BIOLOGY OF PHYSICOCHEMICAL INTERACTIONS AT THE CELL SURFACE

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Poster Sessions

Biology of Physicochemical Interactions at the Cell Surface

Keynote Addresses (Joint)

P 001 PHYSICOCHEMISTRY OF RECEPTOR-MEDIATED CELL FUNCTIONS, Douglas A. Lauffenburger, Departments of Chemical Engineering and Cell & Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Understanding cell functions such as proliferation, adhesion, and migration will require, in addition to identification of molecular components involved, elucidation of the physicochemical mechanisms by which these components operate as an integrated, dynamic system. Regulation of such functions by receptor/ligand interactions depends on parameters characterizing physical and chemical properties of these interactions, and resulting cell behavior can be strongly influenced by quantitative parameter values. Despite the current emphasis on receptor/ligand binding affinity as a central property governing signalling for cell function, a number of additional parameters are often important in determining the response. Among these parameters are included kinetic rate constants for receptor/ligand binding, coupling, and trafficking processes along with mechanical strengths and compliances of receptor/ligand bonds and receptor/cytoskeleton linkages, as well as molecular concentrations. This talk will present a series of examples demonstrating how physicochemical properties of receptor/ligand interactions beyond simple binding affinities are crucial in a variety of cell functions, and how quantitative analysis of these properties can lead to increased mechanistic insights. Since the quantitative parameter values can be altered by genetic or pharmacologic means, an ability to predict consequent effects on cell behavior may be of biotechnological use.

Cell Contact-Dependent Signalling

P 002 **REGULATION OF TYROSINE PHOSPHORYLATION BY THE PLATELET INTEGRIN, $\alpha_{IIb}\beta_3$,** J.S. Brugge[1], E.A. Clark[1], L. Lipfert[1,2], B. Haimovich[3], M.H. Ginsberg[4], J.E.B. Fox[5], S.S. Shattil[2], [1]ARIAD Pharmaceuticals, Inc., Cambridge, MA 02139. [2]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104. [3]Department of Surgery, Univeristy of Medicine and Dentistry of New Jersey, New Brunswick, NJ 08903. [4]Scripps Research Institute, La Jolla, CA. [5]Childrens Hospital Oakland Research Institute, Oakland, CA 94609.

We have previously shown that fibrinogen binding to its integrin receptor, $\alpha_{IIb}\beta_3$, is required for thrombin-induced tyrosine phosphorylation of multiple platelet proteins. The platelet system has been valuable in dissecting the intracellular signaling events that transduce integrin-regulated changes in cell behavior. We have found that $\alpha_{IIb}\beta_3$ regulated tyrosine phosphorylation could be separated into two distinct events: 1) the phosphorylation of p140 and several proteins of M_r 50-72 Kd (p50-72), which is induced by dimerization of integrin receptors with fibrinogen, and 2) the phosphorylation of p95/97 and the protein tyrosine kinase, $p125^{FAK}$, which is dependent on fibrinogen-induced platelet aggregation and a second costimulatory event. The induction of tyrosine phosphorylation of all of these proteins was inhibited in cytochalasin D treated platelets, suggesting that actin-dependent cytoskeletal complexes may couple the integrins with tyrosine kinases and their substrates. At least three classes of tyrosine protein kinases appear to participate in these events: $p125^{FAK}$, which is activated following platelet aggregation; $pp60^{src}$, which is activated independent of $\alpha_{IIb}\beta_3$ but redistributes to integrin-regulated cytoskeletal complexes after platelet aggregation, and Syk, which is phosphorylated on tyrosine following dimerization of $\alpha_{IIb}\beta_3$ by fibrinogen.

P 003 *Abstract Withdrawn*

P 004 GROWTH FACTORS, FOCAL ADHESIONS AND MEMBRANE RUFFLING. Anne Ridley[1], Christine Ellis[2], Annette Self[3], Hugh Paterson[2] and Alan Hall[3]. [1]Ludwig Institute for Cancer Research, University College School of Medicine Branch, 91 Riding House Street, London W1P 8BT, [2]Institute of Cancer Research, Chester Beatty Laboratories, London, and [3]MRC Laboratory of Molecular Cell Biology, University College London, U. K.

One of the earliest cellular responses to many extracellular factors is reorganization of the actin cytoskeleton. In Swiss 3T3 cells, growth factors such as lysophosphatidic acid (LPA) rapidly induce the formation of focal adhesions and actin stress fibres, while PDGF and EGF primarily stimulate the accumulation of polymerized actin at the plasma membrane forming membrane ruffles. Recent evidence has implicated the Ras-related proteins Rho and Rac as key signal transducers in these responses: Rho is required for growth factor-induced stress fibre formation, whereas membrane ruffling is dependent on Rac proteins. Several proteins that act as GTPase activating proteins (GAPs) for Rho-related proteins have been identified, including RhoGAP, Bcr, p190, 3BP-1 and n-chimaerin, and these could act either as targets or down-regulators of Rho or Rac function. We have investigated the specificity of three of these GAPs, Bcr, p190 and RhoGAP, for different members of the Rho family, using the GAP domains of each protein. In vitro, p190 has a striking preference for Rho as a substrate, and when microinjected into Swiss 3T3 cells it inhibits stress fibre formation but not membrane ruffling induced by growth factors. BcrGAP accelerates the GTPase activity of Rac but not Rho in vitro, and specifically inhibits membrane ruffling in vivo. Finally, RhoGAP has a marked preference for the Rho-related protein G25K/CDC42 in vitro, but also inhibits Rho-mediated responses in vivo. These results suggest that p190, Bcr and RhoGAP may play specific roles in signalling pathways through different Rho family members.

The mechanisms underlying Rho-mediated stress fibre formation were further investigated by analysing the role of other signals known to be activated by LPA. Neither activation of PK-C, increased intracellular Ca^{2+}, decreased cAMP levels or Ras activation appear to mediate stress fibre formation. Instead, a genistein-sensitive protein kinase is required for both LPA- and Rho-induced stress fibre formation and for the clustering of phosphotyrosine-containing proteins at focal adhesions. These results imply that a tyrosine kinase lies downstream of Rho in this signal transduction pathway.

Basic Principles: Control of Tissue Development (Joint)

P 005 EXTRACELLULAR MATRIX ORGANIZATION AND CELL SIGNALING DURING WOUND REPAIR, Frederick Grinnell, Ying-Chun Lin, and June He, Dept Cell Biology and Neurosci, Univ Texas Southwestern Med Sch, Dallas, TX 75235

At the end of wound healing, cells switch from a proliferative to non-proliferative phenotype and begin to regress. We studied the possibility that this switch is regulated by mechanical factors. Compared to cells in mechanically stressed collagen matrices, fibroblasts in mechanically relaxed matrices showed a decreased proliferation response to platelet derived growth factor (PDGF). This change was found to depend on decreased capacity of PDGF receptors to autophosphorylate. When mechanically stressed collagen matrices were switched to mechanically relaxed conditions, we observed rapid inactivation of PDGF receptors along with transient ectocytosis of annexin-containing vesicles and release of cell surface fibronectin. Under these conditions, cytoplasmic cyclic AMP (cAMP) increased 10-20 fold in 10 min, and there was a 3 fold elevation of protein kinase A activity. The increase in cAMP depended on stimulation of adenylyl cyclase rather than inhibition of phosphodiesterase, and arachidonic acid (AA) was found to be an upstream component of the signaling pathway. Phorbol activation of protein kinase C (PKC) enhanced the cAMP response. On the other hand, down-regulation of PKC by overnight phorbol treatment blocked the signaling pathway upstream of AA. Finally, extracellular Ca^{2+} was required for AA release and subsequent signaling events. These data suggest that mechanical interactions between cells and their surrounding matrix can regulate cell proliferation by modulating the activity of growth factor receptors. Switching fibroblasts from mechanically stressed to relaxed conditions activates a Ca^{2+} dependent, PKC regulated, adenylyl cyclase signaling pathway.

P 006 SIGNAL TRANSDUCTION BY EXTRACELLULAR MATRIX UTILIZES BOTH CELL SHAPE-DEPENDENT AND CELL SHAPE-INDEPENDENT PROCESSES, Calvin Roskelley and Mina J. Bissell, Lawrence Berkeley Laboratory, Berkeley CA, 94720.

The differentiated state of the mammary epithelium is exquisitely sensitive to changes that occur within the glandular microenvironment. A critical component of this microenvironment is the extracellular matrix (ECM). In three-dimensional culture, mouse mammary epithelial cells undergo complex morphogenetic re-arrangements and they begin to differentiate over a period of days in the presence of ECM and lactogenic hormones. On the other hand, when cell monolayers were first primed with ECM and then treated with prolactin, synthesis of the milk protein ß-casein was initiated within hours. Thus, the latter experimental protocol allowed us to examine the functionally important aspects of cell-ECM interactions in isolation. ECM-priming was associated with a concomitant cellular rounding and clustering which led us to ask two specific questions: 1) Are changes in cell shape necessary for differentiation? 2) Are additional signals initiated by cell-ECM contact required? Agents that prevent cell rounding or force cell spreading, such as phorbol esters and fibronectin, strongly inhibited differentiation when added during ECM-priming. However, when the cells were forced to round and cluster independently, using the non-adhesive substratum polyhema, this inhibition was abrogated. Conversely, genestein treatment during priming had no effect on morphology but differentiation was inhibited, and this inhibition could not be reversed by polyhema. These data suggest that both cell shape-dependent changes, which rely on an intact protein kinase C pathway, and cell shape-independent changes, which are sensitive to alterations in tyrosine phosphorylation, are critical for transducing signals emanating from the ECM. As we have already shown that $ß_1$-containing integrin receptors are involved, the challenge ahead will be to identify the precise molecular nature of these pathways. (Supported by the Office of Health and Environmental Research of the Department of Energy and NCI Canada).

P 007 ROLE OF ADHESIVE RECEPTORS IN REGULATING KERATINOCYTE DIFFERENTIATION AND STRATIFICATION, Fiona M. Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London. WC2A 3PX, UK.

Within the epidermis proliferation takes place in the basal cell layer that is attached to the basement membrane. Basal cells that become committed to differentiate withdraw from the cell cycle, detach from the basement membrane and move into the first suprabasal layer. Thereafter cells undergo terminal differentiation as they move through the suprabasal layers until finally they are shed from the surface of the skin.

Adhesion of basal keratinocytes to the basement membrane is mediated by receptors of the integrin family. Studies with cultured keratinocytes have shown that the β_1 integrins are subject to both transcriptional and post-translational regulation (1,2) and that different subpopulations of basal cells can be distinguished on the basis of integrin expression and function. Keratinocytes with characteristics of stem cells express the highest levels of β_1 integrins, while transit amplifying cells (which have a lower self-renewal capacity and higher probability of differentiating) express lower integrin levels (3). The β_1 integrins on the surface of basal cells that are committed to differentiate become inactive and are no longer capable of binding to basement membrane proteins; this helps ensure that committed cells are selectively expelled from the basal layer. On initiation of terminal differentiation, transcription of integrin genes is inhibited, N-linked glycosylation and transport of immature integrin subunits is inhibited and receptors on the cell surface are endocytosed.

Integrins not only mediate keratinocyte adhesion and stratification; they also regulate the onset of terminal differentiation. Plating keratinocytes on a substrate that inhibits spreading acts as a stimulus for differentiation. Differentiation is regulated by the proportion of β_1 integrins on the cell surface that have bound ligand rather than by cell shape per se (4, 5).The differentiation-regulatory function of integrins is independent of receptor clustering and cytoskeleton assembly (6). The aim of current experiments is to identify the intracellular signalling pathways by which integrin-ligand binding regulates keratinocyte behaviour.

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Physicochemical Factors in Cell Contact, Adhesion and Migration

P 008 TRACTION FORCES EXERTED BY LOCOMOTING FISH KERATOCYTES ON ELASTIC SUBSTRATA, Ken Jacobson,*# Tim Oliver,* Akira Ishihara,* and Juliet Lee*, *Department of Cell Biology and Anatomy and #Lineberger Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-7090.

Cell locomotion involves a complex, dynamic interplay between protrusion, retraction, and adhesion. Adhesions allow the transmission of traction forces from the cytoskeleton to the substratum which cells require in order to translocate. Little is known about the sizes and organization of traction forces required for cell locomotion. We have developed a new assay in which the traction forces exerted by rapidly moving fish keratocytes are reported by two dimensional displacements of small beads embedded in a thin, flexible rubber substratum. Traction forces are observed at the rear half of the cell, oriented perpendicular to the cell edge. The largest of these forces in estimated to be about 2 mdynes. No traction forces were found to be associated with the rapidly extending front edge of the cell. In a separate assay, individual keratocytes were able to exert a maximum force of approximately 5 mdynes based on flexure of calibrated microneedles required to block or redirect keratocyte movement. The size and distribution of traction forces registered in the bead displacement assay depends on the varying ratios of lamellar contractility relative to cell-substratum adhesion strength at different positions along the cell margin. In the front half of the lamella, strong contractility is resisted by large close contacts visualized by interference reflection microscopy. Near the equator of the cell, large traction forces are observed where lamellar contractility is balanced by cell-substratum adhesion strength. At the rear edge, lamellar contractility exceeds cell-substratum adhesion leading to maximum retraction. Thus, the pattern of traction forces generated by locomoting keratocytes can be related to the movement of the whole cell. Supported by NIH GM 35325.

P 009 EXTRACELLULAR REGULATION OF CELL INTERACTIONS BY POLYSIALIC ACID, Urs Rutishauser, Departments of Genetics and Neurosicences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The encounter of two cells in the formation of stable adhesion represents a highly constrained process in which receptor-receptor apposition must be precisely matched in the presence of a myriad of other molecular and physical entities. Under these conditions, it might be anticipated that the regulated presence of a large, bulky substance would have a dramatic impact on the efficiency of such encounters. In our studies of the neural cell adhesion molecule (NCAM), we have observed that the developmentally-regulated addition to NCAM of a very large carbohydrate, polysialic acid (PSA), has inhibitory effects on a wide variety of cell-cell and cell-matrix interactions that do not directly involve adhesion mediated by NCAM itself. These observations, along with physical studies of PSA and the demonstration that its presence can increase membrane-membrane distances, have prompted the hypothesis that NCAM and PSA constitute a push/pull mechanism for regulation of cell interactions. Studies of PSA function in the live embryo have shown that PSA expression is directly associated with many important developmental processes, including the guidance and targeting of axons, migration of muscle cells, and activity-dependent plasticity in the architecture of neural tissues.

P 010 MATHEMATICAL MODELS OF RECEPTOR-MEDIATED DIRECTED CELL MIGRATION, Robert T. Tranquillo[1], Richard B. Dickinson[1], and [2]Wolfgang Alt, Chemical Engineering & Materials Science, Univ. of Minnesota, [2]Theoretical Biology, Univ. of Bonn

How the composition of extracellular matrix and the spatial distribution of its components influence the extent and direction of cell locomotion via receptor-specific adhesion is of great interest in embryogenesis and tissue engineering. There is evidence that gradients of adhesion molecules influence the direction of cell migration (haptotaxis). Whether these responses are the result of an intracellular signaling process or simply a passive adhesion differential is presently unknown. A stochastic model of an idealized cell undergoing adhesion receptor-mediated movement is developed to ascertain the consequences of statistical fluctuations in binding of adhesion receptors[1], where an increase in bound adhesion receptors is assumed to lead to a proportionate increase in traction exerted on the substratum (the passive adhesion differential hypothesis). It is used to address how the magnitude of adhesion may affect cell movement indices such as mean cell speed and persistence time, where movement is modeled as rigid body translation and rotation determined by the pattern of traction. Also considered is how different modes of directed movement (e.g. biased turning and direction-dependent speed) contribute to haptotaxis in a gradient. Model predictions of biased movement are given explicitly in terms of parameters which determine the quantity and spatial distribution of cell surface adhesion receptors as well as in terms of the concentration gradient of adhesion ligand.

Another model which makes explicit account for the generation of force within the cortical actin network is developed to ascertain the consequences of statistical fluctuations in binding of chemotactic receptors, where an increase in bound chemotactic receptors is assumed to lead to an increase in the regulated level of F-actin. In contrast to the haptotaxis model, however, adhesion leading to transmission of force (i.e. traction) is not yet accounted for in terms of the dynamic distribution of bound adhesion receptors. However, model simulations in uniform concentrations and spatial gradients of chemotactic factors yield insight into the synergistic dynamics of chemotactic-modulation of F-actin kinetics, rearward cortical flow, and adhesion-mediated cell turning.

[1]Dickinson, R. B. and R. T. Tranquillo, "A Stochastic Model for Cell Random Motility and Haptotaxis Based on Adhesion Receptor Binding Fluctuations," J. Math. Biol. 31(6), 563-600 (1993).

Physics of Molecular Interactions at the Cell Surface

P 011 PHYSICS OF CELL ADHESION, Gilles Kaplanski[1], Anne Pierres[2] and Pierre Bongrand[2], [1]Service de Médecine Interne, Hôpital de Sainte-Marguerite and [2]Laboratoire d'Immunologie, Hôpital de Sainte-Marguerite, BP 29, 13277 Marseille Cedex 09, France.

Contact between many cells and adhesive surfaces may result in the formation of strong bonds resisting disrupting forces as high as several tens of nanonewton. This process involves several phenomena such as lateral redistribution of surface molecules, reorganization of submembranar cytoskeletal elements, surface deformations in order to form extensive contact areas. These events are dependent on cell activation which may be difficult to model with a biophysical approach. However, the critical step of adhesion may be the formation of the first few bonds following contact formation. It is suggested that this may be described with a three-parameter model, including i) the probability of formation of a first bond (k1+), ii) the dissociation rate of the first bond ((k1-) and iii) the probability of additional bond formation (kb+).

In the present report, we describe an experimental approach allowing quantitative determination of these parameters. This consists of monitoring the motion of cells driven against adhesive surfaces with low shear flow (applied wall shear rate is a few second-1) and monitoring the formation and duration of transient cell stops with computer-assisted videomicroscopy. Parameter k1+ is related to arrest frequency, parameters k1- and kb+ may be derived from the distribution of arrest durations. It is suggested that k1+ is dependent on the structure of cell surface protrusions and extracellular matrix elements, whereas k1- is dependent on the rigidity of cell-surface interaction molecules more than the cell size and mass. Experimental data concerning interaction between human granulocytes and endothelial cell monolayers (1), adhesion of murine lymphoid cells to antibody-coated surfaces(2) and uptake of flowing opsonized erythrocytes by adherent macrophages (3) are presented. It is concluded that both specific bonds and ill-defined nonspecific interaction play a substantial role in initiating contact, but adhesive bonds play a major role in initiating durable adhesion.

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(2) - O. Tissot, A. Pierres and P. Bongrand. Monitoring the formation of individual cell-surface bonds under low shear hydrodynamic flow. Life Science Advances, in press (1993).
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P 012 SUBMICROSCOPIC PHYSICAL ACTIONS IN CELL-CELL ADHESION, Evan Evans, University of British Columbia, Departments of Pathology and Physics, Vancouver, Canada.

Adhesive interactions are central processes in numerous biological functions like tissue assembly (development) and identification and removal of alien organisms in immune defense. The consequences of cell adhesion are more than surface bonding. Adhesion usually initiates signalling pathways to activate and modulate the cell interior so that it becomes more/less rigid, motive, or strongly linked to the substrate structure. Although the phenomenological features of cell adhesion are well recognized, little is known about the submicroscopic physical actions that implement important these biological functions. Key questions to be addressed in physical tests are: Is adhesive strength governed by ligand-receptor bonds or linkages to cytoskeletal structure? Does receptor anchoring to the cytoskeletal structure change with binding of different ligands to the receptor? What are the mechanisms involved when cells separate from substrates? Even if thermodynamic properties of receptor-ligand binding are known, the density of attachments local to an adhesive contact and the origins of molecular detachment remain indeterminant in traditional tests of adhesion strength.

The major task is to establish methods to image bonding and rupture of a single molecular complex under extremely weak forces. Futhermore, submicroscopic actions at a biological interface are expected to exhibit strong fluctuations and marginal determinism. Therefore, experiments must be configured to take advantage of fluctuations. Study of fluctuations provides immediate insight into the range and statistics of single bond formation, the structural properties and modulation of receptor-cytoskeletal linkages, and the dynamics and failure of single attachments under stress. Design of a physical method to probe single bonds and expose stochastic features of submicroscopic interactions between cell interfaces will be described along with state of the art technology being developed to implement this new realm of study.

P 013 FORCES AND MOLECULAR MECHANISMS OF RECEPTOR-MEDIATED INTERACTIONS BETWEEN MODEL MEMBRANES, Deborah Leckband, Dept. of Chemical Engineering, State University of New York at Buffalo.

The forces governing the non contact interactions (D>4Å) between soluble and membrane associated proteins and ligand functionalized model membranes were directly measured with the surface forces apparatus. Using homogeneously oriented membrane immobilized monolayers of streptavidin and anti-fluorescyl IgG Fab' fragments, we directly quantified long-ranged receptor-ligand attractive forces that purportedly enhance binding to biotin and fluorescein derivatized membrane surfaces, respectively. The constituent intermolecular forces determining overall force *versus* distance profiles were identified for the two receptor-ligand pairs. The kinetics of protein association with receptor functionalized membranes are governed by both electrostatic and non electrostatic forces.

The molecular mechanisms determining the strength of receptor-mediated membrane adhesion were elucidated with membrane immobilized streptavidin and biotin as a model cell surface ligands and receptors, respectively. By direct force measurements, we investigated the influence of membrane fluidity, biotin surface density, and the intrinsic receptor-ligand affinity on the mechanism of receptor-mediated membrane detachment and on the inter membrane adhesive strength. The adhesive strength varies linearly with the number of streptavidin-biotin cross-bridges, indicating that streptavidin does not bind cooperatively. Receptor-mediated adhesive strength decreased by nearly an order of magnitude the membrane fluidity was decreased by reducing the temperature below the lipid chain melting temperature. The reduced adhesion is attributed to reduced molecular surface mobility. Restricted mobility inhibits molecular reorientations which align receptor-ligand pairs on opposing membranes. Fewer bonds subsequently form, resulting in attenuation of the total adhesion.

The adhesive strength is also governed by the force associated with the cohesive failure mechanism and not necessarily by the intrinsic bond energy. Using a homologous series of biotinyl-lipid analogs with streptavidin affinities ranging from $<10^4M^{-1}$ - $10^{15}M^{-1}$, we identified the molecular mechanism of cross-bridge failure as a function of the receptor-ligand affinity. At affinities $>10^4M^{-1}$, failure occurred by pull-out of the lipid anchor rather than by specific bond rupture. These findings are consistent with hypothesis that the tensile force to detach membranes is determined by the energy gradient of the detachment mechanism. The total adhesive force is therefore $F_{ad}=F_c*N_b$ where N_b is the number of bonds, and the critical detachment force $F_c = -\nabla E$ where E is the 'bond' energy. For comparable lipid pull-out and specific bond energies, disruption of short-ranged (ca 4Å) bonds therefore requires substantially greater force than lipid pull-out where the effective bond length is *ca* 14Å-the lipid chain length. Assuming a linear relationship between energy and bond length, we demonstrated that preferential bond rupture occurs when $F_{bond}/F_{lipid} < 1$. These results suggest that the cell adhesive strength will be independent of the intrinsic receptor affinity above a critical low affinity threshold, if membrane receptors not anchored by additional mechanisms such as multiple attachments or cytoskeletal association.

Biomaterials in Tissue Engineering: From Natural to Synthetic (Joint)

P 014 POLYMERS FOR PROMOTING AND RESISTING CELL ADHESION IN CARDIOVASCULAR TISSUE ENGINEERING, Jeffrey A. Hubbell[1,2], Jennifer L. Hill-West[2], Sanghamitra M. Chowdhury[1], Paul D. Drumheller[1], and Marvin J. Slepian[3], [1] Department of Chemical Engineering and [2] Department of Biomedical Engineering, University of Texas, Austin, TX 78712, [3] University Heart Center, University of Arizona - VA Medical Center, Tucson, AZ 85723.

Cell adhesion plays an important role in healing in injured vessels and in artificial vascular grafts. Adhesion proteins, which are constituents of the vessel wall or are adsorbed to the surface of a vascular graft, determine the adhesive events occurring during exposure to blood. Two approaches in the control of cell adhesion are presented, one involving the promotion of cell-type specific adhesion to polymeric vascular graft materials, and the other involving the blockade of platelet adhesion and aggregation upon the surfaces of injured blood vessels. Adhesion peptides that have been chemically or physicochemically incorporated into a polymeric material have been used to promote adhesion of endothelial cells to vascular graft materials. Many examples exist on the incorporation of RGD peptides into polymeric surfaces. Work has also been done with the synthetic laminin peptide YIGSR, for which a platelet receptor has not been identified. This would provide the opportunity to promote the adhesion of blood vessel wall cells to a graft material while avoiding blood platelet adhesion. However, for this to occur, adhesion proteins must not adsorb to the graft surface, which would result in signals for cell adhesion other than the incorporated peptide. Toward this end, copolymers of acrylic acid and monoacrylyl-poly(ethylene glycol 1900) (poly(AA-co-PEG)) were synthesized, with various amounts of acrylic acid and mono-acrylyl-PEG. These polymers were further incorporated into a substrate of crosslinked trimethylolpropane triacrylate (TMPTA) as an interpenetrating polymer network (IPN). A poly(AA-co-PEG) with 85% AA incorporated into an IPN with linear, nonacrylated PEG 4000 (0.2 g poly(AA-co-PEG), 0.2 g PEG, 1 g TMPTA) was found to be highly resistant to fibroblast adhesion with incorporated control RDG peptide, but highly adhesive with immobilized RGD peptide. Materials such as this provide a path to highly specific cell adhesion in the vasculature, where tissue responses may be controlled by the incorporated peptides without complication by potentially adsorbing proteins. Controlling cell adhesion is also important in tissue engineering of the native blood vessels. During the therapeutic process of balloon angioplasty, the vessel wall is damaged and becomes thrombogenic. Thrombosis upon the vessel wall is thought to play a causational role in restenosis, the thickening of the intima by smooth muscle cell migration and matrix secretion. Adherent platelets generate thrombin and release platelet-derived growth factor, which may be involved as stimulatory signals. Direct contact of angioplasty-injured vessels with blood was blocked in rabbits by a degradable hydrogel barrier upon the arterial surface. A prepolymer of PEG 10000 with oligomeric lactic acid extensions at both ends and with acrylate end-caps was interfacially photopolymerized by illumination with green light after staining the vessel lumen surface with a solution of eosin Y. The mean ratio of the cross-sectional area of the intima to media was reduced from 1.7 in untreated animals to 0.3 in treated animals ($p<0.003$) 14 d after injury and treatment, more than 10 days after the disappearance of the hydrogel barrier.

P 015 SYNTHESIS OF POLYMERIC SUBSTRATES AND CHARACTERIZATION OF CELL-POLYMER INTERACTIONS, W.M. Saltzman, J. Belt, W. Dai, C.E. Krewson, T. Livingston, M.R. Parkhurst, and M. Shin, The Johns Hopkins University, Department of Chemical Engineering, 3401 N. Charles Street, Baltimore, MD 21218.

For many decades, polymeric materials have been used as structural supports for cultured cells. Although most contemporary cell culture studies utilize a substrate composed of a synthetic polymer (typically surface-modified polystyrene), no general principles regarding the interactions of vertebrate cells with synthetic polymers have emerged. This situation limits the engineering of tissue replacements, so there is considerable interest in developing new polymers and characterizing their interaction with cells.

The interaction of cells with polymers has been studied at three levels of complexity: i) adhesion and spreading of cells on the surface of a polymer; ii) growth, motility, and orientation of cells on the surface of a polymer ; iii) motility, aggregation, and organization of cells within a polymer gel or matrix. Cell adhesion to polymers has been quantified by using flat, homogeneous polymer films that are purely synthetic [1, 2] or modified by covalent coupling of proteins or peptides [3], carbohydrates [4, 5], or glycolipids [6]. In short term studies, cell adhesion and spreading usually vary predictably with surface chemistry. Changes in cell growth or motility are more subtle and less predictable [2, 7], probably because cells modify the polymer surface during longer periods of observation. Rates of cell motility on a surface can be altered by changing the density of adsorbed extracellular matrix (ECM) molecules [8]. Similarly, rates of cell migration through gels of ECM molecules depend on concentration [9, 10]. Regulation of the composition or isotropy of the gel can result in changes in cell aggregation, process outgrowth [9], or cell orientation. These results have been obtained using gels composed primarily of naturally occuring polymers; the development of synthetic polymer gels that produce this same behavior would be a significant advance.

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P 016 THE INTERACTION OF MAN-MADE SURFACES WITH BIOLOGICAL SYSTEMS. George M. Whitesides, Amit Kumar, Gabriel Lopez, Hans Biebuyck, Paul DiMilla, Ralph Haerter, and Kevin Prime, Department of Chemistry, Harvard University, Cambridge MA 02138.
Self-assembled monolayers (SAMs) of alkanethiolates on gold and silver provide organic surfaces with well-defined structures. The functional groups presented at the solid-liquid interface by these SAMs can be readily controlled and modified by organic synthesis; they can be patterned in two dimensions; they can be made optically opaque or transparent, depending on technique of fabrication. This talk will outline the techniques used to make SAMs, and illustrate the application of appropriately functionalized SAMs to the study of the adsorption of proteins on surfaces, and to the study of the adhesion and control of cell growth by attachment to surfaces.

Kinetic Effects at the Cell Surface

P 017 DYNAMICS OF LIGANDS BINDING TO AND CROSSLINKING OF IMMUNO-RECEPTORS, Barbara Baird,* Byron Goldstein,+ Richard Posner,# Kala Subramanian,* Behnam Hashemi* and David Holowka,* *Cornell Univ, Ithaca NY, +Los Alamos National Lab, Los Alamos NM, #Northern Arizona Univ, Flagstaff AZ.
We are developing systematic methodology for measuring ligand-receptor binding events and relating them to observed cellular responses with the aid of theoretical models. Fluorescence measurements of defined monovalent and multivalent DNP-ligands binding to anti-DNP IgE in solution and IgE-receptor complexes on the surface of RBL mast cells has allowed the determination of kinetic and equilibrium parameters and has revealed features of the crosslinking process and crosslinked states that may be physiologically important. A detailed investigation with the symmetric bivalent ligand $(DCT)_2$-cys showed that the equilibrium distribution of crosslinked aggregates does not correlate with stimulated degranulation and points to the possibility that, for this ligand, crosslinks must break and form continuously to maintain a cellular response. This model is consistent with a surprisingly slow binding and crosslinking process observed with the multivalent ligand $DNP_{17}BSA$. Separate studies with a T cell line showed that the Ca^{2+} response stimulated by anti-TCR attached to cell-size beads is inhibited by competing anti-TCR Fab in solution, providing further support for the importance of continual formation of crosslinks in sustained activation of immune cells.

P 018 PHYSICOCHEMICAL ASPECTS OF LEUKOCYTE INTERACTIONS WITH SURFACES DURING FLOW, Daniel A. Hammer, School of Chemical Engineering, Cornell University, Ithaca, New York, 14853.

Leukocyte adhesion under hydrodynamic flow is required for the proper transit of cells to specific locations within the body during the immune response. We have measured the adhesion of two different leukocyte cells lines under hydrodynamic flow, and shown that they are dynamically very different. In one, we measured the adhesion of bovine neutrophils to LPS-stimulated endothelium under flow. For physiological shear stresses, the rolling velocity is approximately 1 % of the free stream velocity of the fluid, and the velocity of motion exhibits a significant non-zero variance. In the other, we have developed a model system where we measured the adhesion of IgE-sensitized rat basophilic leukemia (RBL) cells to antigen-coated substrates. Our antigen is dinitrophenol (DNP). RBL cells do not roll on DNP-coated substrates for any combination of flow rate, antigen-substrate density, or IgE cell surface density. Instead, these cells move at the free stream hydrodynamic velocity, and suddenly come to a stop; since these cells seem to exist in one of two states, we call this behavior "binary". The RBL cell system allows us to systematically test several fundamental tenets of adhesion; for example, we have shown that higher receptor-ligand reaction rates lead to higher levels of adhesion, and that adhesion under flow is independent of receptor-ligand affinity.

However, a fundamental question remains as to why these two systems, which are so chemically (in receptor-ligand rates and affinities of reaction, ligand density, receptor expression) and morphologically similar (in size, surface roughness, and density) display dissimilar types of adhesion. We show that differences in receptor-ligand bond mechanics (molecular stiffness, reactive compliance) or cell microstructural mechanics (microvilli stiffness and viscosity) can explain the differences in these two systems, using a modified version of a method for simulating cell adhesion under flow developed in our laboratory (Hammer and Apte, 1991). The mechanical coefficients we find necessary (such as bond reactive compliance and microvilli stiffness) to give these two distinct types of adhesion have yet to be verified independently.

Basic Principles: Mechanical Forces as Regulators of Biological Activity (Joint)

P 019 ENDOTHELIAL GENE REGULATION BY BIOMECHANICAL FORCES, Nitzan Resnick[1], Bauer E. Sumpio[2], and Michael A. Gimbrone Jr.[1], Vascular Research Division, Dept. of Pathology, Brigham and Women Hospital Boston, MA, and Vascular Surgery division, Yale University, New Haven,CT.

Vascular endothelial cells are exposed to hemodynamic forces generated by flowing blood. These forces, which include wall shear stress and cyclic strain, have been shown both in-vitro and in-vivo to modulate the morphology and function of the endothelium. Recently several groups have demonstrated that, in well defined in-vitro systems both shear stress and cyclic strain can modulate endothelial gene expression. In particular,our group has demonstrated that PDGF-B chain gene transcription is induced by physiologic levels (10 dynes/cm^2)of laminar shear stress. We have defined a region within the PDGF-B chain promoter that is responsible for the induction of the gene by shear stress, and called this the "Shear Stress Responsive Element" (SSRE)[1]. This promoter element binds shear-stress inducible nuclear proteins from endothelial cells, as demonstrated by gel shift analysis. Computer analysis of the promoters of several other endothelial genes that are responsive to shear stress revealed a core sequence within the SSRE that exists in all of them. In recent set of experiments, using gel shift analysis and mutated SSRE probes we have better defined the SSRE showing that indeed the core sequence (GAGACC) is a functional binding site. Moreover, hybrid promoters containing the SV40 promoter (which is not responsive to shear stress) coupled to the SSRE sequence (as taken from the PDGF-B chain promoter), were inducible by shear stress in bovine aortic endothelial cells, thus verifying the fact that the SSRE is both necessary and sufficient for gene induction by laminar shear stress. SSRE probes taken from other shear stress responsive genes formed similar DNA-nuclear protein complexes as observed with the PDGF-B chain probe, suggesting that the SSRE is functional in these genes as well. Since cyclic strain has also been found to modulate endothelial gene expression, we tested the presence of SSRE-binding-proteins in nuclear extracts made from endothelial cells exposed to this hemodynamic force. We have found that the SSRE does bind to nuclear proteins from endothelial cells exposed to cyclic strain (24% strain, 60 cycles/min), that this binding peaks at 30 min. after the exposure of the cells to cyclic strain, is dependent upon the level of strain and on the cell type (endothelial cells vs. smooth muscle cells). These results thus suggest that biomechanical forces act on the endothelium through a common-cell-type-specific mechanism to activate gene transcription. (supported in part by PO1-HL36028.)

[1]Resnick, N., Collins, T., Atkinson, W., Bonthron, D., Dewey C.F. and Gimbrone M.A., Jr. Platelet derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive-element. Proc. Natl. Acad. Sci. USA 90:4591-4595, 1993.

P 020 THE REGULATION OF VASCULAR ENDOTHELIAL BIOLOGY BY FLOW, Gabriel Helmlinger, Olivier Thoumine, Theodore F. Wiesner, and Robert M. Nerem, Bioengineering Center, Georgia Institute of Technology, Atlanta, GA 30332-0405

The biology of vascular endothelial cells (EC) is regulated by a variety of factors including the flow environment in which the endothelium resides. Over the past decade, much has been learned about the influence of flow through cell culture studies. In these a cultured EC monolayer is exposed to a sudden onset of a steady, laminar flow. Although this is not truly a physiologic model of flow, considerable insight has been gained. What we now know is that flow, and the associated shear stress, influences EC shape, its cytoskeletal structure and the resulting mechanical properties of the cell, and the extracellular matrix, including the distribution of focal contact proteins. The ability to proliferate also is affected, with cell proliferation being decreased by flow, and it has been shown that the synthesis and secretion of certain substances by EC is influenced by flow, with this effect extending to the gene expression level. More recent results from our laboratory indicate that EC are influenced differently by the sudden onset of pulsatile flow as compared to steady flow. In these experiments a non-reversing pulsatile flow, a reversing pulsatile flow, and a purely oscillatory flow with a zero mean component have been studied. These all employ a sinusoidal waveform with a 1 Hz frequency. From these studies it is clear that such properties as cell shape, the localization of F-actin, and the extracellular matrix differ depending on the specific pulsatile flow environment. Although the exact mechanisms involved in the recognition of the onset of flow and the associated shear stress are unclear, the elevation of intracellular calcium appears to be an important second messenger in the transduction of the signal. There are slow oscillations present in the flow-induced calcium signal, the frequency of which depends on the composition of the media. Particularly noteworthy is the increase in the frequency of these calcium oscillations for pulsatile flow as compared to steady flow. This alteration in the frequency content of the calcium signal suggests that the temporal nature of this signal may be essential to the information content provided the cell and to the ability of EC to discriminate between different flow environments. The ability to tissue engineer a blood vessel may be dependent on understanding the influence of flow and the mechanical environment imposed, as well as the signaling mechanisms involved.

Receptors that Influence Cell Junctions and Polarity

P 021 DOMAINS IN CELL SURFACE MEMBRANES, Michael Edidin[1], Michael Sheetz[2], and Lisa Hannan[1], [1]The Johns Hopkins University, Baltimore, MD21218 and [2]Duke University, Durham, NC.

Cell surface membranes are patchy. They contain domains whose properties differ from the average properties of a given membrane. We have been interested in two types of membrane domains: domains formed by barriers to lateral mobility of membrane proteins, and domains formed by clustering of membrane lipids and lipid-anchored proteins. The first type of domain was resolved from fluorescence photobleaching recovery (FPR) experiments. Recovery of fluorescence depended upon the area bleached. This is not expected if the membrane is a continuum, but is expected if it is broken into domains which are stable during the time (minutes) of an FPR experiment. Comparison of the lateral mobility of a set of mutant membrane glyco-proteins, using FPR and measurements of the barrier-free-path of these proteins when labeled with colloidal gold particles, shows that the barriers to lateral mobility lie in the cell cytoplasm 2-3 nm below the bilayer surface.

Though not constrained by cytoplasmic barriers, lipid-anchored proteins of polarized epithelial cells appear to form clusters when they are first delivered to the cell surface. These clusters are detected by FPR, and by resonance energy transfer measurements. These results are consistent with observation on the detergent insolubility of mature lipid-anchored proteins and with the hypothesis that polarized delivery of membrane glycolipids and lipid-anchored proteins and cholesterol required their clustering in endomembranes. We have recently explored the requirements for cholesterol and prenylation on clustering and delivery of the model lipid-anchored protein, gD1-DAF in MDCK cells. Cholesterol deprivation and inhibition of cholesterol biosynthesis profoundly depress clustering, as defined by detergent insolubility, and block delivery of gD1-DAF to the cell surface.

P 022 CELL-CELL ADHESION AND DEVELOPMENT OF EPITHELIAL CELL POLARITY, W. James Nelson[1,2], Lindsay Hinck[1,2], Inke Näthke[1], Jackie Papkoff[2,3], and James A. Marrs[1], [1]Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA 94305-5426; [2]Cancer Biology Program, Stanford University, Stanford CA 94305; [3]Sugen Corp., Redwood City, CA 94063.

Cell-cell adhesion is at the top of a molecular cascade of protein interactions that leads to the remodelling of epithelial cell structure and function. Cadherins are a family of calcium-dependent cell adhesion proteins that regulate epithelial cell interactions. The function of cadherins is modulated by a group of cytoplasmic proteins, termed catenins (α-, β-, plakoglobin), that bind to the highly conserved cytoplasmic domain of cadherins. We are interested in the regulation of assembly and function of the catenin/cadherin complex, and the role of cell adhesion in the development of cell surface polarity of epithelial cells. Studies of the assembly of the catenin/cadherin complex show that assembly of α-catenin probably occurs at the cell surface after delivery of β-catenin/cadherin or plakoglobin/cadherin complexes. Assembly of β-catenin appears also to be modulated by expression of Wnt-1. In Drosophila, the homologs of Wnt-1 (*wingless*) and β-catenin (*armadillo*) are segment polarity genes that participate in a signal transduction pathway important for cellular boundary formation in embryonic development, but functional interactions between these proteins are unknown. We find that Wnt-1 expression post-transcriptionally regulates the accumulation of β-catenin and plakoglobin. In addition, binding of β-catenin to cadherin is stabilized, resulting in a concomitant increase in the strength of calcium-dependent cell-cell adhesion. To examine the consequences of cell adhesion on cell organization, we have analyzed the role of cadherins and membrane-cytoskeleton assembly in regulating Na/K-ATPase distribution in kidney and choroid plexus epithelial cells. In polarized epithelial cells the cell surface distribution of the same subunits of Na/K-ATPase may be restricted to either the apical (eg. choroid plexus and retinal pigmented epithelium) or basal-lateral (eg. kidney) membrane domain. Results show that in the kidney, E-cadherin mediated adhesion and induction of membrane-cytoskeleton assembly is sufficient to restrict Na/K-ATPase distribution to the lateral membrane domain. In choroid plexus and retinal pigmented epithelium, Na/K-ATPase and membrane-cytoskeleton proteins are also co-localized indicating that the membrane-cytoskeleton may restrict Na/K-ATPase. However, B-cadherin, the predominant cadherin expressed in the choroid plexus and retinal pigmented epithelium, appears not to have the potential to influence Na/K-ATPase distribution. Interestingly, transfection of E-cadherin, but not B-cadherin, into retinal pigmented epithelial cells and fibroblasts results in co-expression and co-localization of Na/K-ATPase, ankyrin and fodrin at sites of E-cadherin induced cell-cell contacts. Together, these results demonstrate that regulation of E-cadherin expression and function plays critical roles in determining the strength of adhesion between cells and the subsequent reorganization of proteins in the development of epithelial cell surface polarity.

Cell Membrane Dynamics and Compartments

P 023 A ROLE OF MYOSIN II IN THE LOCOMOTION OF *DICTYOSTELIUM* AMOEBAE, Patrick Y. Jay, Carmela Pasternak, and Elliot L. Elson, Washington University Medical School, St. Louis, MO.

The role of conventional myosin (myosin II) in amoeboid locomotion has been a puzzle since it was discovered that mutant *Dictyostelium* cells which entirely lacked myosin II continued to crawl, although at a slower rate than wild type cells (1). Hints about the function of myosin II in these cells were obtained from observing its role in the rearward motion of cell surface particles. One form of rearward transport occurs during the "capping" of crosslinked surface proteins. Although myosin II null cells do not cap (2), there is nevertheless a form of myosin II-independent systematic rearward transport on these cells (3). Detailed measurements showed that the rate of particle transport was similar at the leading edges of both wild type and mutant cells. Near the rear, however, transport was faster in the former than in the latter, suggesting that the contribution of myosin II was exerted preferentially at the rear of the cell. Deformability measurements have also shown a myosin II-dependent differential stiffening at the rear of locomoting *Dictyostelium* cells. These observations led to the hypothesis that myosin II is required for detaching the tail of the cell from its adhesion to the substratum to allow further forward motion. This was tested by measuring the rates of locomotion wild type and of myosin II null mutant amoebae on susbrata of different adhesivities. As predicted by the hypothesis, the locomotion velocities of the mutant cells were more strongly inhibited than those of wild type cells by high substratum adhesiveness. On highly adhesive substrata the mutant cells were almost immobile while the wild type cells continued locomotion at a substantial rate. Hence, on these substrata myosin II is practically indispensible for locomotion. In addition it was observed that on substrata of high adhesiveness, "ultra thin" lamellae formed due to the contractile force generated by myosin at the rear of the wild type cells. These lamellae were not seen on the mutant cells. Taken together these observations confirm that a major function of myosin II in the locomotion of *Dictyostelium* amoebae is to detach the tail from the substrate and thereby permit further forward progress of the cell. These results also emphasize the importance of the strength with which a cell adheres to the substratum in regulating its rate of locomotion.

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2. C. Pasternak, J.A. Spudich, and E.L. Elson, Nature, **341**, 549-551 (1989).
3. P.Y. Jay and E.L. Elson, Nature **356**, 438-440 (1992)

P 024 CELL MIGRATION: ROLE OF GRADED CYTOSKELETAL ATTACHMENTS AND ENDOPLASMIC CONTRACTION.
Michael P. Sheetz and Chaya Nanavati.

The migration of fibroblastic cells in vitro involves the dramatic rearward pulling of material on the dorsal surface whereas the cell pulls itself forward on ventral attachments. In contrast, the leading edge of the lamellipodium extends and individual glycoproteins are seen to move forward to the leading edge. In these phenomena there is no detectable bulk flow of membrane or lipid relative to the cell outline. At the leading edge there is preferential attachment of crosslinked integrins to the cytoskeleton and such cytoskeletal attachments are lost at the rear of the cell (Schmidt et al., J. Cell Biol. in Press). Such graded attachments are essential for the cell to be able to release and migrate forward. In regard to the site of force generation for movement, we postulate that motors at the boundary of the endoplasm and ectoplasm are pulling on the cortical actin. Because the cortical actin is anchored to the external matrix more strongly at the front of the cell than at the rear, contraction by the endoplasmic structure will pull the cell forward. Such a model has important implications for the nature of the glycoprotein attachments to the cytoskeleton and the regional differences in membrane structure.

Receptory Signalling and Activation; Extracellular Matrices and Substrates

P 100 RHO-GDI IS A PHYSIOLOGICAL INHIBITOR OF CHANGES IN CELL MORPHOLOGY.

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Growth factor-treatment or microinjection of the Ras-related proteins-Rac, Rho or Cdc42Hs into serum-starved cells, induces distinct changes in the actin-based cytoskeleton. Rho-GDI prevents the dissociation of GDP from, and inhibits the GTPase activity of, Rac, Rho and Cdc42Hs. Post-translational modifications of these GTP-binding proteins are required for them to interact with Rho-GDI *in vitro*. Furthermore, Rho-GDI is found in complex with Rac, Rho and Cdc42Hs in the cytoplasm of mammalian cells. From these biochemical activities it is difficult to predict the cellular role of Rho-GDI. Rho-GDI has been shown inhibit Rho-mediated cell motility and affects cell morphology. To investigate its cellular role further we have microinjected Swiss 3T3 cells with recombinant Rho-GDI purified from *Escherichia coli*. We will present data showing the effects of Rho-GDI injection on growth-factor induced changes in cell morphology. These data suggest that Rho-GDI is a physiological inhibitor of Rho, Rac and Cdc42Hs signalling pathways.

P 101 THE ROLE OF INTRACELLULAR CA^{2+} IN INTEGRIN- AND CADHERIN- MEDIATED ADHESION OF MDCK CELLS, Brigitte Angres, Michael D. Sjaastad and W. James Nelson, Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305-5426

The coordinate regulation of cell-cell and cell-substratum adhesion in epithelial cells is necessary to maintain the structural and functional integrity of an intact epithelium and to facilitate cell movement in development and wound-healing. Previous studies indicate that these functions are mediated through the association of integrins and cadherins with the actin cytoskeleton and various accessory proteins. In an epithelial model system, MDCK cells, we are investigating the regulation of integrin- and cadherin-mediated adhesion through the cytoskeleton and second messenger systems. The application of a centrifugal force-based adhesion assay was used to analyze very fine-tuned differences in adhesion. Using a RGD peptide as a substrate for integrin-mediated adhesion, a non-linear relationship between peptide density (1-7μg/ml coating concentration) and strength of adhesion indicates a cooperative adhesion mechanism that is presumably facilitated by the association of integrins with the cytoskeleton. Using microscopical imaging and microfluometry we find that the initial cellular contact with RGD-substratum induces large transient increases in intracellular free calcium ($[Ca^{2+}]_i$) from ~ 0.1μM to 3μM in ~90 % of cells. By reducing cytoplasmic Ca^{2+} levels with the Ca^{2+} chelator BAPTA (10μM, 45min.), pretreating cells with the Ca^{2+} channel blocker Ni^{2+} (5mM), or by incubating cells in low Ca^{2+} medium (5μM), $[Ca^{2+}]_i$ transients are reduced by 50-90% and adhesion to RGD is diminished by 48-85%. These results suggest that integrin-mediated Ca^{2+}-signalling not only precedes changes in cell structure, function and differentiation, but also plays a role in the cytoskeletal reorganization leading to a self-enhanced strengthening of cell adhesion. To compare regulative mechanisms in cell-substrate versus cell-cell adhesion similar studies are currently being carried out using the extracellular domain of the cell-cell adhesion molecule E-cadherin or a peptide containing the adhesion-mediating HAV amino acid sequence of cadherins as a substrate. Preliminary results show that a lower percentage of cells responds to the HAV-peptide with equivalent $[Ca^{2+}]_i$ transients as shown for RGD. Furthermore, cell adhesion to the HAV-peptide is considerably weaker, indicated by a 3.4 fold decrease in adhesion to a surface coated with a 4 fold concentrated peptide solution. These studies provide new insights into the mechanism of regulation and strengthening of cell adhesion in epithelial cells.

P 102 INFLAMMATORY REACTIONS INDUCED BY NATURAL AND SYNTHETIC CALCIFIED MATERIAL, Gilbert C. Faure, Bruno Rio, Martin Gonzalvez, Daniel Molé and Marie C. Béné, Laboratoire d'Immunologie & Clinique Traumatologique, Faculté de Médecine, Université de Nancy I, Nancy, France.

Long term clinical tolerance, sudden acute flares, and sometimes spontaneous resolution of inflammatory responses are classical yet ill-understood manifestations of articular and paraarticular crystal deposition diseases in humans. Calcified material spontaneously developing in these diseases is similar to synthetic compounds used as reconstructive biomaterials. We investigated the inflammatory mechanisms and cell interactions in surgically removed samples, using immunofluorescence methods applied to frozen-cut sections of the tissue associated with two types of crystal deposits. Carbonated apatite deposits were present in 40 tendon sheaths collected through endoscopic surgery, and 15 samples of synovial tissue contained apatite or calcium pyrophosphate dihydrate (CPPD). In addition, 5 skin samples were biopsied at controlled intervals during the inflammatory reaction that developed after subcutaneous injection of natural and synthetic apatite in the forearm of a healthy volunteer. FITC monospecific antisera were used to investigate immunoglobulin deposits and complement activation. The degree of inflammation and edema was assessed by staining for fibrinogen. Cells in the vicinity of deposits were identified using monoclonal antibodies directed against lymphocytic and myelomonocytic antigens (CD2, CD3, CD4, CD8, HLA class II, CD14, CD15, CD54, CD9, CD71). Vascularization was assessed after staining for factor VIII. Immune complexes appeared to be involved in none of the tissues studied, but fibrinogen suffusions were detected within nerve fibers in a few samples. C3 deposits were observed among the finely granular apatite material of 10 samples, without clear correlation with clinical symptoms. Cells encountered in 8 tendon sheath samples appeared to be mostly of the macrophagic lineage, activated and expressing the CD4 molecule. Similar cells surrounded CPPD tophi. These cells differed morphologically and phenotypically from the polymorphonuclear cells accumulated around the synthetic and natural injected crystals. Different patterns of inflammatory reactions can thus be induced by natural and synthetic calcified material, possibly depending on the anatomy of the site of deposition or implant.

P 103 OVEREXPRESSION OF N-CHIMAERIN RAC/CDC42 GTPase ACTIVATING DOMAIN (GAP DOMAIN) LEADS TO A REDUCED DIFFERENTIATION POTENTIAL OF NIE-115 NEUROBLASTOMA CELLS

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NIE-115 neuroblastoma cells were stably transfected with cDNAs coding for human and rat n-chimaerin, a protein which has a phorbol-ester binding N-terminal domain and a C-terminal domain with Rac/Cdc42Hs-GTPase (GAP) activity. Expression of a 20 kDa C-terminal (GAP domain-containing) protein, $p20^{nc}$, resulted in cells which displayed a reduced ability to differentiate following DMSO or sodium butyrate treatment, but did not affect differentiation induced by other factors. Furthermore, morphological differentiation was specifically affected by $p20^{nc}$ expression, while other differentiation endpoints such as reduction of DNA synthesis or altered expression of enzymic markers, were not affected. These results implicate Rac and/or Cdc42Hs in pathways affecting neural morphology.

P 104 CROSSLINKING OF ICAM-1 INDUCES EXPRESSION OF THE EARLY ACTIVATION MOLECULE CD69 ON MONONUCLEAR LEUKOCYTES.

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Cell adhesion molecules were first described as accessory molecules, simply to bridge one cell to another. It has recently been shown that some of these molecules also are involved in transmembrane signal transduction. We show that crosslinking of ICAM-1 on the cell membrane with anti-ICAM-1 mAb and $F(ab')_2$ fragments of goat anti-MIgG in the presence of sub-optimal levels of FMLP induces an oxidative burst from human peripheral blood mononuclear cells (PBMC). This oxidative burst is abolished with the depletion of CD14 positive cells (monocytes). Furthermore, the crosslinking of ICAM-1 on PBMC produces a costimulatory signal resulting in upregulation of the early activation molecule CD69. Expression of CD69 was not induced when sICAM-1 was added to compete for the anti-ICAM-1 mAb. However, sICAM-1 had no effect on CD69 upregulation induced by the crosslinking of other cell surface antigens such as CD18. Furthermore, soluble products released from ICAM-1 crosslinked PBMC induced the expression of CD69 on other naive PBMC. The crosslinking of ICAM-1 on the cell surface induces costimulatory activating signals and also results in the release of exogenous products that may induce activation of surrounding cells(as indicated by the upregulation of CD69).

P 105 ACTIVATION OF LFA-1 BY Mg^{++} IS C KINASE-INDEPENDENT, HENCE PROBABLY EXTRACELLULAR,

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The functional affinity of integrins for their cognate ligands, and hence the strengths of the resulting cell adhesions, can be increased or decreased within one minute by receptor-regulated conformational changes not involving a change in integrin density on the cell surface. Many integrins are capable of being activated via protein kinase C (PKC) with phorbol esters (PhE), and PKC may be one physiological pathway of activation. Recently it was reported that the Mg^{++}-dependent leukocyte integrin LFA-1 (CD11a/CD18) can be activated by another means, removal of Ca^{++} (with EGTA) in the presence of ample Mg^{++}. We have confirmed this with a variety of human and mouse T and B cell lines using two assays, homotypic aggregation and heterotypic adhesion of lymphocytes to ICAM-1 transfected cell monolayers. MgEGTA increases adhesion strength by an order of magnitude or more. Near-complete inhibition of MgEGTA-activated adhesion is achieved with antibodies to LFA-1 or ICAM-1. If MgEGTA activates solely by changes in metal cation binding directly to the extracellular domains of LFA-1, the resulting adhesion should be much less dependent on PKC than is PhE-activated adhesion. We report here that MgEGTA-activated adhesion is insensitive to the PKC-selective inhibitor calphostin C (CPC) at concentrations which profoundly inhibit PhE-activated adhesion. Adhesion in MgEGTA plus CPC remains inhibitable by antibodies to LFA-1 or ICAM-1. This is consistent with extracellular activation by MgEGTA, which may be useful in elucidating regulatory mechanisms.

P 106 EFFECTS OF CALCIUM, c-AMP, AND ABA ON THE FLUIDITY OF SPIN LABELED CHARA CELLS, Girma Mitiku and Ambar Jain, Department of Horticulture and Illinois EPR Research Center, University of Illinois, Urbana, IL 61801

Attention has been focused recently on the effects that second messengers and plant harmones exert on plant membrane associated functions[1]. Indeed, Ca^{2+} is known to have multiple effects on model liposome membranes and natural membranes; such effects include changes in permeability and lipid fluidity[2]. An adequate understanding of how second messenger Ca^{2+}, c-AMP (cyclic adenosine monophosphate) and ABA (abscisic acid) regulate various plant membrane associated function require the elucidation of the underlying structural alteration induced by these compounds. The electron spin resonance spectroscopy (ESR) of nitroxide spin label is used in the studies of intracellular environment. The spin label is used to obtained information about fluidity. The comparison is made in the presence and absence of Ca^{2+}, c-AMP and ABA on the viscosity of nitroxide in Chara membranes

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P 107 REDOX SIGNALLING MEDIATED BY THIOREDOXIN AND REQUIREMENT OF AN INTACT REDOX ACTIVE FORM OF THIOREDOXIN. John E. Oblong, Pamela Y. Gasdaska, Margareta Berrgren, and Garth Powis, Arizona Cancer Center, 1515 N. Campbell Ave., Tucson, AZ, 85715

Thioredoxin (Trx) and the flavoenzyme thioredoxin reductase (TR) comprise a ubiquitous redox system. Human Trx (12 kDa) contains the highly conserved active site sequence Trp-Cys-Gly-Pro-Cys-Lys. The suggested intracellular functions of the Trx/TR redox system include reduction of enzymes such as ribonucleotide reductase and regulation of the redox state of transcription factors such as AP-1 and NF-κB. Trx has been shown to be identical to the adult T-cell derived leukemic factor (ADF), an autocrine growth factor. Recombinant human Trx added to the growth media stimulates DNA synthesis and cellular proliferation of cultured cells. In order to better understand the thiol chemistry of the mammalian Trx/TR redox system, mutants of human Trx were produced by site-directed mutagenesis in which the active site cysteine residues were replaced by serine residues individually (C32S and C35S) and in combination (C32S/C35S). All three mutants failed to stimulate cellular proliferation, suggesting that a redox active form of Trx was essential for growth stimulation. C35S and C32S/C35S were found to be competitive inhibitors of the reduction of human Trx by human TR with K_i values of 1.8 and 6.7 μM, respectively. C32S did not inhibit TR due to apparent aggregation of the oxidized species. Examination of all mutant forms of Trx by CD spectroscopy revealed significant differences in the secondary structures when compared to Trx and there were detectable changes in the CD spectra when Trx, C35S, and C32S/C35S were incubated with TR. An 8-mer peptide of the active site sequence was able to function in an insulin reduction assay but failed to stimulate cellular proliferation at concentrations of 100 μM, suggesting that the structural information required for the mitogenic effect is contained in more than just the active site. Furthermore, a similar 8-mer peptide with substitutions of the active site cysteines with serine residues also failed to stimulate proliferation, but was able to compete for reduction of DTNB by TR, with a K_i value of 3.8 mM. Supported by CA 48725 and CA 09213.

P 108 CORRELATION OF STRUCTURE AND FUNCTIONAL ACTIVITY OF THE MEMBRANES OF STAPHYLOCOCCI II. CORRELATION WITH RESISTANCE TO ANTIBIOTICS, Oryabinskaya L.I., Golodok A.F., Vinnikov A.I., Department of Microbiology, State University, Dniepropetrovsk, Ukraine, 320625

Structure functional parametres of cytoplasmatic membranes of various strains of staphylococci that contain plasmids resistant to penicillin, tetracycline, erythromycin, chloramphenicol were studied. The formation of resistence to antibiotics was accompanied by changing protein profile of membrans of cells. The increase of membrane lipids portion and redistribution of their content in side to increasment of cardiolipin, carotinoid pigments, triglycerids fractions were observed. These changes correlated with increase of membrane permeability for nucleotides in 1.4-3.0 times, intensification enzymes function of respiratory chain and also membrane - bound ATP-ase. The diminition of quantity of cytochromes a, b and change of theirs spectral characteristic was noted.

We can suppose according the obtained results that the formation of resistance to antibiotics concerns both structure and functional activity of the staphylococci membranes.

P 109 SIMULTANEOUS CROSSLINKING BY TWO BIVALENT LIGANDS RESULTS IN SYNERGISTIC SIGNALLING OF IgE-RECEPTOR COMPLEXES: EVIDENCE FOR THE IMPORTANCE OF CROSSLINKING DYNAMICS, Richard Posner*, Byron Goldstein+, Kala Subramanian#, David Holowka# and Barbara Baird#. *Northern Arizona University, Flagstaff AZ, +Los Alamos National Laboratory, Los Alamos NM and #Cornell University, Ithaca NY.

We have studied the binding of a symmetric bivalent ligand, $(DCT)_2$-cys, to anti-DNP IgE bound to its high affinity receptor ($Fc\varepsilon RI$) on RBL-2H3 cells. This ligand has been shown to efficiently crosslink IgE but does not trigger significant degranulation responses. Several lines of evidence suggest that this ligand preferentially forms stable cyclic complexes containing two bivalent ligands and two IgE antibodies. In the presence of a dimer forming anti-IgE antibody, $(DCT)_2$-cys is a potent stimulant of degranulation. We have compared the dose response curve for degranulation to simulated IgE aggregation curves, ie., curves that predict the equilibrium IgE aggregate size distribution as a function of $(DCT)_2$-cys concentration. Our results suggest that the equilibrium distribution of aggregates does not determine where maximal degranulation occurs. One possibility suggested by these studies is that crosslinks must break and form continuously in order to maintain a cellular response.

P 110 PHYSICOCHEMICAL STUDIES ON THE INTERACTIONS OF THE WASP VENOM PEPTIDE MASTOPARAN WITH THE MEMBRANE OF LIPID VESICLES, Gerhard Schwarz and Uwe Blochmann, Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland

Mastoparan is a positively charged amphipathic peptide of 14 amino acids, being largely hydrophobic on the N-terminal side up to position 10 whereas the remaining C-terminal part is highly polar. This agent has been found to cause degranulation of mast cells, to mimic receptor functions in signal transduction and to induce channel formation when added to planar lipid bilayers. The latter phenomenon is per se a remarkable one in view of the currently favored channel model featuring a bundle of α-helical peptide chains. The present peptide molecule would not be long enough to span the membrane in such a way.

We generally emphasize a quantitative analysis of any effect caused by membrane-active agents in terms of the portion that is actually associated with the membrane. Accordingly the partitioning between the aqueous and lipid environments is always a fundamental issue.

In the case of mastoparan and similar peptides the bilayer associated molecules exhibit a considerable increase of secondary structure. We have measured the resulting circular dichroism signals of aqueous mastoparan and mastoparan X when titrated with unilamellar vesicles of electrically neutral phospholipids (DOPC and POPC). Using a recently developed theoretical procedure these data were converted into association isotherms ("binding curves", i.e. bound peptide per lipid versus the concentration of free aqueous peptide). In spite of the absence of a net charge in the lipid moiety we have observed that the partition coefficient as well as the conformation of the bound peptide are subject to substantial salt effects. The results are discussed on the basis of a general approach for peptide association with lipid bilayers.

In addition we have established an apparent pore formation as reflected by the efflux of a self-quenching fluorescent marker out of the vesicles. From the time course of marker release the rate of pore formation could be functionally related to the membrane associated peptide content. This is interpreted with regard to the rate limiting reaction steps of the underlying molecular mechanism.

P 111 KINETICS OF CROSSLINKING ANTI-DNP IgE AND ANTI-DNP Fab' IN SOLUTION BY SYMMETRICAL BIVALENT LIGAND, Kala Subramanian*, Richard Posner#, Byron Goldstein+, David Holowka* and Barbara Baird*, *Cornell Univ., Ithaca, NY, #Northern Arizona Univ., Flagstaff, AZ, +Los Alamos National Lab., Los Alamos, NM

Aggregation of immunoglobulin E (IgE) bound to its high affinity receptor $Fc\varepsilon RI$ by multivalent ligand (antigen) leads to activation of mast cells and basophils. We are systematically investigating the features of ligand binding and crosslinking that are critical for this process by analyzing defined monovalent and multivalent ligands binding to IgE in solution and IgE-$Fc\varepsilon RI$ complexes on the cell surface. We previously showed that the symmetrical bivalent dinitrophenyl (DNP) ligand N,N'-bis[[ε-[(2,4-DNP)amino]caproyl]-L-tyrosyl]cystine [$(DCT)_2$-Cys] dissociates from bivalent anti-DNP IgE in solution and on the cell surface with a fast (~ 10^{-2} sec^{-1}) and a slow (~ 10^{-3} sec^{-1}) kinetic component. We have now determined that [$(DCT)_2$-Cys] dissociates from monovalent anti-DNP IgE Fab' in solution with a single rate constant (k_{-1} ~ 10^{-3} sec^{-1}) corresponding to the lower value observed with the bivalent IgE. These results together with equilibrium analyses indicate that the dissociation of singly bound [$(DCT)_2$-Cys] accounts for the slower component and the breaking up of small stable rings corresponds to the faster component. The dissociation of [$(DCT)_2$-Cys] from bivalent [$(Fab')_2$] occurs with a single kinetic component similar to that for the Fab' and different from the biphasic dissociation that occurs with IgE, which suggests that the Fc region of this IgE influences crosslinked ring formation with [$(DCT)_2$-Cys]. To simplify the investigation of complicated binding events on the cell surface we have used hybridoma technology to prepare an intact, bispecific IgE that binds DNP and dansyl in separate Fab combining sites. This will provide a monovalent DNP binding site on the cell surface, similar to Fab fragments in solution and it will allow us to extend our analysis to this physiologically important situation.

P 112 ANALYSIS OF INTRACELLULAR SIGNALING IN TRANSENDOTHELIAL MIGRATION OF TUMOR CELLS. **Noriko Toyama-Sorimachi[1], Wang Lianguo[1], Joji, Ando[2], Takashi, Sakurai[3], Masayuki Miyasaka[1]. [1]Department of Immunology, The Tokyo Metropolitan Institute of Medical Science. [2]Department of Cardiovascular Biochanics, [3]Department of Pharmacology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.**

One of critical steps in tumor metastasis is transmigration of intravascular tumor cells through the endothelial junctions into target tissues. Despite of intense investigation over the last ten years, the complex mechanisms underlying this phenomenon has yet to be elucidated. To understand the intracellular signaling pathway involved in transendothelial migration of tumor cells, we modified an in vitro transmigration assay we established previously (N. Toyama-Sorimachi, et al. Eur. J. Immunol. 23;439 1993) by using human pre-B lymphoma Nalm-6 and mouse cultured endothelial cell line KOP2.16, and investigated effects of var us inhibitors of signal transduction on tumor cell transmigration in this assay. In the absence of inhibitors, Nalm-6 bound to the confluent monolayer of KOP2.16, and migrated through the endothelial cell junctions rapidly. However, pretreatment of Nalm-6 with wortmannin, inhibitor for myosin light chain kinase and PI3 kinase, significantly reduced Nalm-6 transmigration. Herbimycin A, a specific inhibitor against tyrosine kinase, weakly inhibited the transmigration, but genistein and FK506 had no effect. When KOP2.16 was pretreated with wortmannin, inhibition of transmigration was also observed. None of these agents inhibited binding of Nalm-6 to KOP2.16, indicating that transmigration can be regulated independently from adhesion. We also present some results to indicate that opening of inter-endothelial junctions can be initiated by cell adhesion.

P 113 FIBROBLAST-POPULATED COLLAGEN MICROSPHERES: ASSAYS OF CELL TRACTION AND WOUND CONTRACTION, Robert Tranquillo, Bruce Bromberek, Alice Moon, and Victor Barocas, Dept. of Chemical Engineering & Materials Science, Univ. of Minnesota, Minneapolis

Despite the significant understanding that has been obtained from the fibroblast-populated collagen lattice (FPCL) assay about cell traction forces, which underlie contraction of dermal wounds and tissue-equivalents, there is a fundamental limitation in quantitatively characterizing them by simply measuring the compaction of a disk of collagen gel in terms of rate or extent as is typically done. The viscoelastic properties of the gel, for example, are highly dependent on the collagen concentration, so the measured compaction driven by the cell traction forces is directly dependent on the initial collagen concentration. We propose and estimate an objective index of traction force for a variation of the FPCL assay based on a fibroblast-populated collagen microsphere (FPCM). This is accomplished using a mathematical model formulated from continuum-mechanical equations[1] that accounts for viscoelastic and traction forces developed in the collagen network leading to the observed compaction. Our estimate agrees with that measured isometrically using a LIVECO Vitrodyne 200.

We also present a technique to create microspheres in which the core is initially devoid of cells to mimic dermal wound healing and contraction, and compare the rate of microsphere compaction with the rate of fibroblast population of the core, which occurs via cell migration from the outer annulus and mitosis. Our continuum-mechanical model of the FPCM traction assay extends to this FPCM wound assay, novel in that it allows an examination of the interplay between gel mechanics and cell population dynamics intrinsic to contraction of dermal wounds and tissue-equivalents.

[1]Tranquillo, R. T., M. Durrani and A. G. Moon, "Tissue Engineering Science: Consequences of Cell Traction Forces," Cytotech. 10, 225-250 (1992).

P 114 INTERACTION OF FIBRONECTIN WITH ITS INTEGRIN CELL SURFACE RECEPTOR: CHARACTERIZATION OF THE SYNERGISTIC SITE OF THE FIBRONECTIN CELL-BINDING DOMAIN, Shin-ichi Aota, Steven K. Akiyama and Kenneth M. Yamada, Laboratory of Developmental Biology, National Institute of Dental Research, NIH, Bethesda, MD 20892

It is well documented that the cell-binding domain of fibronectin interacts with $\alpha 5\beta 1$ integrin receptor via its Arg-Gly-Asp (RGD) site. However, recent experiments suggested that another site besides the RGD sequence is required for full fibronectin activity. We established sensitive assay methods using bacterially expressed short polypeptides that retained the full cell-adhesive activity of fibronectin. When immobilized directly on polystyrene surfaces, the cell-adhesive activity of short fibronectin fragments was found to be artefactually decreased. This problem could be avoided by binding the polypeptides to surfaces coated with non-inhibitory monoclonal antibodies against fibronectin or by assaying soluble polypeptides in inhibition experiments. We then constructed various mutants using the "homology scanning" method, and characterized the secondary site. We found: 1) The amino acid sequence besides the RGD that is required for full activity of fibronectin is short. By substituting the corresponding part of a non-active construct with the amino acid sequence PHSRN, we observed a gain of function. 2) This sequence strikingly enhanced the inhibitory activity of the RGD site in a synergistic manner when it was inserted at a position *cis* to the RGD on the same molecule. 3) Epitope mapping of an anti-fibronectin mAb that inhibits fibronectin-mediated adhesion identified the identical site. The synergistic site appears to be located in a polypeptide loop between two anti-parallel β strands.

P 115 MECHANOTRANSDUCTIONS OF ENDOTHELIAL CELL ADHERED ON THE EXTRACELLULAR MATRIX, Jun K. Chang, Jongwon Kim, Jin H. Kim, Byoung G. Min, Dept. of Biomedical Eng., Seoul National University, Seoul, 110-744, KOREA

Cellular detachment by laminar shear stress was investigated for the adhesion of human umbilical vein endothelial cell (HUVEC) on extracellular matrix (ECM) coated polyurethane (PU). In the experiments, HUVECs were sparsely cultured on the natural ECM which was secreted by human fibroblasts. A parallel plate, channel flow device designed to provide steady, laminar flow was used to expose cultured HUVEC with a known hydrodynamic forces up to 130 dyne/cm^2. Cells detached significantly for first 1 minute after flow starts and the number of detached cells were directly proportional to the magnitude of applied shear stress. Maximum adhesion strength of HUVEC seeded on the natural ECM occurred on the 4th day after seeding. Endothelial cell adhesion is mediated by ECM proteins, especially fibronectin (FN), and their receptors, the integrin. To confirm the role of FN on the receptor-mediated adhesion, the same experiments were taken on the HUVEC cultured on FN-coated (5 μg/cm^2) PU. Maximum adhesion strength of HUVEC seeded on FN was occurred on the 2th day after seeding, and for these two days, adhesion strength of HUVEC on FN was larger than that on natural ECM. Higher adhesion strength implies that immobilized FN offers higher ligand density to integrin than natural ECM. Integrin ß chains physically interconnect FN with F-actin filaments via actin-associated proteins on the inner surface of plasma membrane, elevated ligand binding on integrins evokes the secure rearrangement of cytoskeleton on adhered cell. We observed i) larger cell spreading area, ii) proximal focal contact formation and cell height decrease, and iii) distal directionality of cell process of HUVEC on FN from the image analysis of the flow experiments. FN seems to play a dominant role in the initial cell shape control through the rearrangement of cytoskeleton during adhesion, which results different mechanotransduction to applied stresses. Proximal decrease of cell height can reduce up to 40 % of shear stress acting on the plasma membrane, and this stress-damping reaction seems to preserve the right function of the stress activated ion channel on plasma membrane under large stress conditions. These results indicate that coupling of cytoskeletal rearrangement to auto-stress-damping reaction is involved in the mechanotransduction of adhered cell.

P 116 THE α6β1 INTEGRIN AND MECHANICAL TENSION REGULATE ENDOTHELIAL TUBE FORMATION ON BASEMENT MEMBRANE MATRIX, George E. Davis and Charles W. Camarillo, Department of Pathology, Texas A&M University Health Sciences Center, College Station, TX 77843

When human umbilical vein endothelial cells are cultured on basement membrane matrix (Matrigel), they are known to dramatically rearrange and form endothelial tubes which mimic the early blood vessels seen during angiogenesis in vivo. In this study, we chose to address the role of the integrin family of adhesion receptors in this process as well as to determine the underlying mechanism regulating the rapid and precise tube formation process. Blocking antibodies directed to the β1 integrin subunit completely interfered with tube formation while non-blocking β1 antibodies had no effect. To determine which β1 integrin(s) play a role in the process, individual α chain blocking antibodies were added (i.e. α2, α3, α4, α5, α6). Of these antibodies, only the antibody directed to the α6 subunit was able to block. During tube formation, we observed two major alterations in the structure of the Matrigel which suggested a mechanism for the precision of tube formation. The first was that the Matrigel contracted and lifted off the substrate during tube formation but did not occur if tube formation was blocked (i.e. using β1 subunit antibodies). Secondly, linear distortions become visible in the Matrigel that exactly correspond to the pathways where extending endothelial cell processes are migrating. We speculate that these distortions or pathways result from the generation of mechanical tension between endothelial cells. Support for this conclusion is that endothelial cells cultured on flexible silicon rubber substrates coated with diluted Matrigel are able to mechanically distort the substrate in a pattern reminiscent of the tube formation structure on Matrigel. These distortions on the silicone rubber are completely eliminated following the addition of cytochalasin B which disrupts the endothelial cell actin cytoskeleton (Supported by American Heart Association, Texas Affiliate Grant #92G-025).

P 117 APPLICATION OF SELF-ASSEMBLED MONOLAYERS SUPPORTED ON TRANSPARENT FILMS OF GOLD TO STUDIES OF PROTEIN ADSORPTION AND CELL ATTACHMENT AND GROWTH, Paul A. DiMilla*, Gabriel P. López†, John P. Folkers, Hans A. Biebuyck, Ralph Härter, and George M. Whitesides, Dept. of Chemistry, Harvard University, Cambridge, MA 02138 (Present Address: *Dept. of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213 and †Dept. of Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, NM 87131)

Self-assembled monolayers (SAMs) of ω-functionalized alkane-1-thiolates (*e.g.*, $HS(CH_2)_{10}CH_3$, $HS(CH_2)_{11}(OCH_2CH_2)_6OH$, and $HS(CH_2)_{11}OH$) chemisorbed on 5 nm- and 10 nm-thick films of gold on titanium-primed glass are ideal systems for studying fundamental physicochemical interactions between proteins, cells, and biomaterials: SAMs of alkanethiolates offer more precise control of the molecular composition and structure of a surface than do other commonly-studied biosurfaces. Films of gold that are 5 nm- and 10 nm-thick are sufficiently thin that they transmit visible light, in contrast to the 200 nm-thick, opaque films commonly used as supports for SAMs. Transparency can be increased using a (3-mercaptopropyl)trimethoxysiloxy layer rather than 0.5-1 nm of titanium as an adhesion promoter between gold and glass, but evaporation of titanium is a more reliable and convenient technique than silanization. The thin metallic films also are continuous -- as assessed by X-ray photoelectron spectroscopy -- and electrically conductive. The advancing contact angles of water and hexadecane on methyl-, hexa(ethylene glycol)-, and hydroxyl-terminated SAMs on these films are indistinguishable from those on SAMs on 200 nm-thick gold; the hysteresis in contact angle -- the difference between the advancing and receding contact angles -- is *smaller*, however, on the thinner films of gold. This observation suggests (and scanning tunneling microscopy confirms) that the thin, transparent films are smoother than the thick, opaque films. To demonstrate how optical transparency over visible wavelengths and the flexibility in control of surface properties offered by these surfaces can be combined to address problems in biomaterials, two applications were studied: the detection by transmission absorbance spectroscopy of chromophore-labeled protein adsorbed on methyl- but not hexa(ethylene glycol)-terminated SAMs, and observations of the attachment and growth of MG63 osteosarcoma cells on patterned SAMs on these transparent films using conventional phase-contrast microscopy.

P 118 MANDIBULAR DISPLACEMENT MODULATES GROWTH AT THE CONDYLAR CARTILAGE AND CHANGES AT THE MOLECULAR BIOLOGICAL LEVEL: A LINK BETWEEN THE CLINIC AND LABORATORIAL RESULTS ?, Hajjar D., Camilo Castelo Branco University, Av. 9 de Julho, 5483/103 cep-01407-910, São Paulo, SP Brazil.

According to the work of Petrovic, A.et al.,1982 the mandibular condylar cartilage has a secondary- type of growth that is given by the division of the young cells not surrounded by the cartilaginous matrix: skeletoblasts and secondary-type prechondroblasts. When the second ones begin synthesizing the specific cartilaginous matrix, it usually stops dividing. General extrinsic factors, local extrinsic factors and intrinsic factors of unknown nature, other than genetic ones; can modulate the mandibular condylar cartilage growth rate. Their *in vivo* experiments with rats showed an increase or decrease at the condylar cartilage growth rate when changing the mandibular posture. Some alterations at the molecular biological levels such as: cellular shape and contact, a rise of the Na+ influx and H+ efflux at cellular membrane and at the cytoplasmic pH, high levels of calmodulin and [Ca++, Mg++]-ATPase, [Na+/K+]-ATPase, cAMP, fibronectin, cellular transglutaminase, heparan sulfate remains at low levels. The influence of some hormones regulating the growth amount at the proliferative zone such as tyroxine, GH-IGFs, sexual steroids are also alter. Patients submitted to a change of the mandibular posture treatment, showed the same increase or decrease at the condylar cartilage growth rate. Patients at the clinic were follow with sequential x-rays and photographs that demonstrated, at first level; a change at the posture of the condyle inside the articular fossa. After a variable period of time, related with the patient's age, type of facial growth and malocclusion; a normal position of the condyle inside the articular fossa was observed. Patients treated before or during the pubertal growth spurt had faster results than older ones. In older patients we could still observe the same clinical sings of the youngest ones but not with the same intensity. These changes may probably be related with local molecular biological alterations induced by this type of mechanical forces. One of the factors that might be involved is the autocrin and paracrin action of the growth factors like IGFs.

P 119 MECHANICAL PROPERTIES OF THE EXTRACELLULAR MATRIX INFLUENCE FIBRONECTIN FIBRIL ASSEMBLY, Nancy L. Halliday and James J. Tomasek, Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Mechanical properties of the extracellular matrix (ECM) are proposed to influence cell behavior and biological activity. The influence of the mechanical environment on fibronectin expression and fibril assembly by human adult fibroblasts was evaluated by indirect immunofluorescence and western-blot analysis. Fibroblasts were cultured in hydrated collagen gels with two distinctly different mechanical properties. Cells cultured within a stabilized collagen gel generate tension which is transmitted throughout the matrix (stressed gel). In contrast, cells that are cultured within a collagen gel that is floating freely in the media do not generate tension (relaxed gel). Fibroblasts in the stressed collagen gel develop large bundles of actin microfilaments or stress fibers. Fibroblasts cultured in a relaxed gel do not form stress fibers. Immunofluorescence studies demonstrate that cells cultured in the stressed collagen gel assemble well organized fibronectin fibrils at the cell surface whereas the same cells cultured in relaxed gels form no fibronectin fibrils. Western blot analysis of fibronectin in these two environments indicates that similar levels of fibronectin are being expressed. These results suggest that although the level of fibronectin synthesis is not significantly influenced by ECM mechanics, the assembly of fibronectin into fibrils is modulated by physical environment. The influence of mechanical properties on fibronectin isoform regulation is currently being evaluated by indirect immunofluorescence, western-blot analysis, and northern-blot analysis.

P 120 REGULATION OF CELL CYCLE PROGRESSION IN HEPATOCYTES BY EXTRACELLULAR MATRIX DENSITY AND CELL SPREADING, Linda K. Hansen[1,2], David J. Mooney[1,3], Joseph P. Vacanti[1], and Donald E. Ingber[1,2]; [1]Department of Surgery, Children's Hospital, and [2]Department of Pathology, Harvard Medical School, Boston, MA 02115; [3]Department of Chemical Engineering, MIT, Cambridge, MA 02139.

Cell adhesion to a solid substrate plays an important role in cell growth, although the underlying mechanisms are unclear. This study was undertaken to separate the effects of extracellular matrix density from its ability to promote cell spreading in order to determine their roles in promoting cell cycle progression in primary rat hepatocytes. The degree of cell-matrix contact formation and cell spreading were promoted in parallel by plating cells on dishes coated with a high (1000 ng/cm^2) or low (1 ng/cm^2) density of fibronectin (FN). In contrast, cell-matrix contact formation was promoted in the absence of spreading when cells were grown on dishes coated with a high density (1000 ng/cm^2) of an RGD peptide that binds to extracellular matrix receptors (integrins). The ability of each of these matrix conditions to support cell cycle progression through the G_0/G_1 transition in the presence of growth factors was examined by measuring the expression of the early growth response gene, junB. A dramatic induction of junB mRNA was observed in hepatocytes attached to a high density of either FN or RGD, with maximal expression observed 2 hours after plating. junB induction was not seen on the low FN density, and thus, its expression correlated with density of integrin ligand and was independent of cell shape. Cell attachment to high density of FN and RGD was also sufficient, without cell spreading, to support progression through mid G_1, as shown by induction of ras protooncogene expression 8-12 hours after plating. In contrast, however, attachment to neither high RGD nor low FN is sufficient to promote DNA synthesis; only cells that spread on the high FN density entered S phase. These results indicate that high densities of immobilized matrix ligands act directly, perhaps via activation of integrin signaling mechanisms, to induce cells to progress through the G_0/G_1 transition and mid-G_1, independently of cell shape. However, for cells to progress further through S phase, matrix must also support cell spreading. Cell adhesion in the absence of cell spreading leads to growth arrest in late G_1.

P 121 **REGULATION OF THE INTEGRIN-MEDIATED INTERACTION OF OSTEOGENIC CELLS WITH COLLAGENOUS MATRIX**, Jyrki Heino, Pia Santala, Liisa Nissinen, Terhi Riikonen, Arto Määttä and Hannu Larjava, The Department of Medical Biochemistry and the MediCity Research Laboratory, University of Turku, FIN-20520 Turku, Finland

Osteogenic HOS cells expressed two integrin-type collagen receptors, namely $\alpha1\beta1$ and $\alpha3\beta1$ heterodimers. HOS cells attached to type I collagen in the presence of Mg^{++} (more than 0.5 mM), whereas no cell adhesion was detected in the presence of Ca^{++} (0.1 mM-5.0 mM). Increasing Ca^{++} concentration did not inhibit HOS cell adhesion in the presence of 1.0 mM Mg^{++}. HOS cells can be transformed by using a mutagenic chemical (MNNG) or Kirsten murine sarcoma virus. In both cases a tumorigenic subclone of cells can be isolated (HOS-MNNG and KHOS-NP, respectively). These cells exhibit significantly increased Mg^{++}-dependent cell adhesion to type I collagen. This was shown to be due to the induction of the expression of a third collagen binding integrin heterodimer, namely $\alpha2\beta1$. A hypomethylating agent, 5-azacytidine could not turn on $\alpha2$ expression in HOS cells. Furthermore, transforming growth factor-$\beta1$, especially when combined with interleukin-1β, could increase $\alpha2$ expression several fold in HOS-MNNG cells and in another osteogenic cell line, MG-63, but not in HOS cells. Thus, we suggest that HOS cells have a strong, transformation-sensitive element, other than hypermethylation, suppressing the expression of $\alpha2$ integrin gene. Cytokines seems to regulate $\alpha2$ integrin expression via a different mechanism than malignant transformation. Interstingly, concomitantly with the induction of $\alpha2\beta1$ collagen receptor, both chemical and viral transformation suppressed type I collagen synthesis. Cellular mRNA levels of $\alpha1$(I) collagen were decreased about 85 % and 90 %, in MNNG cells and KHOS-NP cells, respectively. To conclude, the two tumorigenic subclones of osteogenic HOS cells have common features, including decreased production of collagenous matrix and increased ability to bind type I collagen due to induced expression of $\alpha2\beta1$ integrin-type collagen receptor. Given the fact that type I collagen is the major component of bone matrix, these phenomena may be essential for the formation of bone tumors.

P 122 INDUCTION OF CELL DIFFERENTIATION BY EXTRACELLULAR MATRIX: MECHANICAL VS. CHEMICAL SIGNALLING, H.P. Hohn, R. Grümmer, M. Hook, and H.-W. Denker, Institute for Anatomy, University Hospital, D-45122 Essen, Germany

Human choriocarcinoma cells express trophoblast-specific differentiation markers and are, therefore, a useful model to study the differentiation potential of tumor cells and its relevance for malignancy. When BeWo choriocarcinoma cells were maintained on different forms of extracellular matrix, expression of the differentiation marker chorionic gonadotropin (hCG) was stimulated much more (up to 5-fold) when cells were grown on flexible matrix gels as compared to cells grown on rigid/non-flexible substrates of the same chemical composition (coated plastic). This difference was accompanied by a change in morphology from cell monolayers on rigid substrates to cell spheroid-like aggregates of more rounded cells on matrix gels. A similar correlation was observed during experimental modulation of culture morphology of cells grown on plastic: In response to reduction of substrate adhesiveness (plastic coated with different concentrations of poly-HEMA) the cells assumed a more rounded shape and formed aggregates attached to the support. Concomitantly the secretion of hCG was increased up to the levels obtained on matrix gels. Piling up of cells did not seem to be the cause for this difference: Expression of connexins and of E-cadherin was not correlated with hCG production in attached cell spheroids as compared with spheroids in suspension culture and with cell monolayers. Conclusions: A rounded morphology combined with polar attachment to a substrate facilitate cell differentiation. Both are supported optimally only by flexible matrix substrates. These provide molecules for attachment and proper physical characteristics so that cells can assume a cell architecture favorable for differentiation.

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P 123 **VASCULAR SMOOTH MUSCLE CELLS SENSE MECHANICAL STRAIN THROUGH INTERACTION WITH SPECIFIC EXTRACELLULAR MATRIX PROTEINS**

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Cyclic mechanical strain (1 Hz) induces up to a 10-fold increase in DNA synthesis in cultured vascular smooth muscle (VSM) cells and greatly potentiates the mitogenic response to angiotensin II and α-thrombin. We asked whether this response is mediated by interactions with extracelluar matrix proteins. Silicone elastomer dishes, to which a cyclic vacuum could be applied to generate strain, were coated with poly-l-lysine, elastin, collagen type I, vitronectin, or fibronectin. VSM cells on these surfaces were exposed to strain for 48 h and thymidine incorporation was measured. On poly-l-lysine and elastin, the response was less than a 2-fold increase in thymidine incorporation, while on both collagen type I, vitronectin, or fibronectin, thymidine incorporation increased from 5-10 fold. In cells grown on immobilized type I collagen, soluble fibronectin (20-100 μg/dish) reversed the mitogenic response to strain in a dose-dependent fashion. To determine whether this effect of extracellular matrix is mediated by an integrin-type matrix receptor, cells grown on type I collagen were incubated with 100 μg/ml of the "RGD" peptide GRGDTP or an inactive analog, GRGESP. Cells were exposed to strain, thrombin, or PDGF for 48 h and thymidine incorporation was determined. GRGDTP, but not GRGESP, totally eliminated the mitogenic response to mechanical strain. GRGDTP did not cause the cells to detach, and there was no increase in trypan blue uptake. Moreover, the mitogenic responses to thrombin (5-fold increase) or PDGF (3-fold increase) were unaffected by either of the peptides. In previous work, we showed that the mitogenic response to strain is mediated by strain-induced secretion of PDGF. GRGDTP, but not GRGESP, prevented induction of the PDGF-A gene by mechanical strain. Thus, mechanical strain is sensed by VSM cells through an interaction between membrane integrins and specific extracellular matrix proteins at RGD-containing sites in the matrix.

P 124 DEVELOPMENTAL REGULATION OF THE INTERACTION OF α7β1 INTEGRIN AND EXTRACELLULAR MATRIX IN SKELETAL MUSCLE, Stephen J. Kaufman, Woo Keun Song, Hiro Sato and Weigwang Wang, Department of Cell and Structural Biology, University of Illinois, Urbana, IL 61801

The α7β1 integrin heterodimer is a developmentally regulated extracellular matrix binding protein on the surface of differentiating and adult skeletal muscle. As the sole functional laminin-binding integrin on skeletal myoblasts α7β1 mediates the response of these cells to a laminin-rich environment and this includes increased cell mobility, maintenance of proliferation and myoblast localization at the sites of secondary fiber formation. Once fibers form, the α7β1 integrin localizes along their periphery where it likely serves to help associate these cells with the extracellular matrix. It is also localized at myotendinous junctions where it tethers fibers and thereby plays an important role in force generation. We report here that the diversity of functions of the α7β1 integrin at different stages of myogenesis and in the adult is mediated by its capacity to bind multiple ligands and by the developmentally regulated expression of three alternate cytoplasmic domains of the α7 chain. The α7A, B and C isoforms are generated by alternate RNA splicing and the three amino acid sequences thus derived have distinct motifs and membrane signal transducing capacities. The α7B form is expressed on precursor cells and in replicating myoblasts. Two regions in the α7B cytoplasmic domain have homology to those in the catalytic phosphotransfer domain of serine/threonine protein kinases, a sequence of 15 amino acids is homologous to that in many protein tyrosine phosphatases, and there is a potential actin binding sequence and a unique 3-fold DXHP repeat. Binding ligand causes a change in conformation in the α7B cytoplasmic domain and promotes its association with the cell cytoskeleton. Upon terminal differentiation, there is a decline in expression of α7B and commensurate expression of α7A and then α7C.

In addition to binding laminin, the α7β1 integrin also binds to fibronectin and to a lectin, L-14. L-14 is synthesized in replicating myoblasts but it is not secreted until these cells commence fusion. Our experiments demonstrate that L-14 binds to both laminin and the α7β1 integrin and it can effectively inhibit the association of these molecules. Modulation of α7β1 interactions with its ligands by L-14 is selective: L-14 does not bind to fibronectin, nor does it interfere with the binding of α7β1 with fibronectin. A consequence of the onset of L-14 secretion with terminal differentiation is a shift in myoblast interactions away from laminin to fibronectin, and we believe this is significant to further myogenic development.

P 125 USE OF SELF-ASSEMBLED MONOLAYERS OF ALKANE-THIOLATES ON GOLD FOR THE STUDY AND MANIPULATION OF INTERACTIONS OF PROTEINS AND CELLS WITH SOLID SURFACES, Gabriel P. López*, Paul A. DiMilla†, Amit Kumar, Ralph Härter, and George M. Whitesides, Dept. of Chemistry, Harvard University, Cambridge, MA 02138. (Present addresses: *Dept. of Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, NM 87131 and †Dept. of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213).

This poster describes methods for controlling the concentration and spatial distribution of proteins and cells adhering to patterned, self-assembled monolayers (SAMs). Patterned SAMs can be formed by the serial chemisorption of two or more ω-functionalized alkanethiols ($HS(CH_2)_nR$) onto gold. Several techniques (microwriting, micromachining, stamping, UV microlithography) can be used to fabricate the patterned SAMs. The most useful systems of patterned SAMs for studying the adsorption of proteins and for controlling the attachment and spreading of cells are those with areas formed from oligo(ethylene glycol)-terminated thiols (e.g., R = $(OCH_2CH_2)_6OH$) and other areas formed from thiols terminated by non-polar (R = CH_3), polar (R = CH_2OH) or ionic (R = CO_2^-, PO_3H^-, 2-imidazolo) groups. The areas of the patterned SAMs containing oligo(ethylene glycol)-terminated monolayers resist protein adsorption and cellular attachment; other areas allow protein adsorption and cellular attachment to different degrees. Scanning electron microscopy allows characterization of patterns of proteins adsorbed on SAMs of alkanethiolates. The ability to form patterns with dimensions comparable to those of cells, exposing well-defined functional groups, is useful in studying the physical-organic chemistry of cell attachment and growth: partitioning of proteins on adsorption onto surfaces from media; correlation of proteins adsorbed with attachment; influences of local environment on cellular spreading.

P 126 A REDUCTIONISTIC MODEL FOR STUDIES OF THE MATRIX REQUIREMENTS FOR INTESTINAL EPITHELIAL RESTITUTION: TYPE IV COLLAGEN FACILITATES ENTEROCYTE ADHESION TO ALTERNATE MATRIX. R Moore Department of Pathology, School of Veterinary Medicine and Medicine, Tufts University, Boston, MA, 02111

In healing of some wounds, migrating epithelial cells develop the ability to attach to new matrices during healing. We have previously shown in an *in vitro* guinea pig model of intestinal epithelial restitution that type IV collagen (IV) facilitates rapid repair of the intestine. We have additionally shown by a reductionistic approach that isolated enterocytes preferentially attach to type IV. Here we examine the effect of prolonged incubation of isolated native enterocytes suspended in type IV collagen on adhesion to major basement membrane components. Cells were isolated by mechanical vibration and shown to be functionally viable by their ability to volume regulate for several hours in hypertonic media. Isolated cells were suspended in type IV at 0, 12.5, 62 and 125μg/ml for 60 minutes, washed, resuspended at $5x10^5$ cells/ml and incubated on bound type IV collagen, laminin (Ln), fibronectin (Fn) or BSA-coated plastic wells for 30 minutes at 37°C. Cell attachment was determined by counting the number of adhered cells/HPF. Cells suspended in concentrations of type IV<12.5μg/ml attached better to bound type IV than to bound Fn or Ln (14+/-2.3 vs 9+/-1.8 and 1+/-0.3, for cells incubated on bound type IV collagen vs Fn and Ln, respectively). However cells suspended in >12.5μg/ml type IV did not attach to bound type IV. Conversely, at >12.5μg/ml cell attachment to bound Fn was significantly enhanced(9 +/-1.8, 10+/-1.9 vs 38 +/-5.4 cells/HPF, for cells suspended in 0, 12.5μg/ml type IV collagen vs 62μg/ml, respectively; p<.005). Following prolonged suspension in >12.5μg/ml, more cells attached to Fn than cells isolated and immediately incubated on bound Fn for 90 min. (38+/-5.4 vs 10.6+/-1, respectively; p<.005). Similarly, cell binding to Ln following suspension in saturating concentrations of type IV collagen was also enhanced although to a lesser extent (21+/-3.4 vs 1+/0.3 cells/HPF, for cells suspended in 0 vs 125μg/ml type IV collagen, respectively, p<.005). Cells suspended in type I collagen did not exhibit the same enhanced binding to matrix as cells suspended in type IV collagen. These results suggest that cell suspension in type IV collagen activates the adhesive function of enterocytes to fibronectin and laminin. We speculate that in this epithelia activation/deactivation of cell-matrix binding characteristics by type IV collagen may be important for migration/immobilization of cells during repair of epithelial wounds.

P 127 INTRA-EPITHELIAL MIGRATION OF A HUMAN BLADDER CARCINOMA CELL LINE (T24) INTO REGENERATING UROTHELIUM, Johanna M.J. Rebel, Willem I. De Boer, Cornelia D.E.M. Thijssen, Marcel Vermeij, Ellen C. Zwarthoff and Theo H. Van Der Kwast, Department of Pathology, Erasmus University Rotterdam, Postbox 1738, 3000 DR Rotterdam, The Netherlands

Replacement of (regenerating) urothelium by transformed urothelial cells explains the high recurrence rate of human bladder cancer. The influence of extracellular matrix (ECM) on intra-epithelial migration of T24 human bladder carcinoma cells into regenerating organotypic cultures of mouse urothelium was studied. Mouse bladder explants were allowed to regenerate on porous membranes coated with different ECM components. The regeneration rate, differentiation into superficial cells and number of cell layers of the urothelium varied dependent on the ECM coating.

ECM	regeneration rate	differen-tiation	cell layers
-	+	+/-	2-3
Laminin	+++	+	1
Col IV	+	+	3
Fibronectin	+	+/-	2-3

To study if this difference had an effect on the intra-epithelial migration of T24 cells these cells were inoculated adjacent to primary bladder cultures. On day 24 the migration of the T24 cells into the urothelium was studied using image analysis and an antibody against cytokeratin 19 to identify the human T24 cells. On laminin coated membranes the T24 cells had an intra-epithelial migration comparable to the non-coated membranes, while their migration into primary urothelium was reduced on Col IV and fibronectin.

Since migration of T24 cells alone was not influenced by ECM, it is hypothesised that intra-epithelial migration of T24 cells is influenced by ECM induced differences in the primary urothelial outgrowth.

P 128 THE ROLE OF THROMBOSPONDINS IN CNS MYELINATION, Suzanna R. S. Scott-Drew and Charles ffrench-Constant. The Wellcome/CRC Institute of Developmental Biology, Cambridge, CB2 1QR, UK.

The precursor cell for the myelin-forming oligodendrocyte, the O2-A progenitor cell, migrates extensively during prenatal and early postnatal development in the central nervous system (CNS). The adhesive extracellular matrix glycoprotein thrombospondin (TSP) is present within the pathways of migration (O'Shea et al 1990). We have investigated the potential role of TSP in O-2A progenitor migration. TSP purified from human platelets is an adhesive substrate for O-2A progenitor cells in vitro and TSP will promote the migration of these cells. These results support a role for TSP in glial cell migration in the developing CNS. However, the thrombospondin family consists of five distinct but homologous genes and we have found by northern blot that TSP1, TSP2 and TSP3 are expressed during early postnatal CNS development. In situ hybridisation studies are therefore underway to determine exactly which TSPs are present in the migratory pathways. Future experiments will then determine the relationship between the appropriate TSPs and O-2A progenitor cells in more detail by identifying the cell-surface ligands involved.

Funded by the Wellcome Trust.

Receptors and Adhesion

P 200 **MAb AGAINST E2 MOLECULE (MIC 2 GENE PRODUCT) INDUCES HOMOTYPIC AGGREGATION OF CORTICO-THYMOCYTES AND PHOSPHORYLATION OF A 200 KDa PROTEIN** G.Bernard, D.Zoccola, M.Ticchioni, J.P Breittmayer, C. Aussel and A.Bernard
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We have previously described E2 as a 32 kDa transmembrane glycoprotein involved in T cells adhesion since anti-E2 mAb reacting with defined epitopes block spontaneous T cells rosettes and since anti-E2 mAbs induce exposure of phosphatidylserine at thymocyte cell surface. We show here that anti-E2 mAb inhibiting rosettes and no other anti-E2, trigger aggregation of certain T cells lines and no other cell lines tested. Among normal cells, anti-E2 mAb exclusively induce CD4+ CD8+ (double positive) thymocytes homotypic aggregation and not peripheral T cells. This phenomenon is temperature, energy and Mg^{++} dependent and requires an intact cytoskeleton, aggregation phenomenon is accompagnied by phosphorylation of a 200 kDa protein after 5 minutes exposure to anti-E2 mAb. Therefore, the adhesion pathway triggered by anti-E2 is likely to involve an integrin, since E2 mediated adhesion has no temperature or cations requirement and since they are triggered by anti-E2 mAb blocking rosettes. The event we described here probably represent an important adhesion phenomenon required at a critical stage of T cell differentiation.

P 201 THE ROLE OF CADHERINS IN CALCIUM INDUCED STRATIFICATION OF HUMAN KERATINOCYTES Vania M.M. Braga, Kairbaan Hodivala and Fiona Watt. Keratinocyte Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, WC2A 3PX, London, U.K.

When human epidermal keratinocytes are cultured in medium containing a low concentration of calcium ions (0.1 mM) the cells are prevented from stratifying and initiate terminal differentiation while still attached to the culture substrate. On raising the calcium ion concentration (to 1.8 mM), differentiating cells selectively detach from the substrate and form a suprabasal layer. Differentiating keratinocytes in low calcium medium express functional integrins, but lose their receptors during calcium-induced stratification. Stratification and the loss of integrins from differentiating cells is inhibited by a combination of antibodies to E- and P-cadherin. We are currently investigating how cadherins may regulate integrin expression in keratinocytes. Within 5 minutes of raising the level of calcium ions cadherins and phospho-tyrosine containing proteins become concentrated at cell-cell borders, followed 10 minutes later by β1 integrins and vinculin. The increase in the extracellular calcium ion concentration results in decreased Triton X100 solubility of cadherins and vinculin, suggesting an increased association with the actin cytoskeleton. However, the solubility of β1 integrins and paxillin remains unchanged after the switch to high calcium medium. Disruption of the actin cytoskeleton by treatment with cytochalasin D prevents the localisation of β1 integrins to cell-cell contacts, but does not affect the redistribution of E-cadherin. Further experiments are in progress to analyse whether any of the changes that occur in response to calcium ions play a role in the selective loss of integrins from differentiating cells

P 202 FORMATION OF HOMOGENEOUS CROSS-LINKED LATTICES BETWEEN LECTINS AND GLYCOPROTEINS IN MIXED SYSTEMS. Fred Brewer, Dipti Gupta, and Dipak Mandal, Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461.

Lectin binding to the surface of cells leads to cross-linking of glycoconjugate receptors, including glycoproteins and glycolipids, which, in many cases, is related to a variety of biological signal transduction and recognition processes including mitogenesis. We have observed that many naturally occurring cell surface oligosaccharides and synthetic analogs are multivalent and capable of binding and precipitating with specific lectins (cf. Bhattacharyya, L., Ceccarini, C., Lorenzini, R., and Brewer, C. F. (1987) J. Biol. Chem. 262, 1288-1293). These cross-linking interactions lead to a new source of binding specificity: namely, the formation of homogeneous, homopolymeric carbohydrate-lectin cross-linked lattices (Bhattacharyya, L., Khan, M. I., and Brewer, C. F. (1988) Biochemistry 27, 8762-8767). Our recent studies show that many of these lattices are crystalline, and that structural information can be obtained using electron microscopy, x-ray diffraction, and molecular modeling techniques. We have also recently demonstrated that similar highly organized cross-linked lattices form between glycoproteins and both plant and animal lectins including the 14 kDa β-galactoside specific lectin from calf spleen. Quantitative precipitation analyses of binary mixtures of a variety of glycoproteins in the presence of the mannose-specific lectin concanavalin A or the galactose-specific 14 kDa calf spleen lectin provide evidence that each glycoprotein forms a unique homopolymeric cross-linked lattice with a specific lectin which excludes the lattice of other glycoproteins. These results are discussed in terms of the possible biological recognition properties of lectins and glycoproteins as receptors.

P 203 **THE AMINO ACID SEQUENCE OF THE MURINE DCC TUMOR SUPPRESSOR GENE.** Helen M. Cooper, Penny Armes, Joanne Britto, Ian Faragher, and Andrew F. Wilks. Ludwig Institute For Cancer Research , P.O.Box Royal Melbourne Hospital, Victoria, Australia 3050.

Colon cancer is one of the most prolific and malignant forms of human cancers. Recently, mutations have been identified in the DCC (Deleted in Colon Cancer) gene in over 70% of all colon carcinomas studied. Since it is mutated at both alleles in most carcinomas, the DCC gene appears to be a tumour suppressor gene. In general, these mutations are not observed until later stages of tumorigenesis suggesting that the loss of expression of the DCC gene product influences the metastatic and/or invasive phenotype of colorectal carcinoma. The DCC gene product shares strong homology at the amino acid level to members of the immunoglobulin supergene family. Many of these proteins are known to be homophilic cell adhesion molecules (eg. N-CAM, L1-CAM, Fasciclin I and III).

To date, only a partial sequence of the human DCC gene product has been reported (Fearon et al, 1989). The published sequence comprises 750 amino acids which encode 4 Ig-C2-like domains followed by 3 fibronectin type III repeats. Here we report the complete amino acid sequence of the murine DCC gene product derived from a mouse brain cDNA library. The murine DCC cDNA contains 4 Ig-C2 domains and 6 fibronectin type III repeats followed by a transmembrane and cytoplasmic domain. The mouse DCC amino acid sequence is 96% homologous to the reported human sequence. Since the DCC gene product is so highly conserved between mouse and man it is likely that DCC will play a central role in regulating aspects of cellular proliferation and/or differentiation.

Fearon, E., Cho,K.R., et al (1989) Science 247:49-56.

P 204 **Expression Cloning of a Functional Glycoprotein Ligand for P-Selectin** Dale Cumming*, Dianne Sako*, Xiao-Jia Chang*, Karen M. Barone*, Gloria Vachino*, Gray Shaw*, Trudi M. Veldman*, Kevin M. Bean*, Tim J. Ahern*, Bruce Furie+, Dale A. Cumming*, and Glenn R. Larsen*. *Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA. 02140 and + Center for Hemostasis and Thrombosis Research, Division of Hematology and Oncology, New England Medical Center and the Department of Biochemistry, Tufts University School of Medicine, Boston, MA. 02111.

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

P 205 IDENTIFICATION OF A LARGE COMPLEX CONTAINING THE INTEGRIN α6β1 LAMININ RECEPTOR IN NEURAL RETINAL CELLS, Ivan de Curtis and Giuliana Gatti, Department of Biological and Technological Research, H S.Raffaele, Milano, Italy.

The integrin α6β1 is a laminin receptor involved in adhesion and neurite extension of retinal neurons on laminin. The present study was carried out to identify possible interactions between the α6β1 receptor and cellular proteins involved in integrin signaling and function. We have used a biochemical approach based on solubilization of retinal neurons cultured on laminin with nonionic detergents, followed by centrifugation on sucrose velocity gradients. Analysis of the distribution of the α6 and β1 integrin subunits in the gradients showed that a large complex containing α6β1 is preserved by octylglucoside, but not by Triton X-100 extraction. Cytoskeletal proteins known to localize in adhesion plaques are not components of the complex. Culture of retinal neurons on BSA, a non-adhesive substrate, partially affected the gradient distribution of the receptor after octylglucoside extraction. Analysis of the gradient distribution of two alternatively spliced isoforms of the α6 subunit, α6-cytoA and α6-cytoB, showed differences in their sedimentation properties, which may reflect functional specificities of the two isoforms. Furthermore, a set of polypeptides whose tyrosine phosphorylation was enhanced by culture on laminin (M_r 59, 80, and 98) colocalized with α6β1 on the gradients after octylglucoside solubilization, suggesting possible functional interactions of the tyrosine-phosphorylated molecules with the laminin receptor.

P 206 EVIDENCE FOR CALMODULIN BINDING TO THE CYTOPLASMIC DOMAINS OF TWO C-CAM ISOFORMS

M. Edlund and B. Öbrink.
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C-CAM (Cell-CAM 105) is a transmembrane cell adhesion molecule belonging to the immunoglobulin superfamily. It occurs in a variety of tissues, and mediates intercellular adhesion in rat hepatocytes by a homophilic binding mechanism. Regulation of C-CAM might involve the intracellular protein calmodulin, which has been demonstrated to bind to purified liver C-CAM in a Ca^{2+}-dependent manner. Experiments with proteolytically derived peptides indicated that calmodulin binds to the cytoplasmic domain of C-CAM. To establish the binding region for calmodulin we have produced fusion proteins between a bacterial maltosebinding protein and the cytoplasmic domains of the two major isoforms of C-CAM. These isoforms have cytoplasmic domains of 10 and 71 amino acids, respectively. Calmodulin binding was determined by a gel overlay assay employing ^{125}I-labeled calmodulin. The results demonstrate that calmodulin can bind in a Ca^{2+}-dependent manner to both the large and the small cytoplasmic domains of C-CAM.

P 207 ROLE OF ICAM-3 IN LYMPHOCYTE ADHESION

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Recently ICAM-3 has been identified as third intercellular adhesion molecule that binds LFA-1. Like ICAM-1 and ICAM-2 ICAM-3 belongs to the immunoglobulin superfamily but is expressed solely on lymphoid cells, in contrast to ICAM-1 and ICAM-2. To investigate the role of ICAM-3 in lymphocyte adhesion, mouse L-cells were transfected either with ICAM-1, ICAM-2 or ICAM-3 cDNA alone, or with combinations of cDNA's of the various ICAM's.

We determined whether binding of LFA-1 to ICAM-2 and ICAM-3 requires activation of the LFA-1 molecule as has been reported for adhesion to ICAM-1. This was done by investigating the capacity of different stimuli, known to promote LFA-1/ICAM-1 adhesion, to induce LFA-1 mediated binding to L-ICAM-2 and L-ICAM-3 cells. Different LFA-1 activating stimuli, like CD2 and CD3 triggering, PMA activation, Mn^{2+}, and different LFA-1 activating antibodies were investigated. To determine whether lymphocyte binding to L-ICAM-2 and L-ICAM-3 cells is dependent on cell activation and differentiation, adhesion assays were performed with resting T cells, as well as activated T cells. The results demonstrate that T cells can bind ICAM-2 and ICAM-3, however the strongest adhesion is observed to ICAM-1. Activation of LFA-1 is required for binding to ICAM-2 as well as ICAM-3. Certain LFA-1 activating antibodies induce T cell binding to ICAM-1, whereas they inhibit T cell binding to ICAM-2 and ICAM-3. Furthermore the results demonstrate that clustering of LFA-1 on the cell surface facilitates LFA-1 mediated adhesion to ICAM-1, ICAM-2 and ICAM-3.

P 208 A TRANSMEMBRANE FORM OF THY1 PROMOTES MORPHOLOGICAL DIFFERENTIATION OF AN INSULINOMA PANCREATIC CELL LINE

, Gomez, S., Olive, S., Tiveron. M. C. and Rougon, G. Laboratoire de Genetique et Physiologie du Developpement. UMR C 9943. Case 907, 13288 Marseille Cedex 9, France.

Adhesive interactions mediated by molecules of the immunoglobulin (IgG) superfamily are important for the spatio-temporal positioning of cells. They also modulate tissue-specific gene expression, cell multiplication and differentiation. However, the mechanisms whereby an external-binding event is coupled to an intracellular response are not elucidated. In this study, we examined the role of a GPI versus a transmembrane anchor on the expression and molecular interactions using the THY 1 adhesion molecule. We transfected insulin secreting pancreatic beta cells (RIN) constituvely expressing N-CAM and the TrK receptor. They are able to grow neurone-like extentions in response to nerve growth factor (NGF) (1)The cells were transfected with a cDNA encoding THY1 in either its GPI form or a transmembrane chimera made of the extracellular part of THY1 and the intracellular part of N-CAM 140 kDa. In contrast to the parental or the THY1-GPI transfected cells, the cells transfected with the chimeric form enter into a differentiated state characterized by a slow division and production of long processes. The growth of these extentions is abolished by cell-cell contact. Interestingly, no morphological change is observed in fibroblasts transfected with either the GPI or another transmembrane form of THY 1 (2). Moreover, at steady state the chimeric protein is found at the cell surface while the GPI form of THY 1 is also found inside vesicules, which could be endosomes/lysosomes. These results suggest that RIN cells possess specific factors involved in differentiation and that the intracellular domain of N-CAM play a role in this event probably involving protein-protein interactions both at the cell surface.and intracellularly

(1) Proc. Natl. Sci. (1993), 90: 5781-5785
(2) J. Immunonol (1990), 20: 1491-1495.

P 209 VARIABLE EXPRESSION OF αv-INTEGRINS IN INFLAMMATION,

Kirsi Haapasalmi, Laura Nikkari, Marja Mäkelä, Jyrki Heino, Veli-Jukka Uitto and Hannu Larjava, Universities of British Columbia, Vancouver, Canada, and Turku, Finland.

Integrins are cell surface anchored dimeric glycoproteins which mediate cell adhesion to extracellular matrix. The αvß5 and αvß6 integrins function in binding either vitronectin or fibronectin, respectively. In the present study, we investigated the expression of these integrins in synovial and periodontal tissues with different types of inflammatory reaction. Tissue biopsies were collected from the following sources: normal, osteoarthrotic, and rheumatoid synovia, chronically inflamed and healing periodontium. Frozen section were immunostained for the localization of αvß5 and αvß6 integrins with specific antibodies followed by rhodamine-conjugated secondary antibodies. The αvß5 integrin was expressed in the lining cell layer of normal synovia and in the proliferating lining cell layer of osteoarthrotic synovia. Although the lining cell layer showed proliferating features also in the rheumatoid synovia specimens, the expression of αvß5 integrin was almost totally downregulated. The αvß6 integrin was not expressed by any of the synovial specimens studied. Samples from chronically inflamed or healing periodontium did not express αvß5 and αvß6 integrins with one exception. Migrating epithelial cells of periodontal wound epithelium expressed the αvß6 integrin although the rest of the epithelium was negative. The results indicate that the αvß5 integrin is normallly expressed in synovial lining but not in healthy or inflamed human periodontium. Furthermore, the expression of αvß5 integrin is differently controlled in two etiologically different synovial inflammation. In addition, the αvß6 integrin is only seen in periodontal epithelial cells during wound healing. It is conceivable therefore that the expression of these integrins is controlled by the cell phenotype and the type of inflammation.

P 210 AN EFFORT TO IDENTIFY GENES FOR PROTEINS REGULATING INTEGRIN LFA-1. Håkan Hedman and Erik Lundgren. Department of Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden.

The *Leukocyte Function Associated Antigen-1* (LFA-1) belongs to the integrin family of cell surface receptors. LFA-1 mediates adhesion between cells by interacting with its counter-receptors ICAM-1, 2 and 3. A functionally important feature of LFA-1, and other integrins, is their ability to rapidly change between an active, ligand binding state, and an inactive, non-binding state. Only little is known about the mechanisms behind this regulation. In an effort to learn more about the LFA-1 regulating mechanisms, we have taken a genetic approach. HPB-ALL lymphocytes have been mutagenized and selected for aberrant regulation of LFA-1. This was achieved by selecting cells by virtue of their ability to bind magnetic beads coated with rICAM-1. Thus, cell lines have been established that are unable to activate LFA-1 in response to phorbol esters, and other cell lines that are unable to deactivate LFA-1 in response to the protein phosphatase inhibitor calyculin A. The goal is next to genetically complement the aberrant HPB-ALL clones with cDNA from normal cells, and thereby identify genes involved in LFA-1 regulation.

P 211 ADHESION OF HUMAN COLORECTAL CARCINOMA CELLS TO CARCINOEMBRYONIC ANTIGEN REQUIRES INTACT MICROTUBULES AND ENERGY PRODUCTION, Seiichi Ishii, Rosilyn Ford, Alexander Nachman, Peter Thomas, Glenn Steele Jr. and J. Milburn Jessup, Laboratory of Cancer Biology, Department of Surgery, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02115

Carcinoembryonic antigen (CEA) mediates adhesion of human colorectal carcinoma (CRC) cells by homophilic binding of the molecule. We determined the involvement of cytoskeleton, energy production and phosphorylation in the binding of CRC cells to CEA. Three human CEA-positive CRC cell lines (KM-12c, CCL188 and CX-1) bound significantly to CEA by 15%, 15% and 22%, respectively, in a 90 min solid-phase adhesion assay. Inhibitors of microtubule-formation (200 μM colchicine (Colch.) and 1 μM taxol) blocked all lines binding to CEA by 24% to 78% (Table) while an inhibitor of microfilament (10 μM cytochalasin D: Cyt. D) did not show a consistent effect on CEA-binding (Table). Inhibition of glycolysis by 100 mM oxamic acid (Oxamate) and incubation in low temperature (4°C) significantly blocked adhesion to CEA by 50% to 84% (Table). Inhibitors of phosphorylation (10 μM staurosporine (Stauro.) and 1 mM H-7) increased adhesion to CEA (Table) while the inhibitors blocked adhesion to laminin and collagen (data not shown).

Table. Inhibition of Adhesion to CEA* by Inhibitors of Cytoskeletons, Energy Production and Phosphorylation

	% Inhibition of Adhesion to CEA by Following Inhibitors						
	Colch.	Taxol	Cyt. D	Oxamate	4°C	Stauro.	H-7
KM-12c:	30	45[a]	(+25)	50[a]	82[a]	(+114)	(+73)
CCL188:	27	24	12	84[a]	62[a]	(+44)	ND
CX-1:	78[a]	66[a]	30	66[a]	ND	(+52)	(+16)

*Inhibition of adhesion by each inhibitor was tested in a solid-phase adhesion assay with the presence of the inhibitors during the incubation period. Viability of the cells was not altered by 90 min incubation with the inhibitors by trypan blue dye exclusion. Figures in each parenthesis with a + sign indicates increased adhesion to CEA by an inhibitor. ND: not done. [a]Significant inhibition ($P < 0.01$) versus % cells bound to CEA without inhibitors.

These data indicate that CEA-mediated adhesion depends on energy production and the presence of intact microtubules but not phosphorylation or microfilament-formation. These results suggest that maintenance of CEA-mediated adhesion requires the continuous recruitment of new CEA molecules to the cell membrane involved in the binding.

P 212 HOMOTYPIC ADHESION MEDIATED BY CARCINO-EMBRYONIC ANTIGEN (CEA) IN COLORECTAL CARCINOMA CELLS INVOLVES CD44 AND NCA. J. M. Jessup, P. Thomas, R. D. Ford, S. Ishii. Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA 02215.

CEA and nonspecific crossreacting antigen (NCA) are immunoglobulin supergene family members that support homotypic cell adhesion when transfected into cells that do not aggregate spontaneously. We (Int. J. Cancer 55:262 ,1993) have shown that monoclonal antibodies to epitopes in the N-terminal and A1B1 domains of both CEA and NCA inhibit the adhesion of human colorectal carcinoma cells and CHO cells transfected with CEA to solid phase CEA. Zhou et al. (J. Cell Biol. 122:951, 1993) have reported that an N-terminal:A3B3 domain interaction causes homotypic aggregation. However, access to an A3B3 epitope is limited since T84.66, a relatively high affinity antibody to an A3B3 epitope, displays weak membrane binding to viable, unfixed human colorectal carcinoma cells. Further, homophilic binding is weak because purified CEA does not bind to solid-phase CEA in 0.15M PBS, pH 7.2, under conditions in which CEA-expressing cells bind readily to solid-phase CEA. We sought to determine whether accessory binding proteins promoted CEA-mediated adhesion by chemical crosslinking studies and antibody competition analysis. Studies with a photoactivatable cross-linker identify a 90 kDa binding protein on the surface of KM-12c and CCL 188 human colorectal carcinoma cells that binds to CEA. Monoclonal antibodies to epitopes restricted to NCA or CD44 that have 90 kDa isoforms block adhesion to CEA by 50 - 90% at concentrations of 5 - 10 μg/ml. Digital video microscopy indicates that 45 - 55% of CD44 and NCA molecules are colocalized with CEA on the cell membrane. While CEA-expression is required for adhesion to solid-phase CEA, NCA may stabilize CEA:CEA bonds by enhancing both *cis* and *trans* interactions. CD44 does not bind CEA but may stabilize its *trans* binding through its own homophilic binding properties as well as its ability to anchor other cell membrane adhesion proteins.

P 213 RELATIONSHIP BETWEEN RECEPTOR/LIGAND BINDING AFFINITY AND ADHESION STRENGTH, Suzanne C. Kuo & Douglas A. Lauffenburger, Department of Chemical Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Receptor-mediated cell adhesion is a central phenomenon in many physiological and biotechnological processes. For example, affinity-based cell separation processes take advantage of the specific interaction between the receptors on a cell and the complementary ligand on a surface. Mechanical strength of adhesion is generally presumed to be related to chemical affinity of receptor/ligand bonds, but no experimental study has been previously directed toward this issue. Here we investigate the dependence of receptor/ligand adhesion strength on bond affinity.

We have chosen 10-micron diameter latex microspheres as model "cells." This provides for relatively uniform properties, easy variation of receptor densities, and removal of additional complicating factors of real cells, such as cytoskeletal interactions and receptor diffusion. Our system uses IgG antibody as "receptors" on the surface of the beads and protein A (SpA) as the complementary surface ligand. Protein A specifically binds the Fc region of IgG. Equilibrium binding constants for the antibody/protein A interaction are measured using fluorescence flow cytometry. Adhesion strengths are measured under conditions of laminar shear flow using the Radial Flow Detachment Assay (RFDA), which imposes a range of shear stresses across a ligand-coated glass disc. A spectrum of animal species sources for IgG permits examination of three decades of SpA/IgG binding affinity. Our results for this model system demonstrate that adhesion strength varies with the logarithm of the binding affinity, consistent with a prediction from the theoretical model by Dembo *et al.* (Proc. Roy. Soc. London B234:55 [1988]).

P 214 MoAb BD31 DEFINES A NOVEL GPI-ANCHORED GLYCOPROTEIN CONTROLLING INTERCELLULAR ADHESION IN NORMAL AND NEOPLASTIC EPITHELIA. Pier Carlo Marchisio, Mauro Rabino, Maria Prat, Ottavio Cremona, Paola Savoia and Livio Trusolino, Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, 10126 Torino, Italy and DIBIT, Istituto Scientifico H San Raffaele, 20132 Milano, Italy.

MoAb BD31, raised to a line of gastric carcinoma cells, reacts with intercellular boundaries of human transformed cells originating either from carcinomas or from sarcomas growing in epithelial-like clusters as well as in primary cultures of human epithelial and endothelial cells. BD31 also reacts with intercellular rims of most normal and transformed epithelial tissues and is particularly abundant in glands and fast-growing epithelia. It is absent in nervous and muscle tissues as well as in blood and in mesenchyme-derived cells. Confocal analysis indicates that BD31 antigen is located in discrete dots at cell-cell contact boundaries and absent in basal and apical domains of cultured and *in situ* epithelial cells. MoAb BD31 precipitates a 100 kDa protein from cells labeled with ^{35}S-methionine or ^{3}H-glucosamine as well as from ^{125}I surface labeled cells. This glycoprotein is resistant to trypsin in the presence of Ca^{2+} and releases an 80 kDa fragment typical of epithelial cadherins; the lack of cadherin COOH-terminal domain and protein removal by phosphoinositide-specific phospholipase indicate its membrane-anchoring by a glycosyl-phosphatidylinositol (GPI) moiety. Its extracellular domain, however, is not recognized by a MoAb to E-cadherin (HECD-1). MoAb BD31 reacts with a functionally relevant epitope and, when added to confluent cells, induces loss of cell-cell adhesion, cell scattering and appearance of migratory cell phenotypes. This GPI-anchored molecule may be a glycoprotein specifically enriched at junctions located at cellular lateral domains and with a tissue distribution similar to E-cadherin. Also in view of its functional role, it may regulate intercellular adhesion by a mechanism involving membrane-associated phospholipases.

This investigation was supported by AIRC (Associazione Italiana per la Ricerca sul Cancro) e by CNR target project ACRO (Applicazioni Cliniche della Ricerca Oncologica)

P 215 IMMUNO-EM LOCALIZATION OF BETA-1 INTEGRIN IN WET-CLEAVED FIBROBLASTS, Alexandra M.L. Meijne, Constance A. Feltkamp and Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.

Using immuno-EM, we have studied the localization of beta-1 integrins on chicken embryo fibroblasts (CEF) that had spread for 3 h on fibronectin in the absence of serum. Cells were "wet-cleaved", which yields a ventral substrate-associated membrane with associated cytoskeleton. Beta-1 integrins, detected with cytoplasmic domain-specific antibodies, were present at the cell margin, in discrete clusters at the periphery of adhesion plaques and in clusters over fibronectin fibrils, that could often be seen beneath the cells. Beta-1 integrins were not detected at the central parts of adhesion plaques.

When cells were treated with cycloheximide (3 h before trypsinization and during spreading), fibronectin fibrils, stress fibers and adhesion plaques were not formed, and beta-1 integrins accumulated in large lamellipodia-like marginal areas. Substrate-attached fibronectin was removed from these areas, but this did not cause dispersion of the beta-1 integrins. Talin and vinculin accumulated at these sites, as in normal adhesion plaques in non-treated cells.

These observations show that the formation of a multimolecular structure containing an accumulation of talin, vinculin and integrins, as in normal adhesion plaques, can occur in the absence of stress fiber and fibronectin fibril formation.

Addition of soluble fibronectin during cell spreading in the presence of cycloheximide reversed the effect of this drug: fibronectin fibrils and adhesion plaques were formed. This suggests that under these conditions fibronectin fibrils are required for adhesion plaque formation.

The concentration of beta-1 integrins at the periphery of adhesion plaques and their absence from the center of the plaques, indicate that other membrane components mediate the attachment of the center of the plaque to the substrate.

P 216 SHEAR STRESS UPREGULATES FUNCTIONAL ICAM-1 EXPRESSION IN CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS, Tobi Nagel, Nitzan Resnick, William J. Atkinson, C. Forbes Dewey, Jr., and Michael A. Gimbrone, Jr., Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115, and Fluid Mechanics Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139.

Hemodynamic forces induce numerous functional changes in vascular endothelium, many of which reflect alterations in gene expression. We have recently identified a cis-acting transcriptional regulatory element, the "shear stress response element" (SSRE), present in the promoters of several shear stress responsive genes, that may represent a common pathway by which biomechanical forces influence gene expression. In this study, we have examined the effect of shear stress on endothelial expression of intercellular adhesion molecule-1 (ICAM-1), which contains the SSRE in its promoter, and E-selectin (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both of which lack the SSRE. Cultured human umbilical vein endothelial cells subjected to a physiological level of laminar shear stress (10 dyne/cm^2) in a cone and plate apparatus for up to 48 hours showed an increased surface expression of immunoreactive ICAM-1, compared with static (no flow) cultures, which was correlated with increased adhesion of the JY lymphocytic cell line. Upregulated ICAM-1 message was detectable by Northern blot analysis as early as 2 hours after the onset of shear stress. In contrast, E-selectin and VCAM-1 message and cell surface protein were not upregulated at any time point examined. A 27 bp fragment of the ICAM-1 promoter, containing the SSRE sequence, formed a specific complex with nuclear extracts from shear-stressed endothelial cells when used as a probe in gel-shift assays. The correlation of the shear responsiveness of ICAM-1 with the presence of a functional SSRE in its promoter provides further evidence suggesting that this transcriptional regulatory element may play a role in endothelial adaptations to flow in vivo. (Supported by NHLBI grant P01-HL-36028)

P 217 STRUCTURAL AND CELL-TYPE SPECIFIC ELEMENTS CONTRIBUTE TO THE FUNCTIONAL PROPERTIES OF THE αL/β2 INTEGRIN , *Ruggero Pardi and §Jeffrey R. Bender, *Scientific Institute San Raffaele and University of Milan, 20132 MILAN, Italy and §Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven-CT, 06510 USA .

Leukocyte integrins display a low- and a high-avidity state for their ligands. Transition between the two states is induced by cell activation and appears to use protein kinase C activation as a common pathway downstream the triggering stimulus. In addition to this inside-out signalling required to increase ligand avidity, leukocyte integrins have been shown to transduce activation signals and costimulate cell proliferation and differentiation. In this study we expressed the cDNA's coding for the two subunits of the αL/β2 integrin in several β2 integrin-negative cell types and performed mutagenesis studies to identify the structural elements required for the above mentioned functional properties of the adhesion receptor complex. Upon engagement of the ligand, the transfected αL/β2 integrin induces a locomoting phenotype in cells normally displaying a static, adherent phenotype. Integrity of both the alpha and the beta subunit cyoplasmic domains is required for this function, although the region comprising the 8 most amino terminal residues of the alpha subunit cytoplasmic domain seems to be more relevant for the coupling of the receptor to the effector(s) of the signalling process. On the contrary, two regions comprising residue 733 thru 741 and 747 thru 769 in the beta subunit cytoplasmic domain are responsible for connetcting the receptor to the actin cytoskeleton. The integrin-cytoskeletal connection, which is inducible in leukocytes upon activation, appears to be constitutive in adherent cells such as epitheliod cells and fibroblasts.

In conclusion, both subunits' cytoplasmic domains are required for proper functioning of αL/β2 integrin although, as shown for other integrins, the alpha subunit accounts for receptor-associated functions implying signalling, and the beta subunit is mainly responsible for the anchoring of the receptor to the cell cytoskeleton.

P 218 STRUCTURE/FUNCTION STUDIES OF P-SELECTIN GLYCOPROTEIN LIGAND, Gray Shaw, Karen M. Barone and Deborah Pittman, Genetics Institute Inc. 87 Cambridge Park Drive, Cambridge, MA 02140

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

P 219 DISTINCT REGIONS OF THE CYTOPLASMIC DOMAIN OF THE INTEGRIN β6 SUBUNIT ARE REQUIRED FOR LOCALIZATION TO FOCAL CONTACTS AND FOR STIMULATION OF EPITHELIAL CELL PROLIFERATION.
Dean Sheppard, Aileen Chen, Ann Weinacker and Michael Agrez. Lung Biology Center, Department of Medicine, University of California, San Francisco.

The integrin αvβ6 is a fibronectin receptor that is principally expressed in epithelial cells during organogenesis and following tissue injury, and in malignant tumors derived from epithelial cells. Heterologous expression of the full-length β6 subunit causes new β1-integrin independent cell attachment to fibronectin in the epithelial derived cell line SW480, but not in Chinese Hamster Ovary cells (CHO). SW480 cells transfected with β6 cDNA also demonstrated markedly augmented proliferation in 3 dimensional collagen gels. The cytoplasmic domain of β6 is composed of a 41 amino acid region that is highly homologous to other integrin β subunit cytoplasmic domains, and a unique 11 amino acid C-terminal extension. Deletion of the C-terminal extension had no effect on focal contact localization or cell adhesion in SW480 cells, but eliminated the effects of β6 on cell proliferation. Several different deletions of portions of the conserved 41 amino acid region all abolished focal contact localization and β6-mediated cell attachment. These data suggest that first 41 amino acids of the β6 cytoplasmic domain are required for focal contact localization and cell attachment, whereas the C-terminal 11 amino acids appear to be uniquely important for β6-mediated cell proliferation.

P 220 ROLE OF INTEGRIN β1 CYTOPLASMIC DOMAIN IN SIGNALLING AND CELL ADHESION. Guido Tarone, Paola Defilippi, Fiorella Balzac, Francesco Retta Chiarella Bozzo, and Lorenzo Silengo. Dipartimento di Genetica, Biologia e Chimica Medica, Università di Torino, Torino, Italy.

Interaction of integrin with ECM components trigger tyrosine phosphorylation of intracellular proteins. Using human endothelial cells we showed that adhesion to matrix proteins or to integrin antibodies stimulates tyrosine phosphorylation of a group of proteins of 100-130 kDa and of a 70kDa protein. Tyrosine phosphorylation occurs within 30 seconds from adhesion and remains high as long as the cell adhere to fibronectin. Several β1 complexes, including α3/β1, α5/β1 and α6/β1, are equally capable to induce tyrosine phosphorylation of the 100-130kDa and of the 70kDa proteins. These data indicate that the cytoplasmic domain of the α subunit does not modulate the ability of β1 to activate this signalling pathway. The role of β1 cytoplasmic domain in cell adhesion and signalling was further investigated by analyzing a naturally occurring variant of β1 integrin (β1B) with a distinct cytoplasmic domain. The β1B expressed in CHO cells forms heterodimers with the endogenous α3 and α5 subunits and binds fibronectin in an RGD-dependent manner. The β1B complex, however, are unable to trigger tyrosine phosphorylation of the 100-130kDa proteins. Analysis of cell adhesion indicated that the β1B variant behaves as a dominant negative mutant. In fact, CHO expressing β1B spread poorly on fibronectin and laminin and are strongly inhibited in migration. Moreover, β1B does not localize to focal adhesion, indicating that this protein can not organize actin cytoskeleton. Thus the cytoplasmic domain of β1B does not trigger tyrosine phosphorylation signalling and actin organization and does not support cell adhesion and migration. These data indicate a critical role of integrin β1 cytoplasmic domain in cell-matrix adhesion. (supported by grants from the CNR, P.F. ACRO and from the AIRC).

P 221 A SPLICE VARIANT OF α6 INTEGRIN IS ASSOCIATED WITH MALIGNANT CONVERSION IN MOUSE SKIN TUMORIGENESIS, Tamar Tennenbaum[1], Adam B. Glick[1], Adam J. Belanger[1], Richard Tamura[2], Vito Quaranta[2] and Stuart H. Yuspa[1], LCCTP[1], NCI, Bethesda, MD 20892 and Department of Cell Biology[2], Scripps Research Institute, La Jolla, CA 92037

Surface receptors of the integrin family are important for physiological and pathological processes in skin including proliferation,differentiation and malignant transformation. The epidermal basal cell specific integrin α6β4 is focally expressed suprabasally in benign mouse skin tumors (papillomas) and is diffusely expressed in carcinomas, associated with an increase in the proliferating compartment. To evaluate the molecular basis for altered α6β4 expression, mouse skin papillomas and carcinomas were produced by chemical carcinogens or by introduction of specific oncogenes into normal keratinocytes. In the latter model, keratinocytes transduced with a v-ras^{Ha} oncogene produce papillomas in vivo while co-transduction of v-ras^{Ha} and v-fos produces carcinoma cells. In carcinomas(n=16), transcripts of both the α6 and the β4 integrin subunits were increased relative to normal skin and papillomas(n=8). Further analysis by RT-PCR and DNA sequencing revealed that while papillomas (n=8) express α6A transcripts, carcinomas (n=17) expressed both α6A and a splice variant, α6B. There was preferential transcription of α6B in the poorly differentiated v-ras^{Ha}/v-fos transduced carcinomas. Only a single form of β4 transcript was detected. In vitro, primary keratinocytes and several keratinocyte cell lines including, BK1 (normal) 308 and SP1 (papilloma) and PAM 212 and PAM 25(carcinoma) expressed α6A only. However, I7 cells derived from a v-ras^{Ha} /v-fos carcinoma expressed both α6A and α6B transcripts and both proteins were detected on western blots. To examine the link between v-ras^{Ha}/v-fos transduction and α6B transcription, we transduced the v-fos , v-jun and c-myc genes into SP1 papilloma cells which contain a mutated c-ras^{Ha} allele. SP1 cells transduced with a control vector, c-myc or v-jun expressed only α6A transcript and produced papillomas upon grafting. In contrast v-fos transfectants expressed both α6A and α6B and produced carcinomas in vivo. These results establish a link between conversion to malignancy,specific oncogene expression and regulation of the α6β4 integrin. Current studies are aimed at defining the role of α6A and α6B in proliferation and migration of cells undergoing malignant progression and conversion.

P 222 CONTORTROSTATIN, A SNAKE VENOM DISINTEGRIN, HAS TWO RECEPTOR SITES ON HUMAN MELANOMA M24met CELLS BUT ONLY ONE BINDING SITE ON PLATELETS. Mohit Trikha and F.S. Markland. Department of Biochemistry and Molecular Biology and Norris Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033

Integrins are cell surface adhesion receptors that play a critical role in tumor invasion and dissemination. We have purified a disintegrin, contortrostatin, that inhibits integrin mediated cell-matrix and cell-cell interactions. Contortrostatin was purified from *Agkistrodon contortrix contortrix* snake venom using hydrophobic interaction HPLC, two steps on C18 reverse phase HPLC, and cation exchange HPLC. Positive ion electrospray mass spectrometry of native contortrostatin indicates a single isoform of 13,510 daltons. Contortrostatin inhibits human melanoma M24met cell adhesion to fibronectin, vitronectin and to a lesser extent to collagen and laminin. The binding of ^{125}I-labelled contortrostatin to M24met cells and to platelets is saturable and displacable. ^{125}I-contortrostatin binds to at least two sites on human melanoma M24met cells. It binds to a high affinity site with a dissociation constant (Kd) of 1.1 (± 0.7) nM and 96,000 (± 39,000) sites per cell, and to a lower affinity site with a Kd of 41 (± 13) nM and 480,000 (± 90,000) sites per cell. Contortrostatin, an inhibitor of the integrin receptor, GPIIb/IIIa, mediated platelet aggregation, binds to a single site on platelets with a Kd of 37 nM and 100,000 binding sites per platelet. In conclusion, contortrostatin is a potent inhibitor of integrin mediated interactions. It binds to two receptor sites on M24met cells but to only one site on platelets. Supported in part by the National Cancer Institute Grant Number RO3CA54861 (FM).

P 223 AFFINITY AND KINETIC ANALYSIS OF THE INTERACTION OF THE CELL-ADHESION MOLECULE CD2 WITH ITS LIGANDS CD48 AND CD58, P. Anton van der Merwe, Marion H. Brown, Simon J. Davis, and A. Neil Barclay, MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, OX1 3RE, UK

The T lymphocyte cell-adhesion molecule (CAM) CD2 is known to interact with the structurally-related CAMs CD58 (LFA-3) and CD48. All three proteins contain two immunoglobulin superfamily (IgSF) domains in their extracellular regions and the structure of extracellular portion of CD2 has been determined by X-ray crystallography. Mutagenesis studies have identified one face of the NH_2-terminal IgSF domain of CD2 as the ligand binding site. Soluble, monomeric forms of these proteins were used for a detailed affinity and kinetics analysis of their interaction on a BIAcore™ apparatus. This instrument allows interactions to be followed in real time using the optical phenomenon of surface plasmon resonance to detect protein binding. Using this approach the affinity of the rat CD2 - CD48 and human CD2 - CD58 interactions were measured as Kd 60-90 μM and Kd 10-20 μM, respectively. These very low affinities were the result of extremely rapid dissociation rate constants which were measured as *at least* 3 and 6 s^{-1}, for the rat CD2 - CD48 and the human CD2 - CD58 interactions, respectively. This study represents the first measurement of the kinetics of CAM interactions and our results indicate that these molecules interact with a much lower affinity than previously reported. Very low affinity interactions seem likely to be common for CAMs involved in the transient cell interactions typical of leucocytes because very fast dissociation rates may be required to allow for detachment of cells.

P 224 E-CADHERIN, FAST PLASMA MEMBRANE MOVEMENTS AND CELL SPREADING, Nicolas A.F. van Larebeke, Lionel Gohon, Pilar Navarro and Mark E. Bracke. Laboratory for Experimental Cancerology, University Hospital, Gent, Belgium.

First, we observed an inverse correlation between expression of E-cadherin and intensity of fast plasma membrane movements (FPMM) for cell lines derived from a mouse mammary tumor, normal murine mammary gland and mouse epidermis. Secondly, E-cadherin expressing (**E**$^+$) transfectants were compared with parental cell lines and/or control transfectants lacking E-cadherin expression (**E**$^-$): (**E**$^+$) HaCa4-E54 (Navarro et al. 1991) showed less FPMM than (**E**$^-$) HaCa4 mouse squamous carcinoma, HaCa4-neo and HaCa4-E58; (**E**$^+$) NM-f-*ras*-TD-CAM5 (Vleminckx et al. 1991) less than (**E**$^-$) NM-f-*ras*-TD-G1 (mouse mammary gland); among mouse foetal MO4 transfectants (obtained from Yan Gao), mouse mammary tumor RAC-5E and canine kidney MDCK-ras-f transfectants (Vleminckx et al. 1991), on the contrary, (**E**$^+$) cell lines did not show less FPMM than (**E**$^-$) ones. NM-e-*ras*-MAC1 cells (Vleminckx et al. 1991), transfected with a plasmid encoding E-cadherin-specific antisense RNA, showed more FPMM than NM-e-*ras* or control NM-e-*ras*-G1 cells. A monoclonal antibody to E-cadherin, added 24h or more after plating, induced FPMM and scattering in (**E**$^+$) MCF-7/AZ, but not in (**E**$^+$) MCF-7/6 human breast carcinoma cells. Given at plating this antibody inhibited cell-cell adhesion and cell spreading of MCF-7/AZ and MCF7-6 cells. Our results suggest that E-cadherin contributes, in some circumstances, to the control of cell cortex motility and of cell spreading.

P 225 PHYSICAL MEASUREMENTS OF BILAYER-SKELETAL SEPARATION FORCES, Richard E. Waugh, Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The fluid character of the phospholipid bilayer makes it essential that the bilayer be provided with the structural support of a membrane-associated skeleton for it to be stabilized in the living system. Using red blood cells as a model system, we have developed a physical approach for assessing the strength of the bilayer-skeletal interaction. The approach involves the formation of small cylinders of lipid bilayer (tethers) from the cell surface under controlled conditions. Observations of the distribution of fluorescence labelling of membrane lipids and of the integral membrane protein band-3 indicated that the tether formation process involved the separation of membrane bilayer from membrane skeleton and a segregation of the lipid from the skeletally-entangled band-3, which remained confined to the cell body. To measure the forces required to effect this separation of bilayer from the cell, the cell was held in a micropipette, and a polystyrene bead covalently coated with rabbit anti-human red blood cell IgG was stuck to the surface. The bead was trapped in the forked tip of a microcantilever and the cell was withdrawn forming a tether between the cell body and the bead. The deflection of the microcantilever provided a measure of the pulling force. The force was measured as a function of time for a fixed velocity of tether formation, and an exponential increase in the force over time ($t \sim$ 1 min.) to a nearly steady value was observed. When the tether was held at fixed length, an exponential relaxation of the force to a finite and persistent value was observed ($t \sim$ 5 min.). Thus the force consisted of both dynamic and quasi-equilibrium contributions. Based on our experience forming tethers from lipid bilayer vesicles, we estimated that the steady-state, quasi-reversible contribution to the force from the presence of the membrane skeleton was $\sim 3 \times 10^{-11}$ N. We are now in a position to evaluate potential mechanisms for the stabilization of the bilayer-skeletal interaction and to assess the effects of chemical and structural alterations on the strength of this association. The results of these studies will have important implications for assessing mechanisms for the detatchment of adherent cells from surfaces.

P 226 INHIBITION OF THE STIMULATED UPREGULATION OF MAC-1-DEPENDENT NEUTROPHIL ADHESIVENESS BY THE CELL ACTIVATION INHIBITOR CI-959, Clifford D. Wright and John A. Kennedy, Immunopathology Department, Parke-Davis Pharmaceutical Research, Div. of Warner-Lambert Co., Ann Arbor, MI 48105

CI-959 [5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[b]-thiophene-2-carboxamide, monosodium salt] has previously been reported to inhibit adherence of human neutrophils to TNFα- and LPS-stimulated human umbilical vein endothelial cells. This work further evaluates the effect of CI-959 on leukocyte adherence by examining the effect of the compound on Mac-1-dependent adherence. Treatment of neutrophils with N-formyl-methionyl-leucyl-phenylalanine (FMLP)(10^{-8}M) for 1 hour at 37° C resulted in a 49% increase of β-2 integrin-dependent adherence to keyhole limpet hemocyanin (KLH)-coated plastic. CI-959 inhibited the FMLP-stimulated neutrophil adherence with an IC_{50} of 0.02 μM. In addition, CI-959 inhibited chemotaxis of neutrophils in response to FMLP in the Mac-1-dependent chemotaxis-under-agarose assay with an IC_{50} of 3.1 μM. In comparison, flow cytometric analysis shows a 1.5 fold increase in cell surface expression of Mac-1 on stimulated neutrophils. Mac-1 expression was not inhibited by CI-959 at concentrations up to 100 μM. The ability of CI-959 to inhibit stimulated Mac-1-dependent adhesion without affecting Mac-1 upregulation suggests that CI-959 may inhibit Mac-1-dependent neutrophil adherence by modulating the stimulated increases in Mac-1 avidity.

P 227 **Carbohydrate Specificity of Receptor Sites of *Viscum Album* Toxic Lectin***

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The carbohydrate specificity of toxic lectin-I from *Viscum album* was studied by quantitative precipitin, precipitin-inhibition, and hemagglutination-inhibition assays. The results indicated that ML-I has a broad range of affinity for Gal α, β linked sequences. The galabiose (**E**, Galα1→4Gal) sequence, a receptor of the uropathogenic *E. coli* ligand, was one of the best disaccharide inhibitors tested. The lectin also exhibited affinity for **Lac** (Galβ1→4Glc), **T** (Galβ1→3GalNAc), **I/II** (Galβ1→3/4GlcNAc) and **B** (Galα1→3Gal) sequences. Galα1→4Gal and Galβ1→4Glc are frequently occurring sequences of many glycosphingolipids located at the mammalian cell membrane, such as intestinal and red blood cell surface membranes available for ligand binding and toxic attachment. This finding provides important information concerning the possible mechanism of intoxication of cells by the mistletoe preparation.

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P 228 **SHEAR EFFECTS ON PROTEIN BINDING AND PLATELET AGGREGATION**, Zheming Xia, Mony M. Frojmovic, Department of Physiology, McGill University, Montreal, PQ, Canada, H3G, 1Y6

Platelet aggregation occurs under various shear rates and requires the participation of adhesive proteins such as fibrinogen (Fg) and activated membrane receptors such as GPIIb-IIIa. Shear rates can affect both collision frequencies and capture efficiencies of polymer- particle and particle-particle interactions, while shear stresses may also directly "activate" proteins like Fg. In order to begin a dissection of these effects, we use a microcouette where constant shear rates can be varied from 1 - 1000 s^{-1}, and sub-sample (20 μl) from a total volume of 400 μl via a collection port for kinetic studies of protein adsorption and platelet activation/ aggregation using flow cytometry and particle counting. Although others have found that flow dependent adsorption of albumin depends on polymer chemistry, theoretical considerations predict that proteins such as albumin, Fg, and IgM, with diameters < 50 - 100 nm, will adsorb to surfaces driven only by diffusion. This was experimentally confirmed for adsorption of (i) FITC-labelled albumin onto carboxylated latex spheres (2 μm), or (ii) FITC-labelled IgM, PAC1, a monoclonal antibody binding to the activated GPIIb-IIIa receptor on fixed, activated (AF) platelets, for G = 0, 300, and 700 s^{-1}. Thus any effects of shear on protein binding kinetics are expected only for shear-sensitive proteins, such as von Willebrand factor and/or its receptor on platelets. The capture efficiency (α) of platelets was next evaluated as a function of receptor occupancy, by pre-incubating AF-platelets with FITC-Fg for varying times and concentrations. α is remain maximal (~ 0.24) for of 25 - 75% Fg occupancy, otherwise decreasing rapidly. The data suggests that Fg on occupied receptors cross-links to free, activated receptors, with 50% decrease in α for ≤ 2% occupancy (a few hundred bridging molecules).

P 229 MECHANICAL ANALYSIS OF CELL ADHESION IN MICROPIPETTE EXPERIMENTS

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Detailed mechanical analysis is required in order to understand the stress distribution within the contact area of two adherent cells and the work done to separate them at each stage of the detachment process. The knowledge of adhesion force and energy provides the global constraints for the study of the molecular dynamics of the adhesive receptors, as the continuously distributed stress and energy must be derived from the statistical means of the binding force and energy of the adhesion molecules. We present a continuum mechanics model for the analysis of cell adhesion in micromanipulation experiments. In these experiments, individual cells are manipulated by two micropipettes through aspiration to adhere to one another and to be detached after conjugation has been formed. Since the aspiration pressure and the pipette displacement are controlled and the cell deformation is measured, it is possible to derive the adhesion force and energy as well as the mechanical properties of the cell. In the present study, the cell is modeled mechanically as a liquid drop with a prestressed cortical tension. Analytical solutions have been obtained for the cell deformation due to adhesion and separation under micropipette aspiration. Explicit expressions for the adhesion force and the surface adhesion energy density have been derived as functions of the mechanical properties and the geometric parameters of the deformed cell. Experiments were performed to test the validity of the model, which showed that the liquid drop model was a good approximation for soft cells under slow micromanipulation. The model was utilized to analyze experimental results of separation of individual adherent HL-60 promyelocytic cells from cultured Kaposi's sarcoma cells by micromanipulation. The adhesion force and energy as well as the prestress cortical tension at various stages of the adhesion/separation process were calculated from geometric measurements from sequential images of the micropipette experiments. Studies for the micromechanics of the adhesion molecules are currently underway to incorporate the information obtained from the present continuum model. (Supported by NSF Grants Nos. 9210684 and 9350370, Whitaker Foundation Biomedical Engineering Research Grant, and Emory/Georgia Tech Biomedical Technology Research Grant)

Cytoskeleton/Cell Surface Membranes

P 300 DISTRIBUTION OF BASOLATERAL MEMBRANE DOMAINS IN ISOLATED POLARIZED INTESTINAL EPITHELIAL CELLS IS TEMPERATURE DEPENDENT. T. Albers and R. Moore, Dept. of Pathology, School of Veterinary Medicine and Medicine, Tufts University, Boston, MA 02111

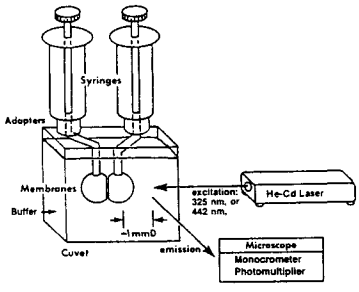
Maintenance of membrane domains in polarized epithelia, such as the intestine, is dependent on the ability to restrict movement of proteins within the plane of the membrane. Commonly used techniques to harvest polarized epithelial cells from native tissue or cultured cells may not take into account the effects of temperature on maintenance of membrane domains in the face of disrupted tight junctions and cell-matrix interactions. In order to determine the effect of temperature on localization of polarized proteins in isolated cells, we examined the distribution of Na^+/K^+-ATPase and α-L-fucose glycosylated proteins in isolated, native guinea pig intestinal epithelial cells and compared their distribution to that in intact epithelium. Cells were isolated by mechanical means at 4°C and 37°C, and distribution of Na^+/K^+-ATPase and α-L-fucose, basolateral and apical membrane proteins respectively, was determined by using immunohistochemistry and biotin-avidin lectin histochemistry. Na^+/K^+-ATPase, restricted to the basolateral membrane in intact epithelium, was exhibited in both apical and basolateral domains of 71% of single cells isolated at 37°C. However, cells isolated at 4°C displayed a distribution more typical of intact epithelium, with 73% of single cells exhibiting exclusively basolateral localization Na^+/K^+-ATPase. α-L-fucose glycosylated proteins, restricted to the apical membrane in intact intestinal epithelium, maintained the apical distribution in 100% of single cells isolated at 37°C. These results suggest that the distribution of Na^+/K^+-ATPase, a protein restricted to the basolateral membrane domain, may be altered when cells are isolated at 37°C. In contrast, α-L-fucose glycosylated proteins, which are restricted to the apical membrane domain of intact intestinal epithelium, are not altered in cells isolated at 37°C. These observations may be an important consideration when evaluating data in which isolated polarized cells are used to examine function of proteins normally restricted to membrane domains.

P 301 CYTOPLASMIC REGIONS OF MURINE $I\text{-}A^k$ MODULATE PROTEIN ROTATIONAL DIFFUSION RATES. B.G. Barisas, W.F. Wade*, N.A. Rahman and D.A. Roess, Departments of Chemistry and Physiology, Colorado State University, Ft. Collins, CO 80523 and *Department of Microbiology, Dartmouth Medical School, Lebanon NH 03756

M12C3 and K46J murine B cell lymphomas were transfected to express $I\text{-}A^k$ Class II Major Histocompatibility Complex molecules exhibiting various truncations in the cytoplasmic regions of the α- and β-chains. Previous measurements of $I\text{-}A^k$ lateral diffusion have shown that α-chain carboxy-terminal amino acids and β-chain amino acids proximal to the plasma membrane are important in restricting $I\text{-}A^k$ lateral motions. To better assess potential interactions of $I\text{-}A^k$ with other membrane and cytoskeletal elements, we have examined the rotational diffusion of $I\text{-}A^k$ using time-resolved phosphorescence anisotropy methods. Wild-type $I\text{-}A^k$ exhibits rotational correlation times (RCT) ranging from 50 μs at 4°C to 11 μs at 37°C suggesting free rotational diffusion of $I\text{-}A^k$ molecules or complexes. The rising anisotropies observed indicate that antibody conjugation occurs at highly specific sites. The α-6/βwt M12C3 and α-12/βwt, αwt/β-12 and α-12/β-12 K46J carboxy-truncated proteins exhibit RCTs of 45 μs, 17 μs, 36 μs and 18 μs, respectively. Similar RCTs are obtained for $I\text{-}A^k$ on any particular M12C3 transfectant whether rotation is probed using erythrosin conjugates of 39J anti-α chain mAb or 10.2.6 anti-β chain mAb. Apparent $I\text{-}A^k$ rotation on K46J transfectants varies somewhat according to whether α or β chain-specific mAbs are used as probes. This could reflect differences in mAb or erythrosin orientation relative to the Class II molecule rotation axis or $I\text{-}A^k$ chain hybridization with K46J intrinsic $I\text{-}A^d$, though such hybridization has been reported to be thermodynamically unfavored. The faster rotation of α-12/βwt (17 μs) relative to α-6/βwt (45 μs) and to αwt/βwt (50 μs) suggests that residues between the C-terminus of the α-chain and the plasma membrane modulate $I\text{-}A^k$ interactions with other membrane proteins or cytoskeletal elements. Supported in part by NIH grants AI26621 (BGB) and AI31160 (WFW).

P 302 GLYCOLIPID-DEPENDENT CHANGES AT THE ADHESIVE JUNCTION BETWEEN MEMBRANES. Gregory J. Brewer, Southern Illinois University School of Medicine, Springfield, IL 62794-9230

To investigate contact and ganglioside-dependent changes between adherent cell surfaces, macroscopic spherical lipid-bilayers were formed at the tips of two syringes and moved into contact.



If 4 mole% gangliosides are included with egg phosphatidylcholine in the membrane, there is a 3-fold increase in conductance across the junctional membrane. This ganglioside-dependent contact sensation is associated with physical-chemical changes in the junction: (1) a less polar environment detected by fluorescent emission from PRODAN, (2) increased concentration and restricted mobility of fluorescent paranaryl derivatives of gangliosides GM1 and GD1a, (3) a doubling of surface potential detected with nonactin-mediated conductance and (4) a 3-fold increase in junctional capacitance. These results support a ganglioside-dependent mechanism of adhesion and contact sensation involving entropic dehydration.

P 303 INHIBITING TYROSINE SULFATION OF THE GP Ib-IX COMPLEX DECREASES ITS ADHESIVE PROPERTIES BUT NOT ITS CELL SURFACE EXPRESSION. Jing-Fei Dong, Chester Q. Li, and José A. López, Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA 94141

Shear forces cause platelets to aggregate by inducing von Willebrand factor (vWf) to bind the platelet glycoprotein (GP) Ib-IX complex. This phenomenon is mimicked *in vitro* by adding peptide inducers (ristocetin or botrocetin) to platelets suspended in plasma. We have recently shown that heterologous cells expressing recombinant human GP Ib-IX also aggregate in the presence of vWf and ristocetin. In other studies, we showed that the ligand-binding subunit of the complex, GP Ibα, is sulfated at tyrosine residues. Here we present evidence that this modification has an important role in cell aggregation mediated by the GP Ib-IX complex, but has no role in its plasma membrane expression. We examined aggregation of transfected L and CHO cells expressing the entire human GP Ib-IX complex (αβIX cells) or only GP Ibβ and GP IX (βIX cells), at low ($1x10^5$ cells/ml) and high ($1x10^6$ cells/ml) cell densities. To induce cell aggregation, 0.5-ml aliquots of a cell suspension were mixed with 400 μl platelet-poor plasma (as a source of vWf) and 1.4 mg/ml ristocetin, and shaken on a rotary shaker at 1–6 cycles/sec. Aggregation of αβIX cells began within 5 min of adding plasma and ristocetin and plateaued after 45 min; βIX cells treated identically did not aggregate during the test period. In contrast, when αβIX cells were tested after a 24-h incubation in sulfate-free medium containing 5 mM sodium chlorate and 0.2 mM guaiacol (a condition that prevents sulfation), they failed to aggregate at low cell density. At high density, the cells aggregated to a similar extent as did sulfate-replete cells but disaggregated rapidly when shaking ceased. Cells grown under both conditions expressed equivalent levels of GP Ib-IX on their surfaces, as demonstrated by flow cytometry using an anti-GP Ibα monoclonal antibody. These data suggest that tyrosine sulfation increases the binding affinity of GP Ibα for vWf. Further, the observation that cells rapidly disaggregate when shaking stops suggests that sulfation may be important for maintaining a shear-induced conformation of GP Ibα that allows it to interact with vWf.

P 304 MEMBRANE FUSION INHIBITED BY A *trans*-DOMINANT MUTATION IN THE HIV-1 ENVELOPE GLYCOPROTEIN gp120-gp41, Hannah F. Elson and Robert Blumenthal, Membrane Structure and Function, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Membrane fusion, which is involved in many important cellular processes, can be studied in simple model systems such as viral mediated fusion, where mutations in the fusogenic proteins may be more easily available. Human immunodeficiency virus type-1 (HIV-1) envelope glycoprotein gp160 (or gp120-gp41), expressed on the surfaces of cells, can produce syncytia with receptor CD4-containing target cells. A recombinant vaccinia virus was used to express a mutation in the HIV-1 envelope glycoprotein. In this mutant, the second amino acid from the N-terminal end of gp41 is a charged glutamic acid instead of a nonpolar valine. Normally, when recombinant vaccinia viruses expressing wild-type HIV-1 envelope protein infect a lymphocyte cell line lacking CD4, the cells express the HIV-1 envelope glycoproteins gp120-gp41 and are able to fuse with $CD4^+$ T lymphocytes. Expression of the mutant envelope glycoprotein gp120-gp41.2 produces no fusion between infected cells and $CD4^+$ T lymphocytes. When both viruses infect CD4-negative cells simultaneously, there is a dose-dependent inhibition of fusion with an increasing fraction of virus expressing the mutated gp41.2. Interestingly, when the opposing, or $CD4^+$ target cells are infected with the mutant virus, while CD4-negative cells are infected with virus expressing the wild type envelope glycoprotein, a similar dose-dependent inhibition of fusion is observed. This suggests that the mutated envelope protein does not need to reside in the same membrane as the wild type protein it inhibits. Further studies with this mutant may therefore help us to understand the mechanism of membrane fusion.

P 305 THREE DIMENSIONAL RE-ARRANGEMENT OF THE ENDOTHELIAL CELL CYTOSKELETON UNDER SHEAR STRESS. C. Galbraith, R. Skalak and S. Chien. Department of AMES/ Bioengineering and the Institute for Biomedical Engineering. University of California, San Diego. La Jolla, CA 92093

In response to the application of shear stress on the apical surface of an endothelial cell a variety of electrical and biochemical changes occur, and the cell and its cytoskeleton align with the direction of flow. In order to establish how the endothelial cell responds to shear stress, it is necessary to determine how the shear stress is distributed through the cell. This requires an understanding of the three dimensional arrangement of the cytoskeletal components of sheared and unsheared cells.

Cultured bovine aortic endothelial cells (BAECs) were triple labeled for f-actin in the microfilaments, β-tubulin in the microtubules, and vimentin in the intermediate filaments. The cells were then examined by confocal microscopy, and stereo pair images were reconstructed from the optical sections. BAECs grown under static conditions have a diffuse layer of microfilaments located along the apical membrane and the majority of microfilaments are uniformly organized as either stress fibers or a dense peripheral band located along the basal side of the cell. The intermediate filaments are present throughout the height of the cell. They are highly concentrated around the nucleus of the cell and extend radially toward the cell periphery. The microtubules follow a distribution pattern which is similar to that of the intermediate filaments. When BAECs are exposed to a shear stress of 17 dynes/ cm^2 for 18 hrs. The majority of the cells align in the direction of the flow. The microfilaments in the sheared cells appear to be less dense in the apical region of the cell. The stress fibers orient along the same direction as the cell, and they appear to be more concentrated on either side of the nucleus. The microtubules and the intermediate filaments maintain the same vertical distribution as in the unsheared cells, but they re-align along the long axis of the cell, and the microtubule organizing center is frequently located perpendicularly to the direction of flow.

The microfilaments, microtubules, and intermediate filaments align with the direction of shear stress. These results suggest that all three cytoskeletal components are involved in stiffening the cell against the applied shear stress.

The authors gratefully acknowledge Dr. Mark Ellisman and Mr. Thomas Deernick of the San Diego Imaging and Microscopy Center for providing the confocal microscope and their assistance in obtaining the images. Support for this project was provided by PHS Grants HL07089 and HL43026.

P 306 THE EFFECTS OF MECHANICAL FORCES ON THE MEMBRANE AND AXOPLASM OF THE SQUID GIANT AXON. J.A. Galbraith[1] and P.E. Gallant[2] Marine Biological Laboratory, Woods Hole, MA 02543; and (1) AMES / Bioengineering and Institute for Biomedical Engineering, UCSD, La Jolla, CA 92093-0412; (2) Laboratory of Neurobiology, NINDS, NIH, Bethesda, MD 20892.

The plasma membrane can undergo rapid and large electrophysiological changes in response to mechanical forces. Some of these changes which can be detected electrophysiologically may serve as signals to trigger many of the physiological and pathological changes observed in mechanically stressed cells. To accurately describe the relationship between mechanical forces and membrane changes we subjected isolated giant axons from the squid *Loligo pealei* to controlled uniaxial extensions of up to 30% in 10 milliseconds.

During these protocols, the axons were placed in 10° C artificial sea water and membrane potential, displacement, and force were monitored throughout each experiment. To gauge cytoskeletal alteration, intracellular transport of large and small organelles was observed thirty minutes post stretch using high numerical aperture differential interference microscopy (DIC) with video enhancement techniques.

As the level of imposed stretch was increased, a proportionally greater membrane depolarization and longer recovery time was observed. Five percent stretches produced depolarizations of several millivolts which recovered in less than 60 seconds. Increasing the elongation to 10% generated depolarizations in the range of 10 to 15 millivolts which took several minutes to recover to resting levels. The severity of structural damage and transport loss was also proportional to the magnitude of stretch. Axons receiving the lowest stretches showed no transport or structural changes. Moderately stretched axons had beaded mitochondria that moved at significantly reduced rates. The most severely stretched axons had a totally disrupted axoplasmic structure and no transport.

In several experiments the level of intracellular structural disruption was reduced by exchanging the external solution for one which contained internal ions. This suggested that the most severe intracellular disruptions were caused by external ions which entered the axon as a result of alteration of membrane permeability rather than direct mechanical disarrangement of the cytoskeleton.

Support for JAG provided by UCSD Academic Senate Grant # RT72-G and a grant from the Whitaker Foundation. Actuator and data acquisition system borrowed from R. Myers San Diego VAMC.

P 307 DISRUPTION OF THE *YCR89W* GENE OF *SACCHAROMYCES CEREVISIAE* INCREASES THE RATE OF CELL WALL DEGRADATION IN THE PRESENCE OF ZYMOLYASE: EVIDENCE THAT *YCR89W* AFFECTS THE INTEGRITY OF THE CELL WALL, Chong K. Jue, Seung T. Lim, Charly Cui and Peter N. Lipke, Department of Biological Sciences, Hunter College, 695 Park Ave., New York, NY 10021

The entire DNA sequence of chromosome III of the yeast *Saccharomyces cerevisiae* has been determined by a consortium of 35 European laboratories(1). The sequence is 315 kb long and contains 182 opening reading frames encoding putative proteins of > 100 amino acids. *YCR89W*, located on the right arm of chromosome III, has a FASTA score >200 when compared to the *AGA1* gene which encodes the core subunit of **a**-agglutinin, a cell wall protein of **a** mating type cells. **a**-agglutinin mediates the sexual agglutination of **a** and α mating type cells. From a yeast genomic library, we have isolated a plasmid (p101) containing 960 bp of the N terminus of the structural gene *YCR89W*(*CWA1*). A disruption plasmid, *cwa1*::*URA3*, was constructed by deleting 35 bp of the internal structural gene and inserting the *URA3* gene. W303-1A(*MATa*), W303-1B(*MATα*), and W303-A/B(*MATa/α*) were disrupted by a single step disruption technique. Transformants were confirmed by Southern analysis. Haploid cells harboring the disrupted gene were viable. The growth rates of the mutants were comparable to the wild types. To characterize the null mutant in terms of cell wall phenotype, we used Zymolyase, a cell wall lytic enzyme, to study the rate of cell wall degradation. Kinetic analysis of cell wall degradation showed that *MATa cwa1* and *MATα cwa1* formed spheroplasts faster than the corresponding wild type haploids. Interestingly, the rate of cell wall digestion of the wild type homozygous diploid(*CWA1/CWA1*) was greater than the wild type halploids. The heterozygous diploid(*CWA1/cwa1*) digested only slightly faster than the wild type homozygous diploid. These results suggest that *CWA1* affects the integrity of yeast cell wall.

1. Oliver, S. G. et al. *Nature* **357**, 38-46 (1992).

P 308 β1 INTEGRINS ARE HIGHLY EXPRESSED IN MYOFIBROBLASTS CULTURED FROM CORNEA, S. K. Masur, R. L. Connors, Jr, E. Weinstein, S. Antohi, J. K.-H. Cheung Department of Ophthalmology, Mount Sinai School of Medicine of CUNY, New York NY 10029-6574.

Wound healing in the corneal stroma involves activation of resident keratocytes into fibroblasts and myofibroblast-like cells. Keratocytes placed in culture as cultured fibroblasts (CFs) resemble wounded keratocytes, in that they express the fibronectin receptor, α5β1. To study the myofibroblasts we have used a function-perturbing antibody (anti-β1*) to immunoselect cells which attached to fibronectin (FN) in the presence of anti-β1 (CMFs, corneal myofibroblasts). Both CFs and CMFs attached to FN in energy-and divalent cation-dependent fashion. Both CFs and CMFs expressed α5β1, and their attachment to FN was blocked by anti-α5β1*. In CMFs synthesis and expression of β1-associated integrins and αv-associated integrins was increased*. Morphologically, CMFs were bigger cells with larger and more numerous focal adhesions. Their stress fibers had immunocytochemically detectable α-actin (smooth muscle actin). CMFs required a higher concentration of cytochalasin to inhibit their attachment to FN. CMFs had up to twice as much protein per cell as CFs. CMFs synthesized FN but not collagen I. Whereas CMFs secreted 72 kD type IV collagenase only, CFs secreted interstitial collagenases as well. Furthermore, 72 kD collagenase secretion was inhibited by PMA in CFs but not in CMFs. Thus separating CMFs by immunoselection permits us to evaluate the respective contributions of myofibroblasts and fibroblasts. CMFs firmly attach to matrix via enhanced integrin and α-actin expression. We propose that CMFs contribute to wound apposition and contraction during corneal stroma wound healing while other fibroblasts remodel the stromal matrix.

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*Antibodes generously provided by C.H. Damsky and F. Giancotti.

P 309 GENERATION OF TALIN DEFICIENT CELL LINES USING HOMOLOGOUS RECOMBINATION. Michael Meenaghan, Richard P Grant and D Jasper G Rees, The Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, United Kingdom.

The 270 kD cytoskeletal protein talin is localised into focal contacts in adherent cells, at the cell membrane of activated platelets and to the cell-cell contact zone of T cell-antigen presenting cell couples when the latter are formed in the presence of antigen. At each site it colocalises with members of the integrin family. Talin also binds to vinculin, and sites of interaction have been mapped on both molecules. However the cellular distributions of vinculin and talin are not identical. Talin consists of a globular N-terminal domain which is a member of the Band 4.1 family, and an extended C-terminal rod-like domain, comprising a series of short repeated units. We have previously described the complete sequence of murine talin, and we now describe full length expression constructs of murine talin, and a series of domain specific variants thereof. We have characterised the structure of the 5' end of the mouse talin gene and generated a pair of constructs for use in homologous recombination experiments for the ablation of expression of the talin gene. The constructs consist of the coding sequence for neomycin^R or puromycin^R inserted in frame into the talin gene such that expression of the resistance marker is dependent on the talin promoter and initiation codon. We have used the neomycin^R construct to transfect mouse $10T_{1/2}$ fibroblasts and cytotoxic T cell clones specific for influenza virus hemaglutinin and have isolated clones resistant to G-418. We are analysing these cell lines for the expression of the talin-neomycin mRNA using PCR. Clones which have a mutated talin gene will be selected and used for a second round of homologous recombination using the puromycin construct to obtain lines that are homozygous null for talin expression. Talin deficient cell lines will then be analysed either for CTL activity or adhesion to extra-cellular matrix and the formation of focal contacts as appropriate. We anticipate that the talin deficient T cells will recognise presented antigen appropriately and will proliferate in an antigen dependent fashion, but will not undergo appropriate cytoskeletal responses to achieve the movement of cytoplasmic vesicles which appears to be a critical step in T cell action. In the case of adherent cells, it is predicted that the organisation of the actin cytoskeleton will be severely disrupted in the absence of talin. These lines will then be transfected with the series of cDNA variants in order to analyse the role of specific regions of the protein.

P 310 CELL-CELL SIGNALLING: ACTIVATION OF EGF RECEPTOR KINASE BY MEMBRANE-BOUND LIGAND, Barbara S. Mroczkowski, The Agouron Institute, 505 Coast Blvd. South, Ste. 400, La Jolla, CA 92037

The utilization of growth factors as cell surface ligands may provide a mechanism for accurately specifying the three-dimensional pattern of growth and differentiation in developing organisms. This idea is supported by the observation that mutations which disrupt tyrosine kinase receptor (*i.e. sevenless* and *c-kit* receptor) interactions with cell surface ligands exert pleiotropic effects on development. The observation that the EGF precursor shares a number of structural features with various cell-surface molecules involved in homophilic or heterophilic protein-protein interactions during development and our finding that the intact precursor can functionally interact with the EGF tyrosine kinase receptor *in vitro* has led us to propose that the membrane-spanning form of the precursor may be involved in cell-to-cell communication/signalling during embryogenesis. Our experimental approach entails using the baculovirus/insect cell expression system as a useful model for *in vivo* analysis of functional EGF receptor-precursor interactions at the cell surface. We have expressed biologically active human EGF precursor and the EGF receptor in lepidopteran insect cells. Immunofluorescence and lactoperoxidase iodination of infected cells expressing either the EGF receptor or the EGF precursor indicate that both the ligand and receptor are correctly routed to the plasma membrane. Preliminary experiments indicate that the EGF receptor is capable of interacting with the membrane-anchored growth factor on adjacent cells. More importantly, this interaction results in the activation of the EGF receptor/kinase activity, demonstrating for the first time, that the membrane-spanning EGF ligand is capable of functionally interacting with its receptor at the cell surface. This finding has the important implication that internalization of ligand-receptor complex is not essential for the activation of signalling pathways involved in growth and differentiation and further demonstrates the participation of tyrosine kinase receptors in cell contact-dependent signalling.

P 311 DYNAMICS OF CADHERIN AND CATENIN INTERACTION: TEMPORAL AND SPATIAL REGULATION I.S. Näthke[1], L. Hinck[1,2], J.R. Swedlow[3], J. Papkoff[2,4], W.J. Nelson[1,2]; [1]Dept. Molecular and Cellular Physiology, Stanford University; [2]Cancer Biology Program, Stanford University; [3]Grad. Group Biophysics, University of California, San Francisco; [4]Sugen Corp, Redwood City.

Cadherins, a family of membrane glycoproteins, mediate homotypic, calcium–dependent cell-cell adhesion. Cadherin function is regulated by specific cytoplasmic proteins that interact with the highly conserved cytoplasmic portion of cadherins. These proteins, termed catenins, include α-, β-catenin and plakoglobin (γ-catenin). We are investigating the protein composition of the cadherin-catenin complex and the dynamics of their assembly in MDCK cells by measuring the association of these proteins with the cytoskeleton as determined by the acquisition of TX-100 insolubility. Our experiments analyzing the TX-100 soluble cadherin/catenin pool show that the assembly of α-catenin is regulated independently of β-catenin and plakoglobin. Measuring the migration of newly synthesized α-catenin on sucrose density gradients reveals the presence of a substantial pool of free α-catenin. Our experiments also distinguish two different complexes that contain E-cadherin and α-catenin in association with either β-catenin or plakoglobin. To examine the organization of the complex in the TX-100 insoluble pool we used high resolution immunofluorescence microscopy and chemical crosslinking . We show plakoglobin and E-cadherin do not co-localize in the insoluble pool suggesting that plakoglobin containing E-cadherin complexes are unable to attach to the cytoskeleton. In contrast, E-cadherin, α- and β-catenin co-localize at the Zonula Adherens (ZA) but only α-catenin co-localizes with E-cadherin in the lateral membrane. Additionally, E-cadherin is selectively enriched in the ZA when compared to α- and β-catenin. These observations indicate that the stoichiometry of E-cadherin and catenins varies along the lateral membrane.

In summary our studies demonstrate that: the composition of the cadherin/catenin complex is dynamic; the association of plakoglobin and β-catenin with E-cadherin is mutually exclusive; the association of α-catenin is temporally regulated; and the composition of the complex dictates its incorporation and spatial organization on the membrane.

P 312 PHOSPHOLIPID/ALKANETHIOL BILAYERS AS BIOMIMETIC MEMBRANES. Anne Plant, Department of Biotechnology, National Institiute of Standards and Technology, Gaithersburg, MD 20899.

Alkanethiols spontaneously form a stable monolayer on gold, creating a nearly defect-free hydrophobic surface. Phospholipid molecules interact spontaneously with these hydrophobic alkanethiol monolayers to produce stable phospholipid/alkanethiol planar bilayers, where the hydrophobic acyl chains of the phospholipids interact with the hydrophobic chains of the alkanethiol, and the polar headgroups of the phospholipid are exposed to the aqueous phase. The structures can be identified as bilayers by their impedence and electrochemical response. These supported bilayers exhibit the properties of other model membranes, but their ease of formation, reproducibility, and stability provide a significant advantage compared to planar bilayers prepared by other methods. Preparing bilayer on gold electrodes allows the direct study of ion diffusion and electron transfer through membranes, and thus provides a method of monitoring the events which effect these processes. This model system provides a means of determining, by electrochemical techniques, changes in membrane microstructure which occur in response to temperature, pore-forming proteins, and the multivalent binding of proteins at the membrane interface.

P 313 THE N-TERMINAL DOMAIN OF TALIN: INITIAL STRUCTURAL AND FUNCTIONAL STUDIES. D Jasper G Rees, Richard P Grant, Zi H Rao, David I Stuart, The Sir William Dunn School of Pathology and the Department of Biophysics, University of Oxford, Oxford, OX1 3RE, United Kingdom.

Talin belongs to the Band 4.1 family of cytoskeletal proteins, which includes Band 4.1, the ezrin/moesin/radixin/merlin family, talin and two protein tyrosine phosphatases. With the exception of the phosphatases, these proteins are all localised at the ends of actin filaments and are found under the cell membrane. Radixin has been shown to have barbed end capping activity for actin polymerisation, while talin has been reported to affect the polymerisation of actin *in vitro*. The common sequence relationship in the family is in the N-terminal domain, and we postulate that this domain is involved in the regulation of actin polymerisation.

In addition talin is a substrate for calpain II, and this cleaves talin between the 47 kD N-terminal domain and the C-terminal rod-like tail. We have expressed a fusion protein, in *E.coli,* of maltose binding protein and the N-terminal domain of talin, representing the 47 kD cleavage product. The protein expressed is the correct size and structure by protein sequencing and mass spectroscopy, but appears to have a Na^+ ion tightly bound to it.

We have used this recombinant protein in initial crystallisation studies and obtained crystals under a variety of conditions. We are currently exploring these conditions further to obtain larger crystals suitable for structure determination. These should enable the determination of the 3-dimensional structure of the first member of the band 4.1 family.

We have also studied the role of talin in actin polymerisation: In experiments to determine the initial rates of polymerisation of actin in the presence of increasing concentrations of talin we have found that talin slightly decreases the initial rate of actin polymerisation while not affecting the overall extent of steady state polymerisation. The critical concentration for actin polymerisation appears to be unaffected by talin concentration however. We are extending these observations with the use of recombinant protein.

P 314 MOLECULAR CLONING, mRNA EXPRESSION ANALYSIS AND PROTEIN IDENTIFICATION OF HUMAN pT49, A FIBRINOGEN-LIKE TRANSCRIPT EXPRESSED IN LYMPHOCYTES. Curzio Rüegg[1,3], Stephanie Blum[1,2], Vladimir von Fliedner[2] and Robert Pytela[3]. Centre Pluridisciplinaire d'Oncologie, CHUV[1] and Ludwig Institute for Cancer Research, Lausanne Branch[2], CH-1000 Lausanne, Switzerland, Lung Biology Center, UCSF, San Francisco[3].

The murine pT49 gene was originally identified as a T cell specific transcript using a subtractive cDNA cloning strategy (T. Koyama et al., 1987, P.N.A.S., 84, 1609). We cloned the complete cDNA sequence of human pT49 by screening a small intestine λgt11 cDNA library with a murine pT49 cDNA probe under low stringency conditions. The coding region is 1317 bp long and the deduced protein of 439 amino acid residues is 79% identical to murine pT49. The predicted molecular weight of the mature human pT49 protein, including the 5 potential N-linked carbohydrate groups, is 62 kD. pT49 has a overall homology to fibrinogen β and γ chains, including the conservation of the 5 cysteines required for the assembly of the fibrinogen chains into mature complex. This striking homology suggests that the pT49 molecule may be part of a fibrinogen-like complex. By Northern blot analysis two mRNAs (~4.5 and ~1.5 kb) were detected in human peripheral blood T cells and in the B cell line SLA, but not in a number of leukocytic and non-leukocytic cell lines tested. RT-PCR analysis on FACS-sorted human lymphocytes revealed that the pT49 gene is expressed in both $CD4^+/CD8^-$ and $CD4^-/CD8^+$ T cells. Activation of T cells by PHA resulted in a substantial down regulation of pT49 mRNA expression. Furthermore we are studying pT49 mRNA expression in a number of human T cell clones. To identify the pT49 protein we generated monoclonal antibodies against a synthetic peptide based on the deduced C-terminal sequence. One monoclonal antibody immuno-precipitates a protein complex from metabolic labeled SLA cell lysate. The complex consists of at least two proteins migrating with an apparent molecular weight of 62 and 55 kD in reducing SDS-PAGE. We are currently using this monoclonal antibody to further characterize the pT49-complex. We also generated stable transfectants (CHO, Jurkat and SLA cells) using expression constructs containing the full-length pT49 cDNA fused with a 3' end sequence encoding for a tag peptide. The expressed recombinant protein will be identified and studied using an anti-tag monoclonal antibody.

P 315 CADHERIN EXPRESSION REQUIRED FOR FORMATION AND INTERNALIZATION OF *SHIGELLA FLEXNERI*-INDUCED INTERCELLULAR PROTRUSIONS INVOLVED IN SPREAD BETWEEN EPITHELIAL CELLS, Philippe J. SANSONETTI[1], Joëlle MOUNIER[1], Marie Christine PREVOST[2], René-Marc MEGE[3], 1 - Unité de Pathogénie Microbienne Moléculaire, INSERM U 199 and 2 - Station Centrale de Microscopie Electronique, INSTITUT PASTEUR, 28 rue du Docteur Roux, 75724 PARIS Cédex 15, FRANCE, 3 - INSERM U 153, 17 rue du Fer à Moulin, 75005 PARIS, FRANCE.

Shigella flexneri, a gram-negative enteric pathogen, invades and destroys the human colonic epithelium. After entering epithelial cells via bacterium-directed phagocytosis, bacteria escape the membrane-bound phagocytic vacuole, move intracellularly, and pass from cell to cell. IcsA, and probably other as yet unidentified bacterial proteins, divert actin and associated actin-binding proteins to generate a cytoskeleton-based motor which pushes forward the bacterium. As the moving bacterium reaches the inner face of the host-cell cytoplasmic membrane, a protrusion forms which allows passage of this bacterium into a neighbouring cell. We show here that other host-cell components are diverted by the bacterium to allow this passage. Using S180, a mouse fibroblastic sarcoma cell line which does not produce CAM, S180L and S180cadN, the same cell-line transfected with L-CAM and N-cadherin cDNA respectively, we demonstrate that expression of a cadherin was required for cell to cell spread to occur. Cadherin is required both to achieve proper structural organization of the protrusion, most probably by anchoring the actin tail to the cytoplasmic membrane, and to actively internalize this protrusion by a neighbouring cell, probably by establishing homotypic cell to cell contact. Therefore, in an epithelialized monolayer, such as that formed by S180L cells, protrusions that allow cell to cell passage of bacteria form an extension of the structure of intermediate junctions. This is confirmed by immunofluorescence staining, showing that the major proteins associated with intermediate junctions, L-CAM, α-actinin, vinculin, α-catenin and β-catenin are associated with the protrusions that initiated at these junctions.

P 316 FIBROBLAST MIGRATION SPEED DEPENDS ON THE EXTENT OF INTEGRIN-CYTOSKELETON ASSOCIATIONS, Christine E. Schmidt*, Alan F. Horwitz†, and Douglas A. Lauffenburger*†, Departments of *Chemical Engineering and †Cell and Structural Biology, University of Illinois, Urbana, IL 61801

Cell migration is a phenomenon requiring dynamic adhesive interactions between the internal cell motile machinery and the external substratum, with adhesion receptors, such as integrins, serving as the transmembrane link. The mechanism by which integrin interacts with cytoskeletal elements to give rise to cell movement is poorly understood. In a previous study, we used nanometer-precision motion analysis and laser optical trapping to explore the cytoskeletal associations of β1 integrin and various site-directed integrin mutants in migrating fibroblasts (Schmidt et al., J. Cell Biol., in press). We found that integrin is supplied by a cytoskeletal-dependent rapid transport mechanism to the cell's leading edge where nascent adhesions are formed. In addition, we found that large aggregates of integrin form stable linkages to rearward-moving cortical actin, with stronger linkages existing at the cell front compared to the rear. In our present study, we have examined the effects of integrin-cytoskeletal interactions on (1) whole-cell migration rates and (2) the kinetics of directed transport of integrin to the cell's leading edge. (1) We used low-magnification video microscopy and image analysis techniques to measure cell speeds for the same fibroblasts containing site-directed mutations in the β1 integrin cytoplasmic domain. We found that cells transfected with integrins that interact strongly with the cytoskeleton (as determined from the laser trapping experiments) migrate significantly slower than either untransfected cells or cells transfected with weakly interacting integrins. These results confirm integrin's crucial role in migration and suggest that cellular regulation of locomotion could potentially occur at the level of integrin-cytoskeleton associations. Furthermore, alteration of integrin's interaction with the cytoskeleton could be a potential candidate for targeted manipulation of cell migration in medical applications. (2) We conducted Brownian Dynamics Simulations (BDS) with an added probability for linkage to a moving cytoskeletal element to mimic the directed transport of integrin to the cell's leading edge. These studies reveal relative association and dissociation rate constants for integrin's interaction with the cytoskeletal element that gives rise to this transport. Information on these rate constants could potentially be useful for selecting or engineering molecules that will inhibit integrin-cytoskeletal interactions.

P 317 ROLE OF EXTRACELLULAR CA2+ ON THE CELL SURFACE DISTRIBUTION AND ADHESION OF LFA-1, *Yvette van Kooyk, Pauline Weder, and Carl G Figdor*, Division of Immunology, The Netherlands Cancer Institute Plesmanlaan 121,1066 CX Amsterdam.

The transition of leukocyte-function-associated antigen-1 (LFA-1), expressed on T cells, from an inactive into an activated state depends on the presence of extracellular Mg^{2+} and/or Ca^{2+} ions. We investigated the Ca^{2+} occupancy of LFA-1, reported by an antibody (NKI-L16) that recognizes a Ca^{2+} dependent epitope on LFA-1. We found that Ca^{2+} can be bound by LFA-1 with different affinities (weak or strong). LFA-1 on resting T cells shows weak binding of Ca^{2+}, whereas LFA-1 expressed on activated T cells shows strong binding of Ca^{2+}. In addition we observed that stable binding of Ca^{2+} to LFA-1 is associated with cluster formation of LFA-1 on the cell surface, thereby facilitating the interaction with its ligand. In contrast, if Ca^{2+} is only weakly bound, T cells exibit a dispersed LFA-1 distribution, and hardly respond to stimuli known to activate LFA-1. Of all divalent cations, only Sr^{2+} can replace Ca^{2+} to form the L16 epitope, and to induce LFA-1 cluster formation. Ca^{2+} binding, and clustering of LFA-1 on the cell surface does not change upon activation of LFA-1 by PMA. We furthermore observed that activation of LFA-1 by certain activating anti-LFA-1 antibodies induces the L16 expression, which is associated with a transition of weak binding of Ca^{2+} into strong binding of Ca^{2+} by LFA-1, and an increase in LFA-1 avidity.

These data indicate that high affinity Ca^{2+} binding to LFA-1, as reported by NKI-L16 binding, induces a clustering of LFA-1 on the cell surface, and enhances the avidity of LFA-1 - ligand interaction.

Late Abstract

CHANGES IN MEMBRANE TRIGLYCERIDE LEVELS IN ACTIVATED THYMIC AND SPLENIC T CELLS, M.F. Veale, A.J. Dingley*, G.F. King* and N.J.C. King, Departments of Pathology and Biochemistry*, University of Sydney, NSW 2006, Australia.

Membrane triglyceride levels, detected using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, increase in activated thymocytes, macrophages, B cells, embryonic cells and metastatic cancer cells. This is believed to result in increased membrane fluidity and cell motility which may correlate with the ability of malignant cells to metastasize and immune cells to migrate to tissue sites. The appearance of the triglyceride is associated with activation but is independent of proliferation (1). We have recently shown that triglyceride concentrations in the membrane and precursor metabolites can be estimated using 2-dimensional (2D) ^{1}H-NMR. Results from activated thymocytes showed that the increase in triglycerides was much greater than the corresponding increase of cell size and volume associated with activation (2,3). In this study we used this technique to investigate and compare the kinetic development of the triglyceride spectrum in activated murine T cells from the spleen and thymus. T cells were stimulated with optimal doses of PMA and ionomycin and cultured for 12-120 hours. Membrane triglyceride levels increased in both cell types and the levels were correlated with the period of activation of the cells. ^{1}H-NMR was also used to examine perchloric acid extracts of the cells, enabling analysis of the levels of precursor metabolites such as choline- and ethanolamine-based phospholipid precursors. These increased during activation of thymocytes. 2D ^{1}H-NMR spectra of mature (splenic) and immature (thymic) activated T cells were compared and showed similar increases in membrane triglyceride concentrations up to 72 hours. However, after longer periods of activation higher concentrations were shown for splenic T cells. These results suggest that the levels of the triglyceride molecules and the kinetics of development in the plasma membrane may be different in these two T cell populations.

1. King *et al.* (1991) *FEBS Letters* **287**, 97-101.
2. Dingley *et al.* (1992) *Biochemistry* **31**, 9098-9106.
3. Dingley *et al.* (1993) *ImmunoMethods*, in press.