied in serum-free medium on substrates coated with fibronectin (Fn) and laminin (Ln). Early stages of trophoblast outgrowth (up to 48 h) on Fn are inhibited by anti-Fn and by function-perturbing anti-αV antibodies, whereas at later times further outgrowth becomes insensitive to anti-αV and sensitive to anti-β1 antibodies, indicating that trophoblast cells modulate their interaction with Fn during outgrowth. Trophoblast outgrowth on vitronectin (Vn) is sensitive to anti-αV antibodies throughout the 5 day period examined. Trophoblast outgrowth occurs on Ln and its E8 fragment (long arm), but not on E1 (cross region). This outgrowth is inhibited by anti-E8, anti-Ln, and by the anti-β1 family antiserum anti-ECMr, but not by anti-αV or the function-perturbing GoH3 antibody that recognizes the α6/β1 integrin, a major Ln (E8) receptor. This suggests that trophoblast outgrowth on Ln or E8 is mediated by a different β1 integrin, such as α3/β1.

Parietal endoderm-like cells (PE), which migrate out onto the culture dish after 5-7 days of blastocyst outgrowth, also recognize Fn and the E8 fragment of Ln. Unlike primary trophoblast outgrowth, however, migration of PE on Fn is not affected by anti-αV antibodies, but PE migration is inhibited by the GoH3 antibody, indicating that α6/β1 is the major Ln receptor for these cells.

Finally, the mesoderm of 7.5 d embryos can be explanted and cultured *in vitro* on defined substrates. These cells attach and spread rapidly (1-4 h) on Fn and Vn and more slowly (8-16 h) on Ln and Col IV. Initial functional studies indicate that their interactions with Ln are inhibited by GoH3. These studies demonstrate the feasiblity of dissecting the adhesive and migratory behavior of isolated cell populations from different regions of very early mouse embryos, and reveal that significant diversification and modulation of integrin adhesion receptors accompany development of migratory behavior. (supported by HD26732 and CA42032).

X 018 INTERACTION OF EXTRACELLULAR MATRIX WITH CELL SURFACE RECEPTORS DURING THE DIFFERENTIATION OF DROSOPHILA MUSCLES,

Liselotte I. Fessler, Donald Gullberg, Michael Yuhasz, and John H. Fessler, Department of Biology and the Molecular Biology Institute, U.C.L.A., Los Angeles.

Previously we reported that sarcomere formation requires the integrin α_{PS2}/β_{PS3} in Drosophila embryos and in primary cultures derived from them (1). While primary myospheroid mutant cells, which lack this integrin, attach to a laminin substrate, the adhesion of normal cells to laminin results in a diffuse distribution of integrin around the cell peripheries, with concentration at cell:cell junctions. In contrast, a substrate of vertebrate vitronectin causes the integrin to concentrate into multiple focal contacts. In either case, attached myocytes fuse into multinucleate myotubes and, upon addition of an external ligand, prominent concentrations of integrin arise at the emerging Z-bands. The mixed, differentiating primary cell cultures synthesize a range of Drosophila extracellular matrix molecules, including laminin, collagen IV, glutactin, the proteoglycan papilin and three glycoproteins that we have partly characterized. Their accumulation in concentrated cultures is accompanied by spontaneous formation of striated myotubes. While several of these ECM molecules (collagen, glutactin and a 220 kd protein) also colocate at the Z-bands of adult flight muscles, we do not know whether any of them critically help to initiate sarcomere formation. Furthermore, vinculin and α-actinin colocalize on the cytoplasmic side of the Z-bands. The sequence of linkage of these components is as yet unknown. Wandering hemocytes of embryos are major producers of ECM molecules, and are also prominent in primary cell cultures, but the sources of ECM formation in pupa remain to be defined. The fasciculations and insertions of adult thoracic muscles stain prominently with antibodies to collagen and some other ECM molecules. We attempted to modulate collagen synthesis during metamorphosis with anti-sense RNA constructs targeted to the thoracic musculature. A progression of disturbance leads to substantial thoracic defects.

1. Volk, T., Fessler, L.I., and Fessler, J.H. (1990) A role for integrin in the formation of sarcomeric cytoarchitecture. Cell 63, 525-536.

X 019 A NETWORK OF GENES REGULATING *DROSOPHILA* INTEGRIN FUNCTION, Michael Wilcox, Marcel Wehrli and Lisa Wessendorf, MCR Laboratory of Molecular Biology, Cambridge, UK.

We are using genetic analysis of *Drosophila* integrin function during development, both to complement vertebrate cell biological studies and to gain some insight into how accurately *in vitro* integrin functions reflect the *in vivo* situation. The PS1α gene of the two member β-1-like PS integrin family has been cloned. The PS1- and PS2α subunits have no more homology to each other than each has to various vertebrate α subunits, while the genomic organization of each shows general conservation of intron-exon boundaries when compared to vertebrate α genes, although many introns have been removed. The remaining exons often do not correspond to suggested protein domains. We are now studying mutations in the two α chain genes in order to further analyze their developmental function.

A viable mutation, if^3 in the PS2α gene, *inflated*, leads to blistering in some 20% of adult wings, as a result of faulty apposition of the two surfaces of the developing wing. We are taking a genetic approach to further investigate the role of integrins in this process. Mutational analysis has identified many genes involved in wing development. We have tested a variety of mutant alleles of such genes for their ability to enhance or suppress the phenotype of homozygous if^3 flies, and have identified alleles that exhibit both dominant and recessive interactions (dominant effectors require only one copy of a recessive mutant allele to produce an interaction, while recessive effectors require two copies). Loci which act as dominant effectors, in particular suggest that the wild type products of these genes are involved in integrin function.

We now find that 1) among these mutant alleles, many fail to complement one another and 2) some of *them interact* genetically with alleles of genes encoding other cell surface proteins. For example, alleles of *plexus* (*px*), one of which (px^1), acts as a dominant suppressor of the if^3 phenotype, also interact with alleles of both *Notch* (*N*) and *Delta* (*Dl*), genes involved in several developmental processes including neuroblast specification and segregation, whose products are EGF-homologous surface receptors. Furthermore, px^1 itself is subject to dominant suppression by alleles of *veinlet/rhomboid*, which encodes another putative transmembrane protein. These, and other data, suggest the existence of a network of genes which regulate the function of integrins and other surface components and might serve as a link between the functioning of different cell interactive systems. The genetic data will be discussed and the molecular characterization of some of the genes described.

Epithelial Phenotype and Differentiation

X 020 CELL ADHESION AND THE BASEMENT MEMBRANE IN MORPHOGENESIS OF THE EPIDERMIS, William G. Carter[1,2], Maureen C. Ryan[1,2], Tod A. Brown[1,2], Banu E. Symington[1,2], Susana Gil[1,2], Virginia Sybert[2], Kim B. Yancey[3], [1]F. Hutchinson Cancer Ctr., Seattle. [2]Univ. of Wash., Seattle, WA. [3]USUHS, Bethesda, MD.

We seek to understand the mechanisms of cell adhesion to epiligrin[1], a new glycoprotein complex in epithelial basement membranes (BMs), and how this interaction may regulate polarity and differentiation of epidermal basal cells. Human skin contains a regenerative, squamous epithelium, composed of five stratifying cell layers, basal, suprabasal, spinous, granular, and cornified in order of increasing differentiation and distance from the BM. Basal cells synthesize epiligrin and express three major integrin adhesion receptors, α2β1, α3β1, and α6β4. Epiligrin, immunoprecipitated with MAb P1E1, is composed of three subunits E170, E145 and E135 kDa and co-precipitates with a laminin isoform of 200 kDa. The interaction of epiligrin with α3β1 and α6β4 controls the formation of three cell adhesion structures in basal cells: (i) On the basal surface, focal adhesions contain α3β1 linked to actin stress fibers that mediate initial cell adhesion and migration; (ii) Also on the basal surface, hemidesmosome-like stable anchoring contacts (SACs[2]) contain α6β4 linked to bullous pemphigoid antigen and intermediate filaments that mediate non-migratory anchorage functions; (iii) At apical/lateral membranes, α2β1 and α3β1 function as co-receptors in cell-cell adhesion. A physiological role for epiligrin as an adhesive BM ligand in skin has been established by linkage to inherited junctional epidermolysis bullosis and a form of cicatricial pemphigoid, both pathological adhesion disorders of the basal epidermis. Immunoprecipitation studies suggest that epiligrin is related to the BM antigen recognized by MAb GB3 that is also altered in junctional epidermolysis bullosis[3].

Multiple partial cDNAs encoding the E170 subunit have been identified, cloned and sequenced, and confirm the unique character of epiligrin as a new BM glycoprotein. Using the epiligrin cDNAs, Northern blot analysis of mRNA from keratinocytes transformed with human papilloma virus identified decreased expression of epiligrin mRNA, establishing epiligrin as a transformation-sensitive adhesive ligand. Further, we have used a unique MAb screening protocol to identify cytoplasmic components that are regulated by cell adhesion to epiligrin. Epiligrin was found to control the basal localization of a new 170/135/45 kDa component of the dermal-epidermal junction recognized by MAb P1H8. Sequencing of a partial cDNA encoding the P1H8 antigen identified it as a new cytokeletal component with partial sequence homology to caldesmon a regulatory component of the cytokeleton. Basal cell detachment from epiligrin in the basement membrane and movement into the suprabasal cell layer correlates with increased cell proliferation, decreased integrin and epiligrin expression, and loss of the polarized distribution of the P1H8Ag. We propose that epiligrin in the BM, signaling through the adhesion receptors, regulates the polarization and expression of cytoplasmic components, like the P1H8Ag.

[1] Carter, W. G., Ryan, M. C., Gahr, P. G. (1991) Cell 65, 599-610.
[2] Carter, W. G., Kaur, P., Gil, S. G., Gahr, P. J., Wayner, E. A. (1990) J. Cell Biol. 111, 3141-3154.
[3] Verrando, P., Pisani, A., Ortonne, J. P. (1988) Biochem. Biophys. Acta 942, 45-56.

X 021 DOWN-REGULATION OF INTEGRIN FUNCTION AND EXPRESSION DURING KERATINOCYTE TERMINAL DIFFERENTIATION, Fiona M. Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London. WC2A 3PX, England

The epidermis is the stratified epithelium that forms the outer covering of the skin. Proliferation is largely confined to the basal layer of keratinocytes that are attached to the basement membrane. On commitment to terminal differentiation, keratinocytes withdraw from the cell cycle and are selectively expelled from the basal layer. I shall describe the role of integrins in regulating both the adhesion of keratinocytes to extracellular matrix proteins, and the initiation of terminal differentiation. Immunofluorescence shows that integrins are expressed by all keratinocytes in the basal layer and are largely absent from the suprabasal terminally differentiating layers. The ability of keratinocytes to adhere to a range of matrix proteins is decreased on terminal differentiation. We have examined in detail how function and expression of $\alpha_5\beta_1$ (the keratinocyte fibronectin receptor) are down-regulated during terminal differentiation. On commitment, the ability of the receptor to bind ligand is reduced by a mechanism that involves modulation of pre-existing receptor on the cell surface and is correlated with changes in phosphorylation of several cytoplasmic proteins. On overt terminal differentiation, transcription of the α_5 and β_1 subunit genes is switched off; the levels of the corresponding mRNAs decline; mature receptor is lost from the cell surface and processing of immature, intracellular subunits is inhibited. One signal for initiation of terminal differentiation appears to be reduced occupancy of keratinocyte integrins with their corresponding ligands. Although the adhesive function of the receptors is intimately linked to their function in regulatng terminal differentiation I shall present evidence that the two functions may operate via distinct mechanisms that can be distinguished by several criteria, including a requirement for receptor clustering and microfilament assembly.

Integrins: Structure, Distribution, Ligand Binding

X 100 SITE-SPECIFIC METHYLATION CONTROLS ACTIVITY OF THE α5 INTEGRIN PROMOTER DURING B-CELL MATURATION AND KERATINOCYTE DIFFERENTIATION, Thomas M. Birkenmeier, Elizabeth D. Boedeker, Tasneem Rangwala and Douglas C. Dean, Departments of Internal Medicine and Cell Biology, Washington University School of Medicine, St. Louis, MO 63110

We have recently described the isolation and initial characterization of the the promoter for the α5 subunit of the human α5β1 integrin complex (Birkenmeier, T.M. et al. (1991) J. Biol. Chem. 266: 20544-20549). The α5 gene 5' flanking region lacks both TATA and CCAAT boxes and it is located in a CpG island. This region was an active promoter in transfection assays using the HT-1080 cell line (fibrosarcoma), which expresses α5, but was inactive in the Raji cell line (B cell), which does not express α5. These results indicated that the α5 gene 5' flanking region acts as a promoter that exhibits the expected cell-type specificity. Here we show that the activity of the α5 promoter is controlled by methylation. During B-cell maturation and keratinocyte differentiation, the promoter becomes methylated at two specific sites, which effectively inhibits its activity. Additionally, in vitro methylation of these sites inhibits promoter activity in transfection assays. Treatment of the mature B-cell line Raji, which does not normally express α5, with 5 aza-C (prevents methylation of cytosine residues) caused the cells to express α5, providing further evidence that α5 expression is controlled by methylation.

X 101 CHANGE IN β1-INTEGRIN EXPRESSION ON A HUMAN NEUROBLASTOMA CELL LINE AFTER DIFFERENTIATION WITH CYCLIC AMP, Geert Carmeliet and Jean-Jacques Cassiman, Center for Human Genetics, University of Leuven, Leuven, Belgium

The integrins expressed by the neuroblastoma cell line TR 14, belong to the β1 family, and are mainly α1β1 and α2β1, as detected by immunoprecipitation. Addition of N6,2-0-dibutyryl cAMP at a concentration of 1 mM induces neurite formation in these cells. The phenomenon of neurite outgrowth can be partially inhibited by a functional anti-β1 integrin monoclonal antibody (mAB 13, Yamada) added at the same time as dBcAMP. To investigate the effect of differentiation on integrin expression, surface labeling by lactoperoxidase catalyzed iodination was carried out at different time points during dBcAMP incubation. A biphasic pattern in β1 integrin expression was observed. During the first 24 hours the amount of labeled integrin receptors at the plasma membrane increased slightly. Thereafter a decrease in surface labeled integrins was observed down to only 30 % of the control values. When the cells were cultured in conditioned medium of a glioma cell line (HTB 138), they also formed neurites, but at a slower rate. A decreased surface expression of β1 integrins was also seen in these conditions.

Northern blot analysis showed unchanged levels of mRNA. The rate of synthesis of β1 integrins was equally unaltered when differentiated cells where compared with untreated cells. Shedding of intact β1 integrins in the medium was also excluded as a major cause of decreased surface expression in the treated cultures. Preliminary results suggest that an alteration in the metabolism of the β1 subunit (maturation and/or degradation), might perhaps partially explain the decreased surface expression observed after neurite formation.

X 102 GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO HUMAN αVß3 INTEGRIN. Anan Chuntharapai, Sarah Bodary, Michael Horton, Glenn Hammonds, John McLean and K. Jin Kim. Genentech, South San Francisco, CA, 94080.

Integrins are a supergene family of cell surface glycoproteins that promote cellular adhesion. The integrin αvß3, vitronectin receptor, is a major integrin expressed by osteoclasts. To gain a better understanding of the structural and functional relationship of αvß3, we have generated and characterized monoclonal antibodies (mAbs) to this integrin. BALB/c mice were immunized with 293-15D cells expressing human αvß3 and hybridized with P3/X63-Ag8U1 myeloma cells. Fifteen different mAbs from 1200 positive hybridomas which bound αvß3, were extensively characterized. Four mAbs were shown to bind specifically to αvß3 complex by ELISA as well as FACS analysis. Three of these four mAbs were able to block the binding of vitronectin to αvß3 protein in ELISA. Two other mAbs were shown to bind to the αv portion of αvß3 complex by FACS analysis. Nine other monoclonal antibodies were shown to bind to ß3 subunit by ELISA using transfected cells. Six of these 9 mAbs were able to bind to the native and denatured ß3 subunit by immunoblotting.

We are in the process of investigating the epitopes recognized by these mAbs and the structral and functional relationship of αvβ3 integrin using purified mAbs.

X 103 A TISSUE SPECIFIC REPRESSOR AND ETS BINDING SITES CONTROL EXPRESSION OF THE VLA-4 INTEGRIN GENE, Glenn D. Rosen, Jennifer L. Clodfelter, and Douglas C. Dean, Washington University School of Medicine, St. Louis, MO 63110.

VLA-4 is a member of the β1 family of integrins that mediates both cell-cell and cell-matrix interactions. It is expressed almost exclusively on cells of hematopoetic origin in the adult; expression is also observed in several solid tumors, rhabdomyosarcoma, osteosarcoma and melanoma. We have previously isolated and sequenced the promoter for the VLA-4 gene (Rosen, et al. (1991) PNAS 88: 4094-4098). Here we show that binding sites for the ets family of proto-oncogenes between positions -42 and -76 bp are critical for basal promoter activity. These sites were compared to ets sites from other promoters in a series of gel retardation assays and we conclude that ets sites from the different promoters bind to different subsets of the ets family. We also identifed a silencer between positions -600 bp and -1.0 kb that is active in cells that do not express VLA-4 and inactive in cells that do express VLA-4. We propose that this silencer controls the pattern of VLA-4 expression.

X 104 INTEGRINS WITH AFFINITY FOR DENATURED PROTEIN SUBSTRATES, George E. Davis, Dept. of Pathology, Texas A&M College of Medicine, College Station, TX 77843

During studies of leukocyte- and tumor cell-substrate adhesion in vitro, I have discovered two different instances where cell adhesion is mediated by integrins which preferentially bind to a substrate coated with a denatured versus native protein. Adhesion of monocyte-differentiated HL-60 cells to substrates coated with various proteins including albumins, catalase, transferrin, casein, ovalbumin and ECM proteins is dependent on β_2 integrins. One common feature of these proteins is that their adsorption to plastic may result in protein unfolding and denaturation. Affinity chromatography experiments revealed that the Mac-1 and p150,95 β_2 integrins bound to denatured albumin-sepharose but bound poorly to native albumin-sepharose. This binding was divalent cation dependent and the related β_2 integrin, LFA-1, did not bind to these columns. In a separate study, the A2058 melanoma cell line was found to adhere through distinct integrins to native versus denatured type I collagen. Adhesion to native type I collagen appears to be largely RGD-independent and mediated through several β_1 integrins. In contrast, adhesion to denatured collagen is RGD-dependent and is mediated through the $\alpha_v\beta_3$ integrin. This latter data suggests that the RGD sites may be masked in native type I collagen and that during processes where collagen denaturation occurs, such as tissue injury and inflammation, they may become exposed to allow interaction with $\alpha_v\beta_3$ or other RGD-binding receptors. An exposure of RGD or denatured protein sites during tissue damage may provide alternative signals for cells to distinguish between a wounded versus normal ECM environment.

X 105 THE AXONAL GLYCOPROTEIN TAG-1 CAN MEDIATE HOMOPHILIC BINDING BUT NEURITE OUTGROWTH ON TAG-1 DEPENDS ON NEURONAL INTEGRIN FUNCTION. Dan P. Felsenfeld, Mary A. Hynes, Andrew J. Furley, and Thomas M. Jessell. Center for Neurobiology & Behavior and Howard Hughes Medical Institute, Columbia University. New York, NY 10032

The guidance of axons toward their targets in the developing nervous system is thought to be mediated by interactions between glycoproteins on the surface of the growing neuron and molecular cues in the local environment. The glycoprotein TAG-1 is transiently expressed in embryonic rat spinal cord on a subset of axons. TAG-1 is a member of the Ig superfamily that also contains fibronectin type III (FN III) repeats and promotes neurite outgrowth when used as a substratum for neurons in culture.

Expression of TAG-1 on the surface of non-adherent Drosophila S2 cells promotes cell aggregation. Cells that do not express TAG-1 are excluded from the aggregates, indicating that cell binding is mediated by a homophilic interaction. This raises the possibility that a homophilic interaction may also underlie neurite outgrowth on TAG-1. Since TAG-1 is linked to the membrane by a glycosyl phosphatidylinositol (GPI) anchor, neurons can be stripped of surface TAG-1 by treatment with phosphatidylinositol-specific phospholipase C. We have found that stripping embryonic dorsal root ganglion neurons of TAG-1 does not affect the ability of these neurons to grow on a TAG-1 substratum. Thus, homophilic binding does not appear to be required for TAG-1-mediated outgrowth. The presence of an RGD sequence in the second FN III domain suggested a possible interaction between TAG-1 and integrins. In support of this, neurite outgrowth was blocked in the presence of antibodies that inhibit the function of β1- and β3-containing integrins. These results suggest that neurite outgrowth on TAG-1 is dependent on integrin function, and that TAG-1 can mediate both homophilic and heterophilic interactions.

X 106 EXPRESSION AND FUNCTION MODULATION OF VLA INTEGRINS ON HUMAN NK CELLS, A. Gismondi, F. Mainiero, S. Morrone, G. Palmieri, M. Milella, M. Piccoli, L. Frati and A. Santoni. Dept. Experimental Medicine, University of Rome "La Sapienza", Rome, Italy.

Our study deals with the analysis of the expression, regulation and role of β1 integrins on human NK cells. We have previously shown that fresh human NK cells express VLA-4 and VLA-5 as fibronectin (FN) receptors and VLA-6 as laminin (LM) receptor while they do not express detectable levels of VLA-1, VLA-2 and VLA-3. Here we analyzed the modulation of integrin receptors expression and/or function following short or long term NK cell activation. Culture of NK cells in the presence of lymphoblastoid EBV$^+$ cell lines for 10 days resulted in a rapid modulation of β1 integrins with induction of VLA-1, VLA-2 but not VLA-3 while VLA-6 expression declined. Changes in integrin pattern expression were associated with changes in adhesive function. Cultured NK cells adhered to FN at the same degree of fresh NK cells while, differently from fresh NK cells, they bound also collagen I, but did not adhere to LM. We have also observed that the ability of NK cells to adhere to FN and LM is modulated by short time activation with phorbol esters or CD16 triggering. Rapid NK stimulation did not affect cell surface expression of β1 integrins, while changes in the phosphorylation status of α6 but not of α4, α5 and β1 subunits were found in our experimental conditions. A different kinetics of α6 phosphorylation after stimulation with TPA or CD16 triggering was also observed. Studies are in progress to analyze the signalling pathway involved in the activation of β1 integrin-mediated functions on NK cells.

X 107 **THE INTEGRIN αV CYTOPLASMIC INFLUENCES ITS PROMISCOUS BETA SUBUNIT ASSOCIATION**
Edward Filardo, Brunhilde Felding-Habermann, Diane Sander, and David Cheresh. The Department of Immunology. The Scripps Research Institute. La Jolla, CA 92037.

The ability of a cell to recognize ligands present in the extracellular matrix or on the surface of an opposing cell is determined largely by the integrin α/β subunit pairing expressed by that cell. The αv subunit, expressed on a wide variety of cell types is capable of associating with multiple beta subunits including β1, β3, β5, β6, and β8. In contrast, αIIb is restricted to platelets where it exclusively associates with β3. In order to determine the molecular basis of these α/β subunit pairings, we transfected the αv negative M21-L cell line with cDNAs encoding αIIb or αv. Reconstitution of these cells with αv results in de novo surface expression of functional αvβ3 and αvβ5 heterodimers while αIIb only associates with β3. However, when αv is expressed with a truncated cytoplasmic tail it selectively loses the capacity to recover β5 to the cell surface but maintains its β3 association. Therefore, we conclude that the cytoplasmic tail of αv influences its promiscuous beta subunit association and thus, the integrin repertoire expressed on the cell surface.

X 108 A SYNTHETIC PEPTIDE CONTAINING ARG-GLY-ASP(RGD INHIBITS BONE FORMATION AND RESORPTION, Gloria Gronowicz, Dept. of Orthopaedics, Univ. of Connecticut Health Center, Farmington, CT 06032

The role of integrins in bone formation and resorption was determined in a mineralizing bone culture system by administration of an RGD-containing peptide which interferes with integrin-mediated cell attachment to specific extracellular matrix proteins. Parietal bones were obtained from 18 day fetal rats and cultured in serum-free BGJ medium supplemented with ITS+ (Collaborative Research, Inc.). During 4 days in culture, there is a 2.3 and 2.2 fold increase in calcium content and % bone/unit area measured by histomorphometry, respectively. The addition of 0.1 to 50 μM GRGDSPK (Telios Pharmaceuticals, Inc.) caused a dose-dependent decrease in calcium content with a maximum decrease of 32% with 50 μM. The % bone/unit area was also decreased in a dose-dependent manner with a maximum inhibition of 43% with 50 μM. Osteoblast organization along the mineralizing front was disrupted and the bone was uneven in contour compared to control bones. The control peptide of 50 μM GRADSP had no effect on calcium content, % bone/unit area or bone morphology. No significant difference was found in dry weight or DNA content between untreated and treated bones. General cell toxicity with the peptides was not observed, since the incorporation of ^{3}H proline into collagenase-digestible and non-collagen proteins, and the % collagen synthesized were similar in control and treated bones. Bone resorption was assessed by injecting pregnant rats with 200 μCi/ml of ^{45}Ca, stimulating fetal bone resorption in culture with 50 μM parathyroid hormone (PTH) and counting ^{45}Ca release into the medium. A dose-dependent inhibition of PTH-induced bone resorption was found with 0.1 to 50 μM GRGDSPK and not with the control peptide. PTH-induced bone resorption was completely inhibited by 50 μM GRGDSPK. Osteoclast number was also decreased.

In conclusion, the inhibition of bone formation and resorption by a RGD-containing peptide in a mineralizing organ culture system, suggests that integrins have an important role in osteoblast and osteoclast function.

X 109 THE ADHESION PATTERNS OF HUMAN BONE CELLS TO EXTRACELLULAR MATRIX GLYCOPROTEINS

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In bone, there are at least 6 glycoproteins that contain the RGD sequence: fibronectin (FN), thrombospondin (TSP), vitronectin (VN), type I collagen (COLL), osteopontin (OPN) and bone sialoprotein (BSP).

In the present study, we investigated the effect of these glycoproteins as well as BSP fragments (endogenous and those obtained by enzymatic cleavage of the molecule with chymotrypsin, trypsin and cyanogen bromide) on attachment of primary human bone cells. All examined glycoproteins promoted bone cell attachment in a dose dependent manner. VN, COLL, OPN and BSP when applied in equimolar concentration promoted cell attachment equally, showing maximum effect at concentration of 0.1 uM in the coating solution. TSP and FN were less active at lower concentrations, however, at a concentration of 0.2 uM they showed activity comparable to other glycoproteins. BSP fragments were also active in promoting cell attachment, even though endogenous fragment 1 was lacking the RGD-containing part of the molecule. A chymotryptic fragment of BSP appeared to be most active, comparable to the intact BSP, whereas trypsin, cyanogen bromide and endogenous fragments were less active (60-80% of the activity of the intact BSP). GRGDS oligopeptide blocked attachment to VN, BSP and OPN completely, while it was only partially inhibitory to FN, COLL and TSP. This suggests that RGD-independent adhesion mechanisms may be involved in bone cell attachment to those glycoproteins. Interestingly, fragment 1 of BSP was active in the presence of GRGDS oligopeptide, while the intact molecule and all other fragments were not . These data suggest the presence of a cryptic site within the BSP molecule, which is revealed and promotes cell attachment only upon enzymatic cleavage by endogenous protease.

X 110 INTEGRIN ANALOGS in *CANDIDA ALBICANS*, OTHER *CANDIDA* SPECIES and *SACCHAROMYCES CEREVISIAE*.

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In both yeast and hyphal phase, *Candida albicans* expresses a surface receptor for C3 fragment iC3b which is antigenically, structurally, and functionally related to the mammalian leukocyte integrins CD11b/CD18 (Mac-1; CR3) and CD11c/CD18 (p150,95; CR4). When assessed by flow cytometry with the monoclonal antibodies anti-Mo1-94 or OKM1, surface expression of the integrin analog is highest in *C. albicans*, is significantly reduced in less pathogenic *Candida* species (*C. albicans*>*C. tropicalis*>*C. parapsilosis*>*C. glabrata*>*C. krusei*) and is entirely undetectable on the surface of the non-pathogenic yeast *S. cerevisiae*. This same hierarchy obtains for adhesion to the human epithelial cell line HeLa: *C. albicans* is strongly adherent, while adhesion of related *Candida* species and *S. cerevisiae* is incrementally reduced. Thus, surface expression of the integrin analog correlates both with pathogenicity and adhesion in yeast.

Incubation of *C. albicans* with purified human iC3b reduces adhesion of this yeast to epithelial monolayers by 82%; a 15-mer RGD peptide reduces adhesion by 49%. RGK peptides or RGD peptides which lack the required flanking sequence fail to inhibit epithelial adhesion of *C. albicans*. Synthesis of C3 by the epithelial cell and subsequent post-translational cleavage to iC3b provide a biologically relevant source for this adhesive ligand.

After purification from cytosolic extracts by affinity chromatography with OKM1, the integrin analogs in *C. albicans* and *S. cerevisiae* appear as single bands of M_r 165±15 kD on SDS-PAGE under non-reducing conditions. Minor structural differences are apparent under reducing conditions, with the integrin analog in *C. albicans* electrophoresing as a triplet of M_r 56, 62, and 64 kD and the integrin analog in *S. cerevisiae* showing three distinct bands at 52 kD, 55 kD, and 58 kD. All three bands are recognized by anti-CD11b/c monoclonals in Western blotting. We hypothesize that subtle structural differences in the integrin analog in *S. cerevisiae* contribute to failure of surface expression and absence of adhesion in this non-pathogenic yeast.

X 111 IMPAIRED REGULATION OF COLLAGEN SYNTHESIS IN COLLAGEN GELS AND CHANGE IN PATTERN OF COLLAGEN BINDING INTEGRINS IN SCLERODERMA FIBROBLASTS. Mikael Ivarsson[1], Alan McWhirter[2], Carol M. Black[2], and Kristofer Rubin[1], Dept. of Med. and Physiol. Chem, University of Uppsala, BMC Box 575, S-751 23 Uppsala, SWEDEN and [2]Dept. of Rheumatology, Royal Free Hospital, London U.K.

Systemic sclerosis (SSc) is a generalized connective tissue disease characterized at the ultrastructural level by excess production and deposition of collagen. We have investigated the behaviour of primary SSc fibroblasts cultured in three-dimensional collagen gels and compared it with normal primary fibroblasts. Fibroblasts were isolated from biopsies taken from the affected areas of skin of scleroderma, as well as from normal subjects. Synthesis of collagen type I was monitored by determinations of levels of α1(I) mRNA. In serum-free cultures a population of SSc fibroblasts down-regulated their level of α1(I) mRNA less markedly than a population of control fibroblasts. Furthermore, the SSc fibroblasts contracted collagen gels slower than their normal counterparts. These findings demonstrate that the ability of SSc fibroblasts to interact with the collagen matrix is functionally impaired.

Polyclonal anti-$ß_1$ integrin IgG inhibited collagen gel contraction and impaired the downregulation of collagen synthesis when normal fibroblasts were cultured in free-floating three-dimensional collagen gels. This finding demonstrate that $ß_1$ integrins are important for the functional interaction with the collagen gels. SSc fibroblasts regularly displayed an abnormal but variable pattern of $ß_1$ integrins as revealed by immunoprecipitations of extracts of ^{125}I-labelled cells. The expression of integrin $α_1$-chain was generally lower in SSc fibroblasts than in the corresponding normal cells. Our data is compatible with the hypothesis that SSc fibroblasts have an abnormal regulation of $ß_1$ integrins at their surfaces and that these alterations may be reflected in a disturbed cell-collagen interaction.

X 112 **INHIBITION OF FIBRINOGEN BINDING TO ADP STIMULATED PLATELETS BY β_3 INTEGRIN DERIVED PEPTIDE 217-231. SIGNIFICANCE OF Pro_{219} IN RECEPTOR-LIGAND INTERACTION.** **Elizabeth C. Lasz, Lee Silver, Maciej Trybulec, Shabbir Khan, and Stefan Niewiarowski. Thromb Res Center, Depart Physiol Temple University School of Medicine, Philadelphia, Pa 19140**

We have shown previously that binding of integrin β_3 derived peptides 217-231 and 217-230 (peptide A) to fibrinogen is not inhibited by RGDS but it is inhibited by $\alpha IIb/\beta_3$ complex; at a 500 fold higher molar concentration, β_3 217-231(Y) displaced a part of $\alpha IIb/\beta_3$ complex bound to immobilized fibrinogen. In contrast, mutants of β_3 217-230 (peptide B with Pro_{219} and Glu_{220} substituted with Ala and Gln, respectively; peptide C with Pro_{219} substituted with an Ala) did not bind to fibrinogen and did not displace IIb_α/β_3. In the present study, we compared the effect of β_3 derived peptide, peptide A and its mutants (peptide B and peptide C) on platelet aggregation and ^{125}I-fibrinogen binding to ADP stimulated platelets. The peptides synthesized by solid phase methods were purified to 99% purity by reverse phase HPLC. The purity of peptides was confirmed by mass spec and amino acid analysis. β_3 217-230 inhibited ADP-induced platelet aggregation in a dose dependent manner with IC_{50} of $10^{-5}M$. The inhibitory effect of peptide was partially reversed by an excess of fibrinogen. Binding of ^{125}I-fibrinogen to ADP stimulated platelets was inhibited by $10^{-4}M$ β_3 217-231 in a non competitive manner. The inhibitory activity of peptides B and C was about 10 fold lower suggesting that Pro_{219} is important for this interaction. In conclusion, Pro_{219} is important for the inhibitory effect of β_3 217-230 on platelet aggregation and fibrinogen binding to ADP stimulated platelets. Our data also suggests that the region 217-230 of β_3 is involved in interaction with adhesive ligands.

X 113 REQUIREMENT OF INTEGRIN β3 SUBUNIT FOR CARCINOMA CELL SPREADING AND MIGRATION ON VITRONECTIN. David I. Leavesley, Greg D. Ferguson, & David A. Cheresh, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

FG human pancreatic carcinoma cells fail to express integrin αvβ3 and thus attach to a vitronectin substrate utilizing integrin αvβ5. These cells fail to form focal contacts, spread or migrate on vitronectin but readily do so on collagen in an β1 integrin-dependent manner. Transfection of FG cells with a cDNA encoding the integrin β3 subunit results in the surface expression of a functional integrin αvβ3 heterodimer providing these cells with novel adhesive and biological properties. Specifically, FG cells expressing β3 acquire the capacity to attach and spread on vitronectin as well as fibrinogen with β3 localization to focal contacts. Moreover, these cells gain the capacity to migrate through a porous membrane in response to either vitronectin or fibrinogen. These results demonstrate that the β3 and β5 integrin subunits when associated with αv promote distinct cellular responses to a vitronectin extracellular environment.

X 114 THE EXPRESSION OF INTACT, SOLUBLE, AND MUTANT FORMS OF THE HUMAN INTEGRIN $\alpha_1\beta_1$. E.E. Marcantonio, M.R. Epstein and R. Briesewitz. Departments of Pathology and Anatomy & Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY 10032.

We are investigating the structure and function of the human integrin $\alpha_1\beta_1$ in order to define its role in development and disease. A full length human α_1 construct has been transfected into 3T3 fibroblasts, and stable murine lines expressing α_1 have been isolated. This exogenous α subunit forms heterodimers with the murine β_1 and is efficiently expressed on the cell surface. Furthermore, this human integrin is functional in the 3T3 cells as shown by the ability of this integrin to bind to both type IV collagen and laminin-Sepharose columns. To assess structure-function relationships, we have produced soluble forms of the human α_1 and β_1 by site-directed mutagenesis, and expressed these subunit cDNAs in transient transfection assays in Cos cells. Although soluble human β_1 subunit is found in the media when the corresponding cDNA was used, the secretion of the soluble α_1 subunit was found to be dependent on the dimerization with soluble β_1. Cotransfection of truncated human α_1 cDNA with truncated forms of either the human or avian β_1 cDNA led to efficient secretion of $\alpha_1\beta_1$ heterodimers. We are currently using this system to assay the effects of a variety of mutant forms of this receptor, constructed using PCR-mediated mutagenesis. These mutants include replacement of the α_1 I domain with those from other integrins, deletions of the cysteine-rich domains of both α and β, and point mutants of the metal binding domains of the α_1 subunit. We hope these mutants will help us design peptide inhibitors to study the function of α_1 in vivo, and will allow us to dissect the laminin versus collagen binding domains of this receptor.

X 115 α5β1 INTEGRIN MEDIATES THE BINDING OF FIBRONECTIN'S AMINOTERMINAL MATRIX ASSEMBLY DOMAIN BY FIBROBLASTS WITHOUT BINDING TO FIBRONECTIN, John A. McDonald and Andrew H. Limper, Mayo Medical School, Rochester, MN 55905 and Scottsdale, AZ 85259

The assembly of fibronectin (FN) into fibrils invokes cell binding to two domains, the RGD site recognized by α5β1 integrins (α5β1) and the 29 kDa aminoterminal matrix assembly domain (29 kDa). Previously, we hypothesized that α5β1 integrins initiate FN fibrilogenesis, whereas binding of the 29 kDa was required for fibril elongation. However, the situation is more complex, as antibodies to either the α5 or β1 subunit inhibiting cell adhesion to FN also inhibit the binding of 29 kDa by cells whereas nonblocking anti-integrin antibodies do not. As α5β1 does not bind to 29 kDa directly, these results suggest that FN binding to α5β1 somehow facilitates 29 kDa binding by a distinct "receptor". To test this, we studied the effect of monoclonal antibody N-294 to FN's RGD site and anti-α5 or β1 integrin antibodies which inhibit cell adhesion to FN on binding of 29 kDa by fibroblasts. Binding was inhibited 50 percent by α5 or β1 antibodies. However, even though N-294 interrupted α5β1 binding to FN, it had no significant effect upon 29 kDa binding. To exclude the possibility that N-294 was somehow replacing the requirement for α5β1 in 29 kDa binding (e.g., by binding to FN's integrin binding site), mixing experiments were carried out. Anti-β1 (Mab13 provided by S. Akiyama and K. Yamada) inhibited 29 kDa binding by 50 percent in the presence or absence of N-294. Thus, α5β1 does not have to be bound to FN in order to mediate 29 kDa binding. Our results suggest surprising complexity in the participation of α5β1 in FN matrix assembly.

X 116 IDENTIFICATION OF A FIBRONECTIN SELF-ASSEMBLY SITE THAT IS INVOLVED IN FIBRONECTIN MATRIX ASSEMBLY AND RECONSTRUCTION OF THE SITE IN A SYNTHETIC PEPTIDE, Alex Morla and Erkki Ruoslahti, La Jolla Cancer Research Foundation, La Jolla, CA 92037

The active form of fibronectin is its extracellular matrix form, which allows for the attachment of cells and influences both the growth and migration of cells. The matrix form is assembled by cells and many tumorigenic cells are defective in this regard. Several regions within fibronectin have been shown to play a role in matrix assembly by cells. One region is the RGD-containing cell binding domain. Another region is the amino terminal, 29 kDa, heparin binding domain. A third region is contained within a 56 kDa fragment which encompasses the gelatin binding domain plus the first type III repeat.

To find new regions of fibronectin which are involved in matrix assembly, fibronectin was proteolyzed with chymotrypsin and the fragments were tested for their ability to inhibit matrix assembly. A 14 kDa fragment, corresponding to a region within the first two type III repeats, was found to inhibit matrix assembly. The 14 kDa fragment also bound to fibronectin and inhibited fibronectin self-assembly. Peptides representing the 14 kDa fragment were synthesized and tested. One of these peptides (peptide P1) retained the ability to inhibit matrix assembly. Affinity chromatography of plasma on peptide columns revealed that fibronectin bound specifically to a peptide P1 column. Peptide P1 also inhibited fibronectin-fibronectin association. Peptide P1 inhibited matrix assembly in a manner similar to that of the 14 kDa fragment. Both had a small effect on the initial binding of fibronectin to cell surfaces, but both significantly inhibited the incorporation of fibronectin into the extracellular matrix. Thus, unlike the 29 kDa fragment that blocks an initial event in matrix assembly (fibronectin binding to cells), this peptide may inhibit a later step in matrix assembly, fibronectin-fibronectin alignment prior to disulfide cross linking.

X 117 INTEGRIN α4β1-FIBRONECTIN INTERACTIONS A. Paul Mould and Martin J. Humphries, Department of Biochemistry and Molecular Biology, Stopford Building, University of Manchester, Manchester M13 9PT, United Kingdom

The type III connecting segment of fibronectin contains two cell-binding sites, represented by the peptides CS1 and CS5, both of which are recognized by the integrin receptor α4β1. Using assays measuring the spreading of A375-SM human melanoma cells, we now report that the adhesion-promoting activity of a 29-kDa protease fragment of fibronectin containing the COOH-terminal heparin-binding domain (HepII), but lacking CS1 and CS5, is completely sensitive to anti-α4 and anti-β1 antibodies, suggesting that HepII contains a third α4β1-binding sequence. Examination of the primary structure of HepII revealed a sequence with homology to CS1. A 19-mer peptide spanning this region (designated H1) was found to support cell spreading to the same level as the 29-kDa fragment. H1-dependent adhesion was completely sensitive to anti-α4 and anti-β1 antibodies. When soluble peptides were tested for their ability to block cell spreading on the 29-kDa fragment, a 13-mer peptide comprising the central core of H1 was found to be completely inhibitory. The active region of H1 was localized to the pentapeptide IDAPS, which is homologous to LDVPS from the active site of CS1. Taken together, these results identify a novel peptide sequence in the HepII region of fibronectin that supports α4β1-dependent cell adhesion. We are currently investigating the mode of interaction of CS1, CS5 and H1 with α4β1.

X 118 **Ligand specificity of the platelet fibrinogen receptor, αIIb/β3 and the vitronectin receptor αvβ3**

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The platelet fibrinogen receptor, glycoprotein IIb/IIIa (αIIb/β3) and the vitronectin receptor αvβ3 are highly promiscuous RGD binding integrins. Among the ligands demonstrated to bind to these integrins are fibrinogen, fibronectin, vitronectin and von Willebrand Factor.
The aim of this study is to investigate the affinities with which these ligands bind to the integrins mentioned above.
The integrins were purified to homogeneity from human platelets (αIIb/β3) or human term placenta (αvβ3). Binding of the ligands to the integrins was quantified i) after immobilization of the integrins on microtiter plares in an ELISA-type assay, ii) after incorporation into lipid vesicles and iii) after incorporation into planar lipid membranes. Fluorescence-labeled ligands were used for the binding to integrins incorporated into vesicles or membranes.
Results will be shown which summarize a) competition experiments for the different ligands, b) the influence of divalent cations (Ca, Mg, Mn) on the binding and c) binding constants of the different ligands.

X 119 **FUNCTIONAL MAPPING OF ICAM-2**

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Leukocyte adhesion plays a fundamental role in leukocyte functions. The binding between cells involves participation of several cell surface glycoproteins, and the most important are the CD11/CD18 integrins and their cellular ligands CD54 (ICAM-1) and ICAM-2. It has been shown that ICAM-2 is a cellular ligand for CD11a/CD18. We have earlier synthesized the ICAM-2 DNA by PCR, and expressed it in E. coli and COS-1 cells. The protein made in bacteria has been used to to obtain polyclonal and monoclonal antibodies. By using one of our anti-ICAM-2 MAb (MAb 6D5) we have shown this molecule to be a glycoprotein with an apparent MW of 55 000. It is strongly expressed on endothelial cells and less in hematopoietic cells. In vitro studies has shown that ICAM-2 is constitutive expressed. MAb 6D5 can inhibit the binding of ICAM-2 transfected cells to leukocytes. We have also shown that the epitope for MAb 6D5 is situated in the first domain of ICAM-2. Currently we are studying more closely which parts of ICAM-2 are essential in adhesion. We are going to present data which confirm the importance of the first domain of ICAM-2 for the leukocyte adhesion.

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X 120 LAMININ AS A NOVEL LIGAND FOR THE αVβ3 INTEGRIN R. Pitti, R. G. Hammonds, K.King, J.W.McLean, M. Helfrich, M. Horton, S. Bodary Genentech Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA, 94080, U.S.A.

Osteoclast adhesion to bone is a critical step in the process of bone resorption. To date, two members of the integrin family of cell adhesion receptors have been identified on the osteoclast surface: αVβ3 (vitronectin receptor) and α2β1 (collagen) receptor. In addition to vitronectin, the αVβ3 integrin may serve as a receptor for numerous ligands, including fibrinogen, fibronectin, von Willebrand factor, thrombospondin, osteopontin and bone sialoprotein II. Osteoclasts from a variety of sources have been found to adhere to these proteins. We have generated a recombinant cell line (15D) which expresses high levels of human αVβ3, by transfection of the αV and β3 cDNAs into human embryonic kidney 293 cells. This cell line (15D) express the receptor in a form which binds multiple ligands. In the course of these studies a novel ligand for this receptor has been identified--human placental laminin. Rat osteoclasts, human recombinant (r-)αVβ3 expressing cells and purified r-αVβ3 have been shown to bind human placental laminin. Binding has been found to be sensitive to inhibition by the tripeptide Arg-Gly-Asp, as well as antibodies to the αVβ3 integrin. In contrast merosin as well as laminins purified from other sources, including mouse EHS tumor and rat L2 yolk sac tumor, do not promote binding. The potential role of this novel ligand in biological events mediated by the αVβ3 integrin is under investigation.

X 121 AVIAN INTEGRIN α_v SUBUNIT ASSOCIATES WITH MULTIPLE ß SUBUNITS, R. Rajaraman, Departments of Medicine & Microbiology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

Integrins are a family of receptors that mediate cell-cell or cell-matrix adhesive interactions. These are non-covalent heterodimers of one α and one ß subunit. Some integrins recognize Arg-Gly-Asp (RGD) sequence on their ligands. Vitronectin receptors (VNRs) from human placenta (H-$\alpha_v/ß_3$), chicken embryo fibroblasts (CEF), 9 d chicken embryos (CE) and adult chicken gizzard (ACG) were isolated by GRGDSPK-Sepharose affinity chromatography. The H-, CEF-, CE-, and ACG-VNRs showed one α (160 kd) and one ß subunit (around 95-90 kD). Using polyclonal antisera raised in rabbits against H α_v, $ß_3$ and ACG-VNR-ß, avian VNRs from different sources were characterized by immunoblot and immunoprecipitation techniques in comparison with those of the human system. Anti-H-α_v cross-reacted with the α subunits of CEF-, CE- and ACG-VNRs. In ACG-VNR-α subunit, this cross-reactivity was highly restricted to the smaller cytoplasmic fragment as compared to the large extracellular domain. Anti-ACG-VNR-ß cross-reacted only with CE-ß subunit to a lesser extent, while it did not cross-react with the 90 kD CEF-ß subunit and the H-$ß_3$ subunit. In reciprocal immunoblots anti-H-$ß_3$ cross-reacted only with CEF-ß and to a lesser extent with CE-ß and did not cross-react with the 85 kD ACG-VNR-ß subunit. Immunoprecipitation studies indicated that in human osteosarcoma MG63 cells α_v is associated with $ß_3$ and $ß_1$ subunits. From radioiodinated CEF ectracts anti-ACG-VNR-ß immunoprecipitated α_v along with four different polypeptides with non-reduced M, 120 kD, 110 kD ($ß_1$-like), 90 kD (avian equivalent of H-$ß_3$), and 85 kD (Avian equivalent of H-$ß_5$). Anti-ACG-VNR-ß did not precipitate any band(s) from 35-S labelled embryonic gizzard (ECG), while anti-H-$\alpha_v/ß_3$ precipitated two major bands with non-reduced M, 155 kD and 95 kD. Human anti-$ß_1$and anti-$ß_5$ did not yield any precipitation from CEF. These results indicate that (a) the 90 kD ß subunit found in CEF, CE and ECG are the avian equivalent of human ß3; (b) the 90 kD ACG-VNR-ß subunit is antigenically different from H-$ß_3$; (c) avian α_v associates with at least four different ß subunits and (d) that there are tissue and developmental variations in the expression of avian VNR subunits. The nature of the 90 kD ß subunit of ACG-VNR and the 120 kD ß subunit in CEF is not known at present.

X 122 ISOLATION OF A cDNA CLONE ENCODING THE 170 kDa SUBUNIT OF THE CELL ADHESION MOLECULE EPILIGRIN, M.C. Ryan and W.G. Carter, Basic Science Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Epiligrin is a glycoprotein complex found in most epithelial basement membranes and is a ligand for cell adhesion via integrin α3β1 (Carter et al. 1991, Cell 65, 1-20). In the extracellular matrix of human foreskin keratinocytes, epiligrin is composed of at least three disulfide bonded subunits referred to as E170, E145, and E135 based on molecular size in kDa's. We have isolated a cDNA clone which corresponds to the 170 kDa component of epiligrin. A human keratinocyte expression library was screened using a polyclonal antibody against epiligrin and several cDNA clones were isolated. Two plaque purified clones were expressed and used to affinity purify antibodies from a crude polyclonal antiserum generated against keratinocyte extracellular matrix. The affinity purified antibodies reacted specifically with the 170 kDa component of Mab P1E1 immunopurified epiligrin. Northern blot analysis of mRNA isolated from human foreskin keratinocytes revealed a single transcript which is at least 5.2 kb in size. This transcript is reduced in the mRNA population of keratinocytes transformed with human papiloma virus, confirming the sensitivity of epiligrin to viral transformation. Preliminary sequence analysis of the clones suggest that the 170 kDa subunit of epiligrin is novel cell adhesion molecule.

X 123 A QUANTITATIVE MICROWELL METABOLIC ASSAY FOR CELL ADHESION TO SUBSTRATES AND HAPTOGENIC GRADIENTS. Gordon E Searles, Walter T Dixon. Div Derm Cutan Sci DofAlberta, Edmonton, AB CANADA T6G 2S2

We report a quantitative attachment assay for cells, modified from Lotz et al (J.Cell Biol.109:1795-1805,1991). 96-microwell plates are adsorbed with soluble substrate and blocked with 5% nonfat milk. Epithelial cells in serum-free media are allowed to attach for a specified time. Wells are filled with media and a nitrocellulose(NC) strip is placed on top, clamped, and centrifuged for 8 min at a set speed in an inverted position. Detached cells on the NC are punched into a 2nd microwell plate. 0.5mg/ml MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)in media is incubated for 3h, removed, and DMSO added for 30 min. Absorbance at 540nm is measured, and percent adhesion is the ratio of abs. of adherent/(adherent+nonadherent). Only viable cells containing succinate dehydrogenase are capable of reducing MTT to formazan. Thus, any cell capable of reducing MTT can be used.

Absorbance is not affected by NC, and is directly proportional to cell number. The assay is nondestructive, and highly reproducible. Subtle alterations in adhesion brought on by blocking antibodies or attachment peptides are easily detected.

Attachment to blocked NC containing Western blotted substrates show same adhesion results as the microwell assay. Complex substrate gradients are created in gels and then blotted onto NC and used as substrates, permitting a quantitative assessment of cell attachment to complex substrates.

This assay offers a rapid, reproducible and cost-efficient method using MTT. Advantages over original assay include: 1)omission of radionuclides; 2)multiple assays can be run simultaneously; 3)apparatus commonly available; 4)much higher sensitivity; 5)requires smaller cell numbers; 6)reproducible (SD 5%). The utility of this assay allows the use of any cell type capable of converting MTT to formazan by succinate dehydrogenase, while the simplicity of the centrifugation method allows sensitive determination of adhesive strength for many cell types.

X 124 DIVALENT CATIONS AND THE $\alpha_2\beta_1$ INTEGRIN-COLLAGEN INTERACTION. William D. Staatz and Samuel A. Santoro, Departments of Pathology and Medicine, Washington University School of Medicine, St. Louis, MO 63110.

We have previously shown that the Mg^{++}-dependent adhesion of platelets to collagen is mediated by the $\alpha_2\beta_1$ integrin complex and that this adhesion is inhibited by Ca^{++} in a simple, linear, noncompetitive manner. Platelet adhesion to collagen is also supported by 2 mM concentrations of at least 5 other divalent cations. We have now examined the dependence of platelet adhesion to collagen upon divalent cation concentration for each of 9 divalent cations. Concentrations of Mg^{++}, Ni^{++}, Co^{++} and Cu^{++} between 0.1- and 2 mM supported platelet adhesion to collagen in a concentration dependent manner, and the apparent affinity constants (Ka') for these cations range from 0.28 mM to 0.66 mM. Mn^{++} supported platelet adhesion in a concentration dependent manner between 0.01- and 0.5 mM, with a Ka' of approximately 0.03 mM. Both Fe^{++} and Zn^{++} also supported platelet adhesion to collagen, but their Ka's were above 5 mM. Sr^{++} did not support platelet adhesion at concentrations up to 5 mM. Concentrations of Ca^{++} from 0.1- to 1 mM supported adhesion slightly above background levels, but 10 mM Ca^{++} inhibited 70-80% of the adhesion supported by 2 mM Mg^{++}, Co^{++} or Zn^{++} or by 100 µM Mn^{++} and inhibited Ni^{++}-dependent adhesion by 39%. Ca^{++} did not significantly alter either Cu^{++}- or Fe^{++}-dependent platelet adhesion to collagen. Results from adhesion assays carried out in combinations of Mg^{++} and Ca^{++} indicated that Mg^{++}-dependent platelet adhesion to collagen is inhibited in a simple, linear, noncompetitive manner by Ca^{++}, with a Ki' of 1.5 mM Ca^{++}. In contrast, parallel studies indicated that Mn^{++}-dependent platelet collagen adhesion is competitively inhibited by Ca^{++}, with a Ki' of 1.5 mM. Both the Mg^{++}- and Mn^{++}-dependent platelet adhesions to collagen were inhibited by the α_2 integrin-specific antibody, P1E6. These data suggest that Mn^{++} and Ca^{++} compete for one type of metal-binding site on the $\alpha_2\beta_1$ integrin complex while Mg^{++} binds to a separate site in the complex. The binding of Ca^{++} and Mg^{++} to these two types of metal binding sites may be important for the physiological regulation of cellular adhesion to collagen.

X 125 [^{3}H]SK&F 107260, A NOVEL RADIOLIGAND FOR CHARACTERIZING THE FIBRINOGEN RECEPTOR, α_{IIb}/β_3, OF HUMAN PLATELETS. J. M. Stadel, D. A. Powers, D. Bennett, A. Nichols, R. Heys, F. Ali, and J. Samanen. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

The integrin, α_{IIb}/β_3, plays a critical role in hemostasis by binding fibrinogen to activated platelets during aggregation. The thrombus formed by the platelet plug can also lead to myocardial infarction or stroke. Recent reports show SK&F 107260 (S,S-cyclo-(Mba-(N$^{\alpha}$Me)Arg-Gly-Asp-Man)) and SK&F 106760 (Ac-S,S-cyclo-(Cys-(N$^{\alpha}$Me)Arg-Gly-Asp-Pen)-NH_2) to be potent and selective fibrinogen receptor antagonists. We have now synthesized [^{3}H]SK&F 107260 (85 Ci/mmol) and characterized its binding to α_{IIb}/β_3 partially purified from outdated human platelets and subsequently reconstituted into liposomes. Radioligand binding studies were performed at 25°C for 60 min. In equilibrium saturation binding experiments [^{3}H]SK&F 107260 displayed $K_d = 3.8 \pm 1.9$ nM (N=3) and $B_{max} = 162 \pm 118$ pmol/mg protein compared to ^{125}I-fibrinogen (185 Ci/mmol) which showed $K_d = 40.3 \pm 28.8$ (N=3) and $B_{max} = 180 \pm 156$ pmol/mg protein. In competition binding experiments a potency series of SK&F 107260 > SK&F 106760 > fibrinogen γ-chain peptide (HHLGGAKQAGDV) was observed with K_ds = 3.2, 49, and 7900 nM, respectively. These data agree with the relative potency of these compounds to inhibit ADP-induced aggregation of washed platelets. [^{3}H]SK&F 107260 also bound specifically to the platelet fibrinogen receptor in detergent (1% octylglucoside) solution or immobilized on plastic. The affinity of immobilized α_{IIb}/β_3 for [^{3}H]SK&F 107260 was apparently increased, $K_d = 0.28$ nM. The results of these studies demonstrate the utility of [^{3}H]SK&F 107260 in characterizing the platelet integin, α_{IIb}/β_3 and this radioligand should facilitate the discovery of fibrinogen receptor antagonists.

X 126 KINETICS OF EXPRESSION OF SURFACE ADHESION MOLECULES ON MONOCYTE/MACROPHAGES. Gianna Stent#, J T May• and S M Crowe#. #Macfarlane Burnet Centre for Medical Research, Fairfield 3078. •LaTrobe University, Bundoora 3083. Australia.

OBJECTIVES: (1) To examine the kinetics of expression of the alpha chains (CD11a, CD11b, CD11c) and the common beta chain (CD18) of members of the LFA integrin family, and the LFA-1 ligand CD54 (ICAM-1) on human peripheral blood derived monocyte/macrophages (MΦ). (2) To compare the expression of these molecules on adherent versus suspension-cultured MΦ, T and B lymphocytes, glioma and neuroblastoma cell lines.

METHODS: Monocytes were isolated from buffy coats (n=12) by density gradient centrifugation and adherence. Cells were maintained either in suspension (teflon jars) or adherent to plastic. MΦ were stained at various time points from the day of isolation (d0) up to 42 days with monoclonal antibodies to CD11a, CD11b, CD11c, CD18 and CD54. Analysis was by flow cytometer. MΦ expression was compared with that on freshly isolated PHA/IL2 stimulated and unstimulated T and B lymphocytes, glioma and neuroblastoma cell lines.

RESULTS AND CONCLUSIONS: On d0 surface expression of CD11a, CD11b, CD11c and CD18 was high (92%-94%). There was a decrease in CD11a and CD11b expression over 4 weeks to 45% and 55% respectively, whilst CD11c and CD18 remained fairly constant and high over 6 weeks (86% & 93% respectively). There was a dramatic increase in MΦ expression of ICAM-1 within the first 24 hours of culture (mean 61% to 86%), followed by a gradual decrease in expression over the next 6 weeks. There was no significant difference in the expression of any of these molecules on cells cultured in suspension or adherent to plastic (by the Student's t test). Expression of CD11a and CD18 on T and B lymphocytes exceeded 80%. Stimulated lymphocytes showed 2 fold higher expression of CD54 than did unstimulated cells. Neuroblastoma and glioma cells did not express CD11a, CD11b, CD11c, CD18 or CD54.

X 127 INTERACTIONS OF CHICKEN $\alpha_1\beta_1$ INTEGRIN WITH DIMERIC AND TETRAMERIC COLLAGEN IV FROM NON-LATHYRITHIC GIZZARD Josef Syfrig and Mats PaulssonDept. of Biophysical Chem. Biocenter, University of Basel, 4056 Basel, Switzerland and Maurice E. Müller Institute for Biomechanics, 3010 Bern, Switzerland

The preferred extracellular ligand of the abundant chicken gizzard $\alpha_1\beta_1$ integrin is collagen IV (Syfrig et al., 1991, Exp. Cell Research **194**, 165-173). In this study, collagen IV isolated from human placenta and chicken gizzard by extensive pepsin digestion bound the $\alpha_1\beta_1$ integrin not equally. Human collagen IV, consisting due to species differences in sites sensitive to pepsin of longer fragments than the chicken collagen IV was a better ligand for the chicken $\alpha_1\beta_1$ integrin.

To investigate whether these findings reflect species differences or variances in fragment size, we isolated collagen IV from chicken gizzard by limited pepsin and by limited trypsin digestion. The pepsin digested collagen IV appeared as the tetrameric form in rotary shadowing EM pictures, whereas the trypsin digested collagen IV consisted of dimers.

The two preparations of large fragments of chicken collagen IV were compared in ELISA-style binding assays with collagen IV tetramers isolated from human placenta by limited pepsin digestion. Human collagen IV bound the chicken $\alpha_1\beta_1$ integrin about twice as efficiently as both chicken collagen IV preparations in this assay. The short, cell binding cyanogen bromide fragment CB3 of human collagen IV (Vandenberg et al., 1991, J. Cell Biol. **113**, 1475-1483) was also a ligand for the chicken $\alpha_1\beta_1$ integrin, but its binding activity was weaker than the one of the large fragments of collagen IV from human and chicken.

In inhibition experiments, binding of biotinylated CB3 to immobilized chick $\alpha_1\beta_1$ integrin, was better inhibited by limited with pepsin digested chicken collagen IV than by trypsin digested chicken collagen IV or by human placenta collagen IV. CB3 was a less efficient competitor than the long fragments of collagen IV.

These results suggest that besides the binding site on CB3, additional binding sites are present on the collagen IV molecule. Since the results obtained with long fragments of chicken and human collagen IV in direct binding and in competition are inconsistent, further experiments are necessary to explain the observed differences and to obtain a better understanding of the binding mechanism of the chicken $\alpha_1\beta_1$ integrin-collagen IV interaction. These experiments will be simplified by the now practicable isolation of large fragments of chicken collagen IV from non-lathyritic gizzard tissue.

X 128 KERATINOCYTE EXTRACELLULAR MATRIX MODULATES INTERCELLULAR ADHESION MEDIATED BY INTEGRINS $\alpha_3\beta_1$ AND $\alpha_2\beta_1$. B.E. Symington and W.G. Carter, Fred Hutchinson Cancer Research Center, M477, Division of Basic Sciences, Seattle, WA 98104

Our previous studies suggested that integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ play dual roles in cell-substrate and cell-cell adhesion (Carter et al., JCB 110:1387, 1990). We now provide functional evidence that these integrins are involved in establishing intercellular adhesion (ICA) in epidermal cell lines. We found that P_1B_5, an anti-$\alpha_3\beta_1$ antibody, promotes ICA of transformed cells that synthesize reduced amounts of extracellular matrix (ECM). P_1B_5 F(ab) also stimulates ICA. Long-term growth on ECM derived from human keratinocytes inhibited P_1B_5-induced ICA, suggesting that ECM modulates integrin-mediated ICA. Antibody inhibition experiments suggested that $\alpha_2\beta_1$ on suspension cells interacted with $\alpha_3\beta_1$ on adherent cells. The adhesion of beads coated with radiolabeled $\alpha_2\beta_1$ to epidermal cells or to plastic-immobilized $\alpha_3\beta_1$ was similarly stimulated by P_1B_5 and inhibited by ECM. These results suggest that: a) anti-α_3 treatment of the adherent monolayer induces an interaction between apical $\alpha_3\beta_1$ on the adherent monolayer and $\alpha_2\beta_1$ on the nonadherent cell and b) this process is modulated by the interaction of the ECM with epidermal basal cells. We propose that the interaction of the ECM with epidermal basal cells regulates the role of integrins $\alpha_3\beta_1$ and $\alpha_2\beta_1$ in epidermal ICA and stratification.

X 129 A POINT MUTATION IN INTEGRIN β_1 SUBUNIT BLOCKS BINDING OF $\alpha5\beta1$ TO FIBRONECTIN AND INVASIN BUT NOT RECRUITMENT TO ADHESION PLAQUES, Yoshikazu Takada, Jari Ylanne, David Mandelman, Wilma Puzon, Mark H. Ginsberg, Committee on Vascular Biology, The Scripps Research Institute, North Torrey Pines Road, La Jolla, CA 92037.

The effects of the Asp^{130} to Ala (D130A) substitution in $\beta1$ on the interaction of $\alpha5\beta1$ (VLA-5) with its ligands, fibronectin (FN) and invasin, were examined using human $\beta1$ or $\beta1$(D130A) cDNA transfected Chinese hamster ovary (CHO) cells. Asp^{130} is located in a region which is highly conserved among different integrin β subunits, and Arg-Gly-Asp peptide cross-links to the corresponding region of $\beta3$. We obtained CHO cells expressing human $\beta1$ or $\beta1$(D130A) on the surface associated mainly with hamster $\alpha5$ subunit. The expression of human $\beta1$ or $\beta1$(D130A) was approximately equal to that of endogenous hamster $\beta1$. The D130A substitution blocked the binding of $\alpha5\beta1$ to FN 110K fragment and invasin as judged by ligand affinity chromatography, suggesting that Asp^{130} is essential for ligand binding. $\beta1$(D130A) was, however, detected in the adhesion plaques formed in transfected cells cultured on FN, suggesting that ligand binding is not essential for recruitment of the receptor to adhesion plaques.

X 130 AN INTEGRIN WITH A NOVEL SPECIFICITY BINDS TO THE HIV TAT PROTEIN, Bruce E. Vogel[1], Shu-Jan Lee[2], Flossie Wong-Staal[2] and Erkki Ruoslahti[1], [1]La Jolla Cancer Research Foundation, La Jolla, CA 92037, [2]Department of Medicine, University of California, San Diego, La Jolla, CA 92093

An unexpected feature of the HIV Tat protein is that it can be released from HIV-infected cells and subsequently stimulate the growth of Kaposi's sarcoma cells or be internalized by uninfected cells and transactivate transfected HIV promoter constructs. Studies designed to determine the cellular mechanism mediating these effects have focused on the interaction of Tat with the cell surface. One group has suggested that the Arg-Gly-Asp (RGD) sequence in Tat is required for the binding of Tat to the cell surface, implicating an interaction mediated by a member of the integrin family of cell adhesion receptors. Another study has proposed a type of non-specific endocytosis for the cellular uptake of Tat. Here, we performed cell adhesion assays with Tat and Tat-derived peptides to show that the basic domain of Tat, not the RGD sequence, is required for the interaction of L8 and SK-LMS cells with Tat. Polyclonal antibodies to the vitronectin receptor (VNR) were used to block this interaction, implicating a VNR-related receptor as a mediator of cell binding to Tat. Affinity chromatography with Tat paptides and immunoprecipitation with various anti-integrin antibodies suggest that the vitronectin binding integrin, $\alpha_v\beta_5$, binds to Tat in an interaction atypical for integrins. This binding to Tat is sensitive to 250 mM NaCl and not to EDTA. The data suggest that one mechanism for the interaction of Tat with the cell surface is through the integrin $\alpha_v\beta_5$.

X 131 MOLECULAR MECHANISMS OF CELL ADHESION TO FIBRONETIN. E. A. Wayner[1], A. Garcia-Pardo[2], M. S. Wilke[1], J. E. Schwarzbauer[3], J. B. McCarthy[1]. [1]Department of Laboratory Medicine and Pathology, University of Minnesota, [2]Centro de Investigaciones Biologicas, Madrid, [3]Department of Biology, Princeton University. The $\alpha4\beta1$ integrin is the receptor for the carboxy terminal cell binding domain (CTCBD) of fibronectin which comprises adhesion sites in Hep II and CS-1 in the type III connecting segment or V (for variable) region. LDV is the minimal peptide in CS-1 capable of supporting stable hematopoietic cell (HC) adhesion. However, only those cells which express an active form of the $\alpha4\beta1$ complex are capable of the adhesive recognition of LDV outside the context of intact CS-1 or fragments of fibronectin which contain the CTCBD (38 kDa). The ability of the $\alpha4\beta1$ complex to bind LDV can be altered with a monoclonal antibody to $\beta1$ which specifically activates $\beta1$ dependent function. In contrast, however, the adhesive recognition of fibronectin fragments containing the entire CTCBD (38 kDa) does not require activation of $\beta1$. In the present studies we identify another peptide ligand for $\alpha4\beta1$ present in the Hep II domain of fibronectin. This is peptide I (YEKPGSPPREVVPRPRPGV). This conclusion is based on the following observations: 1) Monoclonal antibodies were made against the 38 kDa fragment and screened for their ability to inhibit T cell adhesion to intact fibronectin. One of these, P4H10, reacted strongly with fibronectin, fragments of fibronectin containing Hep II but not with an 80 kDa fragment containing the CCBD or with CS-1. 2) Using overlapping recombinant fusion proteins derived from the rat fibronectin sequence (deminectins) P4H10 reacted mapped to an amino acid sequence located in the COOH-terminal portion of the type III_{14} repeat. 3) This portion of type III_{14} contains the two adhesive peptides (I and II) previously described and by ELISA, P4H10 reacted with peptide I. Since adhesion of HC to fragments of fibronectin which contain both CS-1 and peptide I does not require activation, it is concluded that it is the cooperative interaction of peptide I and CS-1 (when present) that together promote the high affinity interaction of $\alpha4\beta1$ with this domain. Furthermore, our data strongly suggest that it is the interaction of peptide I with the $\alpha4\beta1$ complex that activates subsequent binding to the LDV sequence in V_{120} isoforms of fibronectin. This is based on the finding that functionally defined Mabs to CS-1 even if they can be shown to react with intact FN and the 38 kDa fragment of fibronectin (P1F11) do not inhibit adhesion of HC to FN or the CTCBD. These data imply that $\alpha4\beta1$ interaction with peptide I is the initial step in promoting cell adhesion to this domain. Therefore, we propose that HC adhesion to intact fibronectin involves two receptors and at least three peptide ligands: peptide I, LDV, RGD.

Regulatory Pathways Involving Integrins; Cytoskeleton - Plasma Membrane Interactions

X 200 REGULATED Ca^{2+} SIGNALLING THROUGH LEUKOCYTE CD11b/CD18 INTEGRIN.
D.C. Altieri, S.J. Stamnes, and C.G. Gahmberg.
The Scripps Research Institute, La Jolla, CA 92037; and University of Helsinki, Finland SF-00170.
The group of β_2 leukocyte integrins coordinate adhesion mechanisms in immune inflammatory responses. The possible participation of these differentiation molecules in early events of transmembrane signalling was investigated. Monoclonal antibody (mAb) cross-linking of CD18, the integrin ubiquitously expressed on all leukocytes, increased by 2-3 fold cytosolic free $[Ca^{2+}]_i$ in monocyte THP-1 cells. "Real time" digitalized imaging in single adherent cell showed that the CD18-mediated Ca^{2+} transient is temporally biphasic, and involves both release of intracellular Ca^{2+} as well as Ca^{2+} influx from the external compartment. Terminal differentiation of THP-1 cells to a mature monocyte-like phenotype, dramatically down-modulated this Ca^{2+} response, and similarly, only a subset of 20-30% of peripheral blood monocytes retained the CD18-mediated Ca^{2+} signalling properties. On blood lymphocytes, CD18 cross-linking increased cytosolic free Ca^{2+} in a subset of resting T cells (15-20%). These cells expressed the CD11b/CD18 heterodimer (Mac-1), and their Ca^{2+} response was completely abrogated during lectin- or antigen-mediated mitogenic activation. Therefore, occupancy of CD18 integrin on leukocytes transduces a Ca^{2+} signal that is critically regulated by the state of cell activation/differentiation and by the preferential assembly with the unique α subunit CD11b.

X 201 β1-INTEGRIN SIGNALLING IN MUSCLE DIFFERENTIATION MODULATES EXPRESSION OF MUSCLE SPECIFIC TRANSCRIPTION FACTORS.
David Boettiger, Hee Yong Yoon, and A. Sue Menko, Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104

Addition of the anti-β1 integrin monoclonal antibody, CSAT, to replicating, primary chicken embryo myoblasts keeps these cells in the replicating state and blocks the normal process of muscle differentiation. When primary myoblasts were put in culture there was an increase in level of myoD mRNA which peaks about 12 hours and then declines to a plateau level by 48 hours. myf5 expression follows the same pattern as myoD for the first 24 hours but it decreases to undetectable levels by 48 hours. Myogenin is not detectable initially but increases by 12 hours to reach a plateau value by 24 hours. Myosin heavy and light chains are first detected following these changes in transcription factors becoming just detectable at 24 hours and rising there after. Cells plated in the presence of CSAT antibody exhibit the same pattern of expression for the first 24 hours, but thereafter myogenin decreases to undectable levels and the later myogenic markers fail to appear. This suggests that the normal integrin based signal is required to establish stable myogenic transcriptional complexes.

In contrast to the block of differentiation induced by addition of soluble CSAT, substrate cross-linked CSAT acted to induce differentiation. This suggests that the presentation of ligand to the integrin receptor is critical but that the exact composition of the ligand is not critical. Furthermore, the initial signal appears to be mechanical. The possibility that this mechanical signal is transmitted was supported by the finding that cytochalasin D also blocked the integrin signal.

X 202 A 23 kDa ZINC-BINDING PROTEIN IDENTIFIED BY 125I-ZYXIN BLOT OVERLAYS.
Aaron W. Crawford and Mary C. Beckerle, Department of Biology, University of Utah, Salt Lake, UT 84112.
Zyxin is a low abundance, α-actinin-binding protein found at sites of cell-substratum and cell-cell adhesion. In order to identify additional zyxin binding proteins we have performed blot overlay binding studies using radioiodinated zyxin. By this technique we have identified an abundant 23 kDa zyxin-binding protein (p23) in avian smooth muscle extracts and have purified p23 from this source. The hydrodynamic properties of the purified protein suggest that it is globular and monomeric. The 51 N-terminal amino acids of p23 exhibit substantial homology (>80% identity) to the human cysteine-rich protein (hCRP), an evolutionarily conserved protein which has an abundance of cysteine and histidine residues organized into two LIM repeats. LIM domains have been proposed to coordinate metal ions and we have found by atomic absorbance spectroscopy that purified p23 binds zinc. The functional significance of the LIM motif is not known, however LIM domains are present in the proteins encoded by two *C. elegans* genes, *lin-11* and *mec-3*, which play important roles in embryonic differentiation and have been implicated in transcriptional regulation (Nature 344: 876-879). In addition, the expression of another LIM-containing gene product, rhombotin (a putative human proto-oncogene product), is developmentally regulated and segmentally restricted in the developing murine central nervous system (Nature 344: 158-160) We have used Western immunoblot analysis to examine the level of p23 throughout avian embryogenesis, and find that it also exhibits developmentally regulated expression. In addition, preliminary data suggest that p23, like zyxin, is localized at the termini of stress fibers near where they associate with the plasma membrane at adhesion plaques in chicken embryo fibroblasts. The presence of a LIM-family member at sites of actin-membrane interaction and cell-substratum adhesion may be important for mediating such responses of cells to extracellular matrix binding as regulated gene expression or cytoskeletal reorganization

X 203 INTERACTION OF INTEGRINS WITH PHOSPHOLIPASE-Cγ AND PROTEIN KINASES,
Shoukat Dedhar, Mumtaz Rojiani, and Kevin Jewell, Cancer Research, Reichmann Research Building, Sunnybrook Health Science Centre, Toronto, Ontario, M4N 3M5
Integrins mediate cell attachment to the extracellular matrix and also promote cell-cell adhesion. However, recent data indicate that integrins also transduce intracellular signals which result in alterations in specific gene expression. Interaction of cells with fibronectin, or clustering of integrins with anti-integrin antibodies result in the tyrosine-phosphorylation of a 120-130 kilodalton protein complex, suggesting that the extracellular occupation of integrins results in a signal transduction event. In order to understand the initial events involved in the signal transduction via integrins, we carried out experiments to determine whether phospholipase C (PLC) enzymes, which generate the second messenger molecules inositol trisphosphate (IP3) and diacylglycerol (DAG), are physically associated with integrins. We found that in human carcinoma cells phospholipase Cγ is associated with integrins, since anti-integrin antibody purification of β1 integrins in the presence of digitonin, resulted in the co-isolation of phosphophase Cγ as determined by Western blot analysis. The reverse experiment, i.e. immunoprecipitation of phospholipase Cγ resulted in the co-purification of β1 integrins. In addition, two protein kinases are also associated with certain β1 integrins since immunoprecipitation of αv, α5, and α6 integrins, followed by in vitro kinase assay on the immunoprecipitate in the presence of γ^{32}P-ATP resulted in the specific autophosphorylation of proteins of 200 kDa and 70 kDa. These data indicate that integrins interact directly with intracellular signal transducing elements such as PLC and protein kinases. We are currently characterizing the kinases and determining which integrins interact with PLC-γ as well as whether PLC-γ is directly activated by integrin substrates.

X 204 *IN VITRO* ANALYSES OF INTEGRIN-CYTOSKELETON INTERACTIONS, C. Michael DiPersio, Jane E. Trevithick and Richard O. Hynes, Center for Cancer Research and Howard Hughes Medical Institute, Department of Biology, MIT, Cambridge, MA 02139.

The binding of integrins both to the extracellular matrix and to the cytoskeleton is critical for normal tissue development and a variety of cell functions; however, the molecular basis of integrin-cytoskeleton interactions is unknown. The integrin $\alpha 3\beta 1$ is reported to mediate cell adhesion to laminin, fibronectin, and collagen and also appears to be important in cell-cell adhesion. The functional versatility of $\alpha 3\beta 1$ may be due, in part, to the existence of two isoforms of the $\alpha 3$ subunit ($\alpha 3A$ and $\alpha 3B$) which have different cytoplasmic domains and appear to be generated by alternative splicing of a common mRNA. We have cloned the A and B isoforms of $\alpha 3$ from chicken, and we are comparing their cell-matrix and cell-cell adhesion functions in transfected cells that lack endogenous $\alpha 3$. Since alternative cytoplasmic domains may provide a mechanism by which cells regulate the binding of integrins to the cytoskeleton, we will also compare the abilities of $\alpha 3A$ and $\alpha 3B$ to bind to cytoskeletal proteins *in vitro*. Although specific intracellular ligands for integrins are not yet clear, the cytoskeletal protein talin is a likely candidate for binding to the integrin cytoplasmic domain. Talin consists of a "tail" domain (M_r=190K) that binds vinculin and an N-terminal "head" domain (M_r=47K) that is homologous to putative membrane-binding domains of several other cytoskeletal proteins, including ezrin and band 4.1. ^{125}I surface-labeled extracts from cells expressing known repertoires of integrins (such as $\alpha 3A\beta 1$ or $\alpha 3B\beta 1$) are being applied to affinity columns of the talin head domain. Immunoprecipitations of column eluates with specific antibodies will allow us to identify distinct integrins that bind to talin. In addition, to address the role of membrane phospholipids in integrin-cytoskeleton interactions, purified integrins will be incorporated into synthetic liposomes and tested for talin binding. These studies should reveal specific binding interactions between integrins and proteins of the cytoskeleton and will contribute to the understanding of how integrins transmit information across the cell membrane.

X 205 ADHESION OF T AND B LYMPHOCYTES TO EXTRACELLULAR MATRIX AND ENDOTHELIAL CELLS CAN BE REGULATED THROUGH THE β SUBUNIT OF VLA. *Carl G. Figdor,, Yvette v. Kooyk, Annemiek J. de Boer, Richard J.F. Huijbens, Pauline Weder, Willeke v. d. Kasteele, Cornelis J.M. Melief and E. v.d. Wiel-v. Kemenade,*, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Investigating the regulation of VLA mediated functions, we found that TS2/16, a monoclonal antibody directed against the β chain of the VLA group of integrins, can induce binding of resting peripheral blood lymphocytes, cloned T lymphocytes, and EBV transformed B cells to extracellular matrix components fibronectin, laminin and collagen but not to fibrinogen. The antibody stimulates VLA-4, VLA-5 and VLA-6 mediated binding. Further-more, it induces VLA-4 mediated binding to VCAM-1 expressed by rTNF-α stimulated endothelial cells, but it does not stimulate homotypic aggregation of cells as described for a number of anti-VLA-4α antibodies (Bednarczyk and McIntyre, 1990; Campanero et al., 1990). Therefore the stimulating activity of this anti-β1 antibody clearly contrasts with that of the anti-VLA-4α antibodies, which induce homotypic cell aggregation but not binding of cells to ECM components or endothelial cells, indicating that TS2/16 may generate different signals.

The observation that also $F(ab')_2$ or Fab fragments of this anti-β1 antibody stimulate binding to extracellular matrix components and endothelial cells excludes the possibility that binding requires receptor crosslinking, or is Fc receptor mediated. Induction of this adhesion is cation and energy dependent and requires an intact cytoskeleton. Although changes in the conformation of VLA integrins induced by this antibody may regulate their functional activity, the dependence on metabolic energy indicates that also intracellular processes may play a role.

X 206 DIFFERENTIAL EFFECTS OF SHEAR STRESS ON ENDOTHELIAL CELL INTEGRINS, Peggy R. Girard and Robert M. Nerem, Bioengineering Center, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332

Exposure of endothelial cells (ECs) to a fluid-imposed shear stress has been shown to modulate their function and structure, including F-actin microfilament organization. In confluent cultures of bovine aortic ECs (BAECs), $\alpha_5\beta_1$ FNR and $\alpha_V\beta_3$ VNR localized in focal contacts in the region of the dense peripheral band of F-actin in cells in a static environment. When subjected to a shear stress of 30 dynes/cm^2 for 24 hr, the $\alpha_5\alpha_1$ FNR redistributed into parallel streaks coincident with the formation of actin stress fibers aligned with the direction of flow. In contrast, the $\alpha_V\beta_3$ VNR was found to concentrate at the "upstream" end of cells colocalizing with stress fiber terminations. An immunoblot analysis indicated that both receptors showed an increase in levels at 5, 24, and 48 hr of exposure to shear stress. In order to examine receptors for collagen and laminin, levels and localization of the integrin subunits α_2 and α_3 were examined. These subunits did not appear to localize in focal adhesions. In static cultures of BAECs, the α_3 subunit appeared to be evenly distributed throughout the cells. Cells subjected to shear stress for 24 hr showed little evidence of a change in localization or levels of α_3. The α_2 subunit was concentrated in a perinuclear location in cells in static culture, perhaps relecting synthesis of the subunit. Following 24 hr of exposure to shear stress, there was a dramatic increase in the perinuclear staining for α_2 compared to static controls. These studies indicate that integrins are differentially regulated in ECs exposed to a flow environment.

X 207 CHANGES IN PLATELET SHAPE CAUSED BY SPREADING ON A FIBRINOGEN-MATRIX CORRELATE WITH INDUCTION OF TYROSINE PHOSPHORYLATION OF PROTEIN SUBSTRATES, Beatrice Haimovich, Sanford J. Shattil, and Joan S. Brugge, Howard Hughes Medical Institute and Department of Microbiology; and Hematology-Oncology Section, Department of Medicine University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Platelets adhere and spread on fibrinogen-coated polystyrene dishes but do not adhere to BSA-coated dishes. This interaction can be blocked by mAb 7E3, specific for platelet glycoprotein IIb-IIIa ($alpha_{IIb}beta_3$). Spreading on fibrinogen caused an increase in tyrosine phosphorylation of multiple platelet proteins as revealed by anti- phosphotyrosine blots. Several of these induced proteins appeared to be distinct from those observed after platelets aggregation with thrombin. Also, in contrast with thrombin activation of platelets, the interaction with the fibrinogen-matrix did not induce secretion of ^{14}C-serotonin. Treatment of platelets in suspension with PGI2 for 1 min prior to their addition to the fibrinogen-coated dishes, did not affect their adherence but partially blocked their spreading. Immunofluorescence analysis revealed the absence of thick actin cables and vinculin aggregates at the tips of the actin cables in the PGI2 treated platelets as compared with the untreated spread platelets. Parallel samples analysed on blots revealed limited induction of phosphotyrosine containing proteins in the PGI2 treated platelets relative to control platelets. Taken together these results indicate that the interaction between fibrinogen-matrix and platelet $alpha_{IIb}beta_3$ causes an increase in tyrosine phosphorylation of multiple platelet proteins and that these phosphorylation events correlate with cytoskeletal changes during platelet spreading.

X 208 FIBRONECTIN AND LAMININ DIFFER IN THEIR ABILITY TO ACTIVATE THE NA+/H+ ANTIPORTER IN HEPATOCYTES. Linda K. Hansen*+, Martin A. Schwartz†, Claude Lechene#, and Donald E. Ingber*+; *Department of Surgery, The Children's Hospital, and Departments of +Pathology and #Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115; †Committee on Vascular Biology, Scripps Institute, La Jolla, CA 92037.

Hepatocytes spread extensively and undergo DNA synthesis upon attachment to high densities of fibronectin (Fn) or laminin (Lm), while they remain round and quiescent on low densities of either molecule. In order to determine if Fn and Lm activate this growth response in hepatocytes through similar pathways, intracellular pH (pHi) levels were examined. We have previously shown that several extracellular matrix (ECM) molecules activate the Na+/H+ antiporter in capillary endothelial cells, resulting in elevated pHi. Freshly isolated rat hepatocytes were cultured in the presence of growth factors (EGF and insulin) on non-adhesive plastic dishes coated with different densities of Fn or Lm. pHi was measured at timepoints following cell plating using the pH-sensitive fluorochrome BCECF and microfluorimetry. pHi in cells on low densities (1ng/cm^2) of either Fn or Lm remained at 7.02 $\pm$ 0.01, consistent with pHi of resting cells. High densities (1000 ng/cm^2) of Fn induced elevated pHi values of 7.08, 7.20, and 7.20, while Lm induced values of 7.00, 7.09, and 7.20 at 4, 22, and 46 hrs, respectively (SE $\pm$ 0.03). This ECM-induced elevation of pHi was inhibited by addition of hexamethylamiloride (HMA), a specific inhibitor of the Na+/H+ antiporter, at a concentration (40 uM) which inhibits DNA synthesis in hepatocytes. The lag in pHi elevation seen on Lm is not due to different times of entry into the cell cycle, since hepatocytes begin to incorporate ^{3}H-thymidine into their nuclei at similar times (30-36 hrs) after cell plating on either Fn or Lm. *Immunofluorescence studies revealed that hepatocytes deposit Fn onto* Lm-coated plates within 24 hrs after plating. These results demonstrate that, while hepatocytes attach, spread, and undergo DNA synthesis similarly on high densities of Fn or Lm, elevation of pHi occurs at different times and appears to be coincident with the presence of Fn in the matrix. This suggests that receptors which mediate initial hepatocyte attachment onto Lm are unable to activate the Na+/H+ antiporter, while later *de novo* deposition of Fn results in elevated pHi through a different class of receptors which are coupled to the antiporter. Studies are currently underway to determine which specific receptors are involved in this signaling pathway.

X 209 THE CD4 MOLECULE REGULATES T CELL ADHESION TO FIBRONECTIN. Rami Hershkoviz, Shmuel Miron, Irun R. Cohen, Ofer Lider. From Internal Medicine T, Ichilov Hospital and the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, and from the Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

The binding of VLA-4, 5 and 6 integrin receptors to protein components of the extracellular matrix is rapidly augmented by the activation of the T cells without, however, any actual change in the level of expression of these VLA receptors. Thus, it is likely that the activation of these integrins must be regulated by T cell surface molecules capable of transducing signals into the cell. We studied the role of the CD4 molecule in the binding of rat CD4$^+$ T cells to fibronectin (FN). We now report that the CD4 molecule appears to play a major role in regulating T cell interactions with FN. This conclusion is based on the following observations: (a) mAb directed against the CD4 molecule inhibited T cell adhesion to FN; (b) down regulation of the CD4 molecule resulted in partial loss of the ability of CD4$^+$ T cells to adhere to FN; (c) a CD4$^+$ T cell clone adhered to both FN while a CD4$^-$CD8$^-$ clone expressing an identical TCR bound weakly to FN; and, (d) treatment of the CD4$^+$ T cells with an inhibitor of the CD4-associated tyrosine protein kinase activity inhibited T cell adhesion to FN.

X 210 HIGH LEVELS OF EPISIALIN MODULATE CELL-CELL AND CELL-EXTRACELLULAR MATRIX ADHESION. J. Hilkens, F. Buijs, J. Wesseling, H.L. Vos & M.J.L. Ligtenberg. Division of Tumor Biology, The Netherlands Cancer Institute (Antoni van Leeuwenhoekhuis), Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Episialin is a high-molecular weight transmembrane glycoprotein with a large mucin-like extracellular domain encoded by the MUC1 gene. The mucin domain contains many proline residues and numerous O-linked glycans which are heavily sialylated, giving the molecule an extended and rigid structure pointing into the extracellular space. The molecule is mainly present at the apical surface of glandular epithelial cells. In carcinomas, the molecule may be distributed over the entire cell surface as a result of disruption of the normal tissue architecture. In additon the expression is often strongly increased as determined by *in situ* hybridisation. We have investigated whether these properties affect cell-cell and cell-extracellular matrix interactions by transfecting a normal mammary epithelial and a melanoma cell line with full length cDNA encoding episialin. Transfectants of both cell lines which express episialin at levels similar to that of carcinoma cell lines show a strongly decreased aggregation capacity and do not adhere to extracellular matrix components as efficiently as their control cells (parental cells and revertants that do not express episialin). Some transfectants grow partly in suspension. In mixing experiments, episialin transfectants are excluded from aggregates formed by the control cells, indicating that high levels of episialin on one of the interacting cells is sufficient to inhibit aggregation. The effect of episialin overexpression on aggregation is probably not only due to the negative charge of its numerous sialic acid residues, since neuraminidase treatment only partially restored the aggregation capacity of the transfectants. We propose that cells expressing episialin, at levels also found on carcinoma cell lines, can prevent proper interaction of cell surface proteins with macromolecules on adjacent cell membranes and in the extracellular matrix, as a result of the large, extended and rigid structure of episialin.

X 211 CHARACTERISTICS OF CO-ACTIVATION OF T LYMPHOCYTES BY ICAM-1, Peter Kuhlman and Adrienne A. Brian, Department of Chemistry and the Cancer Center, Univ. of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0063

The ICAM-1/LFA-1 receptor couple mediate intercellular adhesion which is increased significantly upon lymphocyte activation. It has recently been demonstrated that the LFA-1 molecule is also capable of modulating the degree to which the lymphocyte is activated, and that the adhesive and co-activating properties are separable.

We have assayed a panel of murine T lymphocytes including cloned lines, splenic CD4+ cells, and hybridomas for responsiveness to ICAM-1 in conjunction with soluble mitogens, CD3 monoclonal antibodies, peptide antigens, and bacterial superantigens. Comparing lymphokine production, proliferation, and adhesion across the panel of T cells, we find:

A) a range of sensitivities to ICAM-1 which does not correlate with surface expression levels, with magnitude of adhesion, or with the degree of increase in adhesion upon T cell activation.

B) two classes of responses -- with sub-optimal T cell stimuli, the ICAM-1/LFA-1 interaction increases the maximal level of T cell activation; with stimuli capable of eliciting a full response, the ICAM-1/LFA-1 interaction causes a shift in the dose-response curve to lower concentrations of TCR ligand. In particular, for some T cells, superantigens elicit a weak response which is strongly enhanced by ICAM-1. To investigate this effect, we have assayed phosphoinositide hydrolysis in the presence and absence of ICAM-1 and find that LFA-1 ligation increases the production of inositol phosphates under both optimal (CD3 Ab) and sub-optimal (superantigen) conditions.

X 212 REGULATION OF FIBRONECTIN RECEPTOR DISTRIBUTION, Susan E. LaFlamme, Steven K. Akiyama, and Kenneth M. Yamada, Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892.

To determine the role of each intracellular domain of the fibronectin receptor in receptor distribution, chimeric receptors were constructed containing the human IL-2 receptor (gp55 subunit) as the extracellular and transmembrane domains, in combination with either the α_5 or β_1 intracellular domain of the fibronectin receptor as the cytoplasmic domain. These chimeric receptors were transiently expressed in normal fibroblasts, and their localization on the cell surface was determined by immunofluorescence using antibodies to the IL-2 receptor. The α_5 chimera was expressed diffusely on the plasma membrane. The β_1 chimera, however, colocalized with the endogenous fibronectin receptor both at focal contacts and at sites where extracellular fibronectin fibrils associate with the plasma membrane. The β_1 intracellular domain alone, therefore, contains sufficient information to target the chimeric receptor to regions of the cell where ligand-occupied integrin receptors are concentrated. The finding that the β_1 chimeric protein behaves like a ligand-occupied receptor, even though the β_1 chimera cannot itself bind extracellular ligand, suggests an intracellular difference between occupied and unoccupied receptors, and predicts that the distribution of integrin receptors can be regulated by ligand occupancy. We tested this prediction by providing a soluble cell-binding fragment of fibronectin to cells spread on laminin. Under conditions preventing further ligand adsorption to the substrate, this treatment nevertheless resulted in the relocation of diffuse fibronectin receptors to focal contacts. Similarly, a redistribution of diffuse vitronectin receptors to focal contacts occurred on cells spread on laminin following the addition of the small soluble peptide GRGDS. We conclude that the propensity for receptor redistribution to focal contacts driven by the β_1 cytoplasmic domain alone is suppressed in heterodimeric unoccupied fibronectin receptors, and that ligand occupancy can release this constraint. This redistribution of integrin receptors after the binding of a soluble substrate molecule may provide a direct means of assembling adhesion sites.

X 213 α-CHAIN AND β–CHAIN LFA-1 ANTIBODIES MEDIATE DISTINCT EFFECTS ON LFA-1 DEPENDENT T CELL ADHESION, R. Clive Landis and Nancy Hogg, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K. A panel of 10 β–chain (CD18) and 22 α-chain (CD11a) anti-human LFA-1 mAbs were screened for their effect on T cell adhesion. Adhesion was assessed in both resting and phorbol ester (PdBU) triggered T cells using the models of homotypic aggregation and binding to purified ICAM-1 on plastic. None of the β-chain mAbs (0/10) was capable of directly inducing homotypic aggregation or binding to purified ICAM-1, but all (10/10) were capable of blocking the PdBU-triggered aggregation and binding to ICAM-1. In contrast, 14/22 α-chain mAbs induced homotypic aggregation in resting T cells, but only 1 of these directly induced binding to purified ICAM-1. PdBU-triggered aggregation and ICAM-1 binding were inhibited respectively by 7/22 and 10/22 α-chain mAbs. The nature of the direct aggregation-response induced by the α-chain mAbs was further investigated. For 13 of the 14 mAbs, aggregation was induced independently of the ICAM-1/LFA-1 adhesion pathway and only the single α-chain mAb that induced binding to purified ICAM-1 demonstrated anti-ICAM-1 inhibitable cluster formation. The ICAM-1/LFA-1 independent aggregation response was shown to occur via a non-specific antibody mediated cross-linking or low avidity event between apposing cells, since aggregation did not correlate with expression of the "activation reporter" 24 epitope of LFA-1 and Fab fragments of a representative antibody (mAb 38) were incapable of inducing aggregation. In conclusion, our blocking data obtained with the β-chain antibodies supports a view that the β-chain of LFA-1 may play an important role in determining the conformation of LFA-1 recognised by ICAM-1.

X 214 ENDOGLIN, AN RGD CONTAINING PROTEIN OF *HUMAN ENDOTHELIAL CELLS, BINDS TGF-β1* Michelle Letarte and Sonia Vera,.Division of Immunology and Cancer Research, Hospital for Sick Children and Dept of Immunology, University of Toronto, Toronto, Canada, M5G 1X8

Endoglin is a major glycoprotein of human endothelial cells, a dimer of Mr-170,000 with an accessible RGD tripeptide. We have demonstrated that adhesion of U-937 cells, labelled with the fluorescent dye BCECF, was stimulated 5-20-fold when monolayers of endothelial cells derived from human umbilical vein (HUVEC) were pretreated with antibodies to endoglin. The increased adhesion was inhibited in the presence of RGD but not RGE peptides and by cytochalasin B. Intact IgG were necessary to stimulate adhesion and pretreatment of U-937 cells with anti-Fc receptor antibodies inhibited the stimulation of adhesion. These results suggest that endoglin is involved in the formation of the multivalent complex likely to mediate the RGD-mediated interaction observed between monocytes and HUVEC. The recent cloning of rat betaglycan, the TGF-β–receptor III, revealed that its transmembrane and cytoplasmic domains were 74% identical to the corresponding domains of endoglin and that a 27% overall sequence identity was found between these two integral membrane proteins (Cell, 67:785- and 797, 1991). Furthermore a dimer of Mr-170,000, was identified in bovine endothelial cells by TGF-β1 cross-linking experiments (J. Biol. Chem. 266:20767, 1991). We have now demonstrated by cross-linking and immunoprecipitation experiments that endoglin is a major TGF-β1 binding protein of HUVEC. As TGF-β1 can modulate adhesion, we will now assess the role of endoglin in mediating this effect. Current results indicate that at most 1% of endoglin can be cross-linked by TGF-β1 suggesting that interaction with additional protein(s), might be necessary. As the structures of the high affinity TGF-β1 receptors I and II have not yet been elucidated, their relation to endoglin, and the pathways of activation mediated by the different receptors remain to be established. Our results suggest that endoglin is an important protein of human endothelial cells implicated in the regulation of adhesion and capable of binding and mediating at least some of the effects of TGF-β1.

X 215 INTEGRIN α6β4 IS EXPRESSED IN CELLS OF MESENCHYMAL ORIGIN. Pier Carlo Marchisio °, Ottavio Cremona °, Christine Chaponnier *, and Giulio Gabbiani * °Dipartimento di Scienze Biomediche e Oncologia, Università di Torino, 10126 Torino, Italy, * Département de Pathologie, Université. de Genève, 1211 Genève, Switzerland.

We have characterized the integrins expressed by α-smooth muscle actin (α-SMA)-positive cells in two different pathologic conditions: a) hypertrophic scars, b) Dupuytren disease, two lesions where most fibroblasts express α-SMA at a very high level. We report that: i) myofibroblasts are intensely positive for α5 and ß1, like normal resting fibroblasts and may then produce the prototype fibronectin receptor α5ß1; ii) apparently no other heterodimers of the ß1 subfamily are expressed inside the lesion on the basis of the reactivity with known monoclonal antibodies; iii) a ß3 integrin is present in a cell population that is negative for α-SMA and is presumably found only in fibroblasts; iv) ß4 and α6, found essentially in the epidermis overlying the lesions and in contact with the basement membrane, are coexpressed in α-SMA positive myofibroblasts; v) ß4 and α6 are also coexpressed by smooth muscle cells (SMC) of small vessels of these pathological tissues. Following this observation, we have labeled several normal tissues for ß4 and α6 and in every case we have seen that SMC of small vessels express ß4 and α6. Integrin α6ß4 has been identified in hypertrophic scar and Dupuytren nodule tissue by Western blotting and immunoprecipitated from cultures of myofibroblasts and vascular SMC. The mRNA for ß4 was also found in myofibroblasts and in SMC. Our results are compatible with the possibility that the heterodimer α6ß4 is located in a hitherto unknown junctional complex in relation to stress fibers in myofibroblasts and to myofilaments in SMC of small vessels. Thus, the biological role of α6ß4 should be reconsidered on the basis of its presence in non-epithelial tissues formed by mesenchyme-derived cells. We also propose that a6ß4 represents a novel multifunctional basement membrane receptor that is also linked to microfilaments in non-epithelial cells as well as to hemidesmosomes in epithelia.

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X 216 FIBRONECTIN RECEPTOR (FNR) REGULATION OF ACTIN ORGANIZATION IN CORNEAL FIBROBLASTS

Sandra K. Masur, Kelly Michelson, Jeff KH Cheung, Stefan Antohi and Dianne Applegate, Department of Physiology & Biophysics, Mount Sinai School of Medicine, New York, NY

FNRs have been implicated in the signal transduction pathway of collagenase induction in synovial fibroblasts (Werb et al, J Cell Biol 109: 811, 1989) and corneal fibroblasts (Masur & Antohi, IOVS 32:1071,1991). We have identified in corneal fibroblasts several FNRs ($\alpha_5\beta_1$, $\alpha_3\beta_1$, $\alpha_v\beta_1$). Using function-perturbing rat monoclonal anti-FNR (antibodies against $\alpha_5\beta_1$ or against β_1, generously provided by Dr C. Damsky) we were able to block initial attachment of corneal fibroblasts to FN. Application of the anti-FNRs to attached fibroblasts induces collagenase secretion. In the present study, we report the use of the anti-FNRs to investigate the role of actin as a possible intracellular tranducer of signals, initiated at the FNR. Previous reports have disagreed as to whether or not cells exposed to collagenase inducing agonists acting at the FNR have fewer stress fibers (i.e. less F-actin) compared to non-induced fibroblasts.

To quantitate actin in control corneal fibroblasts and after application of antibodies to FNR we have taken two approaches:

1) Relative G-actin and F-actin were evaluated respectively on extracted cytoskeleton by binding to DNAse I and to NBD-phallacidin (Fechheimer & Zigmond, Cell Motility 3: 349.1983 and Howard & Oresajo, J Cell Biol 101:1078,1985) These measurements indicate that prior to agonist addition, a large proportion of actin was depolymerized (50-70%). 2) On an individual cell basis with fluorescent microscopy, TRITC-phalloidin binding to fixed cells was quantitated photometrically as an index of F-actin content (Condeelis & Hall, Methods Enzymol 196: 486,1991). Within 5 min of application of 250 nM anti-FNR, relative F-actin content decreased by 30 to 40%. Relative F-actin returned to control levels during the 30-60 min exposure to anti-FNR. Cytochalasin D (1 μM), which causes cell rounding, disappearance of stress fibers, and secretion of collagenases, decreased the relative F-actin content by 50 % throughout a 60 min exposure.

We conclude that actin depolymerization and repolymerization occurs in response to binding of ligand to the FNR. Studies are in progress to determine if FNR-transduced actin depolymerization is required for collagenase induction. *Supported by NIH EY09244.*

X 217 IMMUNO-EM LOCALIZATION OF BETA-1 INTEGRIN IN WET-CLEAVED FIBROBLASTS.

Alexandra M.L. Meijne, Diane M. Casey, Constance A. Feltkamp and Ed Roos. Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.

We have used immuno-EM to study the localization of ß1-integrin in chicken embryo fibroblasts that had spread for 3 h on fibronectin in serum-free medium. Cells were "wet-cleaved" which yields a ventral substrate-associated membrane with associated cytoskeleton, that was sometimes removed with actin depolymerization buffer and high salt buffers to improve accessibility.

Antibodies against the cytoplasmic domain of ß1-integrin revealed ß1 to be located in the cell periphery often close to talin. Furthermore, ß1 was abundant above fibronectin fibrils. Adhesion plaques contained ß1. However, it was not distributed all over the plaque like vinculin and talin, but located in patches, in close proximity to the plaques.

Results obtained after application of actin depolymerization and high salt buffers indicate that this observation is not caused by limited accessibility of integrins located under the adhesion plaque.

Our observations indicate that ß1-integrins are located at the periphery of adhesion plaques. If so, other membrane proteins should be responsible for matrix attachment of the center of the plaque.

X 218 IDENTIFICATION OF NOVEL INTEGRIN-ASSOCIATED MOLECULES IN ADULT TISSUES,

John Muschler, Bao Zheng Zheng, and Alan Horwitz, Departments of Biochemistry, and Cell Biology, University of Illinois, Urbana, IL 61801. Purifications of the integrins from adult chicken tissues using a CSAT monoclonal affinity column has led to the discovery of several novel molecules which co-purify with the receptors and do not purify on control columns. Integrin purifications from adult skeletal muscle result in the co-purification of a novel 70 kD protein. Monoclonal antibodies against this protein reveal it to be strictly membrane associated. Integrin purifications from adult brain result in the co-purification of multiple proteins in the 18 to 50 kD range. Antibodies against two of these proteins show them to be located in myofibrils and fibroblasts as well. In fibroblasts these proteins are both associated with the actin cytoskeleton, and both show partially membrane association. In myofibrils one is localized at the z-lines of myofibrils (like α-actinin and spectrin) and is restricted to the membrane, while the other is localized throughout the z-disc. N-terminal amino acid sequence shows most of proteins purified from brain to be novel. Intriguingly, N-terminal sequence of one, a 36 kD protein, has shown it to be identical to the enzyme glyceraldehyde 3-phosphodehydrogenase. This is a major membrane-associated protein of the red blood cell, and known associate of the spectrin-ankyrin transmembrane linkage.

X 219 REGULATION OF SIGNAL TRANSDUCTION BY PLATELET FIBRINOGEN RECEPTOR GPIIb/IIIa IN THE ACTIVATION OF HUMAN PLATELETS BY A STIMULATORY MONOCLONAL ANTIBODY.

Ulhas P. Naik, Bogdan Walkowiak, Yigal H. Ehrlich* and Elizabeth Kornecki, SUNY, Health Science Center at Brooklyn, NY 11203 and *CUNY at Staten Island, NY 10301.

We have developed and characterized a monoclonal antibody (M.Ab. F11), which stimulates human platelets (Kornecki et al. J. Biol. Chem. 265: 10042, 1990). M.Ab. F11 stimulates platelets by binding to two surface proteins (32 and 35Kd). Platelet activation induced by M.Ab. F11 involves specific binding, Ca^{++} mobilization and phosphorylation of 40Kd and 20Kd proteins followed by granular secretion and aggregation. A monoclonal antibody specific to the fibrinogen receptor component GPIIIa (M.Ab. G10) developed in our laboratory inhibits M.Ab. F11-induced and thrombin-induced platelet aggregation and secretion. M.Ab. G10 also inhibits fibrinogen binding to ADP stimulated platelets. M.Ab. G10 inhibited the phosphorylation of both 40Kd and 20Kd intracellular proteins induced by (5ug/ml) M.Ab. F11 but not by 0.2U/ml of thrombin in ^{32}P-labeled platelets under non-stirring condition. M.Ab. G10 also inhibited the mobilization of extracellular calcium by M.Ab. F11 but not by thrombin as determined in aequorin-loaded platelets. These results suggest that the platelet fibrinogen receptor GPIIb/IIIa is involved in the process of signal transduction induced by stimulatory monoclonal antibody M.Ab. F11. The results also suggest that the pathway of activation of platelets by M.Ab.F11 differs from that of thrombin.

X 220 LIGATION OF VLA-4 ACTIVATES PROTEIN TYROSINE KINASE IN T CELLS

Yoshihisa Nojima, David M. Rothstein, Kanji Sugita, Stuart F. Schlossman, and Chikao Morimoto, Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115

The VLA/integrins are a family of heterodimeric adhesion receptors shown to be involved in cell-to-cell and cell-to-extracellular matrix (ECM) interactions. Given recent evidence that VLA molecules can synergize with CD3/TCR pathway to activate T cells, it is important to identify biochemical event(s) generated by these molecules. Here we report that the engagement of VLA-4 on T cells with specific antibodies or its natural ligand activates protein tyrosine kinase activity as detected by anti-phosphotyrosine immunoblotting. The crosslinking of VLA-β1 (CD29) with a specific mAb (anti-4B4) plus anti-mouse Ig, resulted in the rapid tyrosine phosphorylation of a protein with a molecular weight of 105 kD (pp105) in the human T cell line H9, as well as in peripheral resting T cells. The increase in tyrosine phosphorylation of pp105 was specifically mediated by VLA-4, since mAbs against α4 but not against other VLA α chains could induce this phosphorylation. In addition, the binding of T cells with the CS1 alternatively spliced segment of fibronectin (the binding site recognized by VLA-4) induced pp105 tyrosine phosphorylation. Crosslinking the CD3 complex or VLA-4 molecules with mAbs demonstrated that each of these molecules stimulated the tyrosine phosphorylation of unique sets of proteins with different kinetics, suggesting that these 2 receptor systems are coupled to distinct protein tyrosine kinases. Since tyrosine phosphorylation of cellular proteins has been shown to be a crucial biochemical event in cell growth, our findings suggest that the induction of pp105 tyrosine phosphorylation via VLA-4 may play a role in the transduction of activation signals through this molecule.

X 221 USE OF SYNTHETIC PEPTIDES TO MAP PRECISELY THE ALPHA-ACTININ BINDING SITE ON BETA INTEGRIN. Carol Otey and Keith Burridge, Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC, 27599. We have shown previously that alpha-actinin binds to beta-1 integrin in vitro, and we have used four 13-amino acid synthetic peptides to locate the binding site for alpha-actinin within the 48-amino acid cytoplasmic domain of beta-1 integrin. In both column chromatography and solid phase binding assays, alpha-actinin was found to interact with more than one site in the cytoplasmic domain of beta-1 integrin. The primary binding site for alpha-actinin was localized to a region near the membrane, corresponding to residues 758-770 of beta-1 integrin, and an additional binding site was located near the integrin C-terminus. Currently, we are making use of a recent technology, the mimetope assay, to map the alpha-actinin binding sites more precisely. With this technique, short peptides (8 to 12-mers) are synthesized on the ends of polystyrene pins. These pins are then used to bind radiolabeled alpha-actinin in a solid-phase binding assay. Pin technology has made it possible to examine the protein-binding activity of every short sequence in the cytoplasmic domain of beta integrin, and thus to map both primary and secondary alpha-actinin binding sites. Peptides containing "point mutations" have also been synthesized in order to examine the role of specific residues, and the importance of net charge and side chains, in the binding of alpha-actinin to beta-1 integrin. Supported by NIH grants GM29860 and HL44918.

X 222 **INTERACTION OF MULTIMERIC VITRONECTIN WITH HUMAN SMOOTH MUSCLE CELL SURFACE RECEPTORS**, Sibylle Hess, Errol Wijelath, Vijay V. Kakkar, Catherine Demoliou-Mason and Klaus T. Preissner, Haemostasis Research Unit, Kerckhoff-Klinik, MPG, D-6350 Bad Nauheim (Germany) and Thrombosis Research Institute, Cell Biology Section, London SW3 6LR (U.K.)

The adhesive glycoprotein vitronectin (VN) is present in the circulation predominantly in non-multimeric form (=VN-nat), whereas conformational changes result in unfolding and concomitant self-association (=VN-denat) as documented by non-denaturing gel-electrophoresis and monoclonal antibody binding. The latter form of VN exposes high affinity binding sites for glycosaminoglycans and is believed to be associated with the extracellular matrix sites in the vessel wall. Appreciable direct binding of radiolabeled VN-denat to growth-arrested monolayers of human vein smooth muscle cells (SMC) occured in a time-dependent manner and reached steady state within 2 hr, whereas VN-nat demonstrated hardly any binding under these conditions. Differentiation of VN-denat binding to SMC in monolayer indicated specific binding to both, cell surface receptor sites and extracellular matrix. Binding of VN-denat was partially competed by heparin or RGD-containing peptides, indicating the involvement of both the heparin-binding as well as the cell attachment domain of VN. Specific binding of VN-denat to SMC in suspension was also blocked by more than 50% by these competitors indicating possible cooperative interaction of both functional domains of VN-denat with SMC. Immuno-fluorescence studies with both forms of VN revealed the association only of VN-denat with cells in a bright punctate pattern. These findings provide strong evidence for a direct interaction of a particular form of VN with SMC surface and extracellular matrix conponents which may be relevant for the deposition of VN in the normal media or in atherosclerotic lesions. In this microenvironment VN may play a role as modulator of negative regulators of cell growth such as heparin.

X 223 STUDIES ON TWO ALTERNATIVELY SPLICED DESMOSOMAL CADHERINS, Raynor, Karen and Pamela Cowin, Department of Cell Biology, New York University Medical Center, New York, NY 10016

Desmocollins I and II are major glycoprotein components of the desmosomal adhesive core. They have 40% sequence identity in their ectodomain to the cadherin family of cell adhesion molecules. However, their cytoplasmic domains comprise unique sequence identifying them as a distinct subclass within this family (Mechanic, Raynor, Hill and Cowin. 1991. PNAS 88, 4476). The two bovine epidermal desmocollins are produced by alternative splicing of their carboxy-terminus. The unique region of desmocollin I bears some similarity to the catenin-binding domain of the cadherins (Collins et al 1991 JCB 113, 381). We have raised anti-peptide antisera that specifically recognize the unique carboxy-termini of desmocollins I and II. These antibodies detect their epitopes in various stratified epithelia but show some variation in their staining patterns in the different layers of these tissues. These results suggest alternative splicing generates desmocollin isoform diversity in specific layers of these tissues, or, that the carboxy terminal domains become differentially processed or masked by association with other cytoplasmic components during terminal differentiation

X 224 STRUCTURAL AND FUNCTIONAL ANALYSIS OF TALIN.

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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 protein sequence of chicken talin is approximately 95% identical to that of mouse talin. The N-terminus of talin is found to be homologous with the N-terminal domains of the cytoskeletal proteins, band 4.1, ezrin and moesin, and of two protein tyrosine phosphatases across a 300 residue region. We have previously shown that the 47 kD N-terminal domain is cleaved from the C-terminal 220 kD domain by calpain II, and we are currently investigating the role of this domain using protein interaction studies and the transfection of cells with cDNA clones. The C-terminal 2000 residues have been analysed by Fourier transform analysis and this can be resolved a repeat unit consisting of a short α-helix followed by a turn. We predict that this structure will form an extended twisted ribbon structure representing a novel structural motif in cytoskeletal proteins.

We have isolated genomic clones covering approximately 80 kb of the mouse talin gene and are characterizing the structure of the promoter and first exons. This will allow us to undertake homologous recombination experiments to ablate talin expression in T cell lines. These lines will then be used to analyse the mechanisms by which the cytoskeleton is reorganised during antigen dependent interactions of T cells with antigen presenting cells, and the nature of the interactions with membrane proteins involved in this event.

X 225 A NATURAL KILLER CELL PROTEASE THAT AFFECTS CELL-CELL MATRIX INTERACTIONS

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The granules of natural killer (NK) cells and activated T cells are known to contain a number of characteristic proteins including perforin (cytolysin), various serine proteases and some less well defined cytostatic and cytotoxic proteins. We have purified a protein from the granules of a rat NK leukemia (RNK) cell line which reduced the growth and altered the morphology of certain tumor cells, resulting in a rounding of sensitive tumor target cells followed by their aggregation. This effect was not species specific since sensitive tumor cells could be of murine, rat or human origin. The N-terminal amino acid sequence demonstrated that this protein was a novel rat serine protease (RNKP-1). Synthetic substrates were specifically cleaved after aspartic acid residues. The changes in tumor morphology were most pronounced on a rat hepatoma cell line (N1S1) adhered to fibronectin. The predominant effect of RNKP-1 was on fibronectin binding, since no major changes were observed on N1S1 cells adhered to laminin or collagen IV. Other closely related granule serine proteases had no effect on N1S1 cells adhered to fibronectin. Blocking antibodies and synthetic peptides demonstrated that N1S1 binding to fibronectin was dependent on the RGD cell binding domain. Therefore, local release of RNKP-1 by infiltrating NK cells or activated T cells could have pronounced effects on the structure and function of cells in the surrounding tissue due to effects on cell-cell matrix interactions.

X 226 STRUCTURE AND FUNCTION OF pp125^{FAK}: AN INTEGRIN LINKED PROTEIN TYROSINE KINASE?

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Engagement of certain integrins with their extracellular ligand is followed by an increase in tyrosine phosphorylation on intracellular proteins, one of which is a 125kDa protein recognized by the monoclonal antibody 2A7 (Kornberg, Earp, Turner, Prockop and Juliano, PNAS 88:8392 (1991); Guan, Trevithick and Hynes, submitted). Using Mab 2A7 as a probe, we have isolated a cDNA encoding this protein. Based upon its deduced amino acid sequence and enzymatic activity when expressed in *E. coli*, have identified pp125 as a heretofore undescribed protein tyrosine kinase (PTK). pp125 is not a transmembrane receptor, nor a membrane associated PTK like pp60^{src}, and in fact appears to fractionate like a cytosolic protein. Based upon its unique structural organization, and its relatively low level of sequence homology with other PTKs, we have assigned pp125 as the prototype member of a new PTK family. Our preliminary evidence suggests that pp125 partially localizes in structures resembling focal adhesions, hence we suggest the name Focal Adhesion Kinase (*FAK* or pp125^{FAK}). Our current efforts are focussed upon more definitively identifying the intracellular localization of pp125^{FAK}, identifying any proteins with which it might physically associate, and exploring the regulation of the enzymatic activity of pp125^{FAK}, particularly in the context of integrin function.

X 227 THE SIGNAL-INITIATING MEMBRANE PROTEIN CD9 IS ASSOCIATED WITH SMALL GTP-BINDING PROTEINS,

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CD9 is a plasma membrane-located glycoprotein of broad but selective tissue distribution in humans and is a major component of the platelet surface. The function of CD9 is unknown. However, accumulating evidence suggests that it is linked to the generation of intracellular signals leading to cell-cell adhesion, since antibodies to CD9 induce platelets to release their granules and to aggregate and pre-B cells to undergo homotypic aggregation. We demonstrate that from detergent lysates of human platelets monoclonal antibodies to CD9, but not antibodies to GPIIb/IIIa, immunoprecipitate proteins of 25 and 26 kDa which bind [α^{32}P]GTP on nitrocellulose transfers. The binding is specific, since it is inhibited, in a dose-dependent manner, by non-radiolabelled GTP but not by ATP. The GTP-binding proteins do not belong to a Mg^{2+}-sensitive subset, since they are unaffected by the addition of Mg^{2+} over a range from 2μM to 2 mM. The observations demonstrate that CD9 is associated with selected small GTP-binding proteins.

X 228 REGULATION OF FIBRINOGEN BINDING TO $\alpha_{IIb}\beta_3$ IN PURIFIED PLATELET MEMBRANES, Susan S. Smyth and Leslie V. Parise, Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599. The major platelet integrin, $\alpha_{IIb}\beta_3$, binds soluble fibrinogen only after platelet activation. The mechansim by which platelet agonists and protein kinase C-activators convert $\alpha_{IIb}\beta_3$ into a functional fibrinogen receptor is not known. We have developed a membrane system in which regulation of the fibrinogen binding properties of $\alpha_{IIb}\beta_3$ can be investigated. Purified platelet membranes have been prepared by nitrogen cavitation of washed platelets followed by sucrose density sedimentation. Basal fibrinogen binding to the membranes possesses many of the properties of fibrinogen binding to $\alpha_{IIb}\beta_3$ on activated platelets. Fibrinogen binding can be inhibited by the $\alpha_{IIb}\beta_3$ complex-specific MAb 10E5, an RGD- but not an RGE-containing peptide, and a peptide derived from the γ-chain of fibrinogen. Equilbrium binding analysis reveals that the dissociation constant for fibrinogen binding is approximately 10 nM, compared with reported values of 50-500 nM for fibrinogen binding to activated platelets. However, the levels of fibrinogen binding are considerably lower than those reported for stimulated platelets; 10% or less of the $\alpha_{IIb}\beta_3$ complexes in the membranes are capable of binding fibrinogen. Incubation with the $\alpha_{IIb}\beta_3$-activating MAb 62 or pretreatment with 0.05-0.2 μg/ml chymotrypsin increases fibrinogen binding to the membranes. Higher concentrations of chymotrypsin dose dependently decrease fibrinogen binding and sequentially cleave the proteolytically sensitive disulfide loop in β_3 and then produce 80kD and 60kD proteolytic fragments of $\alpha_{IIb}\beta_3$. To address the physiologic mechanism of $\alpha_{IIb}\beta_3$ activation, potential cellular mediators were tested for their capacity to increase fibrinogen binding to the membranes. In contrast to their effects on intact and permeabilized platelets, platelet agonists (ADP, thrombin, U46619) and guanine nucleotide binding protein-activators (NaF, GTPγS) do not stimulate fibrinogen binding to $\alpha_{IIb}\beta_3$ in the membranes, suggesting that agonist-receptor occupancy does not directly activate $\alpha_{IIb}\beta_3$. Moreover, fibrinogen binding to the membranes is not promoted by treatment with phorbol esters or purified protein kinase C and ATP, under conditions where specific protein phosphorylation is observed. These results suggest that platelet activation modulates cytosolic factor(s) that are required for $\alpha_{IIb}\beta_3$ activation. Reconstitution of the fibrinogen binding activity of $\alpha_{IIb}\beta_3$ in membranes may facilitate identification of the intracellular mediator(s) responsible for this event in platelets.

X 229 REGULATION OF CELL-SUBSTRATE ADHESION BY A GTPase IN XENOPUS XTC FIBROBLASTS, Marc H. Symons and Timothy J. Mitchison, Department of Pharmacology, University of California, San Francisco, CA 94143-0450.

Integrin-mediated adhesion of cells to the extracellular matrix (ECM) is crucial at various stages of development and for the maintenance of normal tissues. We have investigated the role of GTPases in the control of cell-substrate adhesion by injection of guanine nucleotide analogs in Xenopus XTC fibroblasts. Injection of GTPgS inhibits ruffling, increases spreading, and inhibits cell rounding caused by application of GRGDSP, a peptide which inhibits the binding of integrins to vitronectin and fibronectin, while GDPβS promotes GRGDSP-induced cell rounding. Injection with GTPγS does not affect cell rounding induced by trypsin/EDTA however, showing that cell contractility is not affected by activation of GTPases. These results provide evidence for the existence of a GTPase activity which in the activated state increases cell-substrate adhesion. The cell might use this GTPase-dependent pathway to detach itself from ECM components at the onset of mitosis. In support of this hypothesis, we observed that photo-induced release of caged GTPγS causes premature spreading of mitotic fibroblasts.

X 230 THE CD29 K20 MAB INCREASES PIP2 DEGRADATION AND PE SYNTHESIS IN CD4+ T CELLS ACTIVATED VIA CD3 OR CD2, Michel Ticchioni*, Claude Aussel**, Claudette Pelassy**, Serge Manié* and Alain Bernard*, *INSERM U343 and **INSERM U210, Faculté de Médecine, 06107 Nice cedex 2, France

Accumulating evidences show that integrins mediate both cell adhesion and signal transmission. We had shown previously that a CD29 mAb, K20, which binds a peculiar epitope of the β1 chain of the Very Late Antigens (VLA), inhibits T-cell activation and proliferation. Moreover, in CD4+ T cells and Jurkat cell line activated with a CD3 mAb, K20 inhibits phosphatidylinositol-bisphosphate (PIP_2) synthesis and diacylglycerol (DAG) formation. Here we show that, on highly purified CD4+ T-cells and Jurkat cell line, cultivated overnight with tritiated arachidonic acid (AA) and subsequently activated with a CD3 mAb or a mitogenic pair of CD2 mAb (GT2 + T11.1), K20 increases PIP_2 degradation up to 75 % whereas it increases phosphatidylethanomanine (PE) synthesis up to 100 %. The simultaneous decrease of PIP_2 and increase of PE may be explained by an inhibition of AA incorporation in PIP_2 and a transfert to PE through an deacylation-reacylation mechanism. These data suggest that trigerring the β1 chain of the VLA with K20 during CD3 or CD2 activation, profundly modifies the membrane phospholipids repartition, especially the PIP_2 /PE ratio in CD4+ T-cells.

X 231 REGULATION OF LFA-1 MEDIATED ADHESION.

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We have made a unique anti-LFA-1 α antibody (NKI-L16) which activates the LFA-1 molecule, possibly by inducing a conformational change. The L16 antibody induces homotypic aggregation of lymphocytes and monocytes and induces adhesion of these cells to endothelial cells and to L cells transfected with ICAM-1. Monoclonal antibody NKI-L16 recognizes a unique Ca^{2+} dependent epitope on LFA-1. Expression of the L16 epitope is low on resting lymphocytes and monocytes, whereas it becomes induced upon *in vitro* activation of several cytokines. Cells that lack the L16 expression can not be triggered to activate their LFA-1 molecule. Thus expression of the L16 epitope seems to correlate directly with the capacity of cells to bind to ICAM-1 transfectants or to ICAM-1 expressed by endothelial cells, suggesting that expression of the L16 epitope is a prerequisite for LFA-1 mediated adhesion. Furthermore we observed that expression of the L16 epitope is not sufficient to induce LFA-1-ICAM-1 interactions, indicating that additional signals are required to induce cell adhesion. LFA-1 can become activated by triggering of different cell surface receptors on lymphocytes, such as CD2, CD3 or MHC class II. Signals from these molecules generate intracellular Ca^{2+} levels, with kinetics that correlate LFA-1 dependent adhesion. Additional evidence for the important role of intracellular Ca^{2+} levels comes from the observation that ionomycin is also able to stimulate LFA-1 mediated adhesion.

X 232 THE ROLE OF THE CYTOPLASMIC DOMAINS OF THE α AND ß SUBUNITS OF INTEGRIN $\alpha_{IIb}\beta_3$ IN CELL SPREADING AND FOCAL CONTACT FORMATION, J. Ylänne, T.E. O'Toole, J.C. Loftus and M.H. Ginsberg, Committee on Vascular Biology, The Scripps Research Institute, La Jolla, CA 92037

To study the role of integrin cytoplasmic domains in ligand binding and post-occupancy phases of cell adhesion, stable Chinese hamster ovary cell lines expressing recombinant human $\alpha_{IIb}\beta_3$ integrins were constructed. Fibrinogen(Fg)-induced adhesion, spreading, and adhesion plaque formation of these cells was dependent on the transfected $\alpha_{IIb}\beta_3$. Truncation of the cytoplasmic domains of the α subunit ($\alpha_{IIb}\Delta 991$) or ß subunit ($\beta_3\Delta 728$) of $\alpha_{IIb}\beta_3$ reduced the capacity of the integrin to mediate cell adhesion to Fg, but did not reduce Fg binding affinity. This suggested that the inhibitory effect of these truncations was on the post-occupancy phase of cell adhesion. The role of integrin cytoplasmic domains in post-occupancy events was confirmed by the finding that recombinant $\alpha_{IIb}\beta_3$ containing either $\alpha_{IIb}\Delta 991$ or $\beta_3\Delta 728$ manifested reduced cell spreading and adhesion plaque formation on Fg. In contrast, a chimeric α_{IIb} construct containing the cytoplasmic domain of α_5 ($\alpha_{IIb}\alpha_5$) promoted both spreading and adhesion plaque formation. $\alpha_{IIb}\alpha_5$ and $\alpha_{IIb}\Delta 991$ specify a high affinity state of integrin $\alpha_{IIb}\beta_3$, but only $\alpha_{IIb}\Delta 991\beta_3$ loses the capacity to support cell spreading and adhesion plaque formation. Moreover, the $\alpha_{IIb}\alpha_5\beta_3$, which was in a high affinity state, but was capable of inducing spreading and adhesion plaque formation, was more efficient in support of cell adhesion to Fg than wild-type $\alpha_{IIb}\beta_3$. These data show that cytoplasmic domains of both α and ß subunits are involved in post-occupancy functions of integrins such as cytoskeletal reorganization and changes in cell shape. Further, different structural elements of the α subunit cytoplasmic domains regulate integrin affinity and post-occupancy events.

X 233 ROLE OF α1β1-MEDIATED ADHESION IN NEURITE OUTGROWTH BY PC12 CELLS, Zhiyuan Zhang and David C. Turner, Department of Biochemistry and Molecular Biology, SUNY Health Science Center, Syracuse, NY 13210

PC12 cells, an established rat line derived from a pheochromocytoma, respond to nerve growth factor (NGF) by differentiating into sympathetic neuron-like cells that extend neurites on collagen or laminin. On collagen, this outgrowth is completely abolished by a monoclonal antibody, 3A3, that inhibits the function of the integrin α1β1. In one PC12 subline, PC12i, synthesis of α1-chains is increased about 10-fold in response to prolonged (approx. 1-wk) treatment with NGF; this increase in α1-protein synthesis reflects increased levels of α1-mRNA. Since β1-chains are constitutively expressed in PC12i cells, α1 synthesis controls the amount of α1β1 heterodimer expressed. Both initial PC12i cell attachment to collagen and the fraction of neurite-bearing cells are markedly enhanced by the prolonged NGF treatment. These results indicate that NGF regulates neurite outgrowth in PC12i cells by modulating expression of the matrix receptor required for growth cone traction. Fibroblast growth factor mimics NGF in inducing α1β1 in PC12i cells, with consequent enhancement of neurite outgrowth; long-term treatment with epidermal growth factor or dibutyryl cyclic AMP does not have these effects. Another subline, PC12c, expresses high levels of α1-mRNA and α1-protein in the absence of NGF treatment. With or without NGF, these cells adhere well to collagen and more than half extend neurites. Dexamethasone treatment of PC12c cells reduces expression of α1-mRNA and α1-protein, with a consequent reduction in attachment to collagen. On surfaces coated with 3A3 at low concentration (1 µg/ml in coating solution), NGF-treated PC12 cells of either subline extend long processes that resemble those seen on collagen or laminin, indicating that interaction of α1β1 with immobilized antibody molecules instead of a natural ligand allows growth cone motility. On surfaces coated with 3A3 at 10 µg/ml, however, PC12 cells extend broad, wavy, processes that are much shorter than those on collagen or laminin. At very high coating densities (100 µg/ml 3A3 in coating solution) the processes are shorter still. These findings suggest that when most of the α1β1 has been immobilized through high-affinity binding to substratum-adsorbed antibody, the cytoskeletal interactions needed for rapid neurite outgrowth cannot occur. (Supported by NIH grant 27409.)

X 234 MUSCLE CELL ATTACHMENT TO THROMBOSPONDIN-1 INVOLVES THE USE OF NOVEL CELL-BINDING SITES, Josephine C.Adams and J.Lawler, Vascular Research Division, Dept. of Pathology, Brigham and Women's Hospital, Boston MA 02115.

Thrombospondin is a homotrimeric glycoprotein which is present in the extracellular matrix of various tissues and is expressed at highest levels during tissue development and wound healing. In vitro, thrombospondin supports cell attachment, promotes cell migration and is mitogenic for certain cell types. It has therefore been suggested that thrombospondin plays a critical role in tissue formation and remodelling. One tissue in which thrombospondin is prominent during development is skeletal muscle; to elucidate the function of thrombospondin in this tissue we are examining the molecular basis of muscle cell attachment to thrombospondin. Cell lines derived from rodent embryonic skeletal muscle synthesise thrombospondin in culture and undergo time and concentration-dependent spreading on thrombospondin. Other cell types use heparin sulphate proteoglycans such as syndecan to bind the amino-terminal heparin binding domain of thrombospondin, while the RGD site is recognised by the αvβ3 integrin. In contrast, attachment of muscle cells is not inhibited by heparin or RGD peptide, alone or in combination, suggesting that the cells are binding to other regions of the molecule. Similarly, an adhesion-perturbing polyclonal antibody raised against αvβ3 integrin does not prevent cell attachment , even though the cells express this integrin. Cell adhesion is prevented in the presence of 0.5mM EDTA. We are using proteolytic fragments of thrombospondin and anti-thrombospondin monoclonal antibodies to map the region(s) of thrombospondin to which muscle cells attach. Thrombospondin-Sepharose affinity chromatography is being used to identify putative thrombospondin receptors. Indirect immunofluorecence data will be presented regarding the distribution of cytoskeletal components in cells attached to thrombospondin.

X 235 **Receptor-mediated trans-membrane signaling via the platelet integrin GPIIbIIIa.** Anthony Pelletier and Arthur Levinson, Genentech Inc. 460 Pt. San Bruno Blvd S. San Francisco, Ca. 94080

We demonstrate that the platelet integrin aIIb bIII is a receptor in a trans-membrane signal transduction pathway. A 293-derived cell line that expresses the integrin IIbIIIa, the fibrinogen (Fbg) receptor, adheres to Fbg within five minutes and shows marked morphological changes by 20 minutes, while the parent cell line does not adhere. We have exploited this novel morphogenic interaction to characterize the biochemical changes that are mediated by this receptor. We found rapid, transient increases in intra-cellular calcium levels initiated within 30 seconds of contact with Fbg-coated plates. This is followed within 5 minutes by the tyrosine phosphorylation of at least one protein, of approximate molecular weight 125KD. Antiserum specific for IIbIIIa also is able to induce both the calcium increase and the tyrosine phosphorylation, and all of the changes are dependent on the expression of the receptor. The tyrosine phosphorylation and the morphological changes are dependent on the increase in the calcium levels: inclusion of 5mM EGTA in the medium prevents the phosphorylation and spreading of the cells; pre-treatment with a cell-internal calcium chelator BAPTA/AM to buffer internal calcium changes prevents cells from spreading and prevents phosphorylation; and addition of EGTA to induced cells reverses the phosphorylation within 5 minutes, without apparent alteration of morphology. The rapid dephosphorylation induced by the addition of EGTA suggests the existence of a tightly regulated balance between a specific kinase and phosphatase. The dephosphorylation itself can be reversed by the restoration of calcium to the medium. This continuous sensitivity to extracellular calcium implies that signalling is continuous while contacts via the integrin are maintained. Thus this adhesion receptor mediates a rapid and reversible trans-membrane signal mediated by influx of extracellular calcium, presumably through a membrane channel intimately associated with the receptor.

Cancer and Metastasis

X 300 COLORECTAL CANCER CELLS EXPRESS MULTIPLE AND VARIED β SUBUNITS IN ASSOCIATION WITH αV INTEGRIN: POSSIBLE CORRELATION WITH A VARIABLE BIOLOGICAL RESPONSE TO ARG-GLY-ASP-PEPTIDES, Michael V. Agrez and Gordon F. Burns, Faculty of Medicine, University of Newcastle, N.S.W., Australia.

The metastatic spread of colon cancer cells is likely to involve extracellular matrix receptors belonging to the integrin supergene family. From studies on other cell types the subfamily defined by the αv subunit can be implicated in this process but the complexity of this subfamily is not yet resolved and it has not been studied in detail in colon carcinoma. We show here that the αv subunit is prominently displayed in sections of normal and malignant colonic epithelium. Among these colonic epithelial cells there was no detectable expression of the $\beta 3$ subunit that associates with αv to form the classical vitronectin receptor. Immunoprecipitation studies from 5 colon carcinoma cell lines revealed that αv was associated with multiple and varied β subunits on the different lines whereas the pattern of $\beta 1$-associated integrins was relatively homogeneous. Of the β subunits associating with αv, one migrated to the position of $\beta 1$ but the others (up to 3 additional subunits) could not be identified. In the three lines tested, none expressed $\beta 3$ by immunoprecipitation analysis, nor did they contain message for $\beta 3$ or $\beta 5$ by Northern blotting analysis. These data suggest that there may be αv-associated β subunits that are specific for colonic epithelial cells or have yet to be identified on other cell types. The possible functional consequences of this complexity was demonstrated when it was shown that synthetic peptides containing the arg-gly-asp (RGD) sequence profoundly influenced the morphology and proliferative capacity of the different cell lines. The effects seen with RGD peptides varied between the cell lines, ranging from 2-fold stimulation to almost complete inhibition of proliferation. These effects of RGD peptide were dependent upon a collagen substrate, and preliminary inhibition experiments with antibody indicated a role for $\beta 1$ integrin. Other possible correlates with receptor expression are under investigation.

X 301 INHIBITION OF CELL ADHESION BY HIGH MOLECULAR WEIGHT KININOGEN, Shinji Asakura, Iwao Ohkubo, and Deane F. Mosher, University of Wisconsin, Madison, WI 53706 and Shiga University of Medical Science, Tsukinowa Seta Ohtsu, Japan.

An anti-cell adhesion globulin was purified from human plasma by heparin-affinity chromatography. Amino acid sequence analysis revealed that the globulin is cleaved (kinin-free) high molecular weight kininogen (HKa). Globulin fractions from normal plasma immunodepleted of high molecular weight kininogen (HK) or from an individual deficient of HK lacked adhesive activity. HKa inhibited spreading of osteosarcoma and melanoma cells on vitronectin and of endothelial cells, platelets, and mononuclear blood cells on vitronectin or fibrinogen. It did not inhibit cell spreading on fibronectin. The protein had the strongest anti-adhesive effect when preadsorbed onto the otherwise adhesive surfaces. Vitronectin was not displaced from the surface by HKa. Uncleaved single-chain HK was less active in blocking cell adhesion than HKa. HKa preadsorbed at pH > 8.0 was inactive, although appreciable amounts of protein bound to surfaces at these pHs. HKa degraded further to release its histidine-rich domain had little anti-adhesive activity. However, isolated light chain of HKa, containing the histidine-rich domain, and recombinant histidine-rich domain had little activity also. Recombinant histidine-rich domain blocked the anti-adhesive activity of HKa. These results indicate that the cationic histidine-rich domain is critical for anti-adhesive activity, probably by directing the binding of HKa to the surface in an "anti-adhesive" conformation. Thus, cleavage of HK by kallikrein results in both release of bradykinin, a potent vasoactive and growth-promoting peptide, and formation of a potent anti-adhesive protein which alters cell morphology when cells spread on ligands for β_3-integrins.

X 302 INFLAMMATORY CYTOKINES INCREASE THE ADHESION/MIGRATION PROPERTIES OF VASCULAR CELL TYPES COMPOSING AIDS-KAPOSI'S SARCOMA LESIONS, Giovanni Barillari, Rita Gendelman, Robert C. Gallo and Barbara Ensoli, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Endothelial and smooth muscle cells are targets of cytokines released by activated immune cells and are involved in physiological events such as inflammation and wound healing. Here we show that inflammatory cytokines also play a role in a proliferative disease of vascular origin, namely Kaposi's sarcoma (KS).

Although rare in the normal population, KS is frequent in patients with AIDS (AIDS-KS). The AIDS-KS lesions are characterized by "spindle-shaped" cells of supposed vascular origin (AIDS-KS "tumor" cells) and by infiltrating leukocytes, fibroblasts and endothelial cells, with evident neoangiogenesis and edema.

We found that the same inflammatory cytokines, earlier found to be elevated in the supernatants of peripheral mononuclear cells and in the sera of AIDS patients, increase the expression of integrin receptors in normal vascular cells. These are at the levels similar to those expressed by AIDS-KS cells. As a consequence of this, cytokine-treated normal vascular cells and AIDS-KS cells show increased adhesion and migration in response to the molecules of the extracellular matrix, as compared to untreated endothelial and smooth muscle cells.

X 303 THE NATURAL HISTORY OF HUMAN BRAIN TUMORS (ASTROCYTOMAS) IS PARTIALLY DICTATED BY THE EFFECTS OF EXTRACELLULAR MATRIX PROTEINS ON TUMOR CELL ADHESION, MIGRATION AND PROLIFERATION. Michael E. Berens, Monique D. Rief, Adrienne C. Scheck and Joan R. Shapiro, Neuro-Oncology Laboratory, Barrow Neurological Institute of St Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, AZ 85013-4496

A profound and unique pathological feature of malignant astrocytoma is the exceptional rarity of metastasis outside the central nervous system coupled with the marked propensity of these transformed cells to invade the brain proper. A characteristic route for local spread of astrocytomas is along white fiber tracts (myelinated fibers). We have shown that proteins comprising the basement membrane of the vasculature (collagen type IV and laminin) strongly promote rapid attachment of astrocytoma cells in monolayer culture. These same proteins, however, were found to exert a concentration-dependent, growth inhibitory effect on astrocytoma cells, in some instances inducing complete cytostasis. Antiproliferative activity of basement membrane matrix proteins may partially explain the absence of systemic metastasis by astrocytomas. Local brain invasion phenomena may also be related to matrix components. Both normal and neoplastic astrocytes are stimulated to migrate by ligands which activate the epidermal growth factor receptor (EGFR), which is frequently expressed and/or amplified in astrocytomas. These ligands include epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). Recently, the CNS matrix molecule tenascin (hexabrachion) was characterized, and found to contain EFG-like domains. Furthermore, production of matrix-degrading proteases by astrocytoma cells is responsive to EGFR ligands. It appears that the presence of specific integrins on astrocytoma cells defines permissive environments for growth and invasion of these tumor cells. Integrins may indicate predictable patterns of astrocytoma growth and invasion, and therefore may serve as diagnostic or prognostic markers.

X 304 USE OF DISINTEGRINS TO CORRELATE CELL ADHESION AND LUNG METASTASES IN MURINE MODEL. Lucia Beviglia, Gwendolyn J.Stewart and Stefan Niewiarowski Dept of Physiology, Thrombosis Research Center, Temple Univ.Sch.Med., Philadelphia, Pa 19140.

Previous studies demonstrated that the disintegrin albolabrin at 5-20 μg per mouse inhibited lung metastases of B16 murine melanoma cells injected into C57BL/6 mice and was at least 2000 times more active in this system than RGDS (Exper. Cell Res 1991, 196, 6). The purpose of this study was to compare antimetastatic effects of two disintegrins: albolabrin and echistatin. RGDD represents a cell recognition site of both disintegrins; it is located on the loop that is conformationally constrained by two S-S bridges in albolabrin and by three S-S bridges in echistatin. Experimental evidence obtained in our and other laboratories suggests that echistatin is a more selective inhibitor of fibronectin receptor as compared to albolabrin. In this study, albolabrin appeared to be a more active inhibitor of murine lung colonization by B16 melanoma cells than echistatin. At 10 μg per mouse albolabrin (N=9) and echistatin (N=10) resulted in 65.7% +/- 5.2% and 41.2% +/- 3.7% ($p < 0.05$) inhibition of the number of lung metastases (counted after two weeks), respectively. Both disintegrins had similar effect on ADP induced aggregation of murine platelets; IC_{50} for albolabrin and echistatin were 58 nM and 53 nM. At a concentration of 20 μg/ml echistatin and albolabrin preincubated with B16 melanoma cells resulted in 68 +/-3.7 % and 48.5% +/-3.9 % inhibition of cell adhesion to fibronectin ($P<0.05$) and in 16.9 +/- 3.4% and 34.2% +/-5.6% ($P<0.05$) inhibition of cell to laminin. Our data suggests that different disintegrins may be useful to identify cell adhesive receptors involved in metastatic process.

X 305 EXPRESSION AND FUNCTION OF LFA-1 ON PLASMA CELLS IN MULTIPLE MYELOMA,
Andries Bloem, Tanja de Gruyl, Henk Lokhorst*, Ad Dekker* and Els Ahsmann, Departments of Clinical Immunology and Haematology*, University Hospital, Utrecht, The Netherlands.
LFA-1 expression on bone marrow derived plasma cells from normal individuals and patients with Multiple Myeloma (MM) was studied using a new moab, F8.8. This moab recognizes the α-chain (CD11a) of LFA-1 as determined by immunoprecipitation. Although the moab F8.8 stains unstimulated T cells with the same mean fluorescence intensity as other anti-CD11a moabs, it proved to be superior in detecting CD11a on malignant plasma cells as compared to reference moabs. No LFA-1$^+$ plasma cells were detected in normal individuals while in MM patients plasma cell LFA-1 expression correlated with the growth fraction of the tumour. LFA-1$^+$ plasma cells from patients and plasma cell lines were tested in a flow cytometric adhesion assay. LFA-1$^+$ plasma cells are capable of binding to activated human endothelial cells. This interaction can partly be inhibited by moabs directed against CD11a, CD18 or the first domain of CD54. This indicates that the LFA-1 molecules expressed on malignant plasma cells is functional and can be used for adhesion. Studies on functional aspects and regulation of adhesion molecules involved in cellular interactions of plasma cells in MM might contribute to understanding of the pathophysiology of the disease.

X 306 INTEGRIN EXPRESSION IN UNDIFFERENTIATED AND DIFFERENTIATED HT-29 AND CaCo2 COLON CARCINOMA CELLS. Claudie Flohil, Winand Dinjens, Fred Bosman, Dept. of Pathology, Erasmus University, P.O. box 1738, 3000 DR Rotterdam, The Netherlands.
For the study of differentiation in gut epithelium the HT-29 and $CaCo_2$ colon carcinoma cell lines are extensively used. Under standard tissue culture conditions the cells are undifferentiated. A variety of conditions, however, induces HT-29 and $CaCo_2$ to differentiate in the direction of columnar resorptive and/or mucin producing cells. We studied the expression of integrin α_2, α_3 and α_6 chains as well as β_1 and β_4 chains by immunohistochemistry on differentiated and undifferentiated cells, assuming that either the pattern of integrin expression or the distribution of integrins on the cell surface might change with differentiation. Furthermore integrin function was tested by adhesion experiments, using substrates coated with types I and IV collagen, laminin and fibronectin with or without prior incubation of the cells with anti-integrin antibodies.
By immunohistochemistry, α_2 and α_3 as well as β_1 were located circumferentially on the surface of undifferentiated HT-29 cells. However, on differentiated cells the apical cell surface lacked integrin immunoreactivity. Undifferentiated HT-29 and $CaCo_2$ cells adhered to laminin, type IV collagen and fibronectin; in differentiated cells laminin binding was reduced. Binding of differentiated cells could be blocked by anti-β_1 antibodies and of undifferentiated cells also by anti-β_4 antibodies.
These experiments indicate that 1. in intestinal epithelial differentiation not the integrin expression as such but rather its topography on the cell surface changes and 2. in these cells α_2, β_1 and β_4 integrin chains play an important role in cell-cell and cell-extracellular matrix adhesion.

X 307 CELLULAR ADHESION AND THE ROLE OF β_1 INTEGRINS IN LYMPH NODE METASTASIS OF BREAST CARCINOMA CELLS. Djoneidi M., Tawil N.,* Carbonetto S.,* and Brodt P. Dept. of Surgery and the Center for Research in Neuroscience, McGill University, Montreal, Quebec, Canada, H3A 1A4.

The role of cellular adhesion in lymph node metastasis was studied with two rat breast carcinoma lines with high and low metastatic potentials to regional lymph nodes. Cells of the highly metastatic carcinoma TMT-081 were significantly more adherent than the non-metastatic MT-W9B cells to cryostat sections of rat peripheral lymph nodes. When adhesion to extracellular matrix (ECM) protein-coated dishes was measured TMT-081 cells attached significantly better than MT-W9B cells to fibronectin but not to vitronectin, laminin or type IV collagen. Adhesion to the cryostat sections (as well as to fibronectin) could be blocked by an RGD containing peptide and by antibodies directed to the extracellular domain of rat β_1 integrin subunit, implicating a VLA integrin receptor in this adhesion. Immunoprecipitation of ^{125}I-labelled cell surface proteins with integrin specific antibodies revealed that while there were no significant differences in the expression of β_1 and α5 on these tumor cells, the metastatic TMT-081 tumor cells expressed significantly higher levels of the α3 subunit. These results suggest that increased expression of integrin $\alpha_3\beta_1$ may facilitate tumor cell adhesion to the lymph node ECM thereby promoting lymph node metastasis.

X 308 BETA-1 INTEGRINS MEDIATE ADHESION OF OVARIAN CANCER CELLS TO EXTRACELLULAR MATRIX (ECM) PROTEINS AND TO PERITONEAL MESOTHELIUM. S.A. Cannistra, J. Niloff, C. Morimoto, J. DiCarlo, C. Ottensmeier, B. Orta. Division of Tumor Immunology, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115.

Epithelial carcinoma of the ovary most commonly spreads by implantation of tumor cells throughout the peritoneal surface of the abdominal cavity. The mechanisms by which implantation occurs are unknown but most likely involve specific adhesion molecules expressed by ovarian cancer cells and by peritoneal mesothelium. In order to determine the role of adhesion proteins in this process, we characterized the expression of several known adhesion molecules by 2 ovarian cancer (OC) cell lines (CAOV-3, OVCAR-3) and by normal mesothelium. Both OC cell lines expressed VLA-1, 2, 3, 5, and 6, α_V, CD44, and CD54. In addition, VLA-4 was expressed by CAOV-3. Normal mesothelium expressed VLA-1, 2, 4, 5, 6, α_V, CD44, and CD54. VCAM-1, ELAM-1, CD18, and CD56 were not expressed by any cell type. Binding studies were performed in order to determine the functional significance of adhesion molecule expression, with the results of one representative experiment shown below (expressed as percent specific binding):

	control				anti-ß1 antibody			
	- CI	LAM	FN	MESO	- CI	LAM	FN	MESO
CAOV:	1 45	34	38	54	1 2	7	12	29
OVCAR:	1 36	29	24	45	1 13	10	9	30

abbreviations: CI, collagen type I; LAM, laminin; FN, fibronectin; MESO, confluent layer of normal mesothelial cells.

Both OC lines showed significant binding to CI, LAM, FN, and MESO. Binding to CI, LAM, and FN was significantly inhibited by the monoclonal antibody 4B4, which recognizes the common ß1 integrin chain. Binding to MESO was also inhibited by 4B4, but to a lesser degree. Binding to MESO was not affected by pre-treatment with hyaluronidase, suggesting that CD44/hyaluronate interaction is not involved. These results suggest that ovarian cancer cell adhesion to peritoneal mesothelium is partly mediated by ß1 integrins, and that ECM proteins may be involved in this process. Identification of adhesion molecules such as ß1 integrins which may mediate implantation of OC cells onto mesothelium may ultimately provide a strategy for inhibiting the spread of ovarian cancer throughout the peritoneal cavity.

X 309 INFLUENCE OF VLA EXPRESSION UPON THE TUMORIGENICITY OF HUMAN LUNG CANCER, Fang-An Chen, B. Anne Croy, Thomas R. Alosco, Richard B. Bankert, Dept. of Molecular Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263. Approximately 80% of all non-small cell lung cancers from patients exhibit a significant increase in the expression of mRNA encoding VLA-2 α-chain and the VLA-2 molecule on the tumor cell surface when compared to normal lung tissue (J.E.M. 173:1111). To determine whether or not an altered expression of VLA affects the growth and/or metastatic potential of the tumor cells, primary cultures of human tumors were established from fresh lung tumor biopsies and clones expressing distinct VLA profiles were established and tested for tumorigenicity and invasiveness in SCID mice. Each clone was tested for the cell surface expression of VLA-1→6. It was determined that from a single biopsy of a squamous cell carcinoma several clones expressing different VLA phenotypic profiles could be generated. Two phenotypically distinct and stable clones were assayed for tumorigenicity. The difference between these two clones with respect to the VLA phenotype was restricted to the expression of VLA-1 & 2. The first clone 2E9 expressed high level of both VLA-1 & 2, while the second clone 1E12 was negative (or low level) for both VLA-1 & 2. No difference was observed in the rate or pattern of growth of these two cells in vitro, and, morphologically, they were indistinguishable microscopically. However, when tumor cells were inoculated intravenously into SCID mice the VLA-1^+,2^+ tumors grew aggressively in the lung, bone marrow, adrenal gland and ovary, while the same number of VLA-1^-,2^- tumor cells produced little or no tumor growth in the recipient mice. The VLA-1^-,2^- clone, however, was tumorigenic since these cells produced tumors in SCID mice following a subcutaneous inoculation. Our results demonstrate that primary human lung tumors are heterogeneous with respect to their expression of VLA integrins, and that the expression of high levels of VLA-1 and/or VLA-2 may alter the tumorigenicity as determined by growth in SCID mice. The possibility that VLA-1 or 2 is directly involved with the homing and subsequent growth of the tumor is being addressed by attempting to block the homing & growth of the VLA-1^+,2^+ tumor using monoclonal antibodies that selectively inhibit the ligand binding sites of these two integrins.

X 310 INTEGRIN $\alpha_{IIb}\beta_3$ EXPRESSION IN TUMOR CELLS OF NON-MEGAKARYOCYTE ORIGIN. Yong Q. Chen, Xiang Gao, Kenneth V. Honn, Department of Radiation Oncology, Wayne State University, Detroit, MI 48202. $\alpha_{IIb}\beta_3$ glycoprotein heterodimer belongs to the integrin superfamily serving as receptors for extracellular matrix proteins such as fibronectin, vitronectin and fibrinogen. It was believed that this integrin was expressed only in cells of megakaryocyte lineage. We previously detected an $\alpha_{IIb}\beta_3$-like protein in several mouse and human tumor cells by using antibodies (mAb10E5, AP-2) against human platelet $\alpha_{IIb}\beta_3$. Recently, we have identified the authentic $\alpha_{IIb}\beta_3$ in mouse amelanotic melanoma (B16a) cells by Northern blotting and immunoprecipitation. To further support our results, total RNA was isolated from HEL and B16a cells, reverse transcribed to cDNA with α_{IIb} and β_3 specific primers. The cytoplasmic domain of α_{IIb} and β_3 cDNAs were amplified by polymerase chain reaction (PCR). The specificity of amplified DNAs was confirmed by Southern blotting using human α_{IIb} and β_3 cDNA probes. The PCR products were then sequenced. Sequencing data show that α_{IIb} from HEL cells is identical to the previously published cDNA sequence, that α_{IIb} from B16a cells has ~80% homology with human α_{IIb} and ~90% homology with rat α_{IIb}. Finally, α_{IIb} from B16a cells has little or no homology with human α_V and other known α subunits. Our results suggest that 1) PCR amplified sequence is the human α_{IIb} counterpart in mouse cells; 2) $\alpha_{IIb}\beta_3$ expression is not confined to megakaryocyte lineage cells.

X 311 INDUCTION OF DIFFERENTIATION IN A NON-ADHERENT SMALL CELL LUNG CANCER (SCLC) CELL LINE COORDINATELY UPREGULATES THE BETA-1 INTEGRIN FAMILY AND EXPRESSION OF AN ADHERENT PHENOTYPE, Walter T. Dixon*, Gordon E. Searles*, J.G. Seehafer** and Andrew R.E. Shaw**, Division of Dermatology* and Division of Oncology**, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

Small cell lung cancer (SCLC) is a significant clinical problem because it undergoes early and extensive metastatic dissemination. Evaluation of the change in integrin expression accompanying the induction of an adhesive phenotype may have relevance to the process of metastatic spread.

The SCLC cell line NCI-H-69 normally grows in suspension but can be induced to both differentiate and attain an adherent phenotype by treatment with bromodeoxyuridine (BrdU). Following continuous exposure to BrdU (12μM) for 6 weeks stable subpopulations of adherently growing cells could be isolated. The parental line expresses a very restricted integrin repertoire, as judged by indirect immunofluorescent flow cytometry (only low levels of $\alpha_3\beta_1$ and $\alpha_6\beta_1$) and very little (less than 5% positive cells) or no detectable α(1,2,4,5,V) or ß(2,3,4,5,). In contrast, BrdU treatment, and conversion to the adherent epithelioid phenotype results in a dramatically different integrin profile. The β_1 family of integrins, in particular, are upregulated to a level where α(2,3,4,5 and V) are all strongly positive as assessed by flow cytometry. In addition, radio-immunoprecipitation with a monoclonal antibody directed against the β_1 subunit indicated a large increase in the amount of immunoprecipitable β_1 in the adherent H-69B subline.

These phenotypically different forms of the same cell line will provide a useful model to investigate the influence of integrin expression on metastatic potential.

X 312 TRANSFECTION OF LFA-1 INTO AN LFA-1 NEGATIVE MUTANT T-CELL HYBRIDOMA RESTORES METASTATIC POTENTIAL, Mariëtte H.E. Driessens, Felix Rodriguez Erena and Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

LFA-1 is necessary for invasion in vitro and metastasis in vivo of a T cell hybridoma. Anti-LFA-1 antibodies inhibit invasion and LFA-1 deficient mutants are virtually non-invasive and non-metastatic. To prove that the reduction in invasion and metastasis was actually due to the lack of LFA-1 we attempted to restore the metastatic potential by transfection of the β_2 cDNA. A transfectant which expressed LFA-1 at 50% of the parental level had limited invasive potential in vitro and showed identical wide spread metastatic distribution as the parental cells, in particular to the liver and kidneys, albeit in only 50% of the mice. Additional transfectants are being generated to substantiate these findings.

The fact that the parental cell line and the transfectant preferably form metastases in liver and kidney could be related to the presence of the LFA-1 counter structures ICAM-1 and ICAM-2. Recently, the high affinity ligand ICAM-1, which is found in inflamed tissues, was shown to be constitutively expressed along microvessels in the liver and in kidney glomeruli. We observed that ICAM-1 is expressed by isolated rat hepatocytes and that the invasion of T cell hybridoma cells into hepatocyte cultures could be inhibited by the ICAM-1 antibody almost to the same extent as by the anti-LFA-1 antibody. This suggests that the preference of lymphomas for metastasis to organs like liver and kidney is determined by this constitutive ICAM-1 expression.

X 313 NEURAL CELL ADHESION MOLECULE (NCAM) EXPRESSION IN HUMAN NEUROBLASTOMAS, Martin Ellis, Dafna Kaner, Mathilda Mandel and Yael Kaufmann, Institute of Hematology, Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv, Israel

NCAM is a heterogeneous cell adhesion molecule involved in adhesion between neurons and between neurons and muscle cells. A number of different isoforms of this protein have been identified which arise from alternative splicing of a single NCAM gene and from the use of different poly A addition signals. Differing levels of NCAM have been detected on the cell surface of a variety of neuroectodermal and non-neuroectormal tumors in man. This observation raises the possibility that NCAM may be involved in neural cell tumorigenicity.

In this work we have analysed NCAM mRNA expression in a small number of primary neuroblastoma tumors as well as neuroblastoma cell lines. Results to date have revealed that the level and pattern of NCAM expression varied from tumor to tumor. One cell line was found to be negative for NCAM expression while the remainder of the cell lines and primary tumors expressed from one to four NCAM mRNA species. Thus heterogeneous expression of NCAM was found with respect to the number of isoforms and to their relative quantities. In order to determine whether this differential NCAM expression is related to the tumorigenicity of neuroblastoma, a greater number of primary tumors needs to be analysed.

X 314 DISTINCT BIOLOGICAL CONSEQUENCES OF INTEGRIN αvβ3-MEDIATED MELANOMA CELL ADHESION TO FIBRINOGEN AND ITS PLASMIC FRAGMENTS Brunhilde Felding-Habermann*, Zaverio M. Ruggeri, and David A. Cheresh. The Scripps Research Insitute, La Jolla, CA 92037, * present address: E. Merck, 6100 Darmstadt, FRG

Fibrinogen/Fibrin and its proteolytic fragments serve as potential adhesive substrates during thrombosis, wound healing and cancer. We examined the biological response of human melanoma cells to fibrinogen and its naturally occurring plasmic breakdown products that are known constituents of the tumor stroma. Plasmin treatment of fibrinogen first results in fragment X which is characterized by removal of the C-terminal portion of the α chain including an RGD sequence (Aα 572-575). Further digestion leads to fragment D comprising primarily an intact C-terminal stretch of the gamma chain containing the platelet adhesion sequence HHLGGAKQAGDV. In a sensitive adhesion assay M21 human melanoma cells utilized integrin αvβ3 to attach to all three of these ligands.However, only intact fibrinogen supported significant cell spreading while fragment X produced minimal spreading and fragment D promoted only adhesion. These results indicate that fibrinogen contains al least two αvβ3-dependent adhesive sites and these promote distinct biological responses of human melanoma cells. The differential functional properties of these ligands directly correlate to their relative binding affintiy for purified αvβ3 as measured in a solid phase receptor assay. These results provide evidence that a single integrin can promote distinct biological signals depending on the molecular nature of the ligand binding event.

X 315 **BRAIN GLIOBLASTOMA TUMOR INFILTRATING LYMPHOCYTE (TIL)-T CELLS ARE DISTINCT FROM PERIPHERAL BLOOD LYMPHOCYTE (PBL)-T ON THE BASIS OF CELL-ADHESION MOLECULE EXPRESSION.**

Marie-Claude Gingras[1], Eugène Roussel[2], Elizabeth A. Grimm[3], Bradley W. McIntyre[4], Janet Bruner[5] and Richard P. Moser[1]. Depts. of Neurosurgery, Thoracic Surgery, Tumor Biology, Immunology, and Pathology, MD Anderson Cancer Center, Houston, Texas, 77030.

To gain understanding of lymphocyte specific migration mechanisms, we addressed the question: Is there any significant difference in the expression pattern of cell-adhesion molecules between autologous PBL and TIL populations? We studied PBL and TIL in 7 patients with untreated glioblastoma. Blood samples were obtained immediately prior to surgery and TIL were extracted from tumor samples by mechanical disruption without the use of enzymes. Freshly isolated TIL and PBL were double labelled for CD3 and Integrins ß1: CD29, CD49a, b, d, e, f, Integrins ß2: CD18, CD11a, CAM: CD2, CD54, CD56, CD58, Selectin:LECAM, carbohydrate receptors: CD15, CD65, CD44, the activation markers CD26 and IL-2 R CD25. Cell populations were analyzed by flow cytometry. Results were expressed as % of total CD3+ T cells. A consistent difference in the expression pattern was observed between the TIL and PBL populations in 11/18 markers. The following table presents the ratio of TIL/PBL means for each marker showing a significant difference.

CD49a	24.8	CD18	0.8	CD54	6.4	CD15	3.7
CD49b	8.7	CD11a	0.8	CD56	2.8	CD65	8.8
				CD58	1.3	LECAM	0.7
CD25	3.0						

>1 increase, <1 decrease, $p<0.05$

Our data demonstrate that TIL-T cells are a distinct population from PBL-T cells. These results suggest that those tumors cause an inflammatory response.

X 316 GLIOBLASTOMA EXPRESSION OF VITRONECTIN AND THE αvß3 INTEGRIN: ADHESION MECHANISM FOR TRANSFORMED GLIAL CELLS, Candece L. Gladson* and David A. Cheresh, Department of Pathology*, University of Alabama at Birmingham, Rm 573 LHRB, Birmingham, AL 35294 and Department of Immunology, The Research Institute of Scripps Clinic, La Jolla, CA 92037

Glioblastoma multiforme, the most malignant astroglial-derived tumor, grows as an adherent mass and locally invades normal brain. An examination of adult cerebral biopsy material for the expression of adhesive proteins that might potentiate adhesion and invasion demonstrated tumor cell-associated vitronectin (5/5). In contrast, vitronectin was not detected associated with glial cells in low grade astroglial tumors (0/4), reactive astrogliosis (0/4), or in normal adult cortex and cerebral white matter (0/5). Also, a wide variety of other adhesive ligands were absent from the glioblastoma tumor parenchyma. This tumor cell-associated vitronectin is likely derived from the glioblastoma cells and not from serum, since cultured human glioblastoma cells grown in the athymic nude mouse synthesize vitronectin. The αvß3 integrin was the only vitronectin receptor identified in glioblastoma tumors *in situ*, and was also not expressed on low grade astroglial-derived tumors, reactive astrogliosis, or on glia or neurons in normal adult cortex and cerebral white matter. In a cell attachment assay, cultured glioblastoma cells attached to the parenchyma of glioblastoma tumor cryostat sections at the sites of vitronectin expression, but failed to attach to normal brain. This adhesion was inhibited by antibodies directed against vitronectin, the αvß3 integrin, and with an Arg-Gly-Asp-containing peptide. These data provide evidence for a cell adhesion mechanism in glioblastoma tumors that might potentiate glioblastoma cell invasion of normal brain, and suggest glioblastoma tumors remodel their extracellular matrix *in situ*.

X 317 IMMUNOLOCALIZATION OF INTEGRINS IN THE NORMAL AND NEOPLASTIC BREAST. Victor E. Gould, George K. Koukoulis, Matti Korhonen, Vito Quaranta and Ismo Virtanen, Rush Medical College, Chicago, IL 60612, Helsinki University, 00170-Helsinki Finland and Scripps Clinic, La Jolla, CA 92037.

We studied, by immunohistochemistry samples of normal breast (NB, n=6), fibrocystic disease (FCD, n=11), and representative benign (n=9) and malignant neoplasms (n=54). Monoclonal antibodies (Mab s) specific for the $alpha_{1\text{-}6, v}$ and $beta_{1,3}$ and $_4$ integrin subunits were applied to cryosections by the ABC method. Selected samples were studied by double immunofluorescence microscopy with the said Mab s and polyclonal antisera to laminin, and myosin. We found that the $alpha_{1,2,3,6,V}$ and the $beta_1$, integrin subunits were detectable in the normal breast parenchyma; myoepithelial cells stained consistently stronger than the basolateral aspect of the luminal cells. This immunoprofile was retained, and in cases enhanced through the spectrum of FCD, in benign tumors, and in ductal and lobular carcinomas in-situ. In most infiltrating ductal carcinomas not otherwise specified, integrin staining tended to decrease except for some cases reacting strongly for the $alpha_V$ subunit. Notably, mucinous carcinomas reacted very strongly for $alpha_{2,3,6,V}$ and $beta_4$ subunits, and, even more so for the $alpha_5$ subunit that was not found in the normal breast. Also, a subset of infiltrating lobular carcinomas stained convincingly for $alpha_{1,3,6,V}$ and $beta_1$ subunits; reactions were conspicuous in delicate but abundant kinetopodia. Our findings indicate that in hyperplasias and in benign tumors, integrin expression patterns parallel those of the normal breast whereas in carcinomas, variations include decrease, enhancement and emergence of certain subunits that are not in the normal breast repertory. Alterations of integrin expression reflect significantly disrupted cell-matrix and cell-cell interactions in virtually all infiltrating breast carcinomas; notably, certain alterations appear to be associated with certain clinically important variants of breast carcinomas. We suggest that the apparent upregulation of certain integrins in some carcinomas may reflect the selection of subpopulations with increased binding capacity which in turn may impact on their invasive and metastatic properties; this, in turn, may help explain certain histological appearances and the associated behaviour patterns shown by some mucinous and lobular carcinomas.

X 318 **Integrins involved in thymus homing and melanoma metastasis** Beat A. Imhof, Patricia Ruiz, and Dominique Dunon, Basel Institute for Immunology, Grenzacherstrasse 487, Basel, Switzerland.

Metastasis of transformed cells involves the detachment of cells from the primary tumor, their transport through the blood circulation or the lymphatics, adhesion to the vascular endothelium, extravasation and their lodgement to secondary tissue sites. Since tumor cells invade normal healthy tissue, they may use vascular adhesion molecules normally involved in homing of circulating hemopoietic cells. Colonization of the thymus by T cell progenitors represents one excellent model for an invasive homing mechanism occurring under developmentally controlled conditions. The mouse progenitor T lymphocyte cell line FTF1 binds in vitro to thymus blood vessels and liver from newborn mice. A monoclonal antibody, EA-1, raised against an embryonic mouse endothelial cell line, blocked adhesion. The antigen recognized by EA-1 was identified by N-terminal sequencing as an α6 integrin and is present on the apical surface of vascular endothelium. Using an in vivo metastasis model the EA-1 antibody blocked experimental lung lesions of B16-129 melanoma cells, either when the antibody is injected into mice 24 hours before the melanoma cells, simoultaneously with melanomas or when melanoma cells are precoated with EA-1 before injection. The role of α6 integrins in metastasis will be discussed. Since this functional antibody crossreacts with human endothelial cells it may be a valuable tool for inhibition of secondary metastasis.

X 319 CYTOTOXIC EFFECT OF AJOENE ON NEOPLASTIC T-CELLS IS POSSIBLY RELATED TO ITS ACTION ON INTEGRINS, Edward V. Karamov, Galina V. Kornilayeva, Tatyana V. Makarova, Alexander V. Tatarintsev, Peter V. Vrzheshch, Andrey A. Schegolev, Dmitriy E. Yershov, Nikolay A. Fedorov, and Ali S. Turgiev, Institute of Virology, USSR Academy of Medical Sciences, Moscow 123098, USSR.

Monoclonal antibodies against the platelet integrin, GP IIb/IIIa, have recently been shown to inhibit tumor cell growth *in vivo* (Boukerche H. et al. Blood, 1989, 14:909). Ajoene, (E,Z)-4,5,9--trithiadodeca-1,6,11-triene-9-oxide, isolated from extracts of garlic (*Allium sativum*) inactivates GP IIb/IIIa allosterically (Apitz-Castro R. et al. Biochem. Biophys. Res. Commun., 1986, 141:145). We studied the effect of synthetic (E,Z)-ajoene on several lines of leukemic (CEM, MT2, MT4) and lymphoid (H9, Jurkat) T-cells. In contrast with the data obtained previously with blood platelets and neutrophils, ajoene exerted some toxic effects on the neoplastic T-cells tested. Judging by the cytocidal activity, leukemia T-cells were much less sensitive to ajoene (EC100 ~500 μM; 72 hrs of incubation) than T-lymhoid tumor cells (EC100 ~50 μM; 72 hrs). It should be noted that CEM cells which were the least susceptible to ajoene in this respect are known to express minute amounts of integrins on their surface (Wawryk S.O. et al. Immunol. Rev., 1989, 108:135). We speculate that the observed toxic effects of ajoene are related to its action on integrins, since viability of T-lymphoid tumor cells may require functionally active adhesion receptors. Our assumption is further strengthened by the recent data from Scharfenberg K. et al. (Cancer Lett., 1990, 52:103) who have described a correlation between the sensitivity of neoplastic cells to ajoene and their tumorigenic potential.

X 320 Changes in Integrin Expression Associated with Human Prostate Cancer Progression. J. David Knox, Virginia Clark, Kit-Sahn Affinito, Lupe Manriquez, Anne E. Cress*, and Ray B. Nagle. Department of Pathology, *Department of Radiation Oncology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

The goal of this study was to determine the cytoplasmic membrane integrin expression in the normal, prostatic intraepithelial neoplasia (PIN), and invasive carcinoma. In order for the neoplastic luminal cells to escape the confines of a normal gland they must first displace the basal cells, then adhere to and migrate through the underlying basement membrane. Integrins have been demonstrated to play a role in cell-cell and cell-matrix adhesion, transmembrane signaling, and the induction of cell motility. Therefore, the integrins being expressed by a particular cell may determine in part the ability of the cell to form a tumor, invade, and metastasize. To test the hypothesis that prostate cancer progression is associated with a change in the profile of integrins being expressed we have used antibodies specific for α_{1-6} and β_{1-4} to stain frozen sections of both normal human prostate, PIN lesions, and various Gleason grade invasive human prostate tumors. By this immunohistochemical analysis we have demonstrated that normal tissue expresses α_1, α_2, α_3, and α_6, in conjunction with β_1, β_2, and β_4. In contrast, the only integrin observed by this method in neoplastic cells was α_6. Integrins are normally transported to the cell surface as heterodimers. The α_6 in the neoplastic cells appeared to be expressed on the cell surface suggesting that the neoplastic cells are also expressing either an altered form of one of the four β subtypes assayed, one of the several other β subtypes that have recently been described, or a novel β integrin. (Supported in part by ACS grant PDT-388.)

X 321 EXPRESSION OF $\alpha6\beta4$ INTEGRIN ON NORMAL UROTHELIAL CELLS AND ON BLADDER CANCERS. M. Liebert, R. Washington, J Stein, G. Wedemeyer, C. Van Waes, T.E. Carey, H.B. Grossman. Section of Urology and Department of Otolaryngology, University of Michigan, Ann Arbor, MI, 48109.

Integrins are a family of transmembrane heterodimers, many of which function as cell-matrix or cell-cell receptors. The $\alpha6\beta4$ integrin is found on most epithelial cells and tumors derived from epithelial cells. On squamous cancers of the head and neck, increased expression of the $\alpha6\beta4$ integrin is associated with shorter time to recurrence and reduced survival. We evaluated the expression of $\alpha6\beta4$ integrin on normal urothelium and bladder cancers using monoclonal antibodies to $\alpha6$ (BQ16), and $\beta4$ (UM-A9). On normal urothelium, $\alpha6\beta4$ was consistently expressed on the basolateral surface in a basement membrane-like distribution. On bladder cancers, $\alpha6$ was always found in association with $\beta4$ expression, and increased expression of $\alpha6\beta4$ was found frequently. Although the function of the $\alpha6\beta4$ integrin is not known, recent studies have suggested that the $\alpha6\beta4$ integrin is associated with the hemidesmosome (an anchoring structure) on normal epithelial cells. We compared the expression of the $\alpha6\beta4$ integrin and collagen VII (another component associated with anchoring structures) in normal ureter and on bladder cancers. Co-staining of the same frozen tissue sections was performed with monoclonal antibodies to $\alpha6$ (BQ16, IgG1), $\beta4$ (UM-A9, IgG2a), and collagen VII (IgG1) and detected using fluorescein-isothiocyanate conjugated antibody to mouse IgG2a and rhodamine-conjugated antibody to mouse IgG1. Sections of normal urothelium and low-grade bladder cancers showed co-localization of $\alpha6$ and $\beta4$ and of $\beta4$ and collagen VII at basement membrane-like areas. However, 16 invasive bladder cancer specimens showed increased cellular staining for both $\alpha6$ and $\beta4$, and of these specimens, 9 also had lost the collagen VII co-localization. These results indicate a dissociation between collagen VII and $\alpha6\beta4$ integrin expression and suggest that a defect in assembly of anchoring structures in some invasive bladder cancers may contribute to the processes of invasion and metastasis. Supported by grant PDT-409 from the American Cancer Society.

X 322 THE $\alpha6\beta4$ INTEGRIN IS A LAMININ RECEPTOR ON INVASIVE COLON CARCINOMA CELLS, Margaret M. Lotz, Edward C. Lee, and Arthur M. Mercurio. Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA 02115

A ligand for the $\alpha6\beta4$ integrin was identified using a cell line, referred to as clone A, that was derived from an invasive colon adenocarcinoma. This cell line, which expresses the $\alpha6\beta4$ integrin, adheres to the E8 and not to the P1 fragment of laminin. The adhesion of clone A cells to laminin is extremely rapid with half-maximal adhesion observed at 5 minutes after plating and it is blocked by GoH3, an $\alpha6$ specific antibody (75-90% inhibition). The $\alpha6\beta4$ integrin binds specifically to laminin-Sepharose columns and not to collagen-Sepharose. $\alpha6\beta4$ is eluted from laminin-Sepharose with 10mM EDTA but not with NaCl or lactose. Clone A cells do not express any detectable $\alpha6\beta1$ as evidenced by pre-clearing and immunoblotting experiments. These data establish that laminin is a ligand for the $\alpha6\beta4$ integrin and suggest that this integrin can function as a laminin receptor. A laminin binding function for $\alpha6\beta4$ has not yet been shown for other cell types that express this integrin. One possible explanation for this discrepancy is that the laminin binding function of $\alpha6\beta4$ is constitutively activated in clone A cells, a situation that may be different in normal epithelial or less aggressive carcinoma cells.

X 323 ROLE OF INTEGRIN RECEPTORS IN THE PROLIFERATIVE RESPONSE OF MELANOMA CELLS TO FIBRONECTIN, Roberta Mortarini[1], Angela Gismondi[2], Angela Santoni[2], Andrea Anichini[1] and Giorgio Parmiani[1]. Division of Experimental Oncology D, Istituto Nazionale Tumori, Milan, Italy and Dip. Med. Sper. Universita' La Sapienza, Rome, Italy.

Quiescent human melanoma cells cultured in serum-free medium proliferated in a dose- and time-dependent fashion to immobilized fibronectin (FN) as indicated by ^{3}H-TdR incorporation, increment of cell number and cell cycle analysis. This reponse to FN was observed with tumor clones isolated from a subcutaneous metastasis, but only when tumor cells expressed the $\alpha5$ subunit of the FN receptor (VLA-5) and independently from the presence of other integrins. Proliferation to FN was inhibited by monoclonal antibodies to the $\alpha5$ and $\beta1$ subunits of VLA-5. Mapping of FN regions responsible for the proliferative signal, performed with different FN proteolytic fragments, indicated that the main signal was provided by the 120Kd α-chymotrypsin fragment containing the RGD sequence. These data indicate that FN can stimulate the proliferation of melanoma cells and that integrins can contribute to mediate the response of tumor cells to this extracellular ymatrix protein.

X 324 INFLAMMATORY RESPONSE IN PRIMARY LUNG TUMOR IS CHARACTERIZED BY A UNIQUE PATTERN OF TIL AND LYMPH NODE INFILTRATING-T CELL EXPRESSION OF CELL-ADHESION MOLECULES AND LYMPHOKINE GENE ACTIVATION.

Eugène Roussel[1], Marie-Claude Gingras[2], Bradley W. McIntyre[3], Elizabeth A. Grimm[4], and Jack A. Roth[1], Departments of Thoracic Surgery, Neurosurgery, Immunology, and Tumor Biology, MD Anderson Cancer Center, Houston, Texas, 77030.

To gain understanding of the inflammatory response generated by primary tumor, we studied cell-adhesion molecule expression by circulating PBL-T cells, resected negative lymph node infiltrating-T cells and tumor infiltrating lymphocytes-T from 10 patients with untreated primary lung adenocarcinoma. Tumor and lymph node infiltrating lymphocytes were extracted from tissue by mechanical disruption without the use of enzymes and they were phenotyped immediately. Our results showed that the TIL-T cell population was highly different from the PBL-T cell population in the proportion of cells expressing the integrins ß1 *CD49a(12.1)[1], CD49b(3.4), *CD49e(0.6), and *CD49f(0.5), the cell-adhesion molecules CD54(7.7) and CD58(1.6), the carbohydrate receptors CD15(4.2), *CD65(13.5), selectin LECAM(0.3), the cell activation markers *CD26(0.4) and IL-2R CD25(1.9). Significant differences between lymph node infiltrating-T lymphocytes and PBL-T in the proportion of cells expressing some of these markers(*) may suggest a presensitization in the lymph nodes. Lymphokine gene activation PCR analysis of tumor and lymph node infiltrating leukocytes detected IL-2 and IL-4 mRNA in the nodes and IL-2, IL-4, and IL-6 mRNA in the tumors. Our data demonstrate that TIL-T cells are a distinct population from PBL-T cells. These result suggest that despite a successful tumor growth, there is an inflammatory response generated by these primary lung tumors.

1. Ratio of TIL/PBL means, $p<0.05$.

X 325 PROLIFERATION AND METASTASIS OF A MURINE MAMMARY CARCINOMA: DEPENDENCE ON GROWTH FACTORS AND CELL MATRIX INTERACTIONS. Kristofer Rubin[1], Arne Östman[2], Bengt Westermark[3], Peter Ekblom[4] and Bruce Elliott[1]. [1]Dept. of Medical and Physiological Chemistry; [2]Ludwig Institute for Cancer Research; [3]Dept. of Pathology, [4]Dept. of Zoophysiology, University of Uppsala, BMC Box 575 S-751 23, SWEDEN.

We have investigated the dependence of cell-matrix interactions for the growth of a murine mammary tumor, SP1, as well as a highly metastatic variant, SP1-3M. SP1 cells adhered to both collagen type I and fibronectin with a $ß_1$ integrin mediated reaction; SP1-3M cells only adhered to fibronectin. No marked difference in the pattern of $ß_1$ integrins was detected by immunoprecipitation of extracted ^{125}I-labeled surface proteins from the two cells. Immunoblotting revealed the presence on both cells of $\alpha_5ß_1$. *In vivo*, polyclonal rabbit anti-$ß_1$ integrin IgG specifically inhibited the formation of pulmonary macrometastases from primary intramammary tumors of SP1 cells but not of SP1-3M cells. Primary tumor growth and the formation of micrometastases, detected by immunofluorescence with anti-tenascin antibodies, were unaffected by anti-$ß_1$ integrin IgG. *In vitro*, DNA-synthesis increased in both cell lines after stimulation with platelet-derived growth factor-BB (PDGF-BB) and basic fibroblast growth factor (bFGF), but not after stimulation by several other growth factors. A 12 to 24 h proliferative response to PDGF-BB occurred on fibronectin, but not on collagen; this difference was not due to differences in numbers or affinities of PDGF ß-receptors. bFGF stimulated increased adhesion and spreading of both SP1 and SP1-3M cells to collagen within 24 h, whereas PDGF-BB was less capable of this effect. SP1-3M cells, but not SP1 cells, displayed a late (3 day) proliferative response to PDGF-BB on collagen. Our data suggest that $ß_1$ integrin-ECM interactions can modulate the proliferation and metastasis of the current murine mammary tumor. (Supported by the Swedish Cancer Foundation).

X 326 FIBRONECTIN EXPRESSION IN KELOIDS, Jill C. Sible and Noelynn Oliver, Department of Anatomy and Cellular Biology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA, 02111

Among the earliest events in wound healing is the deposition of a provisional extracellular matrix rich in fibronectin. In rats, fibronectin expression is stimulated at the level of transcription, and there is also a change in the pattern of alternative splicing. Later in healing, fibronectin levels decline and a permanent, collagenous matrix of the neodermis is produced. The formation of keloids is a pathological consequence of wound healing that affects as much as 1.5% of the American population and 6% of some Black populations. Keloids are benign tumors of connective tissue that grow beyond the boundary of the original wound, and they are rich in extracellular matrix components including fibronectin and type I collagen. Fibroblasts derived from keloids overproduce these proteins in culture. Our laboratory studies regulation of fibronectin expression during wound healing and keloid formation using both *in vitro* and *in situ* techniques. Our studies indicate that keloids and normal human skin contain fibronectin messages including the alternatively spliced EIIIA and V regions but not the EIIIB region which is present during wound healing in rats. Thus, keloids differ from normal skin in the level of total fibronectin expression but not in the pattern of alternative splicing. We have shown that the overexpression of fibronectin by keloid cells *in vitro* is mediated by a transcriptional effect, and we are studying the *cis* elements responsible for this effect by transfecting keloid cells with a reporter gene system.

X 327 INTEGRIN EXPRESSION IN NORMAL AND MALIGNANT ORAL EPITHELIUM. Paul M Speight, Judith Jones, Masaru Sugiyama and Fiona M Watt. Institute of Dental Surgery, Gray's Inn Road, and Imperial Cancer Research Fund, Lincoln's Inn Fields, London, England.

The integrins are heterodimeric receptors which mediate cell-cell and cell-matrix interactions. They facilitate cell migration, and integrin-ligand binding may control cell proliferation and differentiation. Changes in integrin expression are seen in neoplasia and may be associated with the ability of malignant cells to infiltrate and metastasise. The purpose of this study was to determine whether integrin expression is altered in oral epithelial malignancy. 17 squamous cell carcinomas (SCC), 11 epithelial dysplasias, 10 non-specific ulcers and 35 samples of normal oral mucosa from keratinised and non-keratinised sites were snap-frozen in liquid nitrogen. 6μm frozen sections were stained by an indirect avidin-biotin-peroxidase technique using monoclonal antibodies to the integrin subunits $ß_1$,$ß_4$,α_2,α_3,α_4,α_5,α_6 and α_v.
In normal and hyperproliferative epithelium (ulcers) all cases were positive for all subunits except α_4 and α_5. Staining was pericellular and was generally localised to the basal layers. There were few differences between sites although suprabasal expression was increased in non-keratinised epithelium and in the ulcers. $ß_4$ and α_6 showed strong staining concentrated at the basement membrane zone. In SCC staining was variable both within and between cases. Strong suprabasal, pericellular and cytoplasmic staining was seen. 1 SCC was completely negative for α_2 and all cases showed patchy loss of $ß_4$ and α_6 at the basement membrane zone. Loss of integrin expression did not correlate to the degree of tumour differentiation. Dysplasias showed a similar pattern of expression to ulcers but 2 cases showed focal loss of $ß_4$ and α_6. The results show a change in integrin expression in malignant oral epithelium. Loss of integrins may facilitate infiltration of cells across the basement membrane or may signal a loss of control of cell growth and differentiation. The similar changes in some dysplasias may be important prognostic indicators for progression of these lesions.

X 328 P-SELECTIN MEDIATES ADHESION OF SMALL CELL LUNG CANCER CELLS TO PLATELETS,
Jennifer P. Stone, Barbara C. Furie, Bruce Furie, and Denisa D. Wagner, Division of Hematology-Oncology, Tufts-New England Medical Center, Boston, MA 02111
Activated platelets and stimulated endothelial cells express on their surface an integral membrane protein named P-selectin (P-s). P-s mediates adhesion of these cells to a glycoprotein that includes carbohydrate structures containing sialyl-Lewis X antigens on monocytes and neutrophils. Since many cancer cells also express these carbohydrate structures and are known to interact with platelets in the blood, we asked if P-s may mediate this interaction. Several cancer cell lines were initially screened in a rosetting assay where activated or resting platelets were incubated with cancer cells and their adhesion evaluated by light microscopy. Small cell lung cancer (SCLC, NCI-H128) and a neuroblastoma cell line both preferentially rosetted with activated platelets. This interaction was inhibited with polyclonal and inhibitory monoclonal anti-P-s antibodies and was not inhibited with control noninhibitory antibodies nor with antibodies to the platelet integrin receptor GpIIb-IIIa. The binding of the SCLC to activated platelets and purified P-s was further evaluated. In the rosetting assay, the binding between SCLC and activated platelets was inhibited by purified P-s and by EDTA. Binding was not inhibited with Arg-Gly-Asp-Ser peptide further indicating that GpIIb-IIIa did not mediate this interaction. Pretreatment of SCLC with neuraminidase and trypsin also completely inhibited the binding to activated platelets. Fluorescent phospholipid vesicles containing purified P-s bound to SCLC while vesicles without P-s did not bind as observed by fluorescent microscopy and flow cytometry. This binding could also be inhibited with anti-P-s antibodies. In conclusion, we have shown that P-s mediates adhesion of activated platelets to glycoproteins on the surface of SCLC and possibly on other cancer cell lines. This interaction may play a role in the hematogenous spread of SCLC and in the manner activated platelets facilitate metastasis of certain tumors.

X 329 AJOENE INHIBITS EXPERIMENTAL METASTASIS AND IMPLANTATION OF MELANOMA CELLS IN MICE,
Alexander V. Tatarintsev, Peter V. Vrzheshch, Andrey A. Schegolev, Nina S. Saprykina, and Alexey M. Kozlov, Institute of Physical and Chemical Biology, Moscow State University, Moscow 119899, USSR.
Agents interfering with integrin-dependent cell adhesion are known to inhibit both experimental and spontaneous tumor lung metastasis. Ajoene, (E,Z)-4,5,9-trithiadodeca-1,6,11-triene-9-oxide, isolated from extracts of garlic (*Allium sativum*) has previously been shown to block human platelet aggregation by inactivating allosterically the platelet integrin, GP IIb/IIIa (Apitz-Castro R. et al. BBRC, 1986, 141:145). We studied the effect of synthetic (E,Z)-ajoene on murine platelet aggregation, experimental metastasis and implantation of melanoma B16 cells in C57BL/6 mice. Ajoene inhibited ADP-induced aggregation of PRP with an IC100 of 200 μM and abolished lung colonization by B16 cells (pre-treatment with 200 μM ajoene for 30 min; 10^5 cells per mouse intravenously; mice killed 3 weeks postinjection). The observed antimetastatic activity could result from modulation of B16 cell and/or platelet adhesiveness. To evaluate the effect of the compound on tumor cell implantation mice were inoculated subcutaneously with B16 cells (10^6 per mouse) and the volume of the tumors formed was measured 14 and 21 days after the injection. Pretreatment of the cells with ajoene (0-2500 μM; 15 min) led to a dose-dependent inhibition of tumor growth. At 2.5 μM the volume of melanomas was reduced by 49.5%; preincubation with 2500 μM prevented B16 cell implantation. Analogous results have been obtained by Boukerche et al. (Blood, 1989, 14:909) who have described an inhibition of tumor cell growth *in vivo* by an anti-GP IIb/IIIa monoclonal antibody.

X 330 EXTRACELLULAR MATRIX RECEPTORS AND MOUSE SKIN CARCINOGENESIS: ALTERED EXPRESSION LINKED TO APPEARANCE OF EARLY MARKERS OF TUMOR PROGRESSION,
Tamar Tennenbaum, Stuart H. Yuspa, Atul Grover, Vincent Castronovo, Mark E. Sobel, Yoshihiko Yamada, Luigi M. De Luca, Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD 20892
Interaction of cells with the basement membrane is important for cell proliferation and differentiation. Disruption of the basement membrane is an early event during progression of benign tumors to malignancy. Using the techniques of immunohistochemistry and immunofluorescence, we show that cell-matrix interactions via the cell surface integrin receptors α3β1, α5β1, α6β4, the laminin binding protein 67LR and the secreted matrix protein laminin are strictly regulated during differentiation of mouse epidermis. While α6β4 and α5β1 are polarized to the basal surface of basal cells in contact with the basement membrane, α3β1 and the non-integrin receptor 67LR are primarily detected in the cell periphery of suprabasal cells, where cell to cell contacts are found. Sequential changes in expression of matrix receptors occur following multistage carcinogenesis of mouse skin. In an analysis of benign and malignant skin tumors induced by chemical carcinogens or oncogene transduction, we found that α3β1 and α5β1 as well as the non integrin LBP-37 are sequentially down-regulated in the progression from benign to malignant while α6β4 is the predominant receptor expressed in the carcinomas. Tumor expression of α6β4 is not polarized and is dissociated from its colocalized normal partner bullous pemphigoid antigen, which remains restricted to the basement membrane. The changes in matrix receptors are linked to appearance of keratin 13 in suprabasal regions, but always in α6β4 negative cells. The predominance of α6β4 in the proliferating cells during progression is associated with decreased expression of keratin 13 in carcinomas. These results suggest that matrix interactions with its receptors are important determinants of ordered differentiation in normal skin and show characteristic alterations during carcinogenesis which parallel changes in differentiation of the tumors.

Thrombosis, Platelets, Endothelium; Inflammation and Immunity

X 400 STREPTAVIDIN BLOCKS IMMUNE REACTIONS MEDIATED BY FIBRONECTIN-VLA-5 RECOGNITION THROUGH AN RGD MIMICKING SITE. Ronen Alon, Rami Hershkoviz, Edward A. Bayer, Irun R. Cohen, Ofer Lider, and Meir Wilchek. Departments of Membrane Research and Biophysics, and the Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

Streptavidin (StAv) is a biotin binding avidin analogue secreted by the bacterium *Streptomyces avidinii*. We recently reported that StAv contains an RYDS sequence with structural homology to the RGD cell adhesion domain of fibronectin. Competition studies with RGD peptides indicated that StAv bound to cells by way of this site, independent of biotin recognition [Alon, *et al.* (1990) Biophys. Biochem. Res. Comm. 170, 1236]. Since RGD has been shown to to play a key role in integrin-mediated cell adhesion, we propose that StAv may utilize the RYDS site to bind cells and to abrogate adhesion-dependent processes. Indeed, StAv modulates several matrix-dependent interactions of immune cells: immobilized StAv supported activated human $CD4^+$ T cell adhesion in an RGD-specific, $\alpha 5\beta 1$-dependent manner. In addition soluble StAv strongly inhibited T cell adhesion to fibronectin and interfered with its costimulatory effect on proliferative responses and cytokine secretion by T cells. These results suggest that StAv is a novel example of a bacterial protein which utilizes RGD-mimicry to interfere with integrin-mediated immune responses.

X 401 DISSECTION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELL (HUVEC) ADHESION TO VON WILLEBRAND FACTOR (vWF): EVIDENCE FOR A GPIb-LIKE RECEPTOR ON HUVECs. D. A. Beacham, M.A. Cruz and Robert I. Handin, Hematology-Oncology Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. 02115

Previous studies indicate that the RGD-dependent attachment of HUVECs to vWF is largely mediated by the vitronectin receptor (VNR, $\alpha_V\beta_3$), the analog of the GP IIb/IIIa ($\alpha_{IIB}\beta_3$) platelet receptor. Point mutations were introduced into the C-terminal Arg-Gly-Asp-Ser (RGDS) site of vWF to study vWF adhesion to HUVECs and platelets. The single amino acid substitution of glycine to alanine produced RADS-vWF, while an aspartate to glutamate substitution yielded RGES-vWF (Beacham and Handin, 1990; Blood 76, p.446a.) Recent binding studies of vWF to platelets or collagen indicate that the RGDS mutants of vWF are identical to wild-type recombinant vWF (WT-vWF) with the exception that RADS and RGES-vWF no longer bind to GP IIb/IIIa. Moreover, in adhesion assays, attachment of HUVECs to purified RADS and RGES-vWF was reduced and spreading was completely blocked, compared with WT-vWF. To investigate the RGDS-independent component of HUVEC adhesion to RGDS mutant vWF, the AS-7 monoclonal antibody, specific for the vWF binding site on GP Ib/IX, was used. At a concentration of 100 µg/ml, AS-7 inhibited the attachment of HUVECs to RADS-vWF and RGES-vWF by 50%, but had no effect on attachment of HUVECs to WT-vWF or fibronectin (FN). As an independent test of GPIb-vWF interactions, we utilized a truncated GPIb fragment of Mr = 13,000, rGPIbQ221-L318, encompassing the vWF binding domain. rGPIbQ221-L318 reduced WT-vWF attachment to 80% and RADS-vWF to 45% of attachment in the absence of fragment. The effect was concentration-dependent and maximal at 30 µg/ml. These results suggest that a GPIb-like receptor participates in HUVEC adhesion to vWF.

X 402 MRC OX-62: A MONOCLONAL ANTIBODY RAISED AGAINST VEILED CELLS IN LYMPH RECOGNISING AN ANTIGEN WITH INTEGRIN-LIKE CHARACTERISTICS, Mary Brenan, Mike Puklavec, and D. Jasper G. Rees*. MRC Cellular Immunology Unit / Chemical Pathology*, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

Lymphoid dendritic cells comprise a heterogeneous group of migratory cells which are the most potent accessory cells identified at inducing primary T-cell responses. A mouse IgG1 mAb MRC OX-62 raised against veiled cells in lymph has been produced. In lymphoid organs the MRC OX-62 mAb predominantly labelled populations of cells with dendritic morphology in the thymic medulla, T-cell areas and red pulp of the spleen, the sub-capsular sinus, T-cell areas and medulla of the lymph node, inter-follicular areas and epithelial regions of the Peyer's patch and veiled cells in lymph. In non-lymphoid organs the MRC OX-62 mAb labelled cells with dendritic morphology in epithelial and interstitial regions. Concordance of MRC OX-62 antigen labelling was found in most organs but additional la-populations were labelled. Enrichment of dendritic cells assessed by potent stimulator activity in the primary allogeneic MLR was effectively achieved by magnetic sorting after labelling cells with the MRC OX-62 mAb. The MRC OX-62 mAb immunoprecipitated four bands from radioiodinated dendritic cells under both non-reducing and reducing conditions: a major band of approximately 150 kD apparent Mr, a minor band of approximately 120 kD apparent Mr which migrated faster under non-reducing conditions and two other bands <30 kD apparent Mr. The MRC OX-62 antigen shows similarities to members of the integrin family, but is not $\alpha 3$, $\alpha 5$ or $\alpha 6$. Work is in progress to clone MRC OX-62 antigen by PCR using degenerate oligonucleotides to conserved sequences of the α and β subunits.

X 403 PLATELET FIBRINOGEN AND VITRONECTIN IN GLANZMANN THROMBASTHENIA: EVIDENCE CONSISTENT WITH SPECIFIC ROLES FOR GPIIB/IIIA AND $\alpha_V\beta_3$ INTEGRINS IN PLATELET PROTEIN TRAFFICKING, Barry S. Coller, Uri Seligsohn, Sharon M. West, Lesley E. Scudder, and Karin J. Norton, Division of Hematology, State University of New York, Stony Brook, NY 11794-8151, and Institute of Hematology, Tel Aviv University, Ichilov Hospital, Israel.

In order to assess the individual contributions of the platelet GPIIb/IIIa receptor and the $\alpha_V\beta_3$ vitronectin receptor to platelet levels of fibrinogen and vitronectin, we analyzed the platelets from two groups of Glanzmann thrombasthenic patients: Iraqi-Jews, whose platelets lack both receptors, and Arab patients in Israel, whose platelets lack GPIIb/IIIa but have normal or increased numbers of $\alpha_V\beta_3$ vitronectin receptors. The platelets from both thrombasthenic groups had profound deficiencies of fibrinogen, but the defect in the Iraqi-Jewish patients' platelets appeared to be slightly more severe. This indicates that GPIIb/IIIa is the major determinant of platelet fibrinogen, presumably acting by receptor-mediated uptake, and that the $\alpha_V\beta_3$ vitronectin receptor plays little or no role. Arab patients' platelets have normal amounts of platelet vitronectin, whereas Iraqi-Jewish patients' platelets have nearly 5-times as much vitronectin as control or Arab patients' platelets. To account for these data we propose a working hypothesis in which vitronectin is synthesized in megakaryocytes and the $\alpha_V\beta_3$ vitronectin receptor is involved in transport of the protein out of megakaryocytes and/or platelets. Collectively, these observations suggest that in addition to their recognized roles in cell adhesion and the interaction of cells with extracellular proteins, integrin receptors may be important in protein trafficking into, and perhaps out of, platelets.

X 404 Expression of Cell-Surface and Secreted Forms of the Peyer's Patch Homing Receptor LPAM-1. David T. Crowe, Mickey C.-T. Hu, and Irving L. Weissman, HHMI, Beckman Center for Molecular Genetic Medicine, Stanford University, Stanford, CA 94305

The lymphocyte Peyer's patch adhesion molecule 1 (LPAM-1) mediates the adhesion of circulating lymphocytes to the luminal surface of mucosal lymphoid organ high endothelial venules. LPAM-1 consists of an α_4 integrin subunit noncovalently associated with a novel β_p integrin subunit in a Ca^{2+}-dependent manner. We recently isolated the cDNAs for the murine α_4 and β_p subunits and have stably expressed them in the LPAM-1$^-$ murine T cell lymphoma BW5147. A stable LPAM-1$^+$ clone was isolated by screening individual colonies by FACS with subunit-specific antibodies to α_4 (R1-2) and β_p. β_p-specific antisera was generated by immunizing rabbits with a glutathione S-transferase/β_p fusion protein produced in *E. coli*. Immunoprecipitation of iodinated cell-surface proteins with anti-β_p antisera and analysis by SDS/PAGE identified a 160/130 kD heterodimer that required Ca^{2+} for stable subunit association. This pattern of expression is identical to authentic $\alpha_4\beta_p$ present on the Peyer's patch-binding lymphoma TK-1. We are currently testing the *in vitro* and *in vivo* binding properties of the BW5147 LPAM-1$^+$ double transfectant.

We have also produced a secreted form of LPAM-1 by introducing in-frame termination codons upstream of the putative transmembrane domain of both α_4 and β_p. COS cells were transiently transfected with both cDNAs and metabolically labelled with ^{35}S-cysteine. Radiolabelled supernatants of transfected and mock-transfected COS cells were immunoprecipitated and analyzed by SDS/PAGE. Transfected supernatants contained a radiolabelled 150/116 kD heterodimer that was immunoprecipitated by antibodies to either α_4 or β_p. Dimerization of the secreted $\alpha_4\beta_p$ was also Ca^{2+}-dependent. The ability of a secreted $\alpha_4\beta_p$ receptor to bind to Peyer's patch HEV and to block lymphocyte adhesion is being tested.

X 405 COLONY-STIMULATING FACTORS REGULATE INTEGRIN EXPRESSION AND FUNCTION ON CULTURED HUMAN MACROPHAGES, Mark O. De Nichilo and Gordon F. Burns, Cancer Research Unit, University of Newcastle, N.S.W. 2300, Australia.

Human monocytes freshly isolated from blood express surface integrins of the $\beta 1$ and $\beta 2$ subgroups but the vitronectin receptor ($\alpha v\beta 3$) is not expressed until around day 3 of culture. Upon prolonged *in vitro* culture these cells also display αv in association with an additional β subunit which we have termed $\beta 3b$. To determine the factors influencing the relative expression of the different integrins, we cultured the macrophages in the presence of various cytokines. The integrin phenotype of the cells was then examined by Northern blot analysis and by cell surface labeling and immunoprecipitation and SDS-PAGE analysis. At a 12h timepoint it was found that GM-CSF, IL-3 and TGFβ but not M-CSF induced $\beta 3$ message and these treated cells expressed abundant $\alpha v\beta 3$ on their surface. By contrast, M-CSF rapidly (3h) induced $\beta 5$ message with peak expression at around 6 - 12h, and this expression progressively declined over the next 12 hours. On their surface these cells expressed αv only in association with $\beta 3b$. Cells treated with GM-CSF also expressed surface $\beta 3b$, but $\beta 5$ message was not induced until some 24h after cytokine treatment and the possibility is being examined that this is due to GM-CSF-mediated transcription of M-CSF acting as an autocrine factor. The cells treated with GM-CSF, IL-3 and M-CSF all bound vitronectin, fibronectin and fibrinogen by an RGD-dependent mechanism. However, the morphology of the attached cells was strikingly different. Cells treated with GM-CSF but not with M-CSF bound to laminin and collagen. The specific receptors involved in such binding is presently under investigation but the results above suggest that CSF-mediated regulation of integrin expression may be a major mechanism by which these factors influence the inflammatory response.

X 406 INTERACTION OF FIBRINOGEN Bβ15-42 WITH ENDOTHELIAL CELLS AND IDENTIFICATION OF A 130 kDa MEMBRANE BINDING PROTEIN. John K. Erban, Denisa D. Wagner, Division of Hematology/Oncology, New England Medical Center and Tufts University School of Medicine, Boston MA 02111

The binding of fibrinogen to endothelial cells occurs in part through an RGD dependent interaction of the Aα chain with the $\alpha_v\beta_3$ integrin receptor. However, other mechanisms through which fibrinogen and fibrin interact with the endothelial cells remain incompletely understood. Fibrinogen coated microtiter wells support the binding and spreading of endothelial cells which we show can be only partially inhibited by the RGDS tetrapeptide, suggesting other potential binding sites on fibrinogen. Binding of endothelial cells to purified S-carboxymethylated fibrinogen Aα chain was completely inhibited by 0.4mM RGDS tetrapeptide, in contrast to binding to fibrinogen which was only partially inhibited at 0.8mM. Since fibrin formed with thrombin (lacking both fibrinopeptides A and B) but not fibrin formed with reptilase (lacking only fibrinopeptide A) has been shown to induce rapid release of vWf from endothelial cell Weibel-Palade bodies, we were interested to see whether Bβ15-42 sequence of fibrinogen, exposed after fibrinogen is converted to fibrin by thrombin, interacts with an endothelial cell surface protein. Synthetic Bβ15-42 coupled to ovalbumin supported the binding and spreading of human umbilical vein endothelial cells (HUVEC) on plastic at 2 and 24 hours. Neither scrambled peptide coupled to ovalbumin identically nor ovalbumin alone were able to support binding or spreading. To identify a possible receptor for Bβ15-42, HUVEC were surface labelled with ^{125}I, lysed in octylglucoside, and cell lysate was applied to Bβ15-42 Sepharose or scrambled Bβ15-42 Sepharose columns. A 130 kDa protein bound to the Bβ15-42 column but not the scrambled peptide column, and could be eluted with the Bβ15-42 peptide but not with the scrambled peptide, RGDS, Bβ19-26 or Bβ37-56-cys. The 130 kDa protein was not identified recognized by anti-β_1, anti-β_3 or anti-β_5 antibodies, nor were antibodies to CD31, an endothelial cell surface molecule in the immunoglobulin superfamily able to immunoprecipitate the protein. Limited tryptic digestion of the 130 kDa protein and CD31 under identical conditions produced different cleavage products. The 130 kDa protein binds to wheat germ agglutinin coupled to Sepharose but only minimally to Concanavalin-A-Sepharose, establishing it as a glycoprotein. We conclude that a previous unrecognized 130 kDa protein on the surface of endothelial cells interacts with Bβ15-42, a sequence in the fibrinogen molecule which is exposed on conversion to fibrin. This glycoprotein may promote endothelial cell adhesion to fibrin during the wound healing process and is a candidate for a receptor involved in fibrin mediated release of Weibel-Palade bodies from endothelial cells.

X 407 INTERACTIONS OF RECOMBINANT HUMAN FIBRINOGENS WITH PLATELETS. David H. Farrell, *Perumal Thiagarajan, Shaoming Huang, and Earl W. Davie. Department of Biochemistry and *Department of Medicine, University of Washington, Seattle, WA 98115.

Fibrinogen mediates platelet adhesion and aggregation by its interaction with the platelet glycoprotein IIb-IIIa (integrin $\alpha_{IIb}\beta_3$). Peptides containing the amino acid sequence HHLGGAKQAGDV derived from the carboxy terminus of the γ chain (γ 400-411) and the sequence RGD derived from the α chain (α 95-97 and α 572-574) inhibit these interactions. To determine the role of the γ chain in intact fibrinogen, recombinant human fibrinogen and its γ' variant that lacks the HHLGGAKQAGDV sequence have been expressed and isolated from BHK cells. In the γ' chain, the carboxy terminal four amino acids are replaced by a twenty amino acid sequence, thus interrupting the γ carboxy platelet binding sequence. Therefore, we have expressed recombinant human fibrinogen homodimers containing γ' chains (rFbgγ'), as well as homodimers containing γ chains (rFbg) using a mammalian cell expression system in order to study their interactions with platelets. BHK cells were transfected with two expression vectors which encoded, respectively, the α and γ (or γ') chains, and β chain and a dihydrofolate reductase selectable marker. Stable clones were selected in 20 μM methotrexate which secreted rFbgγ' at 4 μg/mg cell protein/day and rFbg at 5 μg/mg cell protein/day. Metabolically-labeled rFbgγ' and rFbg were clottable, as shown by their ability to be incorporated into fibrin clots and be crosslinked by factor XIIIa. In addition, $^{35}SO_4$ was incorporated into the γ' chain, but not the α, β, or γ chains. The sulfated residue was shown to be tyrosine *O*-sulfate. rFbgγ' and rFbg were also purified from serum-free conditioned medium using a two-step affinity chromatography method. ^{51}Cr-labeled platelets bound to purified immobilized rFbgγ' in a dose-dependent manner which paralleled that of rFbg and plasma Fbg. In contrast, rFbgγ' was defective in platelet aggregation, showing a 3 to 4-fold increase in the $E.D._{50}$ compared to plasma Fbg and rFbg, even though it retained the two RGD sequences in the α chain. These results suggest that the α chain RGD sequences are not sufficient to promote complete platelet aggregation, and directly demonstrate that the carboxy terminus of the γ chain of fibrinogen is essential for optimal platelet aggregation.

X 408 SELECTIVE NON-PEPTIDE ANTAGONISTS OF PLATELET $\alpha_{IIB}\beta_3$. Robert J. Gould, George D. Hartman, Charles T. C. Chang, Melissa S. Egbertson, Wasyl Halczenko, William L. Laswell, Robert J. Lynch, Patricia D. Manno, Robert L. Smith, Guixiang Zhang, Paul A. Friedman and Paul S. Anderson. Departments of Biological Chemistry and Medicinal Chemistry, Merck Sharp & Dohme Research Laoboratories, West Point, PA 19486

A novel series of aminoalkyloxyphenyl propionic acid analogues has been found to exhibit micromolar potency, comparable to linear tetrapeptides such as RGDF, for inhibition of ADP-induced, fibrinogen-dependent human platelet aggregation. These compounds are designed as mimics of the arginyl-glycyl-aspartyl recognition sequence found in ligands for $\alpha_{IIb}\beta_3$ and function as such, as demonstrated by their ability to compete with a radiolabelled RGD-containing peptide (^{125}I-L-692,884) for binding to human platelet membranes. The potency of these compounds for inhibition of platelet aggregation correlates with the potency of inhibiting radioligand binding. In addition, these compounds at concentrations as high as 300 μM have little effect on the ability of cultured human cells to attach to the RGD-containing adhesive proteins fibrinogen, vitronectin, or fibronectin. They therefore appear to be specific for the $\alpha_{IIb}\beta_3$ complex of human platelets. Key structural features of these inhibitors include an anionic carboxylate to mimic the aspartyl residue of the RGD motif, a cationic amine to mimic the arginyl residue of the RGD motif, and an optimum orientation and distance between these sites. This was determined by varying aryl substitutions and intervening chain atoms. These compounds represent a class of low molecular weight, nonpeptide inhibitors of the platelet specific integrin $\alpha_{IIb}\beta_3$ and demonstrate the potential to modulate cellular functions by pharmacological regulation of integrins.

X 409 DESIGN AND SYNTHESIS OF NOVEL NONPEPTIDIC RGD SURROGATES AND THEIR INTERVENTION WITH PLATELET AGGREGATION. Noam Greenspoon, Ronen Alon, Rami Hershkoviz, Gerrard Marx, Silvina Federman and Ofer Lider. Departments of Organic Chemistry, Biophysics and Membrane Research and Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

The target epitope of several integrin receptors is composed of the RGD sequence present in matrix associated adhesive proteins. We have designed and prepared a series of nonpeptidic RGD surrogates having a carboxylate group linked to a guanidinium group by a flexible spacer made of 11 atoms. Such compounds (e.g. 5-N-(6-guanidinohexanamide)-pentanoic) acid were found to compete with the PAC1 mAb, which binds GPIIb-IIIa in an RGD-dependent manner. An RGE surrogate containing 12 atoms in the spacer between the two functionalities described failed to inhibit PAC1 binding. The RGD surrogates inhibit platelet aggregation *in vitro* in a dose dependent manner with an IC_{50} of 0.3 mM (similar to that of GRGDSP), suggesting a potential therapeutic use for these compounds. Our results suggest that RGD-specific integrin receptors have two binding pockets: one that binds the carboxyl group and another, the guanidinium group. To gain maximal binding energy the receptor must bind both groups. Only surrogates of the correct atomic spacing between functional groups can acquire the conformation required for a high binding affinity to the integrin sites. Such non peptidic surrogates should prove to be an excellent tool for mapping the conformational specificities of the various ECM proteins.

X 410 NOVEL INVASION MECHANISM THAT IS MICROFILAMENT INDEPENDENT AND REQUIRES COATED PIT FORMATION. D. J. Kopecko*, P. Guerry#, and T. A. Oelschlaeger*, *Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D. C. 20307-5100 and #Enteric Disease Program, Naval Medical Research Institute, Bethesda, MD 20814

Five *Campylobacter jejuni* and 2 *Campylobacter coli* strains were compared to *Salmonella typhi* Ty2 and *Escherichia coli* for their ability to invade a monolayer of cultured human intestinal epithelial cells in the presence or absence of sublethal concentrations of various inhibitors, added 1h prior to invasion assay. Uptake occurred after centrifugation (200g/5') of bacteria onto the monolayer (50:1 ratio) and 2h at 37C in 5%CO_2. After 2h further incubation in the presence of 100ug/ml gentamycin, the number of intracellular organisms was enumerated. *C. jejuni* strains 81176 and VC84 invaded Henle cells, but no invasion was seen with other *Campylobacter* strains. Whereas *S. typhi* invasion was inhibited >99% by ctyochalasin D, but not by inhibitors of tubulin-polymerization or coated pit formation, the 81176 mechanism(s) was dramatically reduced by inhibitiors of coated pit or microtubule formation, but was unaffected by cytochalasin D. Blocking calmodulin activity with 10uM stelazine lowered invasion by 98% and 57%, respectively, for *Campylobacter* and *Salmonella*. Preventing *de novo* bacterial protein biosynthesis with 100ug/ml chloramaphenicol for 30' inhibited uptake by >90% for both *C. jejuni* and *S. typhi*. The observed efficiency of *C. jejuni* invasion (1-2.5% of input organisms) was consistently greater than the low level invasion seen with *Escherichia coli* HB101 ($9X10^{-3}$%) and the very low levels of microfilament-dependent uptake previously reported for *C. jejuni* ($3X10^{-3}$-$3X10^{-5}$%). Most importantly, however, coated pit formation is signifcantly involved in invasion triggered by *C. jejuni*, and, in contrast to *S. typhi*, strain 81176 uptake is not dependent on microfilaments, but requires microtubules. Transmission EM of invaded cells has shown intracellular *Campylobacter* contained within endosomal vacuoles. As opposed to previously reported *Shigella*, *Yersinia*, and *Salmonella* (i.e. ligand-receptor) invasion mechanisms, which are all microfilament-dependent, these data indicate that *C. jejuni* have a novel uptake mechanism in which the bacterial ligand is a protein of short half-life that triggers a microtubule-dependent endocytosis pathway.

X 411 PREVENTION OF DIABETES TRANSFER IN NOD MICE BY TREATMENT OF DIABETIC CELL DONORS WITH Mel 14. Françoise Lepault, Marie-Claude Gagnerault, and Christian Boitard*.CNRS 1461 and INSERM U25, Hôpital Necker - Paris - France.

The nonobese diabetic (NOD) mouse is widely accepted as a relevant model for human autoimmune type I diabetes, which results from the selective destruction of pancreatic insulin-containing beta cells. The onset of the disease (from 12 weeks of age) is preceded by the infiltration of mononuclear cells around and subsequently inside the islets of Langerhans (from 5-6 weeks of age). Infiltrating cells are mainly T cells belonging to both CD4 and CD8 subsets. However, the cell types responsible for beta cell destruction as well as the mechanisms involved in the entry of leukocytes into the pancreas are still undefined.

The observation of HEV-like structures in the pancreas of NOD mice motivated us to study the effects of Mel14 antibody treatment on the transfer of diabetes by spleen cells from diabetic donors into irradiated young recipients.

Mel14 antibody injection induces a dose-dependent down regulation of Mel14 antigen within the following hours. This phenomenon occurs in non-diabetic as well as in diabetic mice. The loss of Mel 14 antigen lasts for at least 7 days in the injected mice, but Mel 14 antigen is rapidly reexpressed when cells are transferred into an untreated recipient.

Treatment of diabetic donor mice with at least 100μg purified Mel 14 significantly reduces the efficiency of T cells to transfer diabetes into untreated irradiated young animals. However, the transfer of diabetes with cells from untreated diabetic mice into recipients injected twice a week with Mel 14 was as successful as in control recipients. These results suggest that the effect of Mel 14 treatment on the development of diabetes in transfer experiments rather concerns modifications of immune functions than the traffic of cells to the pancreas.

X 412 INHIBITION OF INTEGRIN-MEDIATED CELL MATRIX ADHESION AND T CELL MEDIATED IMMUNE RESPONSE BY NONPEPTIDIC ANALOGUES OF RGD. Ofer Lider, Rami Hershkoviz, Ronen Alon, Silvina Federman, Noam Greenspoon. Departments of Biophysics, Membrane Research, Chemistry and Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

The interactions of cells with proteins of the plasma, the interstitial and the extracellular matrix are regulated primarily by a family of cell-surface heterodimeric receptors designated integrins. The target epitope of several integrin receptors is the RGD primary sequence of matrix-associated adhesive proteins. We examined the feasibility of using nonpeptide mimetics of RGD and control RGE. We now report that nonpeptidic molecules that mimic the structure of the RGD sequence, e.g. 5-[N-6 guanidinohexanamide]-pentanoic acid, attach specifically to the RGD-binding domain of the platelet GPIIbIIIa integrin. Moreover, such compounds inhibit $CD4^+$ T cell adhesion to the immobilized cell-attachment site of fibronectin, and tumor cell adhesion to fibronectin and vitronectin, but not to laminin or collagen. Analogues that mimic the RGE sequence had no effect. *In vivo*, an RGD surrogate, but *not the RGE surrogate, inhibited* a T cell-mediated delayed-type hypersensitivity reaction, most likely through inhibition of inflammatory cell-migration. These results suggest that nonpeptidic surrogates constructed to mimic the functional groups of short protein motifs of matrix-related adhesion molecules might be useful as specific probes of integrin receptors. Moreover, such compounds are expected to be a new class of therapeutic agents applied in several pathogenic processes.

X 413 THE SYNTHETIC CYCLIC PEPTIDE G4120 IS A POTENT INHIBITOR OF FIBRINOGEN BINDING TO GPIIbIIIa AND TO PLATELETS, AND OF ADHESION OF PLATELETS TO EXTRACELLULAR MATRIX PROTEINS. Michael T. Lipari, Mary E. Wessinger, Sherry Bullens, Stuart Bunting and Mary A. Napier, Dept. of Cardiovascular Research, Genentech, Inc, So. San Francisco, CA. 94080.

The glycoprotein receptor, GPIIbIIIa, for fibrinogen (Fg) on platelets (plts) has been implicated in critical plt functions including aggregation (AGG) and adhesion (ADH). A synthetic cyclic peptide, G4120, (P. L. Barker, et al., PCT Intl Appl WO91 01331, 7 Feb; 91) is a potent inhibitor of fibrinogen binding to purified GPIIbIIIa (IC_{50}, 1.6 nM), washed plts (IC_{50}, 3.5 nM), plts in PRP (IC_{50}, 31 nM) and for ADP-induced AGG in human PRP (IC_{50}, 150 nM). Binding studies indicate a common site of action on GPIIbIIIa for G4120, Fg and for the RGD-containing snake venom peptide, kistrin. G4120 binds to resting plts (IC_{50}, 330 nM), and the affinity increases ~ 5-fold (IC_{50}, 60 nM) upon activation with ADP. Inhibition by G4120 of ADH to purified extracellular matrix proteins (ECM) in a microtiter format was examined for activated (ADP, 20uM), unactivated, and inactivated (PGE1, 2uM) washed plts. Activated and unactivated plts adhered to each ECM protein tested in a concentration-dependent manner. ADH of activated plts was ~ 3-fold greater than unactivated plts. Inactivated plts generally bound least, but differential effects were observed for different ECM proteins. Two groups of ECM proteins were identified based on the maximum inhibition obtained by G4120: (I) 100% inhibited, Fg, vitronectin, von Willebrand factor and thrombospondin, or (II) 70% inhibited, fibronectin, collagen I and collagen IV. Activated and unactivated plts were inhibited similarly, IC_{50} range of 20-90nM for the different ECM proteins (coated at 3 ug/ml). However, for inhibition of ADH by G4120 to Fg (coated at 10 ug/ml) an ~4-fold lower potency was observed for inactivated plts (740nM) than for activated plts (170 nM), similar to the activation-dependent increase in affinity from binding studies. CONCLUSIONS: The synthetic peptide G4120 (1) is a potent inhibitor of Fg binding to washed plts and to plts in PRP, (2) binds to both activated and unactivated plts, with a shift to higher affinity upon activation, and (3) inhibits ADH of activated, unactivated and inactivated plts to immobilized Fg, with a shift to greater potency by ADP activation or by apparent activation by immobilized Fg and other ECM proteins. These data suggest that activation of platelet GPIIbIIIa results in a conformation change which permits higher affinity interaction of the GPIIbIIIa antagonist G4120.

X 414 CHARACTERIZATION OF THE MOLECULAR INTERACTION BETWEEN LFA-1 AND ICAM-1, Bridget A. Lollo, Vincent T. Moy, and Adrienne A. Brian, Department of Chemistry, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0063

T cells activated by phorbol esters or CD3 crosslinking show enhanced LFA-1 dependent binding to purified, immobilized ICAM-1. This enhanced adhesion may reflect a conformational change in LFA-1 to a higher affinity state upon activation. To study the affinity of the LFA-1/ICAM-1 interaction we have used a recombinant secreted form of murine ICAM-1 to inhibit the binding of radiolabeled Fab fragments of two function blocking anti-LFA-1 monoclonal antibodies. By measuring the dose-dependence of the displacement at equilibrium we determined that LFA-1 molecules on unactivated T cells bind to ICAM-1 with an affinity of 0.5-1 x 10^6 M^{-1}. The affinity of ICAM-1 for LFA-1 on activated T cells cannot be accurately measured by equilibrium displacement experiments because the time required to reach equilibrium is longer than the period of enhanced adhesion avidity that follows T cell activation. Alternatively, we can measure the kinetics of the competitive binding within the first few minutes of activation and by fitting the data to the simultaneous rate equations we can determine the rate constants for the ICAM-1/LFA-1 interaction. Using this approach we estimate that soluble ICAM-1 binds to an unactivated T cell hybridoma with k_{on} = 1.3 x 10^5 $M^{-1}min^{-1}$ and k_{off} = .197 min^{-1}. These rate constants give K_A = 7 x 10^5 M^{-1} in good agreement with the equilibrium measurements. Rate constants on activated T cells are under investigation.

X 415 HEAVY-METAL MODULATION OF THE HUMAN INTERCELLULAR ADHESION MOLECULE (ICAM-1) GENE EXPRESSION Stefano Martinotti[1#], Elena Toniato[1], Antonella Colagrande[1], Vincenzo Flati[1], Lucia Cilenti[1], Isabella Screpanti[2], Luigi Frati[2], Adrian C. Hayday[3], and Alberto Gulino[1]
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The intercellular adhesion molecule 1 (ICAM-1) can be induced on many different cell types by a set of various modulators (IL1α, TNF, LPS, IFN-β), which are released during the inflammatory process. We have investigated the possibility that other factors, related to the stress and bio-physical perturbations of the inflammatory response, may also modulate ICAM-1. Here, we report that heavy metals, in particular zinc, are able to enhance the expression of the ICAM-1 gene on cells actively involved at different levels during inflammation. Kinetic studies of ICAM-1 gene expression shows a maximum level of induction 4 hours after treatment with metals, followed by a rapid decrease to basal levels within 12 hours. The effect on enhanced gene expression is mostly due to a rapid increase of the transcriptional rate as shown by nuclear run-on experiments. In some cell lineage (B lymphoblastoid cells), but not in fibroblasts, the increase in RNA expression seems significantly greater that the subsequent increase in protein expression, suggesting that a further point of post-transcriptional regulation of ICAM-1 occurs and may be linked to the cellular specificity.

X 416 PERTURBATION OF THE LFA-1 MOLECULE TRANSMITS A POSITIVE SIGNAL FOR CYTOKINE PRODUCTION DURING THE ALLOGENEIC RESPONSE. Susan M. McCabe, Gerry Nakamura, Lavon Riddle, Phil Berman and Paula Jardieu, Genentech, Inc., So. San Francisco, CA

The interaction of LFA-1 and its ligand ICAM-1 is thought to mediate T cell activation when costimulated with OKT3 immobilized on plastic. We now report soluble ICAM-1 (sICAM) significantly enhances the induction phase, but not the effector phase of T cell activation in the human one way Mixed lymphocte reaction (MLR). Supernatants from sICAM treated MLR cultures contain significantly higher levels of TNFalpha and Gamma Interferon than control supernatants. Furthermone, the enhanced allogeneic response was specifically inhibited by either the addition of anti-ICAM, anti-LFA-1 or anti-TNFα monoclonal antibodies. Our findings suggest that the interaction of LFA-1 with ICAM-1 results in increased TNF production which in turn stimulates T cell proliferation in the MLR.

X 417 CARBOHYDRATE BINDING PROTEIN 35 (MAC-2): A LAMININ BINDING LECTIN, Arthur M. Mercurio, Hee-Jong Woo, Margaret M. Lotz and Leslie M. Shaw. Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA 02115

Carbohydrate binding protein 35 (CBP35), also known as Mac-2 is an N-acetyllactosamine specific lectin of unknown function found primarily in the cytoplasm of many cell types, though it is also evident in the nucleus and on the cell surface. Possible functions for CBP35 are suggested by recent findings. CBP35 binds avidly to laminin and in many cell types it is the major, non-integrin laminin binding protein. These observations suggest that it may function in concert with integrins in extracellular matrix interactions. A nuclear function for CBP35 has also been postulated. These seemingly disparate functions prompted us to examine CBP35 structure in more detail and to make use of molecular genetics to gain insight into specific cellular functions. The first approach taken was to study the multimeric nature of CBP35. CBP35 purified from cell extracts by laminin affinity chromatography exists as several distinct species (Mr 35,000, 67,000, and 80,000) when analyzed by under non-reducing conditions. Upon reduction, only the 35,000 species is seen. Recombinant CBP35 expressed in *E. coli* forms disulfide linked homodimers (Mr, 67,000). Site-directed mutagenesis indicated that cysteine 186, the single cysteine residue in this protein is required for dimerization. The dimeric form of CBP35 binds to laminin with higher affinity than does monomer. The larger 80kD form of CBP35 is a heterodimer whose structure is being investigated. These structural studies suggest that the apparently disparate functions that have been proposed for CBP35 may be regulated by its ability to form intermolecular associations with nuclear, cytoplasmic, and cell surface proteins. CBP35 is expressed as a function of both monocyte differentiation into macrophages and intestinal crypt cell differentiation into mature enterocytes. In the absence of defined function, it is not known whether this expression is a cause or consequence of differentiation. To address these issues and to gain insight into specific function, we have transfected sense and anti-sense CBP35 cDNA into both monocytes and intestinal crypt cells.

X 418 REGULATION OF HUMAN LYMPHOCYTE SUBSET TRAFFIC DURING INFLAMMATION *IN VIVO*. Colin A. Michie, Kenneth S. Soo, John H. Wyllie, L. Happerfield, L. Bobrow, Peter C. L. Beverley, Human Tumour Immunology Group, Imperial Cancer Research Fund, University College and Middlesex Schools of Medicine, London, England.

Recruitment of lymphocytes into the inflamed human appendix includes a disproportionately large number of T lymphocytes of the CD45RO+ve subset. Using immunohistological techniques and in situ hybridisation, we examined the separate roles of endothelial adhesion, RANTES and HuMip cytokines in this process. Lymphocyte traffic was estimated from the degree of systemic lymphopenia and lymphocyte numbers in the efferent appendiceal lymphatics from 10 patients. Traffic is significantly greater in two situations, in the gangrenous appendix, and in those appendices in which message for the RANTES cytokine is expressed. The upregulation of endothelial molecules including ICAM 1, ELAM and VCAM, or the expression of HuMip cytokine message showed no correlation with the degree of lymphocyte traffic; these molecules were not detected in normal appendices, but were found in all inflamed organs. 'Chemoattractant' cytokines may therefore modulate the degree of lymphocyte recruitment in this *in vivo* model.

X 419 PROLONGED EFFECT OF A SINGLE ORAL DOSE OF ASPIRIN ON HUMAN PLATELET FUNCTIONS AND PLASMA PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) LEVELS, S. A. Mousa, M. Forsythe, J. Bozarth, L. Pease, and T. M. Reilly, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400

The goal of the present study was to investigate the effect and duration of action of a single oral dose (650 mg) of aspirin (n=5 normal healthy male volunteers) on human platelet functions (including aggregation, fibrinogen binding, and PAI-1 release) and on the plasma level of PAI-1. Aspirin demonstrated a rapid onset of action at 2 hours after ingestion inhibited, ex vivo, arachidonic and (AA) mediated platelet functions including a) fibrinogen binding to gel purified platelets; b) platelet aggregation; and c) platelet PAI-1 release. Additionally, peak reduction of plasma PAI-1 level at 2 hours was demonstrated. The effect of aspirin on the ex vivo AA-mediated effects (a-c) was shown to last for up to four days. While aspirin treatment resulted in inhibitory effects against AA mediated platelet activation, it resulted in a rebound effect with respect to other platelet agonists including adenosine diphosphate (ADP) and epinephrine (EPI).

We conclude that a single dose of aspirin has a long lasting effect on AA induced platelet activation, and reduces the plasma level of PAI-1 as well. The rebound effect of platelets in response to agonists such as ADP and EPI suggest the potential usefulness of the combination therapy of aspirin with other antiplatelet drugs, or the potential advantages for other platelet inhibitors such as a GpIIb/IIIa receptor antagonist.

X 420 BLOCKING EFFECT OF MONOCLONAL ANTIBODY DIRECTED AGAINST THE ADHESION MOLECULE (CD18) ON THE INVASION OF MURINE LB LYMPHOMA INTO LYMPHOID ORGANS, David Naor[1], Elimelech Okon[2] and Muayad A.Zahalka[1], Lautenberg Center for Immunology[1] and Hematopathology unit[2], The Hebrew University - Hadassah Medical School Jerusalem 91010, Israel.
The same integrin adhesion molecules used by normal leukocyutes for traffic and localization in inflammation sites, may be used by malignant cells for dissemination. Identifying the adhesion molecules and subsequently blocking them with appropriate antibody may therefore prove useful for controlling tumor spread. This prediction was tested on a spontaneous murine LB T cell lymphoma that expresses the B2 subfamily integrin molecules. The adhesion molecules were identified by fluorocytometry and immunoprecipitation employing anti-CD18 monoclonal antibody (mAb; M18/2). Subcutaneously inoculated LB lymphoma rapidly infiltrates the spleen and the lymph nodes as indicated by histological examination and ^{3}H-thymidine incorporation assay of proliferating LB cells derived from the invaded organs. The normal organization of the lymphoid organs was totally effaced by the infiltrating LB cells. Intravenous injection of anti-CD18 mAb, protein G purified anti- CD18 mAb or its $F(ab')_2$ fragments (but not irrelevant control mAb), totally blocked the invasion of the subcutaneously inoculated lymphoma into the spleen. Whereas intravenously injected anti-CD18 mAb could not block the infiltration of LB cells into the lymph nodes, local subcutaneous injection of this antibody near the lymph nodes partially inhibited the lymphoma invasion into these organs. The ability of anti-CD18 mAb to block the infiltration of LB cells into the spleen emphasizes the clinical potential of this experimental approach. On the other hand, the fact that anti-CD18 mAb only partially blocked the infiltration of LB cells into the the lymph nodes implies the complexity of the invasion process, which cannot be defeated by a single straightforward protocol.

X 421 *CITROBACTER FREUNDII* USES MICROFILAMENT-DEPENDENT AND INDEPENDENT PATHWAYS TO INVADE HUMAN EPITHELIAL CELLS, T. A. Oelschlaeger and D. J. Kopecko, Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D. C. 20307-5100
Two human urinary tract isolates and four lab strains of *Citrobacter freundii* were compared to *Salmonella typhi* Ty2 and *Escherichia coli* HB101 for their ability to invade monolayers of cultured human intestinal Henle 407 or bladder T24 cells in the presence or absence of sublethal concentrations of various inhibitors. Mid-log phase bacteria added at a ratio of 50 bacteria/cell were centrifuged onto the monolayer. Uptake then occured during 2h incubation at 37C in 5% CO_2. New medium containing 100ug/ml gentamycin was added and incubation continued for another 2 h, followed by enumeration of surviving intracellular bacteria. Only the two *Citrobacter* clinical isolates and Ty2 showed significant uptake. The highest recovery (=% of input bacteria surviving gentamycin treatment) for *Citrobacter*, 12.5% and 8.8% for strains 3009 and 3056, respectively, was achieved with T24 cells. *Citrobacter* invasion of Henle cells was inhibited >60% by g-strophantin and monodansylcadaverine, which prevent coated pit formation, and also by cytochalasin D, which leads to depolymerization of microfilaments. An additive effect was seen when both cytochalasin and monodansylcadaverine were used together. In contrast, *Citrobacter* invasion of T24 cells was only inhibited by g-strophantin or monodansylcadaverine and not by cytochalasin. Demecolcine reduced bacterial uptake by >75% with both cell lines for both *C. freundii* strains, indicating the involvement of microtubules. In contrast, the invasion of Henle and T24 cells by Ty2 was only inhibited by cytochalasin D, and not by inhibitors of microtubules or coated-pit formation. Electron microscopic analysis revealed visible uptake by 15 min and *Citrobacter* could be found within defined endocytic vacuoles. These data suggest that the responsible bacterial ligands, of short half-life, interact with different surface receptors and trigger different transmembrane signals which lead to two distinct endocytic pathways: (A) microfilament-dependent, and (B) microtubule-dependent.

X 422 IDENTIFICATION OF AN INTEGRIN RECEPTOR ($\alpha_3\beta_1$) ON *MYCOBACTERIUM AVIUM* COMPLEX (MAC). *Savita P. Rao, K.R. Gehlsen, and *A. Catanzaro. *UCSD Medical Center, University of California, San Diego, CA 92103 and La Jolla Institute for Experimental Medicine, La Jolla, CA 92037 . *Mycobacterium avium-Mycobacterium intracellulare (Mycobacterium avium* Complex or MAC) is an intracellular organism responsible for the highest incidence of disseminated bacterial infection in patients with AIDS. One of the modes of entry into the host is presumed to be either by ingestion or inhalation. It was of interest to study the initial steps in the establishment of the disease. The binding of MAC to extracellular matrix proteins was investigated. A cell adhesion assay using Terasaki plates revealed that MAC adhere to laminin, collagen type I and IV, and fibronectin in a dose-dependent manner, while no significant binding to vitronectin was observed. The adherence of MAC to laminin, collagen I and fibronectin could be inhibited up to 70% when pre-incubated with polyclonal antibodies against a human laminin-binding integrin ($\alpha_3\beta_1$) and upto 90% with antibodies against a human fibronectin receptor($\alpha_5\beta_1$), while only a partial inhibition (30%) was observed with antibodies against a human vitronectin receptor ($\alpha_v\beta_3$). Control antisera did not inhibit this binding. Immunoblot analysis of a sonicate of MAC with polyclonal antibodies against various integrin receptors, showed cross-reactivity with only anti $\alpha_3\beta_1$. Further, binding of MAC to laminin, collagen I and fibronectin could be inhibited upto 70% with 20mM EDTA indicating that the interaction is cation dependent. These results indicate that MAC have a receptor that binds to laminin, collagen I and fibronectin, and may use this integrin receptor to adhere to basement membrane proteins and gain entry into host tissues.

X 423 ROLE OF FIBRONECTIN IN INFLAMMATION: SIGNAL TRANSDUCTION VIA THE FIBRONECTIN RECEPTOR $\alpha5\beta1$. J. Roman, R. Pacifici and R. Perez. Departments of Medicine of the Veterans Affairs Medical Center, Emory University School of Medicine, Atlanta, Georgia and Jewish Hospital, Washington University School of Medicine, St. Louis, Missouri.
Expression of the cell adhesive extracellular matrix glycoprotein fibronectin (FN) is increased during lung inflammation. Using a model of murine lung granulomatous inflammation, we found that FN deposition in the lung was increased during granuloma formation suggesting a functional role. Mononuclear cells within the granulomas may interact with FN as they expressed integrin subunits $\alpha5$, $\alpha3$ and $\alpha4$ revealing the presence of FN receptors of the $\beta1$ integrin family. In order to investigate the possible role of FN in lung inflammation, we examined the effects of FN on inflammatory cell function. Peripheral blood mononuclear cells (PBMCs) express matrix receptors of the integrin family. Ligand binding to one integrin, the collagen receptor $\alpha2\beta1$, induced the production of Interleukin-I (IL-I) by PBMCs as measured by a biological assay. In contrast to the effect of collagen, FN did not induce IL-I production. However, FN potentiated the collagen-induced response as incubation of PBMCs with both collagen type I and FN resulted in a three to four fold increase in IL-I production. Flow cytometric analysis revealed that the expression of $\alpha2\beta1$ on PBMCs was not altered by FN-treatment. The potentiatory effect of intact FN was mimicked by its 120 kDa RGD-containing fragment and inhibited by synthetic RGD-containing hexapeptides and an anti-$\alpha5$ subunit monoclonal antibody suggesting that the effect of FN was mediated via the integrin $\alpha5\beta1$. These observations suggest that matrix components such as collagen and FN may "activate" inflammatory cells by binding to integrin receptors expressed on their surface. Furthermore, we provide evidence for the existance of an intracellular communication between two members of the $\beta1$ integrin receptor subfamily that may modulate cell interactions with the extracellular matrix. These interactions may play a role during experimental granulomatous inflammation and, perhaps, in certain inflammatory lung diseases.

X 424 MACROPHAGE COLONY STIMULATING FACTOR STIMULATES SYNTHESIS AND EXPRESSION OF $\alpha_5\beta_1$ ON MURINE BONE MARROW MACROPHAGES IN A DOSE DEPENDENT MANNER, F. Patrick Ross, Masaaki Shima, Patricia Osmack, Michael Holers, and Steven L. Teitelbaum, Departments of Pathology and Medicine, Washington University School of Medicine, St. Louis, MO 63110.

Macrophage colony stimulating factor (M-CSF) is a cytokine necessary for the survival and differentiation of cells of the monocyte/macrophage lineage. Immature bone marrow macrophages (BMMs) grown with varying amounts of M-CSF adopt different morphologies and the number of adherent cells increases with the concentration of M-CSF. We asked whether this is related to regulation of integrins on the cell surface. BMMs isolated by a combination of Ficoll gradient and adherence to plastic were cultured for 3 days in Teflon beakers in the presence of 1-1000 units per ml of natural purified murine M-CSF. When cells were then allowed to bind to a range of matrix proteins, adherence was seen only on fibronectin (FN) and was maximal at or beyond 5 μg per ml coating concentration. Increasing the amount of M-CSF in the culture resulted in a dose-dependent increase in the number of cells adherent to FN. A polyclonal antibody to recombinant murine $\alpha_5\beta_1$ inhibited attachment to FN by 75-85%. The same cells were cultured with 1-1000 units M-CSF as before and equal numbers were surface labeled and subjected to immunoprecipitation with the antibody 33.11, directed against the cytoplasmic tail of human β_3. The integrin $\alpha_5\beta_1$ was expressed on the surface of the cells in an M-CSF-dependent manner. Metabolic labeling with ^{35}S-methionine/cysteine and precipitation with the same antibody showed that synthesis was also dose-dependent with respect to the cytokine. We conclude that, as part of its pleiotropic actions supporting macrophage function, M-CSF regulates both synthesis and expression of the integrin $\alpha_5\beta_1$ possibly allowing the cells to interact with their microenvironment, which is known to contain fibronectin.

X 425 THYMIC EPITHELIAL CELLS EXPRESS LAMININ RECEPTORS THAT MAY MODULATE INTERACTIONS WITH THYMOCYTES, Joseli Lannes-Vieira, Roger Chammas, Dea M. Villa-Verde, Marcos A. Vannier-dos-Santos, Sandro J. Souza, Ricardo R. Brentani, and Wilson Savino, Department of Immunology, Fundacao Oswaldo Cruz, Rio de Janeiro; Ludwig Institute for Cancer Research, Sao Paulo Branch, Sao Paulo; Laboratory of Ultrastructure and Eletron Microscopy, Federal University of Rio de Janeiro.

The thymus gland is a central organ of the immune system, in which the T cell repertoire is generated. Differentiating thymocytes migrate within the organ, interacting with cells of the thymic microenvironment, particularly the thymic epithelial cells (TEC). Since cell migration in other biological systems is influenced by extracellular matrix (ECM) proteins via specific receptors (ECMR) it seemed worthwhile to dissect thymic cells in terms of their distinct ECMR.

We described herein a laminin receptor (LNR) expressed by murine TEC, as being an α6ß1 integrin. The immunocytochemical distribution, ascertained with anti-α6 and anti-ß1 antibodies revealed that, the ß1 polypeptide, common to all class I integrins, was evidenced in the thymic cortex and medulla. In contrast, the laminin receptor α6 subunit showed an expression restricted to subcapsulary and subseptal areas as well as medullary regions. Immunoelectronmicroscopy revealed a membrane labeling of both α6 and ß1 molecules, including at desmosomal sites. Lastly, immunoblotting and affinity chromatography of extracts from a mouse TEC line, respectively defined a 140 kDa and 110/120 kDa polypeptides as being the α6 and ß1 chains. This LNR expressed on TEC appears to be functional since TEC adhesion, spreading and proliferation were enhanced *in vitro* by LN. Moreover, LNR expression on TEC was upregulated *in vivo* and *in vitro* by hydrocortisone treatment.

A small proportion of thymic lymphocytes (particularly comprised in immature subsets) also expressed α6ß1 LNR, leading to the hypothesis on the existence of LNR-mediated TEC/thymocyte interactions. Interestingly, *in vitro* experiments showed that there is an increase in TEC-lymphocyte adherence in situations where the expression of these molecules, as well as extracellular matrix components, are enhanced. Most importantly, spontaneous *in vitro* thymocyte release by the so-called thymic nurse cell complexes was enhanced by LN and partially blocked by anti-LN, anti-α6 or anti-ß1 antibodies.

Our results suggest that the α6ß1 integrin receptor may modulate TEC behavior and TEC-thymocyte interactions, that can be putatively ascribed as relevant for intrathymic T cell differentiation events.

X 426 PHENOTYPIC ANALYSIS WITH VLA-β1 (CD29) AND VLA-α4 (CD49d) ANTIBODIES IDENTIFIES A DISTINCT SUBSET OF CD4 MEMORY CELLS WHICH EXCLUSIVELY EXPRESSES A POTENTIAL NEW INTEGRIN β CHAIN

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While naive cells represent a phenotypically homogenous population, $CD4^+$-memory ($CD45RO^+$) cells show marked heterogeneity. At least three different subpopulations exist which differ in levels of expression of VLA-β1 and VLA-α4; nevertheless, they all express CD45RO and other apparent memory markers, like CD58 (LFA-3), CD54(ICAM-1) and CD25 (TAC) at comparable levels. In addition to VLA-$\alpha4^{mod}\beta1^{hi}$ and VLA-$\alpha4^{low}\beta1^{hi}$ subsets there is also a distinct VLA-$\alpha4^{mod}\beta1^{mod}$ subset. The existence of the latter is especially intriguing, since the relative predominance of the α4 over β1 suggests a role for additional β chains different from β1. Further phenotypic studies identified a monoclonal antibody which reacts uniquely with this VLA-$\alpha4^{mod}\beta1^{mod}$ subset. Moreover, the recognized antigen shows reciprocity to β1 in its expression on $CD4^+$-memory cells. Structural characterization suggests that the recognized antigen is a new β-integrin-like molecule, with a molecular weight of approx. 106-110kDa. Its physical association with α4 is indicated by both phenotypic and structural data. Like many integrins, expression of this molecule is augmented with T cell activation. It is also present on B cells and B cell lines. Unlike subsets of β^{hi}-memory cells, none of this unique subpopulation expresses the carbohydrate (CLA Ag, HECA-452) which has been postulated to be a skin homing ligand. Based on the phenotypic heterogeneity and known importance of α4 and other integrins in T cell adhesion, migration and costimulation, we postulate that the VLA-$\alpha4^{mod}\beta1^{mod}$ subset will have distinct and unique homing and functional properties.

X 427 REGULATION OF ALPHA3 INTEGRIN EXPRESSION ON HUMAN MICROVASCULAR ENDOTHELIAL CELLS, N.T.Sepp, T.J. Lawley, R.A. Swerlick, Emory University, Atlanta, GA, 30322

Alpha3 is expressed on a variety of cells, including endothelial cells (EC) and epithelial cells. Because alterations in the expression of alpha3 on microvascular EC may play an important role in cell matrix interactions in wound healing and tissue remodelling, we have examined the effects of basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-b), and the proinflammatory cytokines interferon gamma (IFN-g) and tumor necrosis factor alpha (TNF-a) on alpha3 integrin expression by human dermal microvascular EC (HDMEC). bFGF induced a dose and time dependent decrease (50%) in the expression of cell surface alpha3 which is not accompanied by changes in the expression of cell surface beta1. Decreases were evident after 24 hours and were maximal after 72 hours. Doses of bFGF as low as 10 ng/ml induced decreases in expression and maximal effects were seen at doses of 1 ug/ml. Similarly, incubation of HDMEC with TGF-b (1 ng/ml) or TNF-a (500 u/ml) for 72 hours resulted in comparable decreases in alpha3 expression. Interestingly, stimulation with IFN-g (1000 u/ml) resulted in a dose and time dependent increase in alpha3 expression. Increases were noted after 24 hours and maximal increases of 50% were seen after 72 hours. Stimulation of HDMEC with IFN-g antagonized the decreased expression induced by bFGF, TGF-b, or TNF-a. Interestingly, stimulation of large vessel umbilical vein EC with bFGF, TGF-b, or INF-g did not result in changes of alpha3 expression. These data demonstrate that HDMEC integrin expression is regulated by both growth factors and inflammatory cytokines and that this regulation may be important in events requiring changes in microvascular EC interactions with extracellular matrix.

X 428 THE $\alpha 6_A\beta 1$ INTEGRIN IS A REGULATED LAMININ RECEPTOR. Leslie M. Shaw and Arthur M. Mercurio Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA 02115

Mouse peritoneal macrophages do not adhere to laminin unless stimulated with either PMA or cytokines. This adhesion is blocked by the α6 antibody GoH3 but stimulation does not alter α6β1 surface expression. For these reasons, we examined the possibility that the α6β1 integrin is a "regulated" laminin receptor in the sense that its ligand binding function is activated in response to physiological stimulation. The α6β1 integrin extracted from unstimulated cells did not bind to laminin affinity columns even though it is expressed on the cell surface, but it did bind to laminin columns after PMA stimulation. The α6β1 integrin that was eluted from laminin columns could be immunoprecipitated with an antibody specific for the $\alpha 6_A$ splice variant (provided by L. Reichardt). The expression of this splice variant was confirmed by PCR. Additional evidence for the regulated function of $\alpha 6_A\beta 1$ comes from studies on the P388D1 macrophage cell line. We found that this cell line does not adhere to laminin even in response to physiological stimulation and that it does not express any α6 integrin subunit. These properties make this cell line useful for the analysis of α6β1 function by cDNA transfection. Stable $\alpha 6_A\beta 1$ transfectants of P388D1 were generated and analyzed for laminin binding functions. These transfectants did not adhere to laminin constitutively regardless of the level of $\alpha 6_A\beta 1$ surface expression. However, adhesion to laminin was induced by PMA. The time course of adhesion (15-30' maximum) and the percentage of total transfectants that adhered in response to PMA were similar to that observed previously for mouse peritoneal macrophages. These results demonstrate that macrophages "down-regulate" the ability of $\alpha 6_A\beta 1$ to function as a laminin receptor. Functional activity of this receptor is induced only in response to physiological stimulation.

X 429 AVIDITY, SPECIFICITY, AND ANTI-ADHESIVE PROPERTIES OF L-693,966, A HUMANIZED CD18 MONOCLONAL ANTIBODY. Irwin I. Singer*, Douglas W. Kawka*, Soloman Scott*, Diana M. Kazazis*, Bruce L. Daugherty+, Julie A. DeMartino+, and George E. Mark+, Depts. Biochem. Molec. Path.* and Cell & Molec. Biol+., Merck, Sharp, & Dohme Res. Labs., Merck & Co., Inc., Rahway, N.J. 07065.

Rodent monoclonal antibodies (mAbs) to β_2 integrins are potent anti-inflammatory agents. IB4 (mIB4), an anti-CD18 murine mAb, prevents adhesion and extravasation of polymorphonuclear leukocytes (PMNs) through vascular endothelium. However, therapeutic use of mIB4 in man is limited by its immunogenicity. We have constructed L-693,966, a fully CDR-grafted humanized form of mAb IB4 (hIB4). hIB4 and mIB4 were colocalized in leukocytes of various rabbit and Rhesus tissues by immunoperoxidase microscopy. Therefore, humanization has not altered the specific cellular binding properties of IB4. However, while IB4 stained all types of Rhesus leukocytes, it did not label CD11a/CD18 positive rabbit leukocytes. Competitive binding studies of hIB4 on activated PMNs, U-937 monocytes, or washed unfractionated human blood showed hIB4 to be 2-3 fold less avid than mIB4. Alternatively, both hIB4 and mIB4 inhibited the attachment of activated hPMNs or monocytes to human vascular endothelial monolayers with equal potency (IC_{50} = 1 nM). We conclude that hIB4 is functionally equivalent to its native murine counterpart.

X 430 NON-OPSONIN-MEDIATED BACTERIAL ATTACHMENT TO MURINE PERITONEAL MACTOPHAGES. A. R. Sloan and T. G. Pistole, Univ. of New Hampshire, Durham, NH 03824

Direct recognition of potential pathogens by macrophages is considered an important feature of our innate defenses. We have demonstrated attachment of Group B streptococci (GBS) to thioglycollate-elicited, adherent mouse peritoneal macrophages (pMØ) in a serum-free assay system. Monosaccharides and neoglycoconjugates were unable to inhibit this attachment, suggesting that the interaction is not lectin-mediated. Preliminary studies supported the involvement of leukocyte integrins (CD11/CD18) in this interaction. In down-modulation experiments with the monoclonal antibody (MAb) M1/70 against CD11b (α chain of CR3), binding to GBS was inhibited by 32%. In similar studies using MAb M18/2 against CD18 (β chain of the leukocyte integrin family), binding was inhibited by 63%, suggesting that more than one member of the CD11/CD18 family is involved in this binding. When these same MAb were used in solution to inhibit the binding of GBS to adherent pMØ, the inhibition was 25% and 56%, respectively. Streptococcal lipoteichoic acid (LTA) inhibited the binding of GBS to pMØ in a dose-dependent manner. Pre-incubation of pMØ with LTA yielded significantly greater inhibition than that seen in studies in which LTA and bacteria were added simultaneously. In down-modulation studies with LTA 60-70% inhibition of bacterial binding was seen over the LTA concentration range of 30-3000 μg/ml. These data support the roles of streptococcal LTA and leukocyte integrins in non-opsonin-mediated bacterial recognition by pMØ.

X 431 B CELL ATTACHMENT TO EXTRACELLULAR MATRIX COMPONENTS: INVOLVEMENT OF β1 AND β3 INTEGRIN COMPLEXES. Dwayne G Stupack and John A Wilkins, Rheumatic Disease Unit laboratory, Depts of Medicine and Medical Microbiology, University of Manitoba, Winnipeg, Canada. Lymphocyte attachment to extracellular matrix is thought to play a central role in the sequestration of lymphoid populations in various organs and tissues. Integrin mediated adherence processes appear important in recruitment and retention of lymphocytes during normal trafficking as well as during stimulus induced lymphocyte accumulation, such as is observed in chronically inflamed sites. To assess the adherence potential of B cells , a panel of ten B cell lines were assayed for attachment to the extracellular matrix components collagen, fibronectin, laminin and vitronectin. Both basal and inducible adherence potential was assessed. Cellular expression of integrins was evaluated by flow cytometry and SDS PAGE. Utilization patterns were established by blocking monoclonal antibody techniques during adherence to intact protein or proteolytic fragments. Cell specific variation in both expression and utilization of integrins were observed. Five different integrin complexes, belonging to the β1 and β3 integrin families, have been identified as matrix binding structures. Observed adherence patterns suggest the possible involvement of two further unidentified integrin complexes. Circulating B cells express little or no β1 integrin. However, upon stimulation tonsillar and peripheral B cell populations acquired the capacity to attach to fibronectin coated surfaces, concurrent with the induction of expression of β1 and α5 integrin subunits, and an increase in basal α4 expression. Together the results suggest that different B cells may use different integrins to attach to matrix, and that multiple β subunits may be expressed and utilized on a single cell. These results are indicative of specific integrin utilization by different B cells, possibly related to their anatomical distribution or differentiative state.

X 432 MODULATION OF MICROVASCULAR ENDOTHELIAL CELL VNR BY GROWTH FACTORS AND GAMMA INTERFERON. R.A. Swerlick, T.J. Lawley, L.J. Li, S.W. Caughman, K.H. Lee, N.T. Sepp, Emory Univ., Atlanta, GA, 30322.
Modulation of expression of the alphav/beta3 complex (VnR) on human dermal microvascular endothelial cells (HDMEC) may be crucial in wound healing and angiogenesis. Therefore, we have examined the influence of basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-b), and gamma interferon (IFN-g) on the expression of these cytoadhesin subunits. Stimulation of HDMEC with bFGF increased cell surface expression of both alphav and beta3 up to 100% in a dose and time dependent manner (maximal 1 ug/ml, 72-96 hours). Increases in VnR expression were accompanied by changes in cell morphology. These effects were microvascular specific since bFGF did not induce similar changes in morphology or cell surface expression by umbilical vein endothelial cells. Incubation of HDMEC with TGF-b (1 ng/ml), or IFN-g (1000 u/ml), for 72 hours resulted in a 30-50% decrease in VnR expression. Coincubation of HDMEC with bFGF and TGF-b or IFN-g completely inhibited the development of morphologic changes associated with bFGF stimulation and also inhibited the bFGF induced increases in cell surface expression of VnR by 50 and 100% respectively. In northern blot analysis, resting HDMEC expressed minimal levels of beta3 mRNA, but stimulation of HDMEC with bFGF resulted in a 10 fold increase when examined at 48 hours. Increases induced by bFGF were completely abrogated by coincubation of HDMEC with IFN-g, and inhibited up to 70% by coincubation with TGF-b. These data demonstrate that both growth factors and inflammatory cytokines alter the expression of cell surface integrin complexes, that these alterations correlate with changes in cell shape, and that these changes are specific for microvascular endothelial cells.

X 433 PURIFICATION AND CHARACTERIZATION OF THREE PLATELET AGGREGATION INHIBITORS, TRIKHA M, BRUMMER J, TOM DIEK S, MARKLAND F S, DEPARTMENT OF BIOCHEMISTRY, UNIV. OF SOUTHERN CALIFORNIA, SCHOOL OF MEDICINE, LOS ANGELES, CA 90033.
Platelet aggregation inhibitors (plAI) are disulfide rich polypeptides that have been purified from snake venoms. These polypeptides have been shown to be fibrinogen receptor antagonists by binding to glycoprotein IIb/IIIA, an integrin receptor found on platelet membranes, generally through an Arg-Gly-Asp sequence in the plAI. We have purified plAIs from three snake venoms. Contortrostatin, the plAI from the venom of *Agkistrodon contortrix contortrix*, was purified by hydrophobic interaction HPLC, hydroxylapatite HPLC, and C4 and C18 reverse phase HPLC (RP-HPLC). It appears to be a homodimer with a molecular mass of approximately 18 kD under nonreducing and 8 kD under reducing SDS-PAGE. Purpureomaculostatin, the plAI from *Trimeresurus pupureomaculatus* venom, was purified by molecular sieve chromatography using Sephadex G100, C4 and C18 RP-HPLC. The protein has a molecular mass of about 6.5-8 kD under reducing and nonreducing SDS-PAGE. The third plAI, multisquamatin, was purified from the venom of *Echis multisquamatus* by C4 and C18 RP-HPLC. The protein has a molecular mass of approximately 8 kD uder nonreducing and approximately 6 kD under reducing SDS-PAGE. Inhibition of platelet aggregation was tested by using human or canine platelet rich plasma in the presence or absence of plAI. Aggregation was monitored by the addition of 10 µM ADP. A 50% inhibition (IC_{50}) of human platelet aggregation was observed at 0.8 µg/ml of contortrostatin, 3.4 µg/ml of purpureomaculostatin and 0.6 µg/ml of multisquamatin. The IC_{50} of contortrostatin with canine platelets was 2.2 µg/ml. Amino acid analysis reveals that contortrostatin has 70 amino acids with four disulfide bridges. Interestingly, the sequence of contortrostatin appears to begin 10 amino acids downstream of applaggin, the plAI from the venom of *Agkistrodon piscovorus piscovorus* which has 71 amino acids. Contortrostatin may have an insertion and/or a C-terminal extension of nine amino acids. The sequence of the amino terminal 24 residues of purpureomaculostatin and the amino terminal 40 residues of multisquamatin have been obtained. Further characterization of these plAIs is in progress.

X 434 DEVELOPMENTALLY REGULATED EXPRESSION OF THE $\alpha_6\beta_4$ INTEGRIN ON MOUSE THYMOCYTES. Scott Wadsworth, Mark Halvorson and John E. Coligan, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892
We demonstrate that the $\alpha_6\beta_4$ integrin is expressed in a developmentally regulated fashion on immature T lymphocytes in the mouse thymus, but is undetectable on mature T lymphocytes from lymph node, spleen or blood. $\alpha_6\beta_4$ was not induced by mature T cell activation *in vitro*, suggesting it has a thymus-specific function. $\alpha_6\beta_4$ was expressed by 35% of day 13 fetal thymocytes, rose to 75% at days 14 and 15, then rapidly declined to about 20% by birth. In adults, 80% of early $CD4^-$ $CD8^-$ thymocytes were $\alpha_6\beta_4^{hi}$, most or all immature $CD4^-$ $CD8^+$ $CD3^-$ thymocytes were $\alpha_6\beta_4^{lo}$, while only 30% of the intermediate $CD4^+$ $CD8^+$ cells and <10% of mature $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$ $\alpha\beta$ TCR^+ cells were $\alpha_6\beta_4^{lo}$. Up to 65% of $\gamma\delta$ TCR^+ thymocytes were also β_4^+. Within the early $CD4^-$ $CD8^-$ thymocytes, the highest levels of $\alpha_6\beta_4$ were on the $CD44^-$ $IL2R^+$ $CD3^-$ subset (>98% $\alpha_6\beta_4^{hi}$), which is a critical control point in T cell ontogeny. Our data suggest that $\alpha_6\beta_4$ is acquired or at least upregulated after bone marrow-derived pro-T cells enter the thymus. The developmentally regulated pattern of $\alpha_6\beta_4$ on day 13-16 fetal and adult $CD4^-$ $CD8^-$ $CD3^-$ thymocytes further suggest that $\alpha_6\beta_4$ may play a role in early T cell development prior to acquisition of the T cell antigen receptor.

X 435 THE GPIIIa MAb D3 INDUCES A CONFORMATION IN THE GPIIb-IIIa COMPLEX THAT BINDS BOTH SOLUBLE AND IMMOBILIZED FIBRINOGEN ON HUMAN ERYTHROLEUKEMIA CELLS, C. Denise Wall, Karl Studtmann, Jeanne Hermann-Petrin, Michael E. Dockter and Lisa K. Jennings, Departments of Medicine, Biochemistry, and Microbiology and Immunology, University of Tennessee, Memphis, Tn 38163
Platelet glycoprotein IIb-IIIa (GPIIb-IIIa), a heterodimeric, transmembrane protein, binds adhesive proteins on activated platelets. This integrin is also present on the human erythroleukemic cell line HEL. To date, no conventional agonists have been described that can stimulate GPIIb-IIIa ligand binding on nucleated cells. In this study, we induced fibrinogen binding by HEL cells using a GPIIIa monoclonal antibody (MAb), D3, which causes fibrinogen binding on human platelets without cell activation. Using flow cytometry and an anti-fibrinogen antibody-FITC conjugate, we assayed for soluble fibrinogen binding in the presence of fibronectin-free fibrinogen and the appropriate antibodies. A small percentage of cells showed constitutive binding of soluble fibrinogen. Addition of D3 increased the percentage of cells binding soluble fibrinogen by 5-fold (67%). Fibrinogen binding under both conditions was blocked completely by GPIIb-IIIa MAb 7E3. Adherence of HEL cells to immobilized fibrinogen was accomplished by adding 100 µg/ml fibrinogen to tissue culture plates and then blocking with 0.25% BSA. A small percentage of the control cells bound to the fibrinogen plate constitutively, whereas D3 preincubation increased binding 10-fold over the control. Since the vitronectin receptor (VnR) present on HEL cells also binds fibrinogen, blocking experiments using VnR MAb LM609 demonstrated that the increases observed were not due to interaction with the VnR. GPIIb-IIIa MAb 10E5 blocked both the constitutive binding and the D3 induced binding. These data show that the GPIIb-IIIa complex present on the surface of these nucleated cells is competent to bind ligands and has similar ligand receptor activity as platelet GPIIb-IIIa. These results also suggest that the binding of D3 to GPIIIa (a.a. 422-692) plays a role in the induction of the activated conformation of the GPIIb-IIIa complex on HEL cells.

X 436 CATION-DEPENDENT BINDING OF [^{3}H]-SK&F-107260, A CYCLIC ARG-GLY-ASP (RGD) PEPTIDE TO GLYCOPROTEIN IIb/IIIa: COMPETITIVE INHIBTION BY FIBRINOGEN, Fgγ-DODECAPEPTIDE AND CYCLIC RGD PEPTIDES, A. Wong, S. M. Hwang, K. Johanson, J. M. Stadel, D. A. Powers, D. Bennett, R. Heys, F. Ali, W. Bondinell, T. Ku and J. Samanen. SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406

The platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) is a member of the integrin receptor family that recognizes adhesive proteins containing the RGD sequence in a cation-dependent manner. SK&F-107260 (cyclo-S,S-[Mba-(NMe)Arg-Gly-Asp-Man]) is a potent antithrombotic agent that inhibits platelet aggregation by binding to the GPIIb/IIIa. [^{3}H]-SK&F-107260 binds specifically to purified GPIIb/IIIa reconstituted into liposomes. The apparent K_d is 0.0025 ± 0.0003 μM (mean ± SEM, n=8). Fibrinogen, Fgγ-dodecapeptide (HHLGGAKQAGDV), a cyclic RGD peptide SK&F-106760 (cyclo-S,S-[Cys-(NMe)Arg-Gly-Asp-Pen]-NH_2), and a noncyclic peptidomimetic N-[m-(p-amidinobenzamido)]-benzoyl-β-Ala-HCl inhibit the binding of [^{3}H]-SK&F-107260 to GPIIb/IIIa. Their K_i values are 0.28 ± 0.05 μM, 58.0 ± 12.0 μM, 0.175 ± 0.025 μM and 0.023 ± 0.004 μM, respectively. Lineweaver-Burk Plots of the equilibrium binding data suggest that fibrinogen, Fgγ-dodecapeptide and SK&F-106760 are competitive-type antagonists. The noncyclic peptidomimetic, on the other hand, demonstrates mixed-noncompetitive inhibition. Substitution of Ca^{2+} with Mn^{2+} in the buffer system results in increases of both affinity (K_d = 0.001 μM) and maximal binding capacity (2 fold) of SK&F-107260 to GPIIb/IIIa. The noncyclic peptidomimetic becomes a competitive-type antagonist in the presence of Mn^{2+}. Therefore, divalent cations modulate the interactions of ligands with GPIIb/IIIa. Studies of the binding kinetics of [^{3}H]-SK&F-107260 may provide important information for the regulation of GPIIb/IIIa, and the RGD peptides and peptidomimetics will be useful tools to map the ligand binding domain of GPIIb/IIIa.

X 437 IMMUNO-EM LOCALIZATION OF CD18 AND T-CELL RECEPTORS DURING CTL MEDIATED CYTOLYSIS OF LYPHOBLASTOID TARGETS, John Woods, Diana Kazazis, Tom Blake and Irwin Singer, Molecular and Biochemical Pathology, Merck Sharp & Dohme Research Laboratories, Merck and Co. Inc., Rahway, NJ 07065

Conjugates of human cytolytic Q31 T-cells and JY target cells were fixed, frozen and ultrathin sections cut. The distribution of CD18 and T-cell receptors (TCR) was visualized by labeling the sections with either; 1) H52, a monoclonal anti-human CD18, 2) a polyclonal anti-CD18, 3) 8A3, a monoclonal anti-T-cell receptor or 4) an anti-CD3 anti-peptide antibody directed against a cytoplasmic determinant of the CD3 complex, followed by a colloidal gold secondary reagent.

In Q31/JY conjugates CD18, labeled with either monoclonal or polyclonal antibodies, was observed along the plasmamembrane and on microvilli of the Q31 cells only in the region surrounding the zone of contact between the two cell types and within small vesicles near these contact zones. Plasmamembrane distal to contact zones displayed much less label. Also, gold was rarely observed on the closely apposed membranes in within the zone of contact itself. In contrast, in resting Q31 cells CD18 was randomly distributed along the entire cell membrane and was not observed intracellularly. These results suggest that adhesion of Q31 to JY cells results in a redistribution of CD18 from a random pattern into membrane areas near the actual zone of contact followed by its internalization into small intracellular vesicles.

TCR was observed randomly distributed along the plasmamembrane and in intracellular vesicles only on the Q31 partner of conjugate pairs. Like CD18, TCR was not observed within the actual zones of contact. This was true with both the monoclonal probe directed against an extracellular epitope and the anti-peptide probe which recognized a cytoplasmic domain, suggesting that the zone of contact is not simply a restricted space which is inaccessible to our probes. These results suggest that while both CD18 and T-cell receptors play a role in the initial stage of conjugate formation, both are specifically excluded from areas of membrane contact during subsequent events of CTL mediated cytolysis.

X 438 ADHESION AND COSIGNALLING CONTRIBUTIONS OF CTL INTEGRINS, Belen Ybarrondo, Anne M. O'Rourke, and Matthew M. Mescher

The activation of cytotoxic T lymphocytes (CTL) involves the T cell receptor-antigen interaction and the binding of cell surface adhesion receptors to their respective ligands. Recent evidence suggests that the binding of adhesion receptors to ligand on the surface of other cells and in the extracellular matrix may have a dual role. In addition to mediating adhesion, these receptors may provide an activation signal to the CTL. In this study we have investigated one such receptor/ligand system, integrin binding to fibronectin (FN). Binding of the integrins VLA-4 and VLA-5 to FN is activated through the TCR, and the interaction has been shown to provide a costimulatory signal for proliferation of CD4+ T cells upon stimulation with anti-CD3 antibody. We have investigated the activated binding of cloned murine CTL to immobilized FN and the potential contribution of this interaction to the response of mature effector CTL. Stimulation of CTL with soluble anti-CD3 resulted in dramatically increased binding to immobilized FN. Induction of binding was rapid (detectable within 30 minutes) and reached maximal levels by 2 hours. The addition of a second crosslinking antibody resulted in degranulation and release of serine esterase, but only in the presence of immobilized FN. This finding suggests that binding to FN provides an activation signal to CTL. The ability of FN to contribute to the functional response of CTL to antigen was assessed by coimmobilizing FN and alloantigen. When FN was coimmobilized with antigen there was an increase in serine esterase release from alloantigen-specific CTL compared to that seen with alloantigen alone. The effect of FN on CTL activation required that FN and antigen be present on the same surface; when FN was immobilized in microtiter wells and antigen was present on 5μ latex beads FN had no effect on the response to antigen. Although the individual contributions of VLA-4 and VLA-5 are not yet clear, the data indicate that CTL interaction with FN results in delivery of a costimulatory signal for the CTL degranulation response

X 439 **EXPRESSION OF A SOLUBLE FORM OF ENDOTHELIAL LEUKOCYTE ADHESION MOLECULE 1 IN 293, CHO AND COS CELLS**

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Selective adhesion of leukocytes to the endothelial cell in the vessel wall is a critical step in inflammation, lymphocyte homing, and the process of certain vascular diseases. Endothelial leukocyte adhesion molecule 1 (ELAM-1) is one of the three-known selectin molecules, involved in such adhesion events. A truncated cDNA of ELAM-1 molecule (385 amino acids) has been constructed, which is different from all existing forms. Its stable expression in 293 and CHO cells, and its transient expression in COS cells, have all shown the secretion of the recombinant soluble form of ELAM-1 (rsELAM-1), but with differential degree of N-glycosylation (30%, 42% and 37% respectively). Regardless of their various glycosylation, these rsELAM-1 proteins, when immobilized on to Immulon plates, are fully functional as an adhesion molecules for selective binding of HL60, neutrophils and the Colo 205 carcinoma cells. However, rsELAM-1 is only a weak inhibitor of ELAM-1 mediated adhesion, as mentioned previously by Lobb, R.R. et al..

With the mechanism of the transactivation of human CMV promoter by the adenovirus E1A antigen, the expression level of rsELAM-1 protein in 293 cells after a three day's culture is approximately 6 ug per half million cells. This level of expression is even higher than that of CHO/dhfr- cell obtained by the classical G418 selection and serial amplification using methotraxate.

These rsELAM-1 proteins should be valuble for the further study of the role of ELAM-1 expressed on the vascular wall during the inflammatory response.

* This study was partially performed at Stanford University and Cytel Co.

Integrin Expression in Differentiation and Development

X 500 MODULATION OF FIBROBLAST COLLAGEN BINDING β1 INTEGRIN ACTIVITY BY PDGF.

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The relative importance of the collagen binding integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ in the adhesion of primary neonatal rat heart fibroblasts to collagen type I was investigated. $\alpha_1\beta_1$ and $\alpha_2\beta_1$ both functioned as collagen receptors, whereas $\alpha_3\beta_1$ did not. Using cyanogen bromide fragments and the $\alpha_1\beta_1$ specific monoclonal antibody 3A3 it was found that the rat $\alpha_1\beta_1$ bound determinants present at several positions in the native collagen type I triple helix, whereas the rat $\alpha_2\beta_1$ bound a determinant present in the collagen α1(I) CB3 fragment.

A microtiter-plate based assay, allowing for the quantification of fibroblast mediated collagen gel contraction (Gullberg *et al.* Exp. Cell Res. 186:264-272), was used to investigate the role of $\alpha_1\beta_1$ in this process. Platelet-derived growth factor (PDGF), but not basic fibroblast growth factor (bFGF), up to 25 ng/ml, or epidermal growth factor (EGF), up to 100 ng/ml, effectively stimulated collagen gel contraction. In the absence of PDGF rabbit polyclonal anti-β1 integrin IgG at doses down to 20 μg/ml, completely inhibited collagen gel contraction, the $\alpha_1\beta_1$ -specific 3A3 IgG, at doses down to 1 μg/ml, also markedly inhibited collagen gel contraction. Cross-titration experiments revealed that the inhibitory effect of anti-β1 integrin IgG and 3A3 IgG on collagen gel contraction by primary neonatal rat heart fibroblasts could be overcome by increasing the concentrations of PDGF. In contrast, bFGF in doses up to 25 ng/ml was not able to stimulate contraction in the presence of an inhibitory dose of anti β_1-integrin IgG.

The molecular mechanisms responsible for the stimualtory action of PDGF on integrin activity are currently being investigated. Present data indicate that PDGF-stimulation of fibroblasts increases the apperance at the cell surface of β_1-integrins.

X 501 FACTORS AFFECTING EXPRESSION OF INTEGRINS ON HUMAN LUNG FIBROBLASTS

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Integrin levels were determined on lung fibroblasts originally derived from normal or fibrotic regions of human lung tissue were determine by immuno flow cytometry. The effect of cell origin, matrix composition, and cell density on integrin expression were examined. Fibroblasts derived from normal and fibrotic lung tissue were both strongly stained for α_2, α_3, α_5, α_v, and β_1 subunits. Interestingly, α_v was expressed by only 30-40 % of the cells in each line tested indicating heterogeneity within each cell population. In contrast, α_4 and α_6 were high in subconfluent cultures, but decreased to undetectable levels as the cells approached confluency. When cells were cultured on plates coated with various purified extracellular matrix proteins, no significant differences were seen when compared to surfaces coated with bovine serum albumin. In addition, we "wounded" confluent cultures by scraping the culture dishes with a sterile toothpick, and allowed the adjacent cells to migrate into the denuded area. Cells migrating into the wounded area were more intensely stained for several integrin subclasses including α_6, α_4, and β_1, as compared with nearby confluent cells. Other integrins such as α_3, α_{IIb}, and β_3 were not appreciably different in the wound area. These results indicate that integrin levels in cultured cells can be influenced by cell density and/or migratory activity possibly induced by chemotactic factors. These changes may prepare fibroblasts within an area of tissue damage for migration and proliferation within the wounded tissue zone. Supported by NIH HL-39943.

X 502 CONTROL OF DEVELOPMENTAL DECISIONS BY EPITHELIAL CELL CONTACTS, Judith Austin, Cynthia Kenyon, Dept. Biochemistry, UCSF, San Francisco, CA 94143

We are interested in the role of cell contact in determining developmental fate in the epidermis of the nematode *C. elegans*. In particular, we have been examining the role of cell migration and cell contact in the decision of one epidermal blast cell, V5, to produce a neural sensory structure, the postdeirid. V5 is one of a group of cells, V1-V6, that form a narrow stripe of lateral epidermis along each side of the *C. elegans* body and act as epidermal stem cells. These cells divide once per larval stage, producing a lateral epidermal cell and a cell that will join the dorsal/ventral epithelium. After this division, the lateral epidermal cells are no longer in contact with each other. In response, each cell sends out anterior and posterior processes that extend to touch those of the neighboring cells, reforming a continuous lateral epithelium.

V5 normally divides to generate the postdeirid neuroblast early in the second larval stage. However, when the lateral epidermal cells neighboring V5 either to the anterior or the posterior are removed by ablation during the first larval stage, no postdeirid neuroblast is made. Our results indicate that when the cell processes extended by V5 do not contact those of neighboring lateral epidermal cells during a critical period of time, V5 produces an extra epidermal cell rather than a neuroblast. Interestingly, cells at the edge of the gap produced by cell ablation do eventually reconnect, although the distance traversed by their cell processes is longer than normal. Thus growth of these processes is controlled by lack of contact with lateral epidermal cells. Likewise the requirement of V5 for lateral cell contact for the formation of a postdeirid neuroblast indicates that these cell connections control signals affecting this developmental decision. Specifically, the requirement for both anterior and posterior connection suggests that lack of cell contact generates a signal that prevents neuroblast formation.

X 503 PH-30, A PROTEIN ACTIVE IN SPERM EGG FUSION, CONTAINS A POTENTIAL FUSION PEPTIDE AND AN INTEGRIN LIGAND DOMAIN. Carl P. Blobel, Tyra G. Wolfsberg, Chris W. Turck, Diana G. Myles, Paul Primakoff and Judith M. White. Dept of Pharmacology, University of Callifornia, San Francisco, San Francisco, CA, 94143

We report the sequences of both the α and β subunits of PH-30, a protein shown to function in sperm-egg membrane fusion due to the inhibitory action of a cognate monoclonal antibody (Primakoff et al, 1987, JCB 104:141-149). Biochemical characterization of PH-30 revealed that it is an integral membrane complex of two glycoproteins, α and β, that are both made as larger precursors. Processing of the PH-30 β subunit correlates with the acquisition of fertilization competence (Blobel et al, 1990, JCB 111:69-78). The sequences of PH-30 α and β confirm that both proteins are indeed integral membrane glycoproteins, and further that PH-30 α and β are evolutionarily related. PH-30 α contains a short hydrophobic sequence in addition to its transmembane domain that shares features in common with viral fusion peptides. The aminoterminal domain of PH-30 β shares homology with a family of integrin ligands. Therefore, in analogy to known viral membrane fusion proteins, PH-30 has two subunits that are likely to function in binding to (β) and fusion with (α) the target (egg) membrane. PH-30 may therefore represent the first identified cellular membrane fusion protein. These results further suggest that there is a receptor for PH-30, perhaps an integrin, on the egg plasma membrane.

X 504 INTEGRIN-MEDIATED INTERACTIONS DURING MAMMALIAN PRIMITIVE STREAK MESODERM MIGRATION. C. A. Burdsal[1], C. H. Damsky[2,3], and R. A. Pedersen[1,3], [1]Laboratory of Radiobiology and Environmental Health, [2]Dept. of Stomatology, and [3]Dept. of Anatomy, University of California, San Francisco, CA 94143.

During gastrulation in the mouse changes in cell-cell and cell-substratum adhesion have been hypothesized to play a role as mesodermal cells migrate through and away from the primitive streak. Therefore, we have assayed the ability of mesodermal explants dissected from the primitive streak of 7.5 day mouse embryos to attach, spread, and migrate on various extracellular matrix components. Under serum-free conditions, the mesodermal explants attached and spread quickly on substrata of fibronectin or vitronectin (within 1-4 hr) while their attachment on either laminin or collagen type IV was much slower (8-16 hr). Adhesion to all these substrata was completely blocked by the addition to the culture medium of antibodies against integrins (GP-140). Non-immune antibodies applied at the same concentration had no effect. The GP-140-treated explants recovered and attached, spread and migrated normally after the antibodies were washed away. Because the GP-140 antibodies are known to block interactions involving either β1- or β3-containing integrins, these results indicate that these integrins could play a functional role in mesodermal migration during gastrulation in the mouse. In addition, an antibody specfic for β1α6 (GOH 3), which mediates adhesion to laminin, selectively blocks mesoderm attachment to laminin but not to fibronectin. These results indicate that β1α6 is a functional laminin receptor for primitive streak mesoderm cells in the mouse embryo. (Supported by NIH Grants HD26732, US DOE/OHER Contract DE-AC03-76-SF01012, and NIEHS Training Grant ES 07106).

X 505 EXPRESSION AND LOCALIZATION OF ß-1 INTEGRIN IN THE DEVELOPING HEART, Wayne Carver, Bob Price, Dominique Rasso, Thomas K. Borg and Louis Terracio, Dept. of Anatomy, Cell Biology and Neurosciences and Dept. of Pathology, University of South Carolina School of Medicine, Columbia, SC 29208
Little is known regarding the temporal and spatial patterns of expression of extracellular matrix (ECM) components and corresponding integrins in the developing heart. The present study describes the localization of the ß-1 integrin and ECM components, including laminin and interstitial collagen, during rat heart development. While the ß-1 integrin was seen in most cells of the developing heart, including fibroblasts and myocytes, clear differences in the levels of staining for the protein and mRNAs were noted. Most intense staining was typically seen in areas which also stained for ECM components. For instance, ß-1 integrin and laminin were found abundantly in myocytes of the developing myocardium at 12 days of gestation. By 17 days of development, most abundant staining for ß-1 integrin was seen over cells of the cardiac cushions correlating to the development of an extensive network of interstitial collagen. Electron microscopic analysis also illustrated differences in immuno-staining for ß-1 integrin along the surfaces of fetal cardiac myocytes. Staining was seen predominantly in regions of cell-cell contact and in areas where interstitial collagen fibers connected to the myocyte surface. These areas were also found to be intensely labeled by laminin antisera. These studies illustrate the close association of ECM components such as collagen and laminin with integrin receptors in the developing heart.

X 506 STABLE EXPRESSION OF THE HUMAN INTEGRIN ß6 SUBUNIT IN MOUSE NIH 3T3 CELLS, Ric I. Cone and Dean Sheppard, Lung Biology Center, Department of Medicine, University of California, San Francisco, California 94143.
The integrin ß6 subunit, first identified in guinea pig airway epithelial cells, and sequenced from the human pancreatic carcinoma cell line, FG2, associates with the integrin α_v subunit, and mediates adhesion to fibronectin (Busk, Pytela & Sheppard, in press). The ß6 subunit shares 38 - 47% homology with ß1, ß2 and ß3, however, ß6 contains a unique 11-amino acid extension at the carboxyl terminus, not present in the other ß subunits. α_vß6 is present in some but not all epithelial cells, and is not present in the mouse embryonic cell line, NIH 3T3. We have stably expressed the human ß6 subunit in NIH 3T3 cells following transfection of a neomycin-resistant DNA construct containing the ß6 insert under regulation of a dexamethasone (DEX)-inducible promoter. After 24 hr induction with DEX, expression of ß6 was demonstrated, by both metabolic and surface labeling followed by immunoprecipitation with ß6-specific antisera. ß6 was associated with endogenous α_v, present in NIH 3T3 cells, based upon immunoprecipitation of identical bands using either α_v-specific or ß6-specific antisera. NIH 3T3 cells transfected with the ß6 construct undergo significant change in shape following treatment with 1 µM DEX for 24 hours, an effect that can be reversed within 48 hours following removal of DEX. This change in shape was not seen following DEX treatment of NIH 3T3 cells that were not transfected.

X 507 CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF THE PROMOTERS FOR THE LFA1 AND p150,95 INTEGRIN α SUBUNITS. Angel L. Corbí, Manuel Lopez-Cabrera, Alicia Vara and Arsenio Nueda. Unidad de Biología Molecular, Hospital de la Princesa, Diego de León 62, Madrid 28006, SPAIN.

The members of the ß2 integrin subfamily LFA1, Mac-1 and p150,95 mediate key interactions between leukocytes and endothelial cells, which ultimately lead to leukocyte extravasation. The expression of their corresponding subunits is absolutely restricted to leukocytes, and can be regulated by growth factors and differentiation-promoting agents. In order to determine the structural basis for their constitutive and regulated expression we have isolated genomic clones covering the more 5' region of the genes for LFA1α and p150,95α. Primer extension and nuclease S1 assays located several transcription initiation sites, with the major one resembling the initiator sequence described for other genes lacking TATA-box. Sequential deletions and CAT assays have allowed the identification of 150 bp regions which retain the promoter activity. Both promoters are fully active on lymphoid and myeloid cell lines, although their activity can also be detected on non-leukocytic cells. Therefore, additional sequences must confer the leukocyte-restricted expression of LFA1α and p150,95α. By footprinting analysis, we have identified a number of transcription factors which recognize specific sequences within the p150,95 promoter. The footprinting results indicate the existence of a transcription factor which binds to a sequence also found in other leukocyte-specific genes. An additional sequence has been identified which is only protected in p150,95α-expressing cells. The relevance of these sequences for the constitutive and regulated expression of the α subunits of the leukocyte integrins will be discussed.

X 508 EXPRESSION, TOPOGRAPHY AND FUNCTION OF INTEGRIN RECEPTORS ARE SEVERELY ALTERED IN KERATINOCYTES FROM INVOLVED AND UNINVOLVED PSORIATIC SKIN. M. De Luca*, G. Pellegrini*, G. Orecchia+, F. Balzac§, O. Cremona§, P. Savoia§, R. Cancedda*, P.C. Marchisio§. *Ist. Naz.le Ricerca sul Cancro, +Univ. di Pavia, §Univ. di Torino, ITALY.

Psoriasis is a hyperproliferative cutaneous disease of unknown etiology and etiopathogenesis. Alteration of keratinocyte adhesiveness to basal lamina has been proposed as the initial disturbance leading to poorly controlled proliferation. Keratinocyte adhesion to basal lamina and lateral interactions among basal epidermal cells are mediated, besides other molecules, by integrin receptors that are segregated to discrete membrane domains. In this paper, the expression and functions of integrins in psoriatic keratinocytes were examined both in vivo and in vitro. We found that i) in psoriatic keratinocytes the integrin heterodimers alfa2beta1, alfa 3beta1 and alfa6beta4 have lost their polarized distribution on the plasma membrane; ii) the role of these integrins in mediating keratinocyte adhesion in vitro is altered; iii) psoriatic keratinocytes form focal contacts containing both beta1 and beta4 integrins. In normal adult keratinocytes the alfa5beta1 fibronectin receptor is poorly expressed and diffusely distributed on the basal keratinocyte plasma membrane and is not organized in defined adhesive structures. In contrast, psoriatic keratinocytes show a clear fibronectin receptor staining in vivo, and organize alfa5beta1 in typical focal contacts in vitro without any obvious increase of its expression and synthesis. These multiple alterations of integrins are also present in uninvolved keratinocytes from psoriatic patients, suggesting a key role for altered integrin-mediated adhesion in the pathogenesis of this disease.

X 509 COMPARISON OF MOLECULAR MECHANISMS THAT CONTROL VCAM-1 GENE EXPRESSION IN THE ENDOTHELIUM AND DURING MUSCLE DIFFERENTIATION, Michael F. Iademarco, Jay J. McQuillan, and Douglas C. Dean, Departments of Internal Medicine and Cell Biology, Washington University School of Medicine, St. Louis, MO 63110

We have demonstrated that during muscle development VCAM-1 is expressed constitutively in myoblasts but that expression subsides soon after cells fuse into myotubes. Antibodies to VCAM-1 and to its counter receptor VLA-4 inhibit myoblast fusion, suggesting a role for these proteins in muscle differentiation. In contrast to its pattern of expression in developing muscle, VCAM-1 is only expressed in endothelial cells in response to inflammatory cytokines. We have begun studies to examine the molecular mechanisms controlling VCAM-1 expression in these two tissues. Here we show that differences in promoter activity are responsible for the distinct patterns of VCAM-1 expression in the two tissues. Silencer elements restrict VCAM-1 promoter activity in endothelial cells and we show that TNF-a can overcome the effect of these silencers and activate the promoter. Two NFkB sites are required for the tissue-specific activation of the promoter by TNF. Preliminary experiments indicate that TNF causes dissociation of nuclear proteins from the silencer region, suggesting that TNF may have several complementary effects on the VCAM-1 promoter. In the myoblast cell line C2C12, the VCAM-1 promoter is constitutive and there is no effect of TNF on expression. The silencer region is relatively inactive in C2C12 cells and it forms complexes with nuclear proteins that are insensitive to TNF and that migrate more slowly in gel retardation assays than those formed with proteins from endothelial cells. Ongoing experiments should provide additional insight into mechanisms surrounding the intriguing pattern of VCAM-1 expression in different tissues and during development.

X 510 N-CADHERIN SYTHESIS IN AN INSECT CELL LINE USING THE BACULOVIRUS EXPRESSION VECTOR SYSTEM, Julie A. Dickson[1], Duane D. Bronson[2], John J. Hemperly[2], Douglas P. Malinowski[1] and Robert E. Pearson[1], Departments of [1]Molecular Biology and [2]Cell Biology, Becton Dickinson Research Center, Research Triangle Park, NC 27709

N-Cadherin belongs to a family of Ca++ dependent , membrane-associated cell adhesion molecules. It is involved in cell-cell interactions through molecular self-association, and has been shown to interact intracellularly with cytoskeletal molecules. The molecule is found in relative abundance during vertebrate development, but is found only in trace amounts in adult tissues. To overexpress the protein, the N-Cadherin gene, including the native translational start site, was transferred into the baculovirus AcMNPV employing the Bluebac transfer vector. N-Cadherin was produced through the course of infection of the host insect cell line (Spodoptera frugiperda Sf9) with the recombinant virus. A prominant novel band at the expected molecular weight of 130 kD was seen by Coomassie-stained SDS PAGE gels of infected cell lysates. The expressed proteins appeared as multiple bands when analyzed by Western blot using polyclonal antisera. At least two of these bands were determined to be products of differential glycosylation: these two forms were not expressed when the infected cells were grown in the presence of the N-linked glycosylation inhibitor Tunicamycin. Experiments are underway to determine whether the recombinant protein is functionally active.

X 511 DISTRIBUTION OF INTEGRINS IN AVIAN DEVELOPMENT: ALPHA1BETA1 INTEGRIN EXPRESSION IS REGULATED DURING MYOGENESIS AND NEUROGENESIS

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We have examined the spatio-temporal distribution of the alpha1 integrin subunit, a putative laminin and collagen receptor, in avian embryos, using immunofluorescence microscopy and immunoblotting techniques. We used an antibody raised against a gizzard 175-kD membrane protein which we found to be immunologically identical to the chicken alpha1 integrin subunit. In adult avian tissues, alpha1 integrin exhibited a very restricted pattern of expression in smooth muscle cells and in capillary endothelial cells. In the developing embryo, alpha1 integrin subunit expression was detected transiently in both central and peripheral nervous systems and in striated muscles, in association with laminin and collagen IV, as well as in smooth muscle and capillary endothelial cells. alpha1 integrin was absent from virtually all epithelial tissues and was not found in tissues that were not associated with laminin and collagen IV. In the nervous system, alpha1 integrin subunit expression occurred predominantly at the time of early neuronal differentiation. During skeletal muscle development, alpha1 integrin was expressed on myogenic precursors, during myoblast migration, and in differentiating myotubes. alpha1 integrin disappeared from skeletal muscle cells as they became contractile. In visceral and vascular smooth muscles, alpha1 integrin appeared specifically during early smooth muscle cell differentiation and, later, was permanently expressed after cell maturation. These results indicate that (i) the expression pattern of alpha1 integrin is consistent with a function as a laminin/collagen IV receptor; (ii) during avian development, expression of the alpha1 integrin subunit is spatially and temporally regulated; and (iii) during myogenesis and neurogenesis, expression of alpha1 integrin is transient and correlates with cell migration and differentiation.

X 512 **PARTIAL HOMOLOGY BETWEEN THROMBOSPONDIN AND A 195 kDa CALCIUM-BINDING PROTEIN SECRETED BY ADRENOCORTICAL CELLS IN RESPONSE TO ACTH.** Jean-Jacques FEIGE*, Sylvie Pellerin*, D.L. Shi*, Jean Gagnon° & Edmond M. Chambaz*, *INSERM U244, DBMS/BRCE, CENG,85X, 38041 Grenoble Cedex, France.°DBMS/BS, CENG,85X, 38041 Grenoble Cedex, France.

We identified previously α_2-macroglobulin as the major protein secreted by bovine adrenocortical cells in primary culture. We report here that, under ACTH treatment, these cells secrete an additional protein of apparent MW 195 kDa. We named this protein CISP, standing for corticotropin-induced secreted protein. The protein was barely detectable in the conditioned medium of untreated cells. cAMP analogs and adenylate cyclase activators could mimick the induction of CISP secretion by ACTH. The presence CISP in the conditioned medium was first detected after 8h of ACTH treatment, reached a plateau after 24 h and was sustained for at least 96 h. The induction of CISP secretion was not observed in the presence of an RNA synthesis inhibitor (DRB) suggesting that ACTH regulates CISP synthesis at the transcriptional level. Purification of CISP to homogeneity was achieved and sequencing of a 10 amino-acid long tryptic peptide revealed 60 % identity with a corresponding sequence located in the C-terminus of human thrombospondin. Biochemical characterization of the purified protein indicated that CISP is monomeric, sulfated on tyrosine residues, N-glycosylated and that it binds heparin with a strong affinity. Furthermore, it is a calcium-binding protein peptide mapping analysis which revealed that bovine CISP is different from bovine thrombospondin.
Further characterization of this new protein will allow to determine whether it belongs to the family of adhesive proteins.

X 513 TRANSCRIPTIONAL REGULATION OF THE α_{IIb} INTEGRIN (GPIIb) DURING MEGAKARYOCYTIC DIFFERENTIATION OF K562 CELLS, Alan M. Fong and Samuel A. Santoro, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.
When pluripotent K562 cells are treated with 40 nM phorbol 12, 13 dibutyrate, they are induced to differentiate along the megakaryocytic pathway. During this process, there is increased surface expression of the glycoprotein (gp) IIb-IIIa integrin complex and increased steady state levels of gpIIb and gpIIIa mRNA. We have begun to elucidate the molecular mechanisms controlling the differentiation-specific increase in gpIIb expression. Nuclear run off experiments indicate that the increased level of gpIIb mRNA during megakaryocytic differentiation is primarily due to a ten-fold increase in gpIIb gene transcription. Uzan et. al. (J. Biol. Chem. 256: 8932-8939 (1991)) recently identified a 5' flanking region of gpIIb DNA which appears to control tissue specific expression of the gene. We have isolated a 630 base pair segment corresponding to nucleotides -598—+32 by polymerase chain reaction based upon the sequence of Prandini et. al. (Biochem. and Biophys. Res. Comm. 14: 595-601 (1988)). Sequence analysis reveals 100 percent identity with the corresponding published sequence and the absence of a classical AP-1 binding site/phorbol responsive element. The 630 bp 5' flanking segment has been cloned into a plasmid, pCAT-Basic (Promega), containing the chloramphenicol acetyltransferase (CAT) reporter gene, and transfected into uninduced K562 cells or into K562 cells induced to differentiate along the megakaryocytic pathway with phorbol dibutyrate. CAT assays revealed that the 630 bp fragment was capable of driving CAT expression in uninduced K562 cells at a level significantly above control. CAT expression under the control of the 630 base pair fragment was further enhanced in K562 cells induced with phorbol dibutyrate. Thus, the 630 bp 5' flanking fragment contains one or more elements responsible for the differentiation-dependent expression of gpIIb. Studies are in progress to further define the element(s) within the 630 bp fragment which controls the differentiation-dependent expression of the gpIIb gene.

X 514 GENETIC ANALYSIS OF FIBRONECTIN FUNCTION IN MICE, Elizabeth L.George, Helen Rayburn and Richard O. Hynes, Center for Cancer Research and Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139
To facilitate functional analysis of fibronectins (FNs) in the intact animal, we have generated a FN-deficient mouse strain by gene targeting. Expression of the single FN gene was disrupted by insertion of a *neo* gene into the first exon via homologous recombination in embryonic stem cells. Germline transmission of the disrupted FN gene was achieved with three independent clones, and phenotypic analyses have shown no variations among clones. In mice which are heterozygous for the FN-deficient allele, concentration of soluble plasma FN is one half that of wild type litter mates, thus confirming complete disruption of one FN allele. We plan to utilize this phenotype to determine the significance of soluble FN in processes such as wound healing and susceptibility to carcinogens by comparing heterozygotes with wild type animals. Disruption of both FN alleles results in early embryonic lethality, revealing that FN is required for embryogenesis. Homozygous pre-implantation blastocysts are able to hatch from the zona pellucida and are recovered with expected Mendelian frequency. Homozygous post-implantation embryos, however, have not been detected. Of 26 embryos derived from heterozygote intercrosses, all stain positively for FN ($P<0.01$). Analyses are in progress to determine if this FN-deficient lethality is due to inability of trophectoderm cells to invade the uterine epithelium during implantation. Alternatively, FN may be required for cell migration or differentiation within the embryo prior to the egg cylinder stage. To elucidate the functional significance of FN alternative splicing variants in the intact animal we are generating transgenic mice which express defined FN cDNAs. Transgenics will be analyzed for dominant effects of the transgene and will be bred with the FN-deficient strain to determine which FN splicing variants are capable of rescue of the embryonic lethal phenotype.

X 515 GENETIC ANALYSIS OF ALTERNATIVE EXON EIIIB IN FIBRONECTIN, Elisabeth N. Georges, Helen B. Rayburn, and Richard O. Hynes *, Center for Cancer Research and *Howard Hughes Medical Institute, Department of Biology, M.I.T., Cambridge, MA 02139, U.S.A.
Fibronectin (FN) is an adhesive glycoprotein important in many physiological processes: embryogenesis, hemostasis, thrombosis, wound healing. Various forms are derived from a single gene by alternative splicing. Three alternative exons have been found: EIIIA, EIIIB, and V. EIIIA+ and EIIIB+ forms are prevalent in embryos. In adults, they can be included but to a lesser extent. EIIIA and EIIIB are found in cellular FNs but not in plasma FNs.
To analyze the function of the EIIIB exon, we have introduced mutations into the FN gene by homologous recombination. The targeting vector was a FN genomic clone, in which 670 base pairs containing the EIIIB exon and flanking intronic sequences were deleted and replaced by a neo cassette (neomycin phosphotransferase). A TK gene (thymidine kinase gene from Herpes simplex virus) was added to both ends of the construct, to allow counterselection of cells having randomly integrated the plasmid. After electroporation of mouse embryonic stem cells (ES cells), cell clones were screened for homologous recombinants. One positive clone was found, and injected into mouse blastocysts. The male chimaeras obtained were then bred and a heterozygous line carrying the mutation was established. Heterozygous intercrosses have only produced heterozygous or wild-type mice (33 animals examined), indicating that the deletion of EIIIB exon from the FN gene is a homozygous lethal mutation. We are now examining the effects of this mutation in heterozygous animals, as well as in homozygous embryos.
We have also generated a mutation to render the expression of EIIIB constitutive. To that end, a plasmid was constructed in which a cDNA segment including EIIIB was substituted for the corresponding segment in a genomic FN clone. The final construct contains a neo gene, flanked by 5.5 kb of FN sequences, and a TK gene at one end. After electroporation, two ES cell clones were found that had acquired this mutation by homologous recombination at the FN locus. These clones have been injected into mouse embryos, and have produced chimaeric animals which are now being bred.
These mutant strains of mice should provide information on the significance of this alternatively spliced segment of FN.

X 516 CHANGES IN VLA EXPRESSION, STRUCTURE AND FUNCTION ARE CORRELATED WITH MOUSE THYMOCYTE DEVELOPMENT.

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We analyzed β_1 integrin (VLA) expression and function on various mature and nonmature mouse thymocyte populations, compared to mature peripheral T lymphocytes. Two major forms of β_1 were detected, differing in the extent of N-glycosylation and sialylation. The larger (135 kD, NR) more basic β_1 was the only form on fetal and nonmature adult thymocytes. The smaller (120 kD, NR), more acidic β_1 was the only form on peripheral lymphocytes. Mature thymocytes expressed this form as well as a minor, less sialylated, 120 kD β_1. Analyses of potential differences in protein coding sequence by PCR were negative. The 135 kD β chain was paired with α4, 5 and 6 on immature thymocytes, some of which bound ECM proteins. Mature thymocytes, expressing conventional β_1, bound ECM proteins very poorly.

Analyses of thymocyte α chain expression showed that only mature cells expressed VLA-1 and -2. VLA-4 expression was found to be regulated in a complex, stage-specific manner, in contrast to the more homogeneous distribution of VLA-6, which was less sialylated on immature thymocytes. The patterns of VLA-4, -6 and LFA-1 on early thymocytes suggest that these integrins are not involved in homing of bone marrow-derived stem cells to the thymus. Overall, our data show that major differences in VLA expression, structure and function are closely correlated with the developmental stage of T lymphocytes and suggest an important role for VLA molecules in thymic ontogeny.

X 517 REGULATION OF INTEGRIN EXPRESSION IN FIBROBLASTS AND IN OSTEOSARCOMA CELLS BY INTERLEUKIN-1.

Jyrki Heino and Pia Santala, Department of Medical Biochemistry, University of Turku, SF-20520 Turku, Finland

Interleukin-1ß (IL-1ß) is an inflammatory mediator, known to be present at the site of chronic inflammation, for example in the rheumatoid synovium. Here, we show that IL-1ß is a potent regulator of the synthesis of VLA-1 or α1ß1 integrin herodimer in human skin fibroblasts and in MG-63 human osteosarcoma cells. The concentration of IL-1ß needed was 0.5 U/ml and 5 U/ml gave the maximal effect. In average IL-1ß increased α1 expression about 4.5-fold in skin fibroblasts. In MG-63 cells IL-1ß could turn on α1 expression even in conditions where the untreated cells did not express it in detectable amounts. α1ß1 is known to be a laminin and collagen receptor. In our experiments IL-1ß increased MG-63 cell adhesion on laminin and more specifically on E1 (elastase1) fragment of the molecule. This increase could be blocked by using anti-α1 monoclonal antibody.

The effect of IL-1ß on the other ß1-integrins in MG-63 cells was much smaller. It slightly decreased the synthesis of α3 subunit and increased the expression α2 subunit. We have previously shown that transforming growth factors-ß (TGFs-ß) have a similar but much larger effect on α2 and α3 integrin expression in MG-63 cells. Here IL-1ß and TGF-ß seemed to potentiate each other effects on integrin synthesis.

X 518 GASTRULATION AND THE BUDDING OF FEEDING ARMS ARE DISRUPTED BY SMALL LAMININ PEPTIDES

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We hypothesize that the interactions between the extracellular matrix protein-laminin, and cellular receptors signal or guide events during morphogenesis of sea urchin embryos. We examined the development sea urchin embryos cultured with peptide sequences from the cell binding regions of laminin. Control peptides with no peptides added reached the pluteus larva stage in 72 h. Tyr-ile-gly-ser-arg-NH_2 (YIGSR-NH_2), which is part of the central binding region of laminin, blocked archenteron formation when added soon after fertilization. Instead of plutei, mesenchyme blastulae formed in 72 h, which often had spicules. Other analogues of YIGSR-NH_2 such as YIGSE-NH_2, or YIGSK-NH_2 and also GRGDS had insignificant or no effects on development. The effect of YIGSR-NH_2 was concentration and time dependent. When added after the 16-cell stage, but before the blastulae hatched, only gastrulae with no feeding arms or gastrulae with abortive guts formed at 72 h. We are currently injecting peptides and laminin into the blastocoel of sea urchin embryos to investigate whether peptides are prevented from entering the blastocoel in early embryos by occluding junctions.

The peptide, ile-lys-val-ala-val-NH_2 (IKVAV-NH_2) from the long arm of laminin also disrupted the development of the archenteron, while a scrambled sequence of the same amino acids had no effect.

We suggest that YIGSR-NH_2 and IKVAV-NH_2 either compete with endogenous laminin for binding to cellular receptors and thus block the cell/extracellular matrix interactions required for initiation of invagination, or that YIGSR-NH_2 binds strongly to receptors and maintains all cells as ectodermal epithelium preventing the differentiation of the archenteron. The former mechanism is suggested by analogies with mammalian cells that migrate on laminin matrix (Tashiro *et al., J. Biol. Chem. 264*, 16174, 1989), and the later by the identification of laminin in the basal lamina of the sea urchin blastocoel except in the area of archenteron invagination (McCarthy & Burger, *Development 101*, 659, 1987). Gastrulation in sea urchin embryos is disrupted by a number of other agents, including those that block collagen crosslinking (Wessel & McClay, *Dev. Biol., 121*, 149, 1987). Thus laminin may act in concert with collagen and other molecules to modulate archenteron formation.

X 519 **ALTERED GENE EXPRESSION DURING BASAL LAMINA-INDUCED DIFFERENTIATION OF HUMAN ENDOMETRIAL ADENOCARCINOMA CELL-LINE HEC-6.**

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The human endometrial adenocarcinoma cell-line HEC-6 grown in serum-free defined medium (SFDM) on plastic or in a layer of a reconstituted basement membrane (Matrigel™; MG) shows pronounced morphological differentiation in contrast to monolayer growth on plastic observed in media containing dextran-charcoal (DCC) treated serum. Features of differentiation include clustering of cells in SFDM and formation of tumor gland-like structures after the clusters were put in MG. Columnar cells with microvilli and tight junctions at the apical surface enclosed a central lumen. (Vollmer et al., Proc. Amer. Assoc. Cancer Res. 82: 25,1991). In an early response to culture on top of a thick layer of MG, HEC-6 cells assembled to form spatially organized web-like structures within 12-24 hours.

We found an increased population doubling time (DCC: 25 hours, SFDM: 43 hours) and an increased content of progesterone receptor (DCC: 73.0 fmol/mg protein, SFDM: 283.7 fmol/mg protein) associated with the more differentiated phenotype. No estrogen receptor was detected in either sample. After 6 days under each culture condition, patterns of secreted and pericellularly-stored proteins were studied by ^{35}S-labeling and SDS-gel electrophoresis. Although the labeled samples had many bands in common, there were additional bands induced by SFDM and MG. The most obvious one found secreted into the medium was an approximately 85 kD protein expressed by HEC-6 cells cultured in or on MG. A 190 kD protein sensitive to collagenase VII digestion was secreted only in DCC-cultures. SFDM- and MG-culture conditions caused significant alterations in the expression levels of several other proteins. Differences between DCC-, SFDM- and MG-cultures were much more apparent than differences between cultures in or on MG. Our results indicate that the morphologic differentiation of the HEC-6 tumor cell-line induced by SFDM and MG is accompanied by a dramatic change in several cell functions.

X 520 IMMUNOLOCALIZATION OF INTEGRIN $\alpha_6\beta_4$ IN MOUSE JUNCTIONAL EPITHELIUM SUGGESTS AN ANCHORING FUNCTION TO BOTH THE INTERNAL AND THE EXTERNAL BASAL LAMINA, Marketta Hormia, Ismo Virtanen[1] and Vito Quaranta, Scripps Clinic and Research Foundation, La Jolla, CA 92037 and [1]Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland

The localization of the integrin $\alpha_6\beta_4$, a transmembrane adhesion molecule associated with hemidesmosomes, was studied in the junctional epithelium (JE) of mouse gingiva by using monoclonal antibodies in indirect immunofluorescence microscopy. The results showed that the integrin α_6 subunit was expressed in all cells of JE, whether or not they were in contact with basement membranes. In these cells, α_6 was homogeneously distributed on the cell surface. In other parts of gingival epithelium the α_6 subunit was distinctly polarized to the basal aspects of basal cells. The β_4 subunit had a more restricted distribution in JE. It was only expressed in cells facing the internal (between epithelium and tooth surface) and the external (between epithelium and gingival connective tissue) basal laminae. While distinctly enriched on the basal aspects, β_4 staining was also pericellularly distributed in these cells. In adjacent gingival epithelium β_4 was expressed at the basal aspects of basal epithelial cells, but was, in some cells, also expressed on lateral and apical cell surfaces. The basement membrane components, type IV collagen and laminin, could be detected in the external basal lamina and in other basement membranes of gingival epithelium, but were absent from the internal basal lamina of JE. These results indicate that the $\alpha_6\beta_4$ integrin, expressed in mouse junctional epithelium, may have a role in mediating the attachment of the cells to two compositionally different extracellular matrices, the internal and the external basal lamina.

X 521 Transcriptional and post-translational control of $\alpha5\beta1$ expression during terminal differentiation of human epidermal keratinocytes.

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During suspension-induced terminal differentiation of human epidermal keratinocytes, the $\alpha5\beta1$ integrin is down-regulated in two stages. Firstly, the ligand-binding ability of the receptor is reduced, followed several hours later by loss of the receptor from the cell surface (Adams and Watt, Cell 1990; **63**: 425-435). Loss of $\alpha5\beta1$ is associated with a decline in levels of $\alpha5$ and $\beta1$ mRNA (Nicholson and Watt, J. Cell Sci. 1991; **98**: 225-232). We have examined the mechanisms that regulate these events. Pulse-chase experiments showed that, in adherent cells, maturation of $\alpha5\beta1$ was complete within 12 hours. When keratinocytes were placed in suspension to induce terminal differentiation, however, no maturation of α or $\beta1$ subunits occurred. The inhibition of maturation could be mimicked in adherent cells by treatment with an inhibitor of Golgi α-mannosidase IA and IB, indicating that the block occurred prior to Golgi-dependent stages in N-linked glycosylation. Since integrin precursors are never detected on the surface of keratinocytes, we conclude that down-regulation of receptor function during terminal differentiation involves modulation of pre-existing receptor on the cell surface. Further experiments examining mRNA stabilities and using nuclear run-on assays to examine rates of transcription revealed that the decrease in $\alpha5$ and $\beta1$ steady state mRNA levels seen during suspension-induced terminal differentiation is a consequence of decreased transcription of both $\alpha5$ and $\beta1$ genes. Thus, loss of $\alpha5\beta1$ from the cell surface reflects both inhibition of transcription of the subunit genes and inhibition of maturation of newly synthesized subunits.

X 522 **Syndecan Expression in the Control of Integrin Glycosylation, Adhesion on Laminin and Epithelial Transformation.** Markku Jalkanen*#, Jyrki Heino* and Sirpa Leppä*, Department of Medical Biochemistry* and Turku Centre for Biotechnology#, SF-20520 Turku, Finland.

Malignant transformation leading to invasion and metastasis is a complex process that requires multiple changes in cell-matrix interactions. Indeed, the loss of cellular fibronectin receptor of integrin family is frequently seen in transformed cells and transfection of CHO cells with alfa-5 and ß1 integrin subunit inhibits their malignant behavior. On the other hand, transformed cells has been shown to express an altered pattern of integrin receptors and show increased ability to attach to laminin, the major component of basement membranes. Thus, decreased binding to interstitial matrix and increased adhesion on basement membrane are supposed to be important in malignant behavior of cancer cells. Syndecan is also one well-characterized matrix receptor, which via its glycosaminoglycan chains selectivily binds several interstitial components, including collagen type I, III, and V fibrils, fibronectin, *trombospondin and tenascin. Syndecan can also bind basic fibroblast* growth factor, suggesting a function as a modulator of growth factor response. We have recently observed that steroid-induced transformation of mammary epithelial cells leads to suppression of syndecan expression and loss of organized cytoskeleton. Moreover, syndecan re-expression in these cells using steroid-inducible promoter and full length syndecan cDNA restores normal epithelial morphology and cytoskeletal organization. In this study we show that syndecan-transfected S115 cells also abolished the increased adhesion on laminin. Both normal and transformed S115 cells express alpha-3 and alpha-6 integrin subunits together with beta-1 but in transformed S115 cells, unlike in control and syndecan-transfected S115 cells, all integrin subunits reveal higher molecular weight due to additional complex-type N-linked oligo-saccharides. Thus, the loss of syndecan expression, enhanced glycosylation of integrin subunits and increased adhesion on laminin are all connected to steroid-induced transformation of S115 cells. Furthermore, simultaneous changes in syndecan and integrin expression suggest coordinated regulation. We therefore suggest that both families of matrix receptors, syndecans and integrins, are involved in the maintenance of epithelial behavior by influencing the capacity of cells to recognize and bind matrix, organize their cytoskeletal elements and maintain epithelial phenotype.

X 523 PATTERNED DISTRIBUTION OF TENASCIN AND PROTEOGLYCANS DURING HUMAN HAIR FOLLICLE MORPHOGENESIS, Elizabeth D. Kaplan and Karen A. Holbrook, Department of Biological Structure, School of Medicine, University of Washington, Seattle, WA 98195

Hair follicle morphogenesis is an epithelial-mesenchymal interaction which involves cell signalling, migration, proliferation and differentiation. Molecules of the extracellular matrix (ECM) have a functional role in regulating these cellular activities. We have examined the distribution of ECM molecules tenascin, chondroitin-6-sulfate proteoglycan (C6SPG) and chondroitin-4-sulfate proteoglycan (C4SPG) in basement membrane and hair follicle-related mesenchymal cells using immunohistochemical probes and confocal microscopy. Tenascin is visible first in the basement membrane underlying epidermal condensations of presumptive hair follicles. It continues to be expressed preferentially in follicle-associated basement membrane throughout morphogenesis, but at an intermediate stage of development (hair peg stage), the basement membrane at the base of the follicle appears less immunoreactive. There is strong expression of tenascin by mesenchymal cells along the sides of the follicle compared with weak staining of matrix in the presumptive dermal papilla. From the hair peg stage to the time of bulb formation, the interfollicular dermal matrix is transiently tenascin immunoreactive. At later stages, the dermal papilla becomes strongly positive for tenascin, while dermal matrix levels diminish. C6SPG shows a basement membrane localization similar to that of tenascin and is present in basal keratinocytes. C4SPG is observed early in development in a continuous distribution along the dermal-epidermal junction. It diminishes gradually during follicle morphogenesis and thus does not appear to correlate with this event. Our data reveal changes in tenascin and C6SPG matrix composition and distribution during different stages of hair follicle morphogenesis. Differential expression of these molecules may reflect distinct mesenchymal cell populations, or changing epithelial-mesenchymal interactions with progressive development. Work supported by HD17664 and AR07019 from the PHS.

X 524 FIBROBLAST-MEDIATED REGULATION OF INTEGRIN EXPRESSION AND DIFFERENTIATION IN TRANSFORMED HUMAN EPIDERMAL CELLS. Pritinder Kaur & *William G.Carter, IMVS, Adelaide, Australia & *Fred Hutchinson Cancer Research Center, Seattle, USA.

Human papilloma virus transformed human foreskin keratinocytes (FEPE1L-8 cells) were characterized for changes in integrin expression and function in relation to differentiation. In monolayer cultures, there were no qualitative changes in the expression of the integrins α1β1, α2β1, α3β1, α5β1, α6β4 and β1. Further these receptors were functional in mediating specific ligand interactions. Organotypic cultures (OCs) which provide an in vitro model system for the ordered stratification and differentiation of normal keratinocytes (HFK) were used to study the regulation of integrins and various epidermal markers in HFK and FEPE1L-8 cells. OCs were prepared by plating keratinocytes on a collagen gel containing primary human fibroblasts (HFFs), and then raising the gel to an air-medium interface. Unlike HFKs, FEPE1L-8s exhibited a) disorganised stratification and limited differentiation capacity b) invasion into the collagen gel c) overexpression of α3β1, and underexpression of α2β1 and α6β4 integrins. Ordered stratification and spatial regulation of integrin expression could be induced in the FEPE1L-8s by substituting Swiss 3T3 fibroblasts in the collagen gel. Data from cultures grown in the absence of fibroblasts indicate that the HFFs induce the FEPE1L-8 cells to invade into the collagen gel. We conclude that stromal cells play an important role in regulating the a) differential expression of integrins, b) normal stratification and differentiation, and c) invasive behaviour of epidermal cells.

X 525 A PUTATIVE CELLULAR ADHESION MOLECULE INVOLVED IN ADHESION BETWEEN CARCINOMA CELLS AND HEPATOCYTES, Hans Kemperman, Yvonne Wijnands and Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

We use TA3/Ha murine mammary carcinoma cells which readily form liver metastases upon intraportal injection. These cells grow in suspension, do not attach to culture plates and liver endothelium but do adhere to cultered hepatocytes.

Fab fragments prepared from polyclonal rabbit antibodies directed against TA3 plasma membranes inhibited adhesion of TA3 cells to hepatocytes up to 70 %.

Earlier work suggested that a 195 kD surface protein was one of the proteins involved.

The 195 kD protein was purified under denaturing conditions by preparative SDS-PAGE and a polyclonal antiserum was made. Antibodies isolated from this serum did not inhibit TA3-hepatocyte adhesion.

Immunoprecipitation yielded a pattern of bands that was quite similar to that observed with antibodies against the $\alpha_6\beta_4$ integrin. Sequential immunoprecipitation established that the 195 kD protein is in fact the β_4 subunit. FACS analysis showed poor reaction with intact cells, indicating that most antibodies were directed against the very long cytoplasmic domain. We used this polyclonal serum to purify the native $\alpha_6\beta_4$ complex, and affinity-purified antibodies from the original inhibitory anti-TA3 serum on this complex. These antibodies precipitated the $\alpha_6\beta_4$ complex and reacted with intact cells (shown by FACS analysis). Fab fragments of these antibodies inhibited TA3-hepatocyte adhesion to 36 %, suggesting that the $\alpha_6\beta_4$ integrin is involved in this adhesion.

X 526 PROVOCATION OF KERATINOCYTE INTEGRINS STIMULATES CELL DIFFERENTIATION AND BASEMENT MEMBRANE COLLAGENASE EXPRESSION, Leeni Koivisto, Tuula Salo, Jyrki Heino, and Hannu Larjava, Universities of Turku and Oulu, Finland

Integrins of β1 family are present in the basal cell layer of mucosal epithelium. In the process of keratinocyte differentiation β1 integrins disappear and are no longer present in the upper layers of the keratinized epithelium. Basal cells have to detach themselves from the basement membrane prior to their differentiation occurs. This process probably involves proteolytic enzymes such as basement membrane collagenase (92 kDa type IV collagenase). In the present study we have experimentally provocated β1 integrins in keratinocytes and studied the expression of a differentiation marker (involucrin) and 92 kDa type IV collagenase. Provocation was performed by treating the cells in low (0.15 mM) or high (1.2 mM) calcium conditions with a functional antibody against β1 integrin subunit. Expression of involucrin was studied by Northern hybridization and immunostaining, and type IV collagenase by zymography.

Untreated cells expressed α2β1, α3β1, and α5β1 integrins which were found to disappear in areas of stratification in cultures switched for high calcium conditions for up to 8 days. Cells treated with antibody against β1 integrin subunit were found to express elevated level of involucrin mRNA. Involucrin positive cells were also more abundant in the treated cultures. Antibodies against α3 and β1 but not against α2 subunit also stimulated the expression of 92 kDa type IV collagenase. The results suggest that cell differentiation of human mucosal keratinocytes can be regulated by β1 integrins. This process also involves the induction of type IV collagenase expression, an essential phenomenon for keratinocyte detachment prior to the vertical movement. Supported by the Academy of Finland and the Juselius Foundation.

X 527 *Abstract Withdrawn*

X 528 A ROLE FOR VLA-4/VCAM-1 IN MUSCLE DIFFERENTIATION, Glenn D. Rosen[1], Rhonda LaChance[1], Joshua Sanes[2], and Douglas C. Dean[1], Departments of Internal Medicine and Cell Biology[1] and Department of Anatomy[2], Washington University School of Medicine, St. Louis, MO 63110

Interaction between the VLA 4 integrin receptor and its counter receptor VCAM-1 mediates cell-cell interactions that are critical for maturation and function of immune cells. We found previously that the VLA-4 promoter contains E-boxes, which are important for muscle-specific expression of other genes. This led us to examine expression of VLA-4/VCAM-1 in developing mouse muscle. We found that both proteins are present in embryonic muscle but their spacial and temporal pattern of expression differs. VLA-4 is present on embryonic myotubes and VCAM-1 is present on a population of intra-muscular mononuclear cells; expression of both proteins dissipates perinatally. Consistent with these patterns, VCAM-1 is expressed on myoblasts in culture but its expression subsides soon after myoblast fusion. In contrast, VLA-4 is not expressed in myoblasts but its expression is activated soon after cells fuse into myotubes. Antibodies to VLA-4 or VCAM-1 inhibited myotube formation, suggesting that the interaction between VCAM-1 on myoblasts and VLA-4 on myotubes serves to attract myoblasts to the growing myotube. Finally, we examined mechanisms that control expression of VLA-4 and VCAM-1 during muscle differentiation. We show that expression of both genes is regulated at the level of the promoter and we are currently identifying elements that are important for this control.

X 529 EXPRESSION OF INTEGRINS DURING WOUND HEALING, Hannu Larjava, Tuula Salo, Randall H. Kramer and Jyrki Heino, University of Turku, Finland, and University of California, San Francisco

Integrins mediate keratinocyte adhesion to extracellular matrix, to each others, and they are able to control the differentiation of keratinocytes. In healing wounds, keratinocytes migrate through fibrin-fibronectin rich matrix in a process essentially depending on integrins. In the present study we have investigated the expression of integrins in the migrating epithelium in human wounds. Frozen sections of experimental wound tissue (protocol approved by the Ethical Committee of the University of Turku) from day 1, 3, and 7 were used for immunohistochemical detection of various integrins and their ligands. Resting and migrating epithelium expressed the following integrin polypeptides: $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, $\beta 1$, and $\beta 4$. Expression of $\beta 1$ integrins were stimulated at the leading edge of the migrating epithelial sheet. Keratinocytes were not found to express $\alpha 1$ or $\alpha 4$. Migrating front of the epithelium expressed, however, αv and $\beta 3$ integrin polypeptides which were not present in the resting epithelium. Laminin, type IV and VII collagens were absent at the basal aspect of the migrating epithelial cells. Fibronectin was, however, localized in a zone under the migrating cells. Epiligrin, a newly discovered ligand of $\alpha 3\beta 1$ and possibly $\alpha 6\beta 4$, was interestingly present also at the basal surface of the migrating keratinocytes.

In summary, during wound healing the expression of $\beta 1$ integrins is enhanced. In addition, novel αv and $\beta 3$ integrin polypeptides appear at the leading edge suggesting an active role as wound specific receptors. Epiligrin together with fibronectin could function as putative ligands for $\beta 1$ integrins in the wound provisional matrix. Supported by the Academy of Finland and the Juselius Foundation.

X 530 IDENTIFICATION AND EXPRESSION OF BOVINE INTEGRIN BETA-1 SUBUNIT GENES IN PREIMPLANTATION EMBRYOS. Leslie A. MacLaren and Alan G. Wildeman, Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario N1G2W1

The bovine blastocyst hatches on or about day 10 of gestation and the free-floating embryo elongates rapidly in the lumen until implantation begins about day 18. To characterize extracellular matrix receptor gene expression during this transitional period, preliminary experiments were conducted using the polymerase chain reaction (PCR) to amplify cDNAs generated from reverse-transcribed RNA extracted from individual, whole day-17 bovine embryos. Degenerate oligonucleotide primers designed to recognize integrin beta-subunit sequences conserved between subfamilies, as well as between species, were constructed from published information. An amplified cDNA fragment (317 bp) exhibited a high degree of sequence similarity, 85%, with the human integrin beta-1 chain. Additional PCR using primers specific for the beta-1 subunit sequences yielded a larger cDNA that included the 317-base pair fragment and was the length expected for the homologue of the human integrin beta-1 chain. Northern hybridization indicated that the 317-base pair cDNA hybridized with total cellular RNA extracted from a day-17 bovine embryo, but not adult bovine skeletal muscle. PCR using oligonucleotide primers recognizing the 317-base pair cDNA and subsequent blot analysis demonstrated that this gene is transcribed throughout the day-13 to day-21 period of bovine embryo development.

X 531 SILENCERS AND CYTOKINE-RESPONSIVE ENHANCERS CONTROL THE PATTERN OF VASCULAR CELL ADHESION MOLECULE-1 (VCAM-1) EXPRESSION, Jay J. McQuillan, Michael F. Iademarco, and Douglas C. Dean, Department of Medicine and Cell Biology, Washington Univirsity School of Medicine, St. Louis, Mo 63110

Vascular cell adhesion molecule-1 (VCAM-1) was first identified as a cytokine-inducible protein on the surface of endothelial cells. However, subsequent studies have demonstrated that it is also expressed constitutively on lymphoid dendritic cells, bone marrow fibroblasts, and some tissue macrophages. It is the ligand or counter receptor for the integrin receptor very late activation antigen-4 (VLA-4). This receptor-ligand pair mediates adhesion of lymphocytes and monocytes, which both express VLA 4, to cells that express VCAM-1. As a first step in analyzing the molecular events that control the pattern of VCAM-1 expression, we have cloned and begun characterization of the promoter for this gene. Our results indicate that silencers between positions -1.641 kb and -288 bp normally restrict VCAM-1 promoter activity in human umbilical vein endothelial cells (HUVECs). However, treatment with tumor necrosis factor-a (TNF) overcomes the inhibitory effect of these silencers and activates the promoter. Two NFkB sites, which are known to be TNF-responsive in other promoters, are located at positions -77 and -63 bp of the VCAM-1 gene. Deletion of the upstream site eliminated responsiveness, indicating that this site is essential. This activation by TNF is tissue-specific since VCAM-1 promoter constructs containing the NFkB sites were not responsive to TNF in the T-cell line Jurkat, which is known to be TNF-responsive but does not express VCAM-1. These studies suggest that the VCAM-1 promoter will be a useful tool in dissecting mechanisms that control the pattern of VCAM-1 expression.

X 532 THE USE OF A CROSS-LINKING/EXTRACTION TECHNIQUE TO EXAMINE INTEGRINS IN ADHESION PLAQUES AND THEIR RELATED EXTRACELLULAR MATRIX AND CYTOSKELETAL PROTEINS IN MYOGENESIS, A. Sue Menko, Motomi Enomoto, Mindy George-Weinstein and David Boettiger, Department of Microbiology, University of Pennsylvania, Phila., PA 19104

We have developed a technique which allows us to focus on the integrins involved in cell-substrate adhesion and the molecules with which they interact. A non-penetrating cross-linker preserves the interaction of integrins with their extracellular matrix (ECM) ligands and differential detergent extractions leave or remove cytoskeletal proteins. The studies demonstrate that fibronectin is associated with the substrate and is specifically concentrated at adhesion plaques containing both $\alpha 5$ and $\beta 1$ integrin, throughout myogenesis. Laminin is also produced by and associated with the muscle cells throughout their differentiation in culture but it is neither found in the adhesion plaques nor incorporated into the ECM. With this technique we have been able to demonstrate that muscle cells plated on a laminin substrate, proteolytically remove the laminin and incorporate fibronectin into the ECM, within a few hours after plating, whereas a fibronectin substrate remains undigested. In addition, antibodies against $\beta 1$ integrin (CSAT) and fibronectin but not against laminin or vitronectin, inhibit muscle differentiation. The CSAT antibody inhibits myogenesis by binding to $\beta 1$ integrin within the adhesion plaques without completely releasing the interaction between integrin and fibronectin. These studies indicate that $\alpha 5/\beta 1$ integrin and fibronectin must be key players in the signal to initiate myogenic differentiation and in the progressive morphogenesis from a replicating myoblast to a multinucleate myotube.

X 533 INTEGRINS IN THE NORMAL AND HEALING CORNEAL EPITHELIUM, Taru Päällysaho, Timo Tervo, Ismo Virtanen and Kaarina Tervo, Departments of Anatomy and Ophthalmology, University of Helsinki, Helsinki, Finland

The corneal epithelium adheres to the basement membrane and the underlying stroma through the adhesion complex, composed of keratin filaments, hemidesmosome (HD), anchoring filaments, and anchoring fibrils. In response to wounding the HDs dissassemble and the epithelial cells migrate to cover the wounded area. As the migration is complete the HDs segmentally reappear from the wound periphery to the center. The aim of our study was to clarify the role of different integrin heterodimers in the corneal epithelial adhesion. By using monoclonal antibodies against the integrin β ($\beta_{1,3}$, and β_4) and α ($\alpha_{1\text{-}6}$ and α_v) subunits we showed the presence of the β_1, β_4, α_2, α_3, α_6, and α_v subunits in the normal human corneal epithelium. The distribution of the β_4 and α_6 subunits was also studied in normal and healing rabbit corneal epithelium. The $\alpha_6\beta_4$ heterodimer has recently shown to be a component of the HD. In the normal epithelium both β_4 and α_6 were localized in a polarized manner at the basal aspect of the basal cells. In the healing cornea, where the epithelial cells migrate over the bare stroma, the β_4 subunit was absent from the leading edge reappearing in short segments similarly to other components of the adhesion complex. In contrast, the α_6 subunit was present all the way to the migrating tip of the epithelium. Thus, it seems that at the leading edge the α_6 subunit might be associated with the β_1 subunit in contrast with the normal corneal epithelium where it has been reported to be complexed with β_4. The segmental reappearance of the β_4 might suggest that the reformation of the $\alpha_6\beta_4$ heterodimer plays a role in the reassembly of the HD.

X 534 ANALYSIS OF THE ROLE OF THE 14 Kdal SOLUBLE LECTIN DURING MOUSE EMBRYOGENESIS: DEVELOPMENTAL CONSEQUENCES OF GENE DISRUPTION.
Françoise Poirier and Elizabeth J. Robertson. Department of Genetics and Development, Columbia University, New York, NY10032.

Soluble lectins constitute a family of related molecules found in Vertebrates which specifically bind to lactosamine-based structures. Expression of these carbohydrate binding molecules is regulated during development and during differentiation *in vitro*. However, the biological function of soluble lectins remains unclear.

We have examined the pattern of expression of the 14 Kdal lectin during mouse embryogenesis by in situ hybridization and immunohistochemistry. Three major sites of regulation of expression have been found: (i) at the time of implantation, there is a rapid accumulation of the 14Kdal lectin in the cells of the trophectoderm which are going to make contact with the uterine wall and mediate the attachment of the blastocyst, (ii) in the somites, elevated levels of the 14 Kdal lectin are localized in the myotome suggesting a role at the earliest stages of myogenesis, (iii) the distribution of the 14Kdal lectin is also very highly restricted in the central nervous system.

In order to assess the role(s) of this lectin, we have introduced a null mutation in the gene by homologous recombination in ES cells. Cells carrying the targeted allele were injected into blastocysts and we obtained animals transmitting the mutation. The phenotype of homozygotes lacking the gene encoding the 14 Kdal lectin will be presented.

X 535 A NEW INTEGRIN HETERODIMER, $\alpha_v\beta_6$ IN HUMAN PANCREATIC CARCINOMA CELLS. Carla Rozzo, #Guido Gaietta, * Richard N. Tamura and # Vito Quaranta. Pediatric Oncology Research Laboratory, G. Gaslini Institute, 16148 Genova, Italy. # Dept. of Immunology, IMM8, The Scripps Research Institute, La Jolla, CA 92037 and * Telios Pharmaceutical Inc., San Diego, CA 92037.

We recently described the molecular cloning of a new integrin subunit, β_6 (Sheppard et al., 1990). We have produced antisera against two synthetic peptides from the sequence of the β_6 cytoplasmic domain. These antisera recognized, by immunoprecipitation, a heterodimeric complex of $\beta 6$ with a protein resembling an integrin α chain by electrophoretic mobility. These $\alpha\beta_6$ complexes were immunopurified from pancreatic carcinoma cells, and found to be reactive with monoclonal antibodies specific for α_v, but not for other integrin α chain expressed in those cells. Furthermore, by sequential immunoprecipitations, anti-α_v antibodies and anti-β_6 peptides antisera reacted with the same molecular complexes in carcinoma cell lysates. Thus the $\alpha_v\beta_6$ heterodimer is the fifth member of the α_v group of integrins, which includes $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$. By reverse transcription -PCR amplification of mRNA, the distribution of β_6 appeared to be limited to cells of epithelial origin. In addition, $\alpha_v\beta_6$ was immunoprecipitated from several carcinoma cell lines, as well as from normal human keratinocytes. Therefore, the function of $\alpha_v\beta_6$ may be related to the expression of the epithelial phenotype.

X 536 INCREASED CELL SURFACE EXPRESSION OF FUNCTIONAL $\alpha_2\beta_1$ INTEGRIN ACCOMPANIES THE MEGAKARYOCYTIC DIFFERENTIATION OF K562 LEUKEMIC CELLS. Samuel A. Santoro, Scott R. Burger and Sheila Sturgill-Koszycki, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

The pluripotential cell line K562 was employed as a model of inducible integrin expression accompanying cellular differentiation. Differentiation along the megakaryocytic pathway was induced with 40 nM phorbol 12, 13 dibutyrate, whereas differentiation along the erythroid pathway was induced with 120 μM hemin. Megakaryocytic differentiation was associated with a change from the round, non-adherent phenotype of the uninduced cells to a more adherent, partially spread phenotype with increased cell-substrate and cell-cell adhesion. Such changes did not accompany erythroid differentiation. Flow cytometric analysis of integrin and non-integrin cell surface markers revealed that upon megakaryocytic differentiation there was a marked decrease in expression of LeuM1 a myelomonocytic marker; decreased expression of glycophorin, an erythroid marker; increased expression $\alpha_{IIb}\beta_3$ integrin (GP IIb-IIIa); increased expression of the $\alpha_2\beta_1$ integrin cell surface collagen receptor; and decreased expression of the $\alpha_5\beta_1$ integrin fibronectin receptor. The increased expression of $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ was more pronounced in the adherent population than in the non-adherent population of cells induced to differentiate along the megakaryocytic lineage. Increased expression of $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ was specific for megakaryocytic differentiation. When induced to differentiate along the erythroid lineage, the low nearly undetectable level of $\alpha_2\beta_1$ did not rise, and $\alpha_{IIb}\beta_3$ expression decreased to near background level. Phorbol-treated K562 cells, but neither control cells nor hemin-treated cells exhibited the ability to adhere to collagen substrates in the Mg^{2+}-dependent manner characteristic of a process mediated by the $\alpha_2\beta_1$ integrin. A monoclonal antibody directed against the $\alpha_2\beta_1$ integrin specifically inhibited the adhesion to collagen of K562 cells which had been induced to differentiate along the megakaryocytic lineage thus establishing the functional nature of the induced $\alpha_2\beta_1$. K562 cells provide a model which can be used to define the molecular mechanisms controlling regulated integrin expression during cellular differentiation.

X 537 H36 (α7) IS A NOVEL INTEGRIN ALPHA CHAIN THAT IS DEVELOPMENTALLY REGULATED DURING SKELETAL MYOGENESIS. Woo Keun Song, Weigwang Wang and Stephen J. Kaufman, Department of Cell & Structural Biology, University of Illinois, Urbana, Il 61801.

H36 is a 120,000 Da membrane glycoprotein that is expressed in a developmentally regulated fashion during the differentiation of skeletal muscle. H36 cDNA clones were isolated from a λ UniZapXR rat myotube cDNA library and sequenced. The deduced H36 amino acid sequence demonstrates that H36 is a novel integrin alpha chain that shares extensive homology with other alpha integrins that includes: [1] the GFFKR sequence found in all alpha integrins; [2] a single membrane spanning region; [3] conservation of 14 of 18 cysteines; and [4] a protease cleavage site found in the non-I-region integrin alpha chains. The cytoplasmic domain of H36 is unique and additional extensive regions of non-homology futher indicate H36 is distinct from all other alpha chains. In keeping with current nomenclature we designate this alpha chain α7. Immunofluorescence of H36(α7) identifies distinct cells that comprise and distinguish the primary and secondary skeletal muscle lineages. Precursors of primary and secondary myoblasts are present in the 12 day embryonic rat hindlimb bud and do not express H36(α7) or the muscle intermediate filament protein desmin. Primary myoblasts, present from embryonic day 13 to 15, do not express H36(α7) but do express desmin. H36(α7) is first detected during primary myogenesis well after terminal differentiation has begun. In contrast, replicating myoblasts that develop into secondary fibers express both desmin and H36(α7) and at the onset of terminal differentiation, expression of H36(α7) is increased. Northernblots also demonstrate that expression of H36(α7) mRNA is regulated both early in the development of the secondary myogenic lineage and later, during terminal differentiation. H36(α7) is not detected in a variety of cells, including muscle fibroblasts and C3H10T1/2 cells, and it is markedly reduced in developmentally defective and transformed myoblasts. It is in fibers derived from myoblasts obtained by treatment of 10T1/2 cells with azacytidine or transfection with MRF4. Immunoblots and immunofluorescence demonstrate that H36 (α7) chain is, in part, associated with integrin β1, and, that the association of α7 with β1 is developmentally regulated.

X 538 IDENTIFICATION OF MURINE ENDOGLIN. Sylvie St-Jacques, Eichi Ishii, and Michelle Letarte, Division of Immunology and Cancer Research, Hospital for Sick Children and Dept of Immunology, University of Toronto, Toronto, Canada, M5G 1X8.

Endoglin is a membrane glycoprotein predominantly expressed on human endothelial cells and also observed on the syncytiotrophoblast of human term placenta. Endoglin is potentially implicated in RGD mediated cellular adhesion and has recently been identified as a TGF-ß1 binding protein. This growth factor plays an important role in the regulation of adhesion and of cellular proliferation. We now report on the presence of a murine endoglin molecule. Immunofluorescence staining with a polyclonal antiserum to human endoglin showed reactivity with the vascular network in stomach, intestine, heart, muscle, thymus and uterus. In the ovary, the oocytes were intensively stained suggesting the presence of endoglin prior to fertilization. A mouse endothelial cell line (SVEC4-10) was shown by flow cytometry to react with HEC-19, one of 10 mAb to human endoglin. Western blot analysis should confirm the subunit structure of murine endoglin on different tissues and on the endothelial cell line. We have demonstrated by Southern blot analysis that human endoglin cDNA can hybridize to murine genomic DNA. Mouse endoglin cDNA has been generated and amplified from mouse RNA by Reverse Transcriptase and PCR using primers derived from the human sequence. Northern blot analysis with this murine cDNA probe should reveal the quantitative expression of endoglin gene in different tissues. The presence of endoglin in the placenta and on the oocyte, the putative function of this molecule in adhesion, and its interaction with TGFß1 suggest a role for endoglin in adhesion and invasion processes which take place during the early development of the embryo and its implantation in the uterus. To address this question, we propose to study by in situ hybridization the expression of murine endoglin gene during peri-implantation events.

X 539 **Coordinated expression of ECM-degrading proteinases and their inhibitors regulates functional status of mammary epithelia during involution.** Rabih S. Talhouk*, Mina J. Bissell*, and Zena Werb*. *Lab. Of Radiobiol. and Env. Health, Univ. Of California, San Francisco, CA and *Div. Of Cell And Mol. Biol., LBL, University Of California, Berkeley, CA.

Mammary epithelial cells express β-casein in culture only when in contact with a basement membrane. In the mammary gland secretory epithelia dedifferentiate as the gland traverses from lactation to involution. During this latter stage, activity of extracellular matrix-degrading proteinases (ECM-dp) is highest. We asked whether dedifferentiation and loss of β-casein during involution temporally correlates with increased activity of ECM-dp. Expression of ECM-dp [72 kDa gelatinase, Stromelysin (SL), and tissue plasminogen activator (tPA)] was reciprocal to that of β-casein except for a brief interval between days 3-5 of involution. The expression of ECM-dp and β-casein positively correlated when levels of ECM-dp inhibitors (TIMP and PAI-1) were at their peak. These results suggested that epithelial cells maintained β-casein expression as long as ECM-dp activities were low, or inhibited by their respective inhibitors. To test this, two approaches were considered: First, one of the glands of lactating mice was sealed by cauterization thus delaying involution due to prolonged lactational stimuli. Expression of β-casein, SL, and TIMP was determined up to 7 days post sealing. β-Casein mRNA levels were abundant in sealed glands and were similar to that in lactating glands of the same mice. The expression of SL and TIMP mRNA was only detected in sealed but not lactating glands, independent of lactational stimuli. Furthermore, compared to an involuting gland, levels of SL mRNA in the sealed glands were lower while those of TIMP were higher. In the second approach, slow release pellets containing human recombinant TIMP were surgically implanted in the gland during involution when endogenous TIMP levels were declining. Exogenous TIMP delayed regression of alveoli in a dose dependant manner. Alveolar structures in glands implanted with high levels of TIMP (5 and 10 μg) were larger and showed morphological evidence of secretory activity. The above demonstrate that a critical balance between ECM-dp and inhibitors modulates cell function *in vivo*, presumably by regulation of cell/ECM interactions.

X 540 Changes in the distribution and expression of integrin in human corneas with age. V. Trinkaus-Randall, M. Tong, K. Svoboda and A. Cornell-Bell*. Boston Univ Sch. Med. Boston, MA 02118 and *Yale Univ Med Sch New Haven CT 06510

The localization and distribution of integrin subunits was examined with a Bio-Rad MRC 600 confocal laser scanning microscope (CLSM). To date we have examined 25 normal corneas from individuals ranging in age from 4 wk to 94 y. The corneas were frozen, 6 micron sections were cut and double stained with GoH3 indirectly conjugated to FITC and antibodies to either the $beta_4$ subunit or laminin conjugated to Texas Red. $Beta_4$ was present in a continuous pattern along the basal lamina at all 3 age groups. Projections into Bowman's membrane were not detected. $Alpha_6$ was present in a continuous pattern with projections into Bowman's membrane in the middle age group; however in corneas from older individuals the subunit was most intense at the projections. This is shown using 3D reconstructions. The images were merged for the 2 subunits and rotated around the Z axis and overlapped except for the projections. Transmission electron microscopy revealed an intact basal lamina. While no difference was detected with age on a microscope equipped with epifluoresence, dramatic differences in distribution were depicted using CLSM. To determine whether the antigens were present after culture, cells were cultured and examined using immunofluoresence and non isotopic in situ hybridization. While cells from a 41 y person retained the expression of the integrin subunits, cells from a cornea of an 80 y individual did not. In situ hybridization is being conducted to determine whether the expression is a reflection of a large number of cells or of a subpopulation of cells. Supported by NIH grant EY06000 to VT-R and Mather's Foundation grant to AC-B.

X 541 DOWNREGULATION OF TENASCIN EXPRESSION BY ANTIPROGESTINS. Günter Vollmer, Horst Michna*, Kirsten Ebert, and Rudolf Knuppen. Institut für Biochemische Endokrinolgie, Medizinische Universität, Ratzeburger Allee 160, D-2400 Lübeck, Germany. • Schering AG, Müllerstr. 170-178, D-1000 Berlin, Germany.

Studies in hormonally dependent growing tissues of breast and endometrium suggested that expression of tenascin parallels the progression of normal or malignant proliferative alterations of the tissue. However, so far it was unknown whether or not this process is reversible, e.g. if induction of differentiation of tumor cells down regulates tenascin expression. Recently, it had been demonstrated that antiprogestins were potent tumor inhibitors of DMBA- or NMU-induced rat mammary tumors. The mechanism of growth inhibition was believed to be a progesterone receptor mediated induction of terminal differentiation of tumor cells. With the study presented here we addressed the question if terminal differentiation downregulates tenascin expression. With comparative immunolocalization of tenascin in sections of untreated DMBA-induced tumors and hormonally treated tumors we got the following results: 1. Ovariectomy, anti-estrogen (tamoxifen)-, and antiprogestin treatment reduced tumor growth roughly to the same extend. 2. Tenascin-like immunoreactivity was always localized in the extracellular space of the stroma. 3. The entire extracellular space of the stromal mesenchyme was decorated by tenascin immunoreactivity in cases of untreated control tumors. 4. Both ovariectomy and antiestrogen treatment resulted in an increase of the arbitrarily judged staining intensity. 5. Antiprogestin induced inhibition of tumor growth primarily induced a pronounced formation of basal lamina-like structures, to which tenascin immunoreactivity was confined. Secondarily, areas within the tumor became detectable, which were built up by apparently secretory active cells. In these areas of the tumor we failed to immunolocalize tenascin. These results provide further evidence that expression of tenascin reflects malignant proliferative alterations of adult tissues, whereas its down regulation is due to differentiation of the tissue. Additionally, evidence is provided that the mechanism of tumor inhibition by antiprogestins indeed is induction of terminal differentiation of tumor cells.

X 542 GENETIC ANALYSIS OF INTEGRIN FUNCTION IN MICE, Joy T. Yang, Helen Rayburn and Richard O. Hynes, Howard Hughes Medical Institute and Center for Cancer Research, Department of Biology, MIT, Cambridge, MA 02139

To examine the *in vivo* function of $\alpha5\beta1$ integrin, we are presently producing $\alpha5$ integrin-deficient mice by gene targeting techniques, in which disruption of a gene is achieved by homologous recombination of the wild-type gene with a modified gene fragment transfected into embryonic stem cells. In our gene targeting vector, we replaced a DNA fragment containing the translational initiation site in the first exon of the $\alpha5$ integrin gene with a neomycin resistance gene to ensure a complete disruption of the gene. In addition, the thymidine kinase gene of HSV-1 was included in the construct for double selection. We have generated five independent embryonic stem cell lines, in which one of the $\alpha5$ alleles has been disrupted. Double selection enriched the targeted clones by 4 fold, which gives a targeting frequency of 1/164. Chimaeras have been generated from one line, and additional chimaeras are being produced with independent lines. Once we obtain germline chimaeras, the phenotypes of the heterozygous and homozygous offspring will be analyzed. In parallel, we are also generating mutations in the $\alpha4$ integrin gene using similar methods.

X 543 Novel POU-domain transcription factor, Lyc-1 expressed in thymic epithelial cell, Kazunori Yukawa, Teruhito Yasui, Akihito Yamamoto, Takashi Shiku, Hitoshi Kikutani and Tadamitsu Kishimoto, Department of Immunology, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan

POU-domain transcription factors are known to be powerful developmental regulators which specify organ development and cell phenotypes. In order to clarify the roles of POU-domain transcription factors in T cell development, we cloned a novel gene named Lyc-1 which is expressed only in thymus in the hemato-lymphoid system. The amino acid sequence of POU-specific domain of Lyc-1 is identical to those of Oct-1 and Oct-2. However, within POU-homeodomain, 14 out of 60 amino acids are different between Lyc-1 and Oct-2. No homologies were found in linker sequences. Lyc-1 is an octamer binding protein as recombinant protein specifically binds to octamer sequence. By RNase protection assay, Lyc-1 is found to be expressed in thymic stroma. Other tissues such as skin and stomach also express Lyc-1. In situ hybridization indicates that cortical epithelial cells of thymus express Lyc-1. Lyc-1 might be involved in early T cell development by transactivating the genes of some growth factors or cell surface molecules such as integrins which support the differentiation of immature thymocytes. Alternatively, Lyc-1 might be a master key regulator of epidermal development originated from primitive ectoderm. We are now trying to elucidate the role of Lyc-1 in T cell development or embryonic development by overexpressing Lyc-1 in several cell lines, producing transgenic animals and performing gene targeting.

X 544 REGULATION OF INTEGRIN EXPRESSION DURING MEGAKARYOCYTIC DIFFERENTIATION OF K562 LEUKEMIA CELLS. Mary M. Zutter, Alan M. Fong, Hannah R. Krigman, and Samuel A. Santoro, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.
Pluripotent K562 cells have been employed to define mechanisms controlling integrin expression during cellular differentiation. Megakaryocytic differentiation of K562 cells with phorbol 12, 13, dibutyrate is accompanied by increased cell surface expression of the $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ (GP IIb-IIIa) integrins. Analysis of total cellular RNA revealed that upon megakaryocytic differentiation, the level of the 8 kb α_2 mRNA increased markedly from the undetectable level present in the uninduced cells with a time course similar to that of $\alpha_2\beta_1$ cell surface protein. In contrast to the marked increase in steady state level of α_2 mRNA observed upon differentiation, the level of the 4.2 kb β_1 mRNA did not change. Thus $\alpha_2\beta_1$ protein expression appears to be controlled by the level of α_2 mRNA. Expression of the GP IIb-IIIa complex is regulated differently. GP IIIa mRNA was undetectable in the uninduced population but was significantly increased following induction. GP IIb mRNA was detectable at low level prior to induction, but at increased level following differentiation. Thus GP IIb-IIIa protein expression appears to reflect the levels of mRNA of both integrin subunits. The $t_{1/2}$ of α_2 mRNA was relatively short, 4 1/2 hr., in contrast to the $t_{1/2}$ of β_1 mRNA which was greater than 24 hr. The t 1/2 of GP IIb mRNA was approximately 8 hr. The $t_{1/2}$ of GP IIIa mRNA was 12 hr. Nuclear run off experiments were performed to assess the transcriptional activity of the α_2, β_1, IIb, and IIIa integrin genes in uninduced K562 cells and in cells which had been induced to differentiate along the megakaryocytic pathway. The experiments revealed a 20-fold increase in transcription of the α_2 gene upon differentiation, but no change in the low rate of transcription of the β_1 gene. Transcription of the IIb and IIIa genes was also markedly increased. Thus, the increase in $\alpha_2\beta_1$ protein which accompanies the megakaryocytic differentiation of K562 cells is a consequence of the increased steady state level of α_2 mRNA due to transcriptional activation of the α_2 gene. The long-lived β_1 mRNA does not appear to be tightly regulated. In contrast, increased GP IIb-IIIa protein appears due to increased steady-stae levels of both GP IIb and GP IIIa mRNA as a consequence of transcriptional activation of both integrin genes.

Late Abstracts

CHARACTERIZATION OF THE BETA1 INTEGRIN GENE PROMOTER. Piero Cervella, Emilio Hirsch, Cristina Pastore, Lorenzo Silengo and Fiorella Altruda.
Department of Genetics, Biology and Medical Chemistry, University of Torino, Torino, Italy.
We have identifiyed the promoter sequence of the human beta1 gene. This region reveals several interesting features characteristic of housekeeping genes: it lacks a tipical TATA or CAAT box, but it contains potential binding sites for transcription factors Sp1 and AP1. Consistent with these characteristics, the gene contains multiple transcription initiation sites. A DNA fragment of 1000 bp encompassing all the transcription initiation sites shows strong promoter activity when linked to a heterologous reporter gene in transfection assays. Since TGF-beta increases the steady-state RNA level of beta1 integrin in several cell lines tested, we have examinated whether the chimeric gene can be activated in DNA transfection experiments by treatment of cells with TGF-beta. This activation is shown to be mediated by specific sequences located 400 bp upstream to the promoter region. Our data show that, using DNA transfection, TGF-beta acts at trancriptional level by stimulating the activity of the human beta1 promoter. Detailed analysis of this promoter region should provide insight into the molecular events regulating expression of the beta1 gene.
In order to study the temporal and spatial pattern of expression of this gene, transgenic mice expressing the enzyme beta-galattosidase under the control of the beta1 promoter were obtained. The analysis of the beta-galattosidase activity in the embryo is in progress. Supported by grant from CNR, progetto Finalizzato Ingegneria Genetica.

TNFalpha DOWNREGULATES THE EXPRESSION OF THE INTEGRIN ALPHA6/BETA1 ON HUMAN ENDOTHELIAL CELLS. Paola Defilippi, Paola Rossino, Lorenzo Silengo and Guido Tarone, Department of Genetics, Biology and Medical Chemistry, University of Torino, 10126 Torino, Italy.
Cell surface receptors for the extracellular matrix (integrins) mediate the interaction between the endothelial cells and the basal membrane. Endothelial cells explanted from human umbilical vein (HEC) express a large panel of integrins on their surface, including receptors for fibronectin, laminin, collagens and vitronectin. In this paper we show that the treatment with the tumor necrosis factor alpha, TNFalpha, downregulates the expression of the laminin receptor alpha6/beta1 integrin in cultured HEC. The treatment with TNFalpha for more than 15 hours decreases the level of expression of the alpha6/beta1 complex at the cell surface at the 20% of the control value. The downregulation of the alpha6/beta1 integrin is due to a decreased expression of the alpha6 subunit, while the beta1 integrin subunit remains constant. The rate of synthesis of the alpha6 subunit is specifically decreased in treated cells. Northern blot analysis shows that the decreased level of synthesis of alpha6 subunit is due to the downregulation of alpha6 mRNA in TNFalpha treated HEC. We also found that in addition to TNFalpha, interleukin 1 beta (IL-1beta) is able to decrease the expression of the alpha6/beta1 integrin on HEC. We have previously shown that treatment of umbilical vein endothelial cells with TNFalpha induces the expression of the integrin alpha1/beta1 (Defilippi et al., J.Cell Biol., 114, 855-863, 1991), and that a combined treatment with TNFalpha and IFNgamma downregulates the expression of the vitronectin receptor alphav/beta3 (Defilippi et al., J.Biol.Chem., 266, 7683-7645, 1991) These data indicate that TNFalpha regulates the expression of specific integrin complexes on the surface of endothelial cells. This event may play an important role during inflammatory processes. (Supported by a grant of the CNR Progetto Finalizzato BBST).

VLA-2 IS THE INTEGRIN USED AS A COLLAGEN RECEPTOR BY LEUKOCYTES. Joanna Harvey, Rachel Goldman, Nancy Hogg, Macrophage Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX UK.

The β_1 type integrins, VLA-1, VLA-2 and VLA-3 expressed on a number of cell types are known to have specificity for collagen. However, leukocyte VLA receptors (excepting platelets) have not been tested for collagen binding. Further, both VLA-1, VLA-2, and all three β_2 integrins, LFA-1 (CD11a), CR3 (CD11b) and p150,95 (CD11c) contain an I domain which has sequence homology with certain collagen binding proteins. Thus it was speculated that the I domain may have a role in collagen binding. In this study it was found that soluble collagen receptor from T cell lysates, consisting of a heterodimeric complex of 160/130kD, was immunoprecipitated only by a β_1 specific mAb. Of the β_1 receptors on both cultured T cells and freshly isolated PBMC only VLA-2 was able to bind collagen. This finding was supported by the fact that only β_1 and VLA-2α chain specific mabs were able to block the binding of cultured T cells to collagen type 1. These data provide evidence that the β_2 integrins do not bind collagen despite the presence of an I domain and that the collagen receptor on leukocytes is VLA-2.

CLONING AND FEATURES OF THE HML-1 AND PEYER'S PATCH-SPECIFIC LYMPHOCYTE HOMING RECEPTORS, Geoffrey W. Krissansen, Qian Yuan, Wei-Meng Jiang, Euphemia Leung, Paul Mead, Cristin Print, Daniel Hollander and James D. Watson. Department of Molecular Medicine, School of Medicine, University of Auckland, New Zealand.

We cloned the newest integrin β subunit, β_7, and showed that four cysteine-rich repeat sequences in β_7 were homologous to repeats in other integrin β subunits, to domain III of the laminin B chains, and to EGF-like repeat sequences. Expression of β_7 was leukocyte restricted, like its closest family member, β_2. Sequences encoded by cDNA for mouse β_7 were 87% identical with human β_7. The human β_7 gene mapped to chromosome 12q 13.13 and the mouse homologue to the distal part of chromosome 15. The gene coding for the β_7 subunit spans about 11kb and is composed of at least 15 exons. An N-terminal region of 13 contiguous amino acids of mouse β_7 showed identity with the N-terminus of the 120kDa subunit of the M290 antigen, a surface molecule found highly expressed on mouse intraepithelial lymphocytes (IEL). The β_7 integrin expressed on human lymphoblasts was found to be structurally and immunologically related to the human IEL antigen, HML-1. A recent report by Kilshaw and Murant [Eur.J.Immunol. 21:2591 (1991)] has shown that β_7 is present in mouse on most lymphocytes in association with α_4, rather than αM290. $\alpha_4\beta_7$ was shown to be identical to LPAM-1 ($\alpha_4\beta_p$), the Peyer's patch lymphocyte homing receptor. The cloning of the common $\beta_7(\beta_p)$ subunit of the LPAM-1, HML-1 and M290 receptors provides an essential link in understanding how the intestinal mucosal immune system is formed and regulated.

CORRELATION OF ADHESION MOLECULE EXPRESSION AND CYTOKINE RELEASE BY HUMAN EPIDERMAL KERATINOCYTES, Tatiana M. Oberyszyn and Fredika M. Robertson. Department of Surgery, The Ohio State University College of Medicine, Columbus, OH 43210.

Infiltration of leukocytes is a hallmark of cutaneous inflammation. Localization and retention of leukocytes occurs through the expression and binding of integrin molecules to ligands present on both epidermal keratinocytes and dermal endothelial cells. One such adhesion molecule present on the surface of resident epithelial cells is intercellular adhesion molecule-1 (ICAM-1). To examine the potential role of keratinocytes in regulating inflammatory responses, the effect of a protein kinase C specific inflammatory stimulus, 12-0-tetradecanoylphorbol-13-acetate (TPA), on expression of ICAM-1 by cultured human epidermal keratinocytes was examined. Following exposure to TPA (24h; 100 ng/ml), cultured human epidermal keratinocyte expression of ICAM-1 was 61% over that of untreated cells, as measured by analytical flow cytometry and ICAM-1 specific antibody binding. Expression of ICAM-1 remained at similar levels at other time intervals examined (48-96 h) but did not increase over that observed at 24 h. In order to identify signals that may be responsible for migration of leukocytes to a local site of inflammation, keratinocyte-derived soluble cytokine levels were examined. Human epidermal keratinocytes simultaneously expressed ICAM-1 in response to TPA exposure, and released 250-850 pg/ml Interleukin-8, 18-120 pg/ml IL-1-alpha and 50-230 pg/ml tumor necrosis factor-alpha, as measured by ELISA. Adhesion molecule expression coupled with cytokine release suggests that keratinocytes play an important regulatory role in the cutaneous inflammatory response.

THE CD9-PROXIMAL ADHESION SIGNAL IS Na^+-DEPENDENT AND SENSITIVE TO INHIBITORS OF Na^+/Ca^{2+}, AND Na^+/H^+ EXCHANGE, Andrew R.E.Shaw, Edward J. Cragoe, and Lee S. Griffith, Department of Medicine, University of Alberta, Edmonton, Alberta, T6G 1Z2

CD9 is a cell surface glycoprotein associating with the integrin GPIIb/IIIa. We recently reported that $F(ab')_2$ fragments of the anti-CD9 MAb 50H.19 immobilized on polystyrene latex beads induce a platelet aggregation signal. We report here that platelet aggregation initiated by bead-immobilized $F(ab')_2$ fragments of mAb 50H.19 is proportional to the concentration of external Na^+. The basis of the dependency upon Na^+ was investigated using pharmacological inhibitors of Na^+ transport. The aggregation was not affected by the Na^+ channel blocker phenamil, or by ouabain, and veratridine, inhibitors of the Na^+/K^+ ATPase, when employed at concentrations 1 to 2 x their respective K_i. Aggregation was insensitive to staurosporine excluding the involvement of pathways under the regulation of protein kinase C. However, phenamil at the K_i for Na^+/Ca^{2+} exchange, amiloride, and the amiloride analogs HMA, and EIPA at concentrations known to inhibit either Na^+/Ca^{2+} exchange or Na^+/H^+ exchange, as well as the highly specific Na^+/Ca^{2+} antiport inhibitor, CBDMB completely abrogated CD9 induced aggregation. CBDMB did not influence thrombin, or ADP mediated aggregation. We conclude that CD9-induced platelet aggregation has a unique dependency upon Na^+ which does not involve a conventional Na^+ channel, or the Na^+/K^+ ATPase, but is sensitive to inhibitors of Na^+/Ca^{2+} and Na^+/H^+ exchange.

REGULATION OF THE INTEGRIN EXPRESSION AND NEURITE ELONGATION IN DIFFERENTIATING NEURONAL CELL LINES.

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We have previoulsy shown (Exp. Cell Res. 189, 100, 1990) that NGF induces increased expression of the laminin receptor, integrin alpha1/beta1 in the rat neuronal cell line PC12. The binding specificity of alpha1/beta1 integrin for the P1 laminin domain, as demonstrated by affinity chromatography, correlates with the preference of NGF-treated PC12 cells for the P1 laminin domain as neurite promoting substrate. Up-regulation of the alpha1/beta1 laminin receptor also occurs during retinoic acid (RA)-induced differentiation of human neuroblastoma cell lines (SY5Y, IMR32 and SK-N-BE). RA induces a dramatic neurite elongation response in these cells, while other agents, including NGF, CNTF, insulin and PMA, are only poorly effective. None of the latter four agents changed integrin expression in these cell lines. Pulse chase experiments indicated that the synthesis of the alpha1 subunit was specifically increased by RA, while the levels of the beta1 and of the alpha3, a second integrin alpha subunit present in SY5Y cells, were unchanged. Increased expression of the alpha1/beta1 integrin correlates with increased neurite response to laminin. In neurite extension assay on laminin coated substratum about 40% of the undifferentiated SY5Y cells extended processes with mean length of 20μm. After RA differentiation, 94% of cells had processes with mean length of about 40μm. Neurite extension in RA-treated cells was more efficient on laminin than on fibronectin or collagen type I, and was inhibited with beta1 integrin antibodies on all three substrate. In conclusion, these data show that neuronal differentiation is accompanied by a selective up-regulation of alpha1/beta1 in both rat and human neuronal cell lines. Alpha1/beta1 is a receptor for the P1 domain of laminin. P1 domain promotes neurite extension in PC12 cells and is more active than the E8 domain. (supported by a grant of the CNR, P.F. BBST).