EXTRACELLULAR MATRIX IN DEVELOPMENT AND DISEASE

Organizer: A. Harri Reddi

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The Extracellular Matrix in Repair and Development (Joint)

RZ 001 THE THROMBOSPONDINS, A NOVEL FAMILY OF MODULAR GLYCOPROTEINS: STRUCTURE, REGULATION OF EXPRESSION, AND FUNCTION IN CELL-MATRIX INTERACTIONS, Paul Bornstein, University of Washington, Seattle.

It has recently become apparent that the thrombospondins (TSPs) are encoded by at least four homologous genes. TSP1, the commonly recognized protein isolated from platelets, is similar to TSP2 in structure. Both proteins contain NH_2-terminal, COOH-terminal, and procollagen homology domains, and type I (TSP or properdin), type II (EGF-like), and type III (Ca^{2+}-binding) repeats. However, the two TSPs differ in amino acid sequence and in the regulation of their expression. TSP1 is rapidly induced by serum and growth factors. A serum response element (SRE) and a binding site for the transcription factor NF-Y have been shown to mediate the serum response of the human TSP1 gene. On the other hand, TSP2 is far less responsive to serum than TSP1 and lacks the promoter elements that mediate the serum responsiveness of TSP1. TSP3 and TSP4 (previously described as COMP or cartilage oligomeric matrix protein) resemble TSP1 and TSP2 in their COOH-terminal domains and type III repeats, but contain four rather than three type II repeats and lack type I repeats and a procollagen homology. The NH_2-terminal domains of all four proteins are substantially different from one another. The TSPs demonstrate characteristic patterns of expression in the developing and adult mouse. It is therefore likely that each protein subserves a discrete function. We have begun to study the role of TSP1 during the angiogenesis that accompanies endometrial cycling and during the process of cord formation by endothelial cells *in vitro*. Our results suggest that TSP1 plays an inhibitory role in neovessel formation.

RZ 002 AMPHIBIAN REGENERATION AND MAMMALIAIN WOUND REPAIR: A MECHANISTIC LINK, Jerome Gross, Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

The goal of current studies is to make a significant link between an early limiting step in the regenerative process in the amphibian limb and the repair sequences in mammalian skin. We are exploring a working hypothesis which explains a major early event difference between these two on the basis of extracellular matrix dissolution (histolysis) in the amphibian amputation stump tissues, which does not occur in full thickness mammalian skin excision wounds. This collagenolytic process in the amphibian dermis allows the first necessary step for fully differentiated connective tissue cells to dedifferentiate, migrate, reaggregate, proliferate and redifferentiate to form the new limb in the amphibian. In contrast, in mammalian skin wounds, because of the absence of significant dermal matrix degradation in the surrounding dermis, the dermal cells remain dormant. The wound contents are produced by a different population of fibroblasts which synthesize a fibrous matrix but lacks the morphogenetic instructions to regenerate a normal dermis and to induce epidermal appendage formation.

Experiments will be described in which dermal fibrocytes are separately isolated from subcutaneous and granulation tissue fibroblasts and demonstrate some significant functional differences between the dermal cells and the other two cell populations which are similar to each other. Related studies dealing with the mechanism of excision wound closure ("contraction") and scar formation in mammals are part of the overall story.

RZ 003 *Abstract Withdrawn*

Collagens: Chemistry and Molecular Biology

RZ 004 ASSEMBLY AND FOLDING OF RECOMBINANT SUBUNITS OF TYPE III AND TYPE IV COLLAGEN, Klaus Kühn[1], Sari Lukkarila[1], Ralph Golbik[1], Albert Ries[1], Jürgen Engel[2], Karl Tryggvason[3], and Darwin Prockop[4], [1]Max-Planck-Institute for Biochemistry, 8033 Martinsried, Germany, [2]Biozentrum Basel, Switzerland, [3]Biocenter and Department of Biochemistry, University of Oulu, Finland, [4]Jefferson Institute of Molecular Medicine, Phildelphia PA.

The human fibrosarcoma cell line HT1080 transfected with the expression vector pCMVXXNH, containing the entire α1(III) cDNA, produces beside endogenous type IV collagen and fibronectin recombinant intact triple helical procollagen III molecules. They were isolated from culture medium and characterized by PAGE, amino acid analysis, CD and fluorescence spectroscopy as well as with immunological means. Transfection of the human kidney cell line 293 with the α1(III) containing expression vector did not lead to triple helical molecules. The cells secreted instead individual non-triple helical proα1(III)chains, although the proline and lysine residues were fully hydroxylated. Renaturation experiments revealed the ability of the recombinant proα1(III)chains to form triple helical conformations.

Similar results were obtained with 293 cells which were transfected with α1(IV) or α2(IV) containing expression vectors or double transfected with both subunits. The cells secreted fully hydroxylated α(IV) subunits into the medium, but no triple helical homo- or heterotrimers connected by disulfide bridges were observed.

According to *in vitro* renaturation experiments, followed by CD and fluorescence spectroscopy, only the α1(IV) but not the α2(IV) subunit was able to form triple helical structures of low stability. The triple helix formation of a mixture of recombinant α1(IV) and α2(IV) chains at a ratio of 2:1 did not exceed that of the individual α1(IV) chain.

An important step in the posttranslational formation of an intact collagen molecule is thought to be the chain assembly, which includes selection and registration of the α-subunits. According to fluorescence spectroscopy and ultracentrifugation, the tendency of the NC1 domains of both α(IV) subunits to aggregate was low under the *in vitro* conditions used.

It is reasonable to assume that in the 293 cells as well as under *in vitro* conditions, factors are missing which are essential for the correct assembly and triple helix formation of the α-subunits. Putative factors thought to be important for these posttranslational events are under investigation.

RZ 005 COMPARISON OF COLLAGEN STRUCTURE AND ORGANIZATION IN CARTILAGE AND VITREOUS, Richard Mayne[1], Randolph G. Brewton[1], Zhao-Xia Ren[1], Pauline M. Mayne[1] and John R. Baker[2], [1]Department of Cell Biology and [2]Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294.

The mammalian vitreous humor is a useful model system to investigate the structure of collagen fibrils and their potential interaction with non-collagenous macromolecules. Unlike other tissues, the collagen fibrils can be readily isolated from the vitreous by centrifugation, and associated molecules of hyaluronan removed by washing. When the vitreous is examined in the electron microscope after rotary shadowing, the collagen fibrils are of constant diameter, do not appear to branch and are not cross-linked to each other. Several previous studies show that type II collagen is the major collagen of both vitreous and hyaline cartilage. Type IX collagen is also present in vitreous and hyaline cartilage where it is located on the surface of the collagen fibrils and may function to i) stabilize the fibrils, ii) promote interactions between fibrils iii) interact with other macromolecules. In both cartilage and vitreous, type IX collagen is also a proteoglycan with a single chondroitin sulfate chain located at the non-collagenous NC3 domain of the α2(IX) chain. However, type IX collagen from vitreous lacks the non-collagenous NC4 domain which projects from the surface of the fibril. In cartilage, this domain has the potential to interact with other components of the matrix and thereby to stabilize the matrix. Type XI collagen is present in bovine cartilage from nasal septum and consists of α1(XI), α2(XI) and α3(XI) chains which form a single triple helical molecule. For vitreous, similar collagen chains were isolated and characterized by amino acid sequencing of selected tryptic and cyanogen bromide peptides. The results show that the α1(XI) and α2(V) chains are present in vitreous collagen fibrils. This result, together with recent results from other laboratories, shows that the α1(XI) chain is not cartilage-specific, but is present in a variety of non-cartilagenous tissues. It appears that type V and type XI collagen are not separate collagen types, but form part of a type V/type XI family of collagens in which several different chains associate to form stable triple helices. The results also suggest that the genes for type II, type IX and type XI collagens are not coordinately regulated. Each tissue may have different structural requirements and draws on a limited number of collagen genes to form fibrils of different diameter and function. Supported by NIH DE 08228 and EY 09908.

RZ 006 COLLAGEN CHAINS, CROSSLINKS AND TYPES, Karl A. Piez*, Department of Biochemistry and Molecular Biology, Jefferson Medical College, Philadelphia, PA 19107; * Scholar-in-Residence, Fogarty International Center, NIH, Bethesda, MD 20892.

I would like to portray some of the flavor of the decade beginning about 1960 in my laboratory, and some of the consequences of that period, in unabashedly autobiographical form. There was no molecular biology; gel electrophoresis and immunochemistry were in their infancy. Chromatography and protein chemistry were the technologies of the time, and we were at the cutting edge. There was only one collagen type known, but its chain structure and the chemistry of its crosslinks were unknown. Chromatography showed that denatured, soluble collagen had three chains of equal size but of two kinds, designated α1 and α2. There were two α1 chains and one α2 chain in each molecule. Covalently crosslinked dimers (β-components) and trimers (γ-components) of the α chains were present in most samples (1). CNBr cleavage of isolated and purified α chains and β-components showed that there were short nontriplet N-terminal ends on both α chains. These regions contained a lysyl residue that had frequently been converted to an aldehyde. In β-components, two aldehydes formed an aldol condensation product, the first crosslink identified in collagen (2). CNBr cleavage also provided the starting point for the first systematic amino acid sequencing of collagen. Since the CNBr peptide pattern of a collagen was a fingerprint of its primary structure, it became possible to identify genetically distinct collagens. Types II and III collagen were identified in cartilage and skin, respectively (3). Type I collagen, of course, was the one that we had studied until that time. From this beginning, there are now 14 or more collagen types. Amino acid sequences abound, now obtained with relative ease from DNA. The spectrum of extracellur matrix proteins has expanded far beyond the collagens. Lastly, we are beginning to understand supramolecular structures in the extracellular matrix (e.g., see 4) and how they provide a home for cells during development and homeostasis.

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RZ 007 RECOMBINANT ANALYSIS OF COLLAGEN TYPE VI, Rupert Timpl[1], Ulrike Mayer[1], Emanuelle Tillet[1], Ulrich Specks[1], Karlheinz Mann[1], Ernst Pöschl[1], Roswitha Nischt[2], Thomas Spissinger[3], Jürgen Engel[3], and Mon-Li Chu[4], [1]Max-Planck-Institut für Biochemie, D-8033 Martinsried, [2]Department of Dermatology, University, D-5000 Köln, [3]Biozentrum, University, CH-4056 Basel and [4]Department for Biochemistry and Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107.

Collagen VI is a major microfibrillar component and consists of an α1, α2 (each ~1000 residues) and α3 (~3000 residues) chain (s1). The chains form together a triple helical domain of about 100nm length and two large globular domains on each end. The globular domains consist mainly of of ~200-residue motifs homologous to the A domains of von Willebrand factor. Since it is difficult to obtain collagen VI from tissues in native, undegraded form we have resorted to the use of efficient eukaryotic expression vectors and stably transfected human cell clones for producing various parts of the protein. This included the α3 chain part of the N-terminal globe (fragment N9-N2) which consists of eight similar 200-residue motifs. The native folding of individual motifs was shown by ultracentrifugation, electron microscopy and CD spectroscopy (2). Fragment N9-N2 showed binding to hyaluronan, heparin and the collagen VI triple helix indicating its involvement in homo- and heterotypic interactions. It was, however, inactive for other collagenous and non-collagenous ligands and did not promote cell adhesion. Specific antibodies demonstrated the presence of N9-N2 structures in tissues, serum and tissue-extracted collagen. Another domain C5 with similarity to Kunitz type protease inhibitors was predicted for the very C-terminal end of α3 chain. C5 could be produced in substantial quantities and was shown to be secreted in a stable, disulfide-bonded form. A large variety of proteases were, however, not inhibited by the recombinant C5 fragment. C5 could be demonstrated in small amounts in biological samples (serum, fibroblasts) but was virtually absent in tissue-derived collagen VI. In addition, we prepared full length recombinant α1 and α2 chains which were efficiently secreted in a fully hydroxylated form. These chains were unable to form a triple helical structure indicating the requirement of all three chains for the assembly of collagen VI protomers.

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2) Specks, U., Mayer, U., Nischt, R., Spissinger, T., Mann, K., Timpl, R., Engel, J. and Chu, M.-L. (1992) EMBO J. 11, 4281-4290.

Cell Attachment Proteins in Repair and Development (Joint)

RZ 008 SPARC AND TYPE I COLLAGEN ARE FUNCTIONAL PROTEINS IN ANGIOGENESIS, E. Helene Sage, University of Washington, Seattle, WA 98195.

Angiogenesis, the growth of new blood vessels from extant capillaries, is a continuous process throughout most of the embryonic and mature lifespan of vertebrates. Endothelial cells regulate the growth of new vessels, in part, by their contribution to the synthesis and degradation of extracellular matrix (ECM). The ECM, in turn, provides both positive and negative signals to the endothelium. Endothelial cells *in vivo* normally display low rates of replication and are noninvasive, whereas cells participating in angiogenesis secrete ECM-degrading proteases and modulate transcription of extracellular products that include type I collagen and SPARC (secreted protein acidic and rich in cysteine). Experiments with cultured endothelial cells have identified potential functions for SPARC as a morpho-regulatory factor: a) inhibition of cell spreading and disruption of focal contacts, b) modulation of cell shape, c) inhibition of cell-cycle progression, d) subversion of migration and proliferation induced by basic fibroblast growth factor, e) specific binding to platelet-derived growth factor B-chain, f) induction of plasminogen activator inhibitor-1, and g) regulation of ECM proteins that affect vascular morphogenesis. Recently we have found that a Cu^{+2} -binding tetrapeptide, released from SPARC by proteolysis, stimulates angiogenesis *in vitro* and in the chicken chorioallantoic membrane.

Both SPARC and type I collagen are expressed in endothelial cells that comprise the vascular sprouts which invade the murine embryonic brain. In culture, angiogenic endothelial cells initiate transcription of type I collagen genes and assemble a system of highly ordered fibrils which appear to serve as a template for cellular alignment and lumen formation. These endothelial cells, which form cords and tubes *in vitro*, proliferate in response to TGF-ß, whereas subconfluent cells are inhibited. The stimulation might result in part from an interaction with and/or contraction of fibrillar type I collagen by angiogenic cells. Type I collagen might therefore provide a suitable context for selected regulatory factors to facilitate angiogenesis.

Collagen Fibrillogenesis and Assembly

RZ 009 BUILDING AND REMODELLING THE *DROSOPHILA* EXTRACELLULAR MATRIX, Liselotte I. Fessler[1], Katherine Garrison[2], Robert E. Nelson[1], Michael P. Yuhasz[1], Donald Gullberg[1], Yasumitsu Takagi[1], Maureen L. Condic[3], James W. Fristrom[4], and John H. Fessler[1], [1]Molecular Biology Institute and Biology Dept., UCLA, Los Angeles, CA 90024-1570, [2]Shriners' Hospital, Portland, OR, [3]Univ of Minnesota, Minneapolis, MN, [4]UC Berkeley, CA.

Significant deposition of collagen IV into early basement membranes of *Drosophila* embryos is preceded by local accumulation of laminin and the basement membrane proteoglycan papilin. Individual wandering interstitial cells make all three components, but following different expression patterns. The same cells make an exoperoxidase, with the potential for cross-linking of matrix. Another constituent of *Drosophila* basement membranes, the glycoprotein glutactin, is largely made in a diffuse organ, the fat body, but is found in basement membranes throughout the organism. Thus basement membranes arise by sequential deposition and specific accumulation of their components. Subsequently, first reducible, and then nonreducible collagen cross-links form. The proportion of collagen IV in these basement membranes increases with the size and age of *Drosophila* larvae, consistent with a consolidating, mechanical role, while laminin predominates during earlier development. Specialized extracellular matrix also accumulates at the insertion sites of embryonic muscles, where integrin is required to maintain mechanical continuity. Matrix synthesis diminishes, and mRNA levels of collagen IV and laminin drop at the end of the larval period. The metamorphosis that follows in prepupae and pupae requires much rebuilding of extracellular matrix, with degradation and new synthesis proceeding side-by-side. Targeted inhibition of collagen synthesis, with antisense RNA locally expressed during this time in transgenic pupae, initiates a cascade of malformations. Breakdown of preexisting collagen IV, as in the basement membranes of everting imaginal discs, is initiated by a specific cleavage at a site of helix-imperfection of collagen IV. Interestingly, all helix-associated cysteine residues, which can form interchain disulfide links, are to the N-terminal side of this cleavage. Correspondingly, the amino-fragment is released as a high molecular weight, disulfide linked complex. The carboxyl-fragment, which carries the globular NC1 domain, is set free in monomeric and dimeric form, joined in part by a nonreducible link. The results suggest controlled, restricted breakdown of collagen IV matrix during these developmental changes, rather than mere dissolution and destruction.

RZ 010 COLLAGEN FIBRILLOGENESIS: TRACING THE PATHWAY BETWEEN MUTATIONS IN COLLAGEN GENES AND CONNECTIVE TISSUE DISEASE, *Karl E. Kadler, David F. Holmes, ¶Samantha J. Lightfoot and Rod B. Watson, Department of Biochemistry and Molecular Biology, School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT U. K.

A strategy used here to determine the molecular organisation of the matrix is to study how mutations in type I collagen genes alter the biological properties of the protein and thereby manifest themselves as brittleness of bone (in osteogenesis imperfecta, OI) and laxity of joints (in Ehlers Danlos syndrome (EDS) type VII). In studies of EDS type VIIB, we showed that fibrils generated from collagen and abnormal pNcollagen (pNcollagen-ex6, lacking the sequences encoded by exon 6) from fibroblasts of an affected individual were hieroglyphic in cross section and the N-propeptides were located exclusively at the fibril surface. Remarkably, the hieroglyphs could be resolved to near-cylindrical fibrils by treatment with N-proteinase which cleaved only the pNα1(I) chains [1]. Moreover, the fibrils were similar in appearance to those seen in tissues of people with EDS type VIIB. Important questions were: how are partially-cleaved N-propeptides of pNcollagen accommodated by near-cylindrical fibrils in EDS type VIIB, and, do the retained N-propeptides alter the way collagen fibrils interact with other matrix components?

Rotary shadowing EM of individual procollagen molecules (secreted from normal, OI and EDS type VIIB fibroblasts) showed that N-propeptides were folded back onto the main triple helix of the molecule. Quantitative scanning transmission EM (STEM) showed that N-propeptides in hieroglyphs were 'bent-back' and located exclusively to the overlap zone of the fibril *D*-period (*D*, [axial periodicity of collagen fibrils], = 67 nm). In contrast, STEM of fibrils from the dermis of an individual with EDS type VIIB showed that partially-cleaved N-propeptides (in which pNα1(I) and not pNα2(I)-ex6 chains were cleaved) were distributed equally between the gap and overlap zones of the fibrils. The N-propeptides in hieroglyphic and tissue fibrils were axially extended over 33 nm irrespective of their location to the gap or overlap zone. These data exclude the possibility that N-propeptides adopt a random configuration, but rather, that they locate specifically to the gap and overlap zones. This co-distribution of partially-cleaved N-propeptides between gap and overlap zones allows a higher surface packing density of N-propeptides and explains how circularity of large-diameter fibrils can be achieved despite the retention of N-propeptides in tissues of individuals with EDS type VIIB. Numerous connective tissue components, particularly small proteoglycans, bind to collagen fibrils in vitro and in vivo. The location of partially-cleaved N-propeptides in gap and overlap zones in collagen fibrils may disrupt the binding of small proteoglycans and help explain the joint hypermobility and soft tissue complications observed in individuals with EDS type VIIB. Preliminary studies show that the levels of decorin are 4-fold greater in the skin of an individual with EDS type VIIB compared with normal human skin.

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Supported by grants from The Wellcome Trust and the Nuffield Foundation (Oliver Bird Fund). *Recipient of a Senior Research Fellowship award from The Wellcome Trust. ¶Recipient of a Michael Geisman Fellowship from the Osteogenesis Imperfecta Foundation USA.

RZ 011 MINERALIZATION OF COLLAGEN FIBRILS, Wolfie Traub, Talmon Arad, and Steve Weiner, Weizmann Institute of Science, Rehovot Israel

The major vertebrate hard tissues bone, dentin and mineralized tendon are composed of crystals of carbonate apatite (dahllite) in a matrix of type I collagen fibrils, together with small amounts of other macromolecules and water. The crystals are small plates organized in parallel layers along grooves through the collagen fibrils. These grooves arise from contiguous collagen 'gap' regions and are separated by the 67nm D repeat along the length of the fibrils and some 4nm in the orthogonal direction.
In mineralized turkey tendon and bone the crystals form initially in the gap regions, appearing as short needles near the surface of the collagen fibrils at the 'e' bands. The needles grow along the length of the fibrils till temporarily constrained by the boundaries of the grooves. They then grow laterally to form belts, and ultimately push their way out of the grooves into the 'overlap' regions.
The mineralized collagen fibrils with their 3-dimensionally ordered arrays of flat crystals may be regarded as building blocks, assembled in various ways in different mineralized tissues. In turkey tendon they are all generally parallel to the tendon axis, and there is a tendency for adjacent fibrils to be coherently aligned so that their crystals are coplanar. This is even more pronounced in cortical bone where crystals from many fibrils grow together to form continuous planes, but only within the confines of the lamellar sub-structures. In alternate lamellae the orientations of the collagen fibrils and the crystal planes are very different, giving the bone a kind of plywood structure. The relative widths and orientional relations between lamellae appear to vary with the bones mechanical functions.
Preliminary studies have been made of bones from patients affected by osteogenesis imperfecta (O.I.) a condition generally atttributed to mutations in the collagen. Some of the mineralized fibrils appear to have quite normal structures even in severe forms of O.I. However, others have small poorly aligned crystals, sometimes encrusted onto seemingly normal fibrils. Fused crystal aggregates, apparently unrelated to any collagen fibrils, were also seen. These observations could be attributed to a scarcity of collagen in the extracellular matrix rather than to the presence of structurally abnormal molecules, which are less likely to be extruded by the cells than the normal collagen.

Laminin and Elastin

RZ 012 NEURONAL AND MUSCULAR LAMININ VARIANTS: DEVELOPMENTAL EXPRESSION, LOCALIZATION, AND ACTIVITIES, Eva Engvall[1], Ilmo Leivo[2], Hong Xu[1], and Theo Hagg[3], [1]La Jolla Cancer Research Foundation, La Jolla, CA, [2]Department of Pathology, University of Helsinki, Helsinki, Finland, and [3]Department of Biology, University of California, San Diego, La Jolla CA.

Laminins belong to a family of basement membrane-associated proteins which are heterotrimers of a heavy chain (A chain in classical laminin or M chain in merosin) and two light chains (the B chains). All subunits are homologous except that the heavy chains, A and M, have an additional domain at the C-terminus, the G domain. The expression of the subunits is regulated in a cell type specific manner so that each basement membrane contains at least one member of the laminin family, with a subunit composition that may differ between tissues and stages of development. Merosin was discovered as a protein associated with basement membranes of skeletal muscle, peripheral nerve, and placenta. The developmentally controlled expression of laminin variants is strikingly demonstrated in muscle; fetal skeletal muscle contains predominantly laminin (A-B1-B2), while merosin (M-B1-B2) becomes the dominant variant in more mature muscle.
The expression of the merosin M chain is induced around birth in the rat and mouse and reaches maximum at about three weeks of age. Merosin is also expressed in the developing and regenerating liver and in many glandular organs. The M and B chains are the major laminin subunits in the adult central nervous system and are associated with neuronal fibers in several brain regions, while the A chain is almost exclusively found in blood vessels in the brain. The function of merosin is not known. Merosin shares several binding activities with laminin: it promotes cell adhesion and neurite outgrowth and binds to heparin. Unlike the laminin A chain, the merosin M chain is proteolytically processed in tissues. The significance of this processing is not understood, but one hypothesis is that it confers flexibility to basement membranes that need frequent or continous remodeling.

RZ 013 ROLE OR THE BASEMENT MEMBRANE LAMININ IN DEVELOPMENT AND TUMOR GROWTH,
Hynda K. Kleinman, Keizo Yamamura, Soo Han Jun, Derrick S. Grant and Maura C. Kibbey. Laboratory of Developmental Biology, National Institute of Dental Research, NIH, Bethesda, MD 20892.

A basement membrane mixture (Matrigel) has been found in vitro to promote and maintain the differentiation of a variety of epithelial and endothelial cells. For example, salivary gland rudiments form classical branching structures and endothelial cells form capillary-like tubules when cultured on a Matrigel substrate. When normal cells are cultured on this substrate little or no growth is observed, whereas tumor cells show extensive growth and invasion into the matrix. When tumor cells and Matrigel are coinjected, the incidence (i.e. take) and rate of tumor growth are greatly increased and we have been able to establish new human tumor cell lines. Laminin, a major basement membrane component, is important in this process since laminin-derived synthetic peptides have been shown to influence cell differentiation on Matrigel in vitro and tumor growth and metastases in vivo. An SIKVAV-containing peptide from the A chain promotes vessel formation in vitro and angiogenesis in vivo. It also enhances B16F10 melanoma lung colonization five-fold and subcutaneous tumor growth three-fold. Another peptide containing YIGSR from the B1 chain reduced vessel formation in vitro and angiogenesis in vivo and tumor growth and spread in vivo. When daily intraperitoneal injections of YIGSR were initiated after the subcutaneous tumors had grown to 1cm, further tumor growth was reduced. Melanoma cells selected in vitro for adhesion to YIGSR formed larger subcutaneous tumors and more lung colonies than the parental B16F10 cells and the YIGSR non-adherent cells formed smaller tumors and fewer lung colonies. YIGSR and SIKVAV alter tumor growth due to their effects on angiogenesis and also due to direct tumor interactions. In summary, these studies demonstrate the importance of multiple active sites on laminin in different biological processes.

RZ 014 ASSEMBLY OF THE ELASTIC FIBER: A COMPLEX PROBLEM IN DEVELOPMENTAL REGULATION,
[1,2] Robert P. Mecham, [2]Elaine C. Davis, [1]William Parks, [3]Mark A Gibson, and [2]Maria Filiaggi, [1]Departments of Medicine and [2]Cell Biology and Physiology, Washington University Medical School, St. Louis, MO 63110, and [3]Department of Pathology, University of Adelaide, Adelaide, Australia.

The elastic fiber is one of the most complex structures in the extracellular matrix, consisting of two morphologically distinct components: an amorphous core of elastin surrounded by a peripheral sheath of tubular structures 10 to 13 nm in diameter termed microfibrils. While the elastin core imparts to the fiber its elastomeric properties, the function of the microfibrillar component is less clear. In developing elastic tissue, the observation that networks of microfibrils accumulate in the extracellular space before the appearance of tropoelastin has led to the proposal that microfibrils act as a scaffold to organize or direct elastin fibrillogenesis.

Aside from descriptive morphologic studies, direct investigation into the role of microfibrils in elastic tissue has progressed slowly and until recently, little was know about their composition, structure, or synthesis. Using both biochemical and immunohistochemical techniques, several microfibrillar proteins have been characterized. One of the best described is fibrillin, a large 350 kDa glycoprotein that forms an integral part of the microfibril structure. Another of the microfibrillar proteins is a MAGP, a 31 kDa glycoprotein. Other candidate microfibril components include the enzyme lysyl oxidase, glycoproteins of 36 kD and 115 kDa, and a 32 kDa glycoprotein termed AMP.

In elastic tissues, active fibrillogenesis is restricted to the last half of fetal development and decreases during the neonatal period to low adult levels. To determine the developmental expression of microfibrillar components, total tissue RNA isolated from fetal bovine ligamentum nuchae and aorta was hybridized with cDNA probes coding for bovine MAGP, fibrillin 15 (Fib 15), and a unique fibrillin-like protein (FLP). In both aorta and ligament, the pattern of expression of Fib 15 and FLP paralleled the expression of tropoelastin in the tissue. The message level for Fib-15 in the aorta was maximal at 140 days, preceding the peak in FLP and tropoelastin by 10 days. In nuchal ligament the expression of Fib 15 was highest at 200 days, while tropoelastin and FLP continued to increase to 270 days. In contrast to Fib 15 and FLP, the message level for MAGP was constant and remained at a relatively high level with no significant change during development in either tissue. The similar developmental expression of FLP and Fib 15 suggest that both proteins may function as microfibrillar components in elastic tissue. In addition, the observation that mRNA levels for Fib 15 and FLP reach their highest values at or before maximum tropoelastin expression supports the hypothesis that elastin-associated microfibrils function to nucleate elastin fibrillogenesis.

Osteoinduction in Hard Tissue Wound Repair (Joint)

RZ 015 GROWTH FACTOR REGULATION OF FRACTURE REPAIR, Mark E. Bolander[1], Seiya Jingushi[2], Michael E. Joyce[3], and Toshi Izumi[2], [1]Mayo Clinic, Rochester, MN 555905, [2]Kyushu University, Fukuoka, Japan, [3]Washington University, Saint Louis, MO.

The scar that forms during repair of soft tissues injuries has lost much of the highly organized structure characteristic of these tissues prior to injury. Fracture repair, on the other hand, results in the formation of osseous tissue indistinguishable in its structure and function from the original bone. The reformation of normal bone anatomy after fracture appears to be dependent on several distinct cellular events, including intramembranous bone formation and cartilage formation, that occur in the reparative granuloma, or callus, that forms in the tissue adjacent to the injured bone. Decreased cartilage formation in this callus is associated with impaired fracture repair while stimulation of chondrogenesis in impaired-healing conditions improves repair, suggesting that chondrogenesis and endochondral bone formation are critical to the normal fracture repair process[1].

Several growth factors, including acidic Fibroblast Growth Factor (aFGF), Transforming Growth Factor Beta-1 (TGF-ß1), and two members of the TGF-ß superfamily, Bone morphogenic protein 2 and 7 (BMP-2 and OP1), are associated with the initiation of chondrogenesis, chondrocyte maturation, and subsequent endochondral bone formation in the fracture callus [2,3]. Immunolocalization studies identifiy TGF-ß1 and aFGF in pre-chondrogenic regions of the early callus, TGF-ß and osteoprogenitor protein 1 (OP-1) in the cytoplasm of chondrocytes during matrix synthesis, and BMP-2 in undifferentiated cells in the overlying tissues. The intensity of immunostaining for aFGF, OP1 and BMP-2 decreases with chondrocyte maturation, while TGF-ß1 immunostaining changes from the chondrocyte cytoplasm to the extracellular matrix. Northern analysis identifies mRNA for TGF-ß and FGF, but not BMP-2 or OP1, in the fracture callus.

Injection of either TGF-ß, BMP-2, OP1 or aFGF into the subperiosteal region of non-fractured bones stimulated chondrocyte differentiation from cells in the periosteal tissue[4]. The early appearance of chondrocytes and rapid synthesis of cartilage matrix after injection of TGF-ß, BMP-2, and OP1 suggested that these growth factors stimulated chondrogenesis through similar pathways. The appearance of chondrocytes and expression of genes coding for cartilage matrix proteins is delayed until termination of aFGF injections. Tissue formed after injection of TGF-ß, BMP-2, and OP1 was positive when immunostained for aFGF. These observations suggest that cartilage formation during normal fracture repair is regulated by a combination of growth factors, possibly expressed in sequence by cells during the process of chondrocyte differentiation and matrix synthesis. It appears that aFGF and members of the TGF-ß superfamily stimulate chondrogenesis by acting at different points in this pathway.

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RZ 016 EXTRACELLULAR MATRIX AND BONE MORPHOGENETIC PROTEINS: INDUCTION OF PLEIOTROPIC CASCADE OF BONE REPAIR, A.H. Reddi, and Noreen S. Cunningham, Departments of Orthopaedic Surgery and Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore MD 21205

The presence of growth and differentiation factors in bone has been demonstrated by subcutaneous implantation of extracellular matrix which initiates new cartilage and bone morphogenesis. The genes for bone morphogenetic proteins (BMPs) have been cloned and expressed (1). Recombinant BMPs induce endochondral bone formation in vivo. The type of bone formation is dependent on the microenvironment. Predominantly membranous bone formation is observed in implants to alveolar bone. The multistep sequential development cascade consists of chemotaxis, mitosis and differentiation of cartilage and bone. BMPs stimulate osteogenic and chondrogenic phenotypes. Osteogenin (BMP-3) and recombinant BMP-4 are equipotent in chemotaxis, limb bud chondrogenesis, cartilage maintenance and in vivo osteogenesis. During early stages of development of matrix-induced implants, ED-1 and Ia-positive monocytes-macrophages were observed implying BMPs as chemoattractants. Concentration of 10-100 fg/ml (0.3-3 fM) recombinant BMP-4 (also known as BMP-2B) and BMP-3 induce the directed migration of human monocytes (2). Both natural BMP-3 and recombinant BMP-4 stimulated TGF-β_1 mRNA expression in human monocytes. BMPs have cognate receptors as demonstrated by iodinated BMP-2B (BMP-4). The other novel members of the BMP family include osteogenic protein 1 (BMP-7) and BMP-8 (OP-2). Bone morphogenetic proteins are related to the TGF-β superfamily and include three distinct subfamilies: BMP-2, BMP-3 and BMP-7. Native BMP-3 and recombinant BMP-4 bind type IV collagen of the basement membrane. This novel connection may be the long elusive mechanistic explanation for the requirement of angiogenesis and vascular invasion for bone morphogenesis. BMPs may have a role in fracture repair, periodontal surgery, and reconstructive surgery.

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RZ 017 ROLE OF OSTEOGENIC PROTEIN-1 (OP-1) IN GROWTH, DEVELOPMENT AND REPAIR OF BONE, T. Kuber Sampath, Creative BioMolecules, Hopkinton, MA 01748.

Osteogenic Protein-1 (OP-1) is a bone morphogenetic protein member of TGF-ß superfamily (also referred as BMP-7). We recently demonstrated that recombinant human OP-1 induces new bone formation *in vivo* with a specific activity comparable to that of natural bovine osteogenic protein preparations. Preclinical studies using segmental midshaft diaphyseal defects in the long bone of primates and dogs have shown that OP-1 implants fully repair the defects with new bone which is fully functional, both biologically and mechanically. The recombinant human OP-1 implant is currently in human clinical studies for the repair of non-union fractures. In addition, local injection of OP-1 onto periosteal and endosteal surfaces of long bones induces new bone formation and significantly increases bone mass. *In vitro* studies indicate OP-1 acts as a mitogen for bone forming cells, stimulates markers characteristic of osteoblast phenotype, increases the local production of IGF-I and its binding protein BP3 as well as IGF-II, and modulates the action of the calcitropic hormones, PTH and 1,25 dihydroxyvitamin D_3, on bone forming and bone resorbing cells. Immunolocalization and hybridization studies show that OP-1 is involved in embryonic bone formation as well as overall skeletal development, and is synthesized predominantly in kidney in adults and thus may act as an endocrine factor to exert its effects on bone homeostasis.

Proteoglycans

RZ 018 "THE ROLE OF THE SYNDECANS, DEVELOPMENTALLY REGULATED CO-RECEPTORS FOR MATRIX AND GROWTH FACTORS, IN DEVELOPMENT AND WOUND REPAIR", Merton Bernfield, Harvard Medical School, Boston, MA 02115

The cellular microenvironment contains a panoply of insoluble matrix components and diffusible peptides that interact with cell surface receptors to signal cells to change behavior. The syndecans are a family of four transmembrane proteoglycans (PGs) that mediate many of such interactions. The syndecans contain heparan sulfate (HS) chains and extensive amino acid diversity in their extracellular domains and highly conserved short polypeptides that interact with the actin-containing cytoskeleton in their cytoplasmic domains. The chromosomal assignment, exon organization and relationship with a *Drosophila* syndecan indicate that the mouse syndecan family arose from gene duplication. Syndecan-1 HS chains bind a variety of extracellular ligands (e.g. collagens I, III, V, fibronectin, bFGF, etc.) with affinities of ca. 1nM. This binding can immobolize ligands to the cell surface. But the syndecans may also act as co-receptors in which the PG, in concert with other cell surface molecules, comprises a functional receptor complex that binds the ligand and mediates its action. This ternary interaction, best understood for fibronectin and bFGF, provides control mechanisms in addition to those based on ligand affinity.

Syndecan expression is highly regulated, both developmentally and during wound repair. Most information is based on studies of syndecan-1: it appears soon after fertilization during mouse development, localizes on the cells that will form the embryo, and is on developing epithelia which, in turn, induce it transiently on a variety of mesenchymal tissues. It is lost during the transformation of epithelia to mesenchyme. Syndecan-1 exists in discrete isoforms, epithelial and mesenchymal cells, differing in number and size of GAG chains and in HS fine structure. In mature tissues, it is polarized on the basolateral surface of epithelia, but is predominantly intracellular in mesenchymal cells. However, during injury and repair of dermal wounds, syndecan-1 appears on dermal fibroblasts and endothelial cells. This induction correlates with the appearance in early wound fluid of soluble peptide(s) whose activity increases both the level of syndecan-1 mRNA and the half-life of syndecan-1 at the cell surface, and reduces the size of its GAG chains. This activity is unique to wound fluid, specific for mesenchymal cells and not duplicated by any growth factor or cytokine tested. The induction by wound fluid mimics that seen in developing mesenchymal tissues. The highly regulated expression of the syndecans, th: structural variability of their HS chains and their putative co-receptor function suggest they are involved in regulating a variety of cellular responses to the microenvironment.

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RZ 019 HYALURONIC ACID: AN HONORARY PROTEOGLYCAN, Vincent C. Hascall, Bone Research Branch, National Institute of Dental Research, NIH, Bethesda, MD. 20892

Hyaluronic Acid (hyaluronate, hyaluronan) is not synthesized covalently bound to a core protein (1), and therefore does not fit the definition of a proteoglycan. Nevertheless, research on hyaluronic acid (HA) has been intimately associated with the development of proteoglycan research since its discovery by Karl Meyer in the 1930's (2). In spite of its deceptively simple polymeric structure: (glucuronic acid-β1,3-N-acetylglucosamine-β1,4-)$_n$ where n can be >25,000; many details of its biosynthesis, macromolecular properties and biological functions remain elusive. Evidence favors a mechanism of biosynthesis in which: (a) the synthase enzyme(s) is localized at or near a cell surface compartment (3); (b) the HA is elongated by adding an appropriate UDP-sugar to the reducing end (1); and (c) the growing chain is extruded through the plasma membrane into the extracellular compartment (1). Three ongoing research problems relating to different properties of HA (synthesis, catabolism and matrix organization) will be briefly discussed. I. Cumulus cell-oocyte complexes induce HA synthesis and organize an expanded extracellular matrix in response to a gonadotropin surge in pre-ovulatory follicles. Methods have been developed to quantitate HA synthesis and localize it during this fascinating biological process (4). II. In cartilage matrix, HA and the proteoglycan aggrecan are organized in supramolecular aggregates. Metabolism of both macromolecules is co-ordinately regulated in steady state conditions. While most of the proteoglycan (>90%) is released from the tissue in degraded, but macromolecular form, little (<10%) of the catabolized HA is released. This suggests that cell surface HA receptors and HA internalization are key elements in proteoglycan aggregate catabolism (5,6). III. An adipogenic (3T3-L3) mouse cell line produces an exceptionally visco-elastic matrix during differentiation. While HA forms the structural basis for this matrix, the HA concentration in the matrix (<1 μg/ml) is ~500 fold less than required by standard HA solutions to achieve similar levels of viscosity. This suggests that the HA in this and other biological fluids, such as the vitreous of the eye, form extensive, highly organized intermolecular networks (7).

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RZ 020 **STRUCTURE AND FUNCTION OF CARTILAGE PROTEOGLYCANS.**, Dick Heinegård, Dept. Physiological Chemistry, Lund University, P.O. Box 94, S-22100 Lund, Sweden.

The major proteoglycan in cartilage, aggrecan, M_r 3x10^6, has major constituents of protein bound chondroitin sulfate, keratan sulfate, N-glycosidically and O-glycosidically linked oligosaccharides. The molecule binds specifically to hyaluronate, thus linking several proteoglycan molecules in a proteoglycan aggregate. The hyaluronate binding domain, G1, constitutes some 20 % of the roughly 2100 amino acids, is located in the extreme N-terminal part of the proteoglycan,. It binds also to an ancillary protein, the link protein, that it turn binds to the hyaluronate with the same strength (K_D ≈10^{-8}) and specificity for a decasaccharide as the aggrecan. The aggrecan G2-domain is separated from the G1 domain by a short peptide sequence. Following is the keratan sulfate rich domain, which varies in size between species, but in the bovine is made up of some 200 amino acids. The C-terminal part of this region contains 21 highly conserved repeats of an hexapeptide carrying the keratan sulfate chains. The next part of aggrecan, some 1000 amino acid residues, is made up of two domains, CS1 and CS2, carrying the about 100 chondroitin sulfate chains.The C-terminal globular domain shows extensive homology to lectins. This part of the proteoglycan contains an EGF-homology domain which appear to undergo differential splicing. The most apparent function of aggrecan is to provide cartilage with an extreme density of fixed charged groups, thereby retaining water in the tissue and providing a large swelling pressure. This is essential for tissue properties like resilience.

The other major group of proteoglycans in the tissue is 4 much smaller molecules made up of a short and variable N-terminal part, where side chains of dermatan or chondroitin sulfate are attached in decorin and biglycan, while fibromodulin and possibly lumican contain a tyrosin sulfate rich domain. The major central portion of the some 40 000 kDa core protein is made up of some 10 homologous repeats of each some 25 amino acids. that in the case of fibromodulin carry up to 4 N-glycosidically linked keratan sulfate chains. Lumican contains one or two keratan sulfate chains.

Fibromodulin and decorin both, although with different specificities, bind to the fiber forming collagens I, II, III and XI as well as collagen VI.. Biglycan appears to bind only to triple helical collagen VI. Decorin and biglycan have been shown to also bind to fibronectin and decorin also to TGF-ß.

Cartilage matrix contains other molecules carrying glycosaminoglycan chains, e.g. collagen IX, bound along the fibers of collagen II and carrying a single chondroitin sulfate chain. A role of these collagen binding molecules may be to modulate collagen fiber growth and/or collagen fiber surface properties.

Heritable and Autoimmune Diseases

RZ 021 MOLECULAR TARGETS IN THE AUTOIMMUNE BLISTERING DISEASES OF SKIN, Robert A. Briggaman and W. Ray Gammon, Department of Dermatology, University of North Carolina, Chapel Hill.

In this presentation, we will review recent data regarding the cutaneous basement membrane zone (BMZ) and its molecular composition; present accumulating evidence that adherence molecules are the major targets for the autoimmune blistering diseases of skin; and discuss how autoimmunity may contribute to the phenotypic features and pathogenesis of these diseases. A common meeting ground exists here for the basic scientist interested in adherence and matrix molecules and the clinician interested in disease pathogenesis since the normal function of a molecule can be understood best by seeing the results of its dysfunction. The cutaneous BMZ can be structurally and functionally separated into three subdivisions: the BM proper, the keratinocyte cell surface and its special modifications (hemidesmosomes and anchoring filaments), and the sub-BM reticular zone composed of type VII collagen associated anchoring fibrils. In all autoimmune blistering diseases in which molecular targets have been identified, the targets are components of either the keratinocyte cell surface or the sub-BM reticular zone, but never the BM proper. All of these target molecules have a limited distribution to epithelial BM in distinction to the abundant and ubiquitous BM components such as type IV collagen, laminin, entactin/nidogen and heparan sulfate proteoglycans that compose the BM proper. A bullous pemphigoid (BP) antigen designated BP 230 is found intracellularly associated with the adhesion plaque portion of hemidesmosomes. BP 230 has been cloned and sequenced. Its molecular organization contains a globular N-terminal region, a central coiled-coiled alphahelical rod and C-terminal region homologous to desmoplakin I. This molecule is the major target for BP and a new disease, paraneoplastic pemphigus. Another BP antigen designated BP 180 is the major autoantigen in herpes gestationis and in many patients with BP. This molecule has also been cloned and sequenced and consists of intracellular, transmembranous and extracellular collagenous domains. Nicein/epiligrin/kalinin, which are now known to be the same molecule, is located in the lamina lucida associated with anchoring filaments. Epiligrin is a major ligand for intergrins, α6/β4 and α3/β1 present on basal keratinocytes. A newly recognized disease with the clinical phenotype of cicatricial pemphigoid recognizes this molecule as its major molecular target. Type VII collagen has recently been cloned and sequenced and consists of a very large N-terminal domain and a collagenous C-terminal region. Epidermolysis bullosa acquisita and bullous SLE are associated with autoantibodies that recognize epitopes in the N-terminal globular non-collagenous domain of type VII collagen. In each of these disease situations, the target molecules are involved in either keratinocyte-BM or BM-dermal adherence. Conceptually, dysadherence results from either interference with function of the molecules themselves, their immune-mediated injury or both.

RZ 022 IMMUNOGENETICS OF COLLAGEN INDUCED ARTHRITIS IN MICE: A MODEL FOR HUMAN POLYARTHRITIS, Chella S. David, Ph.D. Mayo Clinic, Rochester, MN 55905.

SUMMARY: Collagen induced arthritis is an experimental animal model of inflammatory polyarthropathy that has many features of human rheumatoid arthritis. Type II collagen is the major matrix protein of hyaline cartilage and is a sequestered protein which can be presented as an autoantigen under certain conditions. To induce CIA, type II collagen is injected intradermally with complete Freund's adjuvant. Susceptibility to CIA is dependent on the presence of the trimolecular complex: 1) the arthritogenic epitope on the type II collagen; 2) a class II MHC molecule on the accessory cell presenting the arthritogenic epitope; and 3) T cells expressing specific V chains in their TCRs. Complement and other non-MHC background genes also may play a role in susceptibility to CIA. Both cell mediated and humoral immunity are involved in the pathogenesis of CIA.

To date immmunotherapies that have modulated CIA include use of anti-class II antibodies, anti-lymphokines, and monoclonal antibodies directed against specific cellular markers. All of these therapies are able to modulate disease to some extent but lack the specificity and efficacy to make them practical for widespread use in human disease. Most promising, is the use of monoclonal antibodies directed against specific Vß TCR subsets. This is potentially a very specific and effective therapy because it will affect only the cells involved in disease while leaving the host otherwise immunocompetent. Therapies on the horizon include the use of sythetic peptides with sequences homologous to various regions on the TCR, immunotoxins, and superantigens to modulate the immune response and ameliorate disease.

RZ 023 IMMUNITY TO THE CARTILAGE PROTEOGLYCAN AGGRECAN AND ARTHRITIS, A. Robin Poole[1], Jean-Yves Leroux[1], Carolyn Webber[1], Lawrence C. Rosenberg[2], and Subhashis Banerjee[1], [1]Joint Diseases Laboratory, Shriners Hospital, Division of Surgical Research, Department of Surgery, McGill University, Montreal, Quebec, Canada H3G 1A6, and [2]Orthopaedic Research Laboratories, Montefiore Hospital and Medical Center, New York, NY, USA.

Repeated immunization of BALB/c mice with human fetal aggrecan PG leads to the development of an erosive polyarthritis and spondylitis. Interestingly, patients with ankylosing spondylitis and adult and juvenile rheumatoid arthritis exhibit T cell mediated immunity to this molecule. PG-reactive $CD4^+$ T cell clones have been isolated from patients with ankylosing apondylitis. MHC ($H\text{-}2^d$, $H\text{-}2^k$) as well as non-MHC genes influence the susceptibility to the disease in mice. Both T cell and humoral immunity to PG are expressed in arthritic mice. The importance of the latter is indicated by a requirement for normal complement C5 levels for arthritis development. Onset of the disease is $CD4^+$ T cell dependent. Depletion of $CD8^+$ T cells with antibody *in vivo* enhances disease severity but not incidence nor time of onset. $CD4^+$ T cell lines/clones and hybridomas specific for human aggrecan have been isolated from arthritic mice. They produce lymphokines characteristic of the TH1 subset. The G1 globular domain is often recognized and cross-reaction to link protein has been observed in those T cell lines and hybridomas that recognize G1. This suggests that the epitope(s) recognized by these cells reside in G1 domains which share homology with link protein. The presence of keratan sulfate on aggrecan or G1 can inhibit T cell responses. Its removal by keratanase digestion or cleavage of native G1 by cyanogen bromide can remove this inhibition. An activated PG-reactive T cell line was shown to produce characteristic disease pathology when injected intraarticularly into a naive mouse. These studies implicate the aggrecan molecule as a candidate antigen in immunity to cartilage in rheumatic diseases and reveal how immunity, at the level of the T lymphocyte, can be influenced by the presence of keratan sulfate.

Osteoporosis

RZ 024 EXTRACELLULAR MATRIX, CELLULAR MECHANICS, AND TISSUE DEVELOPMENT, Donald E. Ingber, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We are interested in the mechanism by which extracellular matrix (ECM) molecules regulate cell form and function during morphogenesis. In studies with cultured cells (e.g., endothelial cells, hepatocytes, smooth muscle cells), we have shown that the growth and differentiation-modulating effects of ECM molecules depend largely on their ability to support cell and nuclear spreading. How do cell shape changes alter cell function? Our working hypothesis has been that cell, cytoskeletal, and nuclear form alterations result from changing the balance of mechanical forces that are distributed across transmembrane ECM receptors on the cell surface and that it is this change in force distributions that provides regulatory information to the cell. Recently, we carried our experiments with membrane-permeabilized cells to analyze the mechanism of cell shape determination. These experiments confirmed that the structural stability of the cell and nucleus depends on a dynamic balance of tensile and compressive forces. Mechanical tension is generated within contractile microfilaments via an actomyosin filament sliding mechanism similar to that found in muscle, transmitted across transmembrane ECM receptors (integrins), and resisted by ECM anchoring points. When cytoskeletal tension overcomes the mechanical resistance of the substratum in a spread cell, rapid and coordinated retraction of the cell, cytoskeleton, and nucleus result. These studies suggest that activation of a cellular response does not result from introduction of mechanical loads where there were previously none. Rather, all mechanical loads are imposed on a pre-existing cellular force balance. This finding is also consistent with our concept that integrins act as mechanoreceptors and provide a molecular mechanism for mechanochemical transduction across the cell surface and to the cytoskeleton. To directly test this hypothesis, a magnetic twisting device was developed to apply controlled mechanical stresses directly to specific cell surface receptors without producing global cell shape changes. The transmembrane extracellular matrix receptor, ß1 integrin, effectively transferred mechanical loads across the cell surface and supported a force-dependent cytoskeletal stiffening response whereas non-adhesion receptors (e.g., acetylated-LDL receptor) did not. Force transfer correlated with recruitment of focal adhesion proteins and linkage of integrins to the actin cytoskeleton. Yet the cytoskeletal response to stress involved microtubules and intermediate filaments as well as microfilaments. Furthermore, continuum mechanics analysis revealed that the intact cytoskeleton responded to mechanical stress as if it were a continuous, pre-stressed structural lattice. This behavior was mimicked precisely using "stick and string" tensegrity (tensional integrity) models that contain mechanically-interdependent structural elements that rearrange rather than deform locally in response to stress. These results demonstrate that integrins can act as cell surface mechanoreceptors and transmit mechanical signals to the cytoskeleton. Mechanochemical transduction, in turn, may be mediated simultaneously at multiple locations inside the cell by force-induced rearrangements of both cytoskeletal filaments and associated elements of the cell's metabolic machinery.

RZ 025 CELLULAR APPROACHES TO OSTEOPOROSIS, Gideon A. Rodan, Merck Research Laboratories, West Point, PA 19486.

Osteoporosis is caused by the imbalance between osteoclastic bone resorption and osteoblastic bone formation, usually during increased bone turnover. Osteoclasts are shortlived, multinucleated cells of hemopoietic origin. During resorption they attach firmly to the bone surface, creating a sealed space acidified to pH 4, where lysosomal enzymes degrade the matrix. The α_v/β_3 integrin and its ligand osteopontin have been implicated in this attachment. α_v/β_3 ligands inhibit bone resorption and increase intracellular calcium in isolated osteoclasts. Other agents, which act on osteoclasts, are bisphosphonates, which bind to the hydroxyapatite surface in bone and interfere with the function of osteoclast membranes. The bisphosphonate, alendronate (ALN), modulates membrane permeability to cations and is a very effective inhibitor of bone resorption *in vivo*, both in animals and humans.

A complimentary strategy for treating osteoporosis is stimulation of bone formation by osteoblasts. Osteoblast differentiation is a stepwise process during which phenotype-specific genes are sequentially expressed. No master gene, which initiates osteoblastic differentiation, has been identified yet. Retinoic acid (RA), which induces alkaline phosphatase and PTH responsiveness and upregulates type I collagen expression in predifferentiated cells, increases markedly the expression of the early gene, Egr1/zif268, in those cells, but not in cells which already express the differentiated features. This effect, which is transcriptional via an RA response element, seems to be inhibited in the nonresponding cells by a differentiation-dependent repressor. Another example of differentiation-dependent changes in hormonal regulation of a phenotype-specific gene is glucocorticoid stimulation of osteopontin expression in predifferentiated cells and suppression in the differentiated ones, again apparently due to a transactivating factor, which is however distinct from the previous one, as indicated by gel shifts.

Matrix Function in Repair and Development (Joint)

RZ 026 CYTOKINE INFLUENCES ON ECM METABOLISM DURING WOUND HEALING, Jeffrey M. Davidson, Kenneth N. Broadley, Stephen I. Benn[1], Departments of Pathology and [1]Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232 and DVA Medical Center, Nashville, TN.

Cellular migration and tissue integrity strongly depend upon the appropriate proportions and quantities of extracellular matrix (ECM) components. Effective tissue repair requires the orchestration of a sequence of soluble mediators in the context of a permissive matrix. Cytokines such as TGF-ß have a profound influence on patterns of ECM expression in wounds, and this is further modulated by interactions with other factors present at the wound site. TGF-ß augments ECM production by increasing ECM gene transcripts, decreasing ECM degrading enzyme transcripts, and increasing transcripts for inhibitors of ECM metalloproteinases. TGF-ß1 acts at both the transcriptional and post-transcriptional levels, to produce net accumulation of ECM molecules such as the collagens, fibronectin, and elastin. The mechanism of TGF-ß regulation of elastin production is being studied. Since the predominant effects are upon transcript stability, we are currently seeking evidence for a stabilizing element within the elastin transcript and trans-acting stability factors that are modulated by cytokines such as TGF-ß. The TGF-ß family is implicated in the wound healing process since exogenous TGF-ß augments matrix accumulation and the biomechanical properties of wounds. Others have reported that antibodies to TGF-ß or decorin, a TGF-ß binding protein, can reduce scar formation. In experiments utilizing an antibody neutralizing TGF-ß 1 and 2, we observed that total wound collagen was not significantly reduced; however, there was a doubling of cellularity of the wounds, decreasing the collagen/DNA ratio by 50%. This suggests that TGF-ß 1 and/or 2 may be a negative regulator of proliferation at the wound site. Plasmid DNA with viral promoters driving TGF-ß expression can be transfected directly into granulation tissue to elicit effects similar to those observed with the cytokine. Wound transfection may be a useful tool for evaluating the role of growth factor isoforms and muteins in a biological process. The matrix-accumulating effects of TGF-ß are strongly modulated by the presence and relative proportions of other cytokines. In vitro data show clear antagonism of TGF-α and basic FGF with respect to TGF-ß1 induced production of type I collagen and elastin by fibroblasts and smooth muscle cells. While acute in vivo administration of basic FGF sharply reduces collagen accumulation, co-administration of bFGF and TGF-ß produces a synergistic accumulation of connective tissue, especially in defective wound healing. Supported by grants AG06528,GM378387 and the DVA.

RZ 027 **MOLECULAR COMPLEXITY OF THE CUTANEOUS BASEMENT MEMBRANE ZONE: PERSPECTIVES ON WOUND HEALING,** Jouni Uitto, Angela M. Christiano, Linda C. Chung-Honet, Daniel S. Greenspan*, Kehua Li, and Katsuto Tamai, Departments of Dermatology, Biochemistry and Molecular Biology, Jefferson Medical College, Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107; *Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI.

The cutaneous basement membrane zone (BMZ) consists of a large number of distinct macromolecules that form an intricate network at the dermal-epidermal junction through discrete molecular interactions. We have recently cloned genes encoding two collagenous molecules, characteristic for the cutaneous BMZ. First, Type VII collagen, the major component of the anchoring fibrils, has been elucidated by extensive cloning of the corresponding gene and cDNA. Deduced amino acid sequences revealed that the α1(VII) chain consists of a central collagenous domain which contains 22 imperfections, flanked on the 5'-side by a large non-collagenous domain (NC-1) and on the 3'-side by a smaller NC-2 domain. The chimeric organization of NC-1 revealed modules with homology to cartilage matrix protein, 9 consecutive fibronectin type III domains and the A domain of von Willebrand factor (Christiano *et al.*, Human Mol. Genet. 1:475, 1992). The human type COL7A1 gene consists of over 120 exons in a compact gene of ~30 kb at the chromosomal locus 3p21.

Secondly, we have recently cloned the full-length mouse 180-kDa bullous pemphigoid antigen (BPAG2), an integral component of hemidesmosomes at the keratinocyte-lamina lucida interface. Isolation of overlapping clones, together with 5' and 3' RACE cloning, allowed for delineation of the entire coding sequence of BPAG2. The deduced polypeptide was predicted to contain 1,433 amino acids including a large, 573-amino acid non-collagenous domain (NC-1), and the carboxy-terminal collagenous domain. The collagenous domain consists of 13 separate segments, the largest of them (COL-1) consisting of 242 amino acids. Computer analyses of the deduced amino acid sequence demonstrated the presence of a membrane associated segment, suggesting that BPAG2 is a transmembrane protein. Thus, the mouse BPAG2 cDNA encodes a collagenous polypeptide, and we have recently proposed that this polypeptide be designated as the α1-chain of type XVII collagen (Li *et al.*, J. Biol. Chem., In press).

Type VII and XVII collagens, two newly characterized proteins of the cutaneous BMZ, are predominantly expressed by basal keratinocytes. Therefore, regulation of their expression is critical for establishment of the stable association between the neo-epidermis and the underlying dermis during re-epithelialization of wounds as part of the epidermal healing process.

Transgenic Models in ECM Disease

RZ 028 CONTROL OF COLLAGEN FIBRILLOGENESIS IN TRANSGENIC MICE, Benoit de Crombrugghe[1], Silvio Garofalo[1], Eero Vuorio[2], William Horton[3], Marjo Metsaranta[2], Glenn Decker[1], and Jeffrey Ellard[3], [1]The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, [2]University of Turku, Turku, Finland, [3]The University of Texas Medical School, Houston, TX 77030.

Two major steps control the formation of collagen fibrils. The first consists in the intracellular assembly of triple helical procollagen molecules, whereas the second is the extracellular assembly of the fibrils from their constituent molecules. We have chosen to study the assembly of the collagen fibrils of cartilages largely because the diameter of these fibrils is thin and uniform and because the components of this multiprotein structure are relatively well defined. In two different types of transgenic mice harboring either a mutant or a wild-type α1(II) procollagen gene, expression of the transgene affects a different step in fibril formation. Expression in chondrocytes of transgenic mice of a mutant mouse α1(II) collagen gene, containing a deletion of exon 7, resulted in the virtual absence of collagen fibrils in cartilages presumably because the intracellular step consisting in the assembly of the α1(II) procollagen molecule was seriously disrupted. This drastic decrease in cartilage collagen fibrils had severe consequences on the organization of chondrocytes in the growth plate and caused limb deformities, craniofacial anomalies and neonatal death.

Overexpression of a normal proα1(II) collagen gene in chondrocytes of transgenic mice also produced anomalies, consisting in abnormal, large banded collagen fibrils in cartilages. The amount of these large fibrils was largely proportional to the level of expression of the normal transgene and mice with the highest expression died at birth in respiratory distress. The simplest interpretation to explain the presence of these large fibrils is that overexpression of the wild-type proα1(II) collagen gene caused an imbalance in the concentration of one of the constituents of the fibrils in cartilages and that this imbalance disrupted the mechanism that controls their assembly.

Late Abstracts

GENETIC ANALYSIS OF FIBRONECTIN FUNCTION IN MICE, Elizabeth L. George, Helen Rayburn and Richard Hynes, Center for Cancer Research and Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

As major components of the extracellular matrix, fibronectins (FNs) are involved in cell adhesion and migration in such diverse processes as morphogenesis, hemostasis and thrombosis, wound healing and oncogenic transformation. To facilitate functional analysis of fibronectins in the intact animal, we have initiated a transgenic approach in mice. First, we have generated a FN-deficient mouse strain by gene targeting. Expression of the single FN gene was disrupted by insertion of a *neo* gene into the first exon via homologous recombination in embryonic stem cells. Germline transmission was achieved with three independent targeted clones. In mice which are heterozygous for the FN-deficient allele, the concentration of soluble plasma FN is one half that of wild type littermates. We are currently using this phenotype to determine if concentration of soluble FN affects wound healing by comparing heterozygotes to wild type animals. Disruption of both FN alleles results in early embryonic lethality, revealing that FN is required for embryogenesis. Homozygous embryos are recovered in expected Mendelian frequency prior to and early after uterine implantation. By day 9.5, homozygotes have begun to deteriorate and are absent by day 14. Developmental delay and morphological distortions in homozygotes are first evident at the early head fold stage (day 8.0). By day 8.5 several developmental features are abnormal, including short longitudinal axis, defective neural tube and notochord, absent or defective somites and defective vasculature. Approximately one half of homozygous embryos initiate heart development, but none undergo the process of turning. Further characterization of the embryonic lethal phenotype is in progress. The second component of our transgenic approach to FN function is to determine the functional significance of FN variants generated by alternative RNA splicing. We have generated transgenic mice harboring defined FN splicing variants in which rat FN cDNA is under control of the mouse FN promoter. By breeding these transgenic mice with the FN-deficient mouse strain, we are asking if single FN variants are capable of rescue of the embryonic lethal phenotype. Alternatively, multiple FN variants may be required for embryogenesis. These transgenic mice are also being analyzed for dominant effects of the transgene.

ROLE OF OSTEOGENIN AND RELATED BMPs IN CRANIOFACIAL REGENERATION IN BABOONS, Ugo Ripamonti, Medical Research Council, DRI, University of the Witwatersrand, Johannesburg, South Africa.

The recently isolated and characterized bone morphogenetic proteins (BMPs) induce endochondral bone differentiation in vivo. This provides the potential for controlled initiation of bone repair and regeneration in man. While substantial progress is being made to elucidate the molecular and cellular mechanisms involved in BMP-induced bone differentiation, the morphogenetic potential of BMPs, including osteogenin (BMP-3), is predominantly based on work in rodent models. Information concerning the bone inductive potential of osteogenin and related BMPs in nonhuman primates is a prerequisite for the ultimate clinical application in man. To determine the efficacy of osteogenin (BMP-3) as potential therapeutic initiator of osteogenesis, we have developed an orthotopic model in which large osseous defects (2.5 cm in diameter) were surgically created in the calvaria of adult male baboons (Papio ursinus). Osteogenin was isolated from baboon bone matrix and purified by chromatography on heparin-Sepharose, hydroxyapatite and Sephacryl S-200 (1). Final purification to homogeneity was obtained by electroendosmotic elution from a preparative SDS-polyacrylamide gel, resulting in a single band on a SDS-PAGE with an apparent molecular mass of 30-34 kDa. Baboon osteogenin fractions in conjunction with insoluble collagenous bone matrix induced complete regeneration of the calvarial defects. Osteogenin induced bone formation also when adsorbed onto porous inorganic hydroxyapatite substrata implanted in extraskeletal sites as well as calvarial defects of adult baboons (2). The finding that the biological activity of osteogenin can be induced by both organic and inorganic substrata to obtain predictable phenotypes in primates, may help tissue engineers to design appropriate delivery systems for recombinant human BMPs that are amenable to contouring of form for the therapeutic initiation of osteogenesis.

1. Ripamonti, U., Ma, S., Cunningham, N.S., Yeates, L. and Reddi, A.H. Initiation of bone regeneration in adult baboons by Osteogenin, a bone morphogenetic protein. *Matrix* 12: 369-380 (1992).
2. Ripamonti, U., Ma, S. and Reddi, A.H. Induction of bone in composites of Osteogenin and Porous Hydroxyapatite in baboons. *Plastic and Reconstructive Surgery* 89: 731-739.

STEROIDS MODULATE EXPRESSION OF OSTEOCLAST INTEGRINS, F. Patrick Ross, Xu Cao and Steven L.Teitelbaum, Department of Pathology, Jewish Hospital at Washington University Center, St. Louis, Mo 63110.

We have shown previously that the integrin $\alpha_v\beta_3$ mediates attachment to and resorption of bone by osteoclasts. In addition, we demonstrated that the hormone $1,25(OH)_2D_3$ (D_3) modulates synthesis of the α_v subunit by a translational mechanism. We now report our studies on regulation of the β_3 subunit by D_3 and two other steroid hormones which mediate bone cell function, retinoic acid (RA) and estrogen (E_2). Using a cDNA for avian β_3 which is >85% identical to the human homolog we observed a large increase in steady-state level of β_3 mRNA when osteoclast precursors are treated with either D_3 or RA. Nuclear run-on and actinomycin D studies that D_3 treatment both augments β_3 transcription and stabilizes the gene transcript. RA treatment, like D_3 leads to a marked increase in steady state levels of β_3 mRNA, while not affecting α_v mRNA, suggesting that β_3 is regulating expression of $\alpha_v\beta_3$ on the cell surface. RA+D_3 synergistically enhance β_3 mRNA levels. In contrast to D_3 or RA, treatment with E_2 alone is without effect, but the sex steroid blunts the enhancing action of either D_3 or RA on β_3 mRNA levels. To study further the nature of this control we cloned the promoter region of the β_3 subunit, using a combination of 5′ RACE, primer extension analysis, S1-nuclease protection assays and sequencing to isolate, identify and characterize this element. The most 3′ 1.5 kb of genomic DNA contains AP-1, SP-1, and INR consensus sequences, but no TATA box. In addition, potential retinoic acid and/or vitamin D response elements can be identified. Fragments of the putative promoter region were generated by PCR were cloned into a luciferase reporter gene, followed by transfection into competent cells, resulting in a strong increase in promoter activity. A region between -1500 and -800 bps, when transfected into an avian macrophage-like cell line, confers responsivity to D_3. We conclude that 1) the β_3 integrin subunit probably regulates expression of $\alpha_v\beta_3$ by osteoclast precursors and 2) the gene is regulated by essential bone-seeking steroids.

THE OSTEOGENESIS IMPERFECTA MOUSE (OIM): MATRIX SYNTHESIS IN THE PRESENCE OF ALPHA 1(I) HOMOTRIMER, Jay R. Shapiro, Daniel McBride, Neal Fedarko, David Gray, William Landis, Sandy Marks and Barbara Brodsky, Division of Geriatric Medicine, The Johns Hopkins University, Baltimore, MD, 21224, Harvard Medical School, Boston, University of Massachusetts Medical School, Worcester, and the Robert Wood Johnson Medical School, Piscataway.

Osteogenesis imperfecta (OI), a heritable disorder of connective tissue, is the result of various mutations affecting the proα1(I) or proα2(I) chains of type I collagen. Mice with skeletal defects were first observed at the Jackson Laboratory. Evaluation of these animals revealed multiple fractures with deformities, joint laxity and scoliosis. Osteopenia and excessive numbers of osteocytes are present on light microscopy. Named oim, the disorder is inherited as an autosomal recessive trait. Biochemical evaluation reveals that fibroblasts and osteoblasts from oim fail to secrete proα2(I) chains: instead, proα1(I)3 homotrimer is found in mouse tissues and in matrix formed in tissue culture. Full length proα2(I) mRNA is recovered from cells by Northern blot. The mutation is a G deletion that induces a frame shift in the COOH-terminal exon 52 of proα2(I). TEM of mouse fibroblasts indicates dilatation of the endoplasmic reticulum, presumably because of retained protein. Evaluation of oim α1(I)3 thermal stability describes a depressed melting point of enzymatically predigested type I collagen, as well as early scission of α1(I)3 to a larger and smaller sized component. As in human OI, body weight and body size are both depressed in oim. Cell growth curves and cell doubling times are delayed in oim homozygotes compared to wild type mice and are intermediate in the heterozygotes. Analysis of oim bone proteoglycans reveals an abnormal pattern of excess large chondroitin sulfate proteoglycan and hyaluronate similar to that found in human OI and fetal specimens. TEM of tendon specimens demonstrates decreased type I fibril diameter but a normal banding pattern. X-ray diffraction studies of tail tendon indicate that native lateral packing of molecules and higher order lateral association are disrupted when the α2(I) chain is replaced by a third α1(I) chain. The Achilles tendon mineralizes in oim. Tomographic 3D image reconstruction reveals structural differences in the spatial relationship of mineral to collagen between OI and normally mineralizing tissue. In summary: the oim is a duplicate of human OI that permits investigation of matrix abnormalities in the presence of a proα2(I) mutation.

Collagens & Collagenases

RZ 100 EXPRESSION OF COLLAGEN TYPE I, TYPE IIA AND TYPE IIB IN CARTILAGE IN EXPERIMENTAL OSTEOARTHRITIS.

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Introduction: After canine anterior cruciate ligament transection (ACLT), articular and osteophyte cartilage proliferate extensively. We sought to define the relative abundance in this proliferative repair cartilage of collagen types IIA and IIB mRNA (COL2A, COL2B), to identify the cells producing them and to define if the proliferating articular chondrocytes produce type I collagen mRNA.

Method: Experimental OA was induced in skeletally-mature dogs by right ACLT, the left knees were controls. At 10 weeks post-op, RNA was extracted from articular and osteophyte cartilage from 5 OA and 5 control joints (3). COL2A and COL2B mRNA was analyzed by RT-PCR using human oligonucleotide primers. In 7 dogs, tissues were used for histology and *in situ* hybridization (*ISH*).

Results: Osteophytes developed at the chondrosynovial junction and ligament insertions as an osseous core covered by cartilage or fibrocartilage and a superficial layer of poorly differentiated cells. PCR analysis showed that in OA samples *vs* controls, levels of type IIB were similar, but levels of type IIA were greater than 2X control values. The ratio of IIA to the total type II (IIA+IIB) was 0.72 in OA samples *vs* 0.41 in controls. With *ISH*, chondrocytes with the most abundant pericellular proteoglycan staining, in the center of the osteophyte, had moderate signal with type IIB probes. Those at the periphery with moderate pericellular proteoglycan staining had a strong signal with type IIB probes. Type IIA probes hybridized mainly to the poorly differentiated cells in the superficial layer that were also expressing type I and type IIB mRNA. Some articular chondrocytes at the joint margins clearly showed hybridization to type I collagen.

Discussion: This suggests that type IIB collagen expression was highest in differentiating chondrocytes that actively synthesize matrix. Type IIA collagen expression is present in developing osteophytes, but the cells expressing COL2A were not morphologically typical chondrocytes, but were likely chondroprogenitor cells, *i.e.*, cells with the potential to differentiate into mature chondrocytes. Finally, some articular chondrocytes clearly expressed mRNA for type I collagen.

RZ 101 APPEARANCE OF ALTERNATIVE α2(I) AND α1(III) COLLAGEN TRANSCRIPTS DURING EARLY EMBRYOGENESIS AND CHONDROGENESIS, Sherrill L. Adams, Arthur Cohen, Hyun-Duck Nah, Zeling Niu and Kim M. Pallante, University of Pennsylvania, Philadelphia, PA 19104-6003.

Type I and III collagens are not found in cartilage, although the α2(I) and α1(III) collagen genes are transcribed in this tissue. Transcription of the α2(I) gene in cartilage initiates at a previously undescribed promoter within intron 2, resulting in an mRNA in which exons 1 and 2 are replaced by a cartilage-specific exon. Conceptual translation of the cartilage mRNA identifies a very small (71 amino acid) open reading frame that is out of frame with the collagen coding sequence. Thus these transcripts cannot encode α2(I) collagen, and may encode a noncollagen protein. Secondary structure modeling of the protein predicts an α helix-β turn-α helix, a motif common to many nucleic acid binding proteins, at the amino terminus. *In vitro* transcription/translation of cDNA containing the predicted open reading frame produces a protein with the predicted size of 8 kD, as well as a 35 kD complex between the 8 kD protein and a 160 nucleotide RNA, thus confirming that this protein can bind nucleic acids.

RT-PCR analyses indicate that the cartilage promoter of the α2(I) collagen gene is the first to be activated during embryogenesis, and cartilage-type transcripts have been identified in presomite embryos. *In situ* hybridization analyses have localized these mRNAs to tissues derived from neural ectoderm (myelencephalon, spinal cord and retinal pigmented epithelium). This surprising localization suggests that this alternative α2(I) collagen transcript, which we initially believed to be cartilage-specific, may also play a role in differentiation of neural ectoderm.

Preliminary analysis of the cartilage type III collagen transcripts indicate that they are more than 1500 bases smaller than the authentic type III collagen mRNA, and that exons 1-20 are missing. These results suggest the existence of a cartilage-specific promoter for the type III collagen gene as well. Thus there may be a subset of collagen genes with alternative promoters which, depending on promoter selection, may or may not function either as templates for collagen synthesis.

RZ 102 MOLECULAR ANALYSIS OF CHICKEN SPARC.

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SPARC is a secreted glycoprotein that modulates cell shape and cell-matrix interactions. SPARC levels are increased at sites of somitogenesis, osteogenesis, and angiogenesis in the embryo and during wound repair in the adult. We have cloned and expressed chicken embryo SPARC in *E. coli*. SPARC cDNA was obtained by a novel "two-step" use of the polymerase chain reaction. A 2.2 kbp cDNA species was sequenced by oligonucleotide primer walking, and the deduced primary structure for chicken SPARC was found to be 85% identical to human SPARC. Antisera directed against both carboxy- and amino- terminal sequences of murine and bovine SPARC identified a 42-43 kDa protein in chicken embryo extracts. Chicken SPARC is comprised of 298 amino acid residues which include a 17-residue amino-terminal signal peptide. The mature, secreted form of the polypeptide has a calculated molecular weight of 32,090 and a predicted isoelectric point of 4.5. Two sites of potential attachment of N-linked carbohydrate are present. The primary structure of SPARC was characterized by profile analyses of the databases and by predictions of secondary structure, functional motifs, and hydrophilicity. The results demonstrate that SPARC can be defined by 4 sequence signatures: *(i)* A conserved spacing of 11 cysteines in domain II, *(ii)* a 100% conserved region of 10 residues in domain III, *(iii)* a carboxy-terminal, calcium binding EF-hand, and *(iv)* the pentapeptide KKGHK in domain II. Sequence signatures *i, ii,* and *iii* are also present in the 676 residue "QR1" protein from quail retina (Accession EMBL:M61908) and in the 634 "SC1" protein from rat brain (Accession PIR:JQ0593). SPARC mRNAs in the 10-day chicken embryo are represented by three species as determined by Northern blot analysis: 1.6, 2.2 and 3.0 kb. The relative steady-state levels for the 2.2 kb RNA were determined as aorta ≥ skeletal muscle > calvaria > vertebra > anterior limb > kidney > heart > brain > skin = lung >> liver. The relative abundance of the 1.6 kb and 2.2 kb RNAs varied among these tissues and indicates differential tissue processing. All three RNA species were detected by a DNA probe for the N-terminal half of the coding region. Thus, the 3 RNA species appear to arise from differential 3' splicing and/or differential polyadenylation. Our collective evidence demonstrates that SPARC has been well conserved during vertebrate evolution and supports the hypothesis of a fundamental role for the protein in tissue morphogenesis and in the response to injury. (Supported in part by NIH GM-40711).

RZ 103 COLLAGEN BINDING SPECIFICITY OF ANCHORIN CII AND METABOLIC EFFECT OF INHIBITION OF ITS Ca^{2+}-CHANNEL ACTIVITY, B. Boehm, J. Mollenhauer, M. Hejna, and K.E. Kuettner, Departments of Biochemistry and Orthopaedic Surgery, Rush Medical College, Chicago, IL 60612.

Anchorin CII (ACII) is a collagen-binding protein which forms voltage-gated calcium channels in chondrocyte membranes. It is presumed to allow chondrocytes to detect via collagen fibers mechanical load applied to the tissue. It binds selectively to type II and type X collagens. Previous observations suggest that the binding site is located in the telopeptide region. The nature of the binding site was further investigated using an attachment assay. Purified, iodinated ACII was inserted into liposomes. Multiwell plates were coated with type II collagen and the labelled liposomes were added in the presence or absence of soluble collagen peptides. Bound radioactivity was determined after solubilization of the complex. Collagen peptides of 5-15 amino acids produced by bacterial collagenase and CNBr cleavage inhibited attachment of liposomes to collagen, probably through specific interaction with ACII.

The effect of ACII calcium channel activity on matrix synthesis was investigated by blocking transmembrane Ca^{2+} flow with Zn^{2+}. Bovine articular chondrocytes were incubated with ^{3}H-proline and ^{35}S-sulfate in the presence or absence of 2 mM Ca^{2+} and 0 to 10 uM Zn^{2+}. Isotope incorporation into matrix macromolecules was determined. In the presence of 2 mM Ca^{2+}, ^{3}H-proline incorporation was suppressed. This effect was reversed by blocking the ACII channel with micromolar Zn^{2+}. No suppression was seen in cells cultured in low Ca^{2+} medium. No effect on proteoglycan synthesis was seen suggesting that collagen synthesis in particular may be partially controlled by ACII-regulated intracellular Ca^{2+}.

RZ 104 CORNEAL PROTEOGLYCANS ASSOCIATE WITH TYPE VI COLLAGEN IN DEVELOPING RABBIT CORNEA, Charles Cintron, Toshiaki Takahashi and Claire L. Kublin, Schepens Eye Research Institute/Harvard Medical School, Boston, MA 02114

Keratan sulfate proteoglycan (KSPG) and dermatan sulfate proteoglycan (DSPG) in adult rabbit cornea are associated with collagen fibrils. Recent studies in our laboratory, however, have suggested that proteoglycans may be associated with type VI collagen in the fetal cornea. To determine the ultrastructural location of KSPG and DSPG in the fetal cornea, we developed polyclonal antibodies specific to the core proteins of rabbit corneal KSPG and DSPG and used the antibodies as immunocytochemical probes. We showed that immunogold particles are associated with type VI collagen filaments but not with collagen fibrils in the fetal and neonate rabbit cornea. Similarities in the staining pattern with or without fixation indicated that localization was not an artifact of preparation. Association of corneal KSPG and DSPG to type VI collagen was cytochemically confirmed by using monoclonal antibodies to low-sulfated keratan sulfate glycosaminoglycan (GAG) and chondroitin 4-sulfate GAG, respectively. Characterization of the polyclonal and monoclonal antibodies as mono-specific; exclusive binding of antibodies to type VI collagen filaments; and previous chemical identification of low-sulfated KSPG and DSPG in developing rabbit cornea support our contention that corneal proteoglycans in developing rabbit cornea are associated with type VI collagen.

RZ 105 REGULATION OF PN-1 TARGET PROTEASE SPECIFICITY BY COLLAGEN TYPE IV AND HEPARIN. Frances Donovan, Patrick J. Vaughan, and Dennis D. Cunningham. Department of Microbiology and Molecular Genetics, University of California, Irvine, CA. 92717.

Protease nexin-1 (PN-1) is a 43 kDa serine protease inhibitor found primarily in the brain. In culture, PN-1 is secreted by a number of cell types including astrocytes, smooth muscle cells and fibroblasts. PN-1 released by these cells can inhibit and form complexes with thrombin, urokinase, and plasmin. Once formed, these complexes bind to the cell surface via a receptor that recognizes the PN-1 moiety, and are then rapidly internalized and degraded. Thus PN-1 acts as a local regulator of serine proteases. In contrast to released PN-1, PN-1 bound to the cell surface or extracellular matrix (ECM) is accelerated in its inhibition of thrombin, but is unable to form complexes with urokinase or plasmin. PN-1 is known to bind and interact with at least two extracellular matrix components, vitronectin and heparan sulphate. The acceleration of PN-1/thrombin complex formation is due to heparin, or the chemically similar heparan sulphate, a glycosaminoglycan present on cell surfaces and ECM, whose binding to PN-1 and accelerative effect has been previously investigated. The molecule(s) responsible for the change in protease specificity of PN-1 upon binding to the cell surface or ECM are unknown. The present studies indicates that collagen type IV, in combination with heparin, can mimick the regulation of PN-1 target protease specificity by the ECM. Understanding the ECM components involved in this regulation of PN-1 target specificity may elucidate target proteases of PN-1 in different environments and contribute to understanding the possible physiological roles of PN-1 in wound repair.

RZ 106 POST-TRANSLATIONAL REGULATION OF PROCOLLAGEN GENE EXPRESSION BY CULTURED CARDIAC FIBROBLASTS, E.G. Eleftheriades, A.G. Ferguson, and A.M. Samarel, Department of Medicine, Loyola University of Chicago Stritch School of Medicine, Maywood, IL 60153.

Adaptive changes in the extracellular matrix (ECM) accompany the development of cardiac hypertrophy in response to hypertension. We have recently shown that interstitial collagen accumulation during the initial response to hypertension induction in the rat was not due to increased collagen gene transcription, but rather to a decreased rate of intracellular degradation of newly synthesized procollagens (Eleftheriades et al., *J. Clin. Invest.*, in press, 1993). Previous studies have also shown a transient increase in prolyl hydroxylase activity in cardiac fibroblasts 2 days after hypertension induction. To further investigate the relationship between prolyl hydroxylation and the susceptibility of newly synthesized procollagens to intracellular proteolysis, we studied the effects of ascorbic acid (ASC) deficiency and inhibition of collagen transport on procollagen metabolism by cultured, neonatal rat cardiac fibroblasts. Cells were isolated by sequential collagenase digestion, grown to confluence and subcultured. Subconfluent monolayers were then exposed to serum-free culture medium (DME/F12/PC-1) alone, DME/F12/PC-1 containing ASC (40 μg/ml), or DME/F12/PC-1 containing both ASC and colchicine (0.5 μg/ml). After 24 h, procollagen synthesis, accumulation, and degradation, were analyzed by measuring hydroxyproline content in the cell monolayer, and in the ethanol-soluble and ethanol-precipitable fractions of the culture medium. Type I procollagen and Type I collagen were also localized intracellularly and in the ECM by immunofluorescence microscopy using antibodies specific for the aminoterminal propeptide of α_1(I) and the triple-helical domain of Type I collagen, respectively. Finally, mRNA levels for α_1(I) procollagen polypeptide were assessed by Northern blot analysis. ASC-deficient fibroblasts showed decreased rates of prolyl hydroxylation and total collagen accumulation in the absence of a significant reduction α_1(I) mRNA levels. The fraction of newly synthesized procollagens degraded was substantially increased in ASC-deficient cells (50±6 vs.33±2% in ASC-deficient vs. control cells, $P<0.05$). These findings were associated with increased intracellular accumulation of Type I procollagen and decreased deposition of Type I collagen into the ECM. Inhibition of intracellular transport by colchicine also increased intracellular accumulation of Type I procollagen but had no effect on prolyl hydroxylation, total collagen accumulation or intracellular degradation. We conclude that decreased procollagen hydroxylation due to ASC deficiency increases intracellular accumulation and degradation of newly synthesized procollagen polypeptides. These observations suggest that increased procollagen hydroxylation may be responsible for the decreased rate of intracellular degradation and disproportionate collagen accumulation in the early stages of cardiac hypertrophy.

RZ 107 ED-A AND ED-B ISOFORMS OF FIBRONECTIN SHOW A STAGE RELATED DIFFERENCE IN DISTRIBUTION IN DUPUYTREN'S DISEASE. N.L. Halliday*, G. Rayan+, and J.J. Tomasek*. *Department of Anatomy, and +Department of Orth. Surg., University of Oklahoma Health Science Center, Oklahoma City, OK 73190.

Different fibronectin (FN) isoforms arise via alternate splicing of a single gene transcript in a tissue and cell specific manner. Monoclonal antibodies recognizing extra domain A (ED-A) and extra domain B (ED-B) fibronectin were used to evaluate the presence, distribution, and levels of these isoforms in Dupuytren's diseased and normal palmar fascia. Dupuytren's disease (DD) is a pathological condition in which the palmar aponeurosis becomes shortened leading to irreversible flexion of one or more digits. Previous studies have demonstrated that the disease progresses through three distinct histological stages: proliferative, involutional, and residual. Immunolocalization studies show abundant extracellular fibronectin fibrils, including ED-A and ED-B FN, in proliferative and early involutional stage Dupuytren's diseased tissue. However, the ED-B fibronectin appears less abundant and more restricted in its distribution as compared to ED-A FN or total FN. Total FN and ED-A FN are significantly reduced in late involutional and residual DD tissue while ED-B FN is no longer present. In contrast, normal palmar fascia shows no fibronectin present at fibroblast cell surfaces. Western blot analysis confirms results seen with the immunofluorescence. These results indicate that ED-A and ED-B fibronectin are present in Dupuytren's diseased fascia, and show stage related differences in distribution. The expression of ED-A and ED-B fibronectin does not appear to be co-regulated in Dupuytren's disease. All antibodies used were a generous gift from Luciano Zardi, Laboratory of Cell Biology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy. (Supported by Bennett Research Fund)

RZ 108 REGULATION OF T CELL ADHESION TO EXTRACELLULAR MATRIX (ECM) PROTEINS BY CALCIUM MOBILIZATION, Perry J. Hartfield, Richard D.R. Camp and Malcolm W. Greaves, Professorial Unit, St. John's Institute of Dermatology, St. Thomas' Hospital, UMDS, London, SE1 7EH, U.K.

Regulated adhesion of T cells to ECM proteins probably plays a pivotal role in T cell migration. Cell adhesion to ECM components is mediated by members of the β1 subfamily (also known as VLA proteins) of the integrins. Exposure of lymphocytes to a variety of stimuli can rapidly and dramatically augment β1-mediated cell-ECM interactions without an apparent increase in receptor expression, suggesting that changes in either the affinity of the receptor for its ligand or changes in the organization of the adhesion receptors at the cell surface results in enhanced cell adhesion. The present study investigates the role that cellular Ca^{2+} mobilization plays in the affinity modulation of T cell adhesion to the ECM proteins, fibronectin (FN), laminin (LN) and collagen type IV (CO). Levels of T cell adhesion to FN, LN and CO were all substantially increased when Ca^{2+} was mobilized from extracellular sources, through the action of the Ca^{2+} ionophores, ionomycin (I) and A23187, and from intracellular sources, through the action of the endoplasmic Ca^{2+}-ATPase inhibitors, thapsigargin (Tg), cyclopiazonic acid (CPA) and 2,5-di-(*tert*-butyl)-1,4-hydroquinone (BHQ). To elucidate the Ca^{2+} signalling pathway mediating T cell adhesion, activated binding to FN was further investigated. T cell adhesion to FN was inhibited in the absence of extracellular Ca^{2+}, but not by the Ca^{2+}-channel blocker, nifedipine. In addition, caffeine, a ryanodine receptor stimulus, did not regulate T cell adhesion to FN, suggesting that activated T cell adhesion may be initiated via $InsP_3$-linked Ca^{2+}-mobilization. Experiments using a VLA β1 chain specific mAb (4B4) demonstrated that T cell binding activated by Ca^{2+} mobilization was mediated by VLA integrins, as it was completely inhibited by mAb 4B4, and blocking studies with RGD-peptides confirmed that VLA-5 largely mediated activated adhesion to FN.

RZ 109 THE DIRECT ANALYSIS OF COLLAGEN GENE EXPRESSION IN HEALING HUMAN WOUNDS

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The collagen family of extracellular matrix proteins has a central role in the wound healing response. We have developed a rapid reverse transcriptase-polymerase chain reaction amplification technique (RT-PCR) for routinely analyzing the differential expression of the α1(I), α2(I) and α1(III) procollagen genes in 6mm punch biopsy specimens obtained from acute and chronic wounds at varying timepoints in healing. The data obtained using this technique demonstrates that α1(I), α2(I) and α1(III) procollagen are being expressed throughout the healing of both acute and chronic wounds. In the acute wounds the Type I and Type III procollagen genes are being expressed at higher levels between injury and wound closure than in normal skin, whilst in the chronic wounds although all three genes are being expressed there is an increase in the expression of α1(III) collagen as compared with normal skin. We are developing a quantitative RT-PCR assay to determine absolute levels of mRNAs encoding Types I and III procollagen in which a defined quantity of a deletion mutant of either procollagen mRNA is included as an external standard in the RT reaction. The α1(I) or α1(III) procollagen cDNAs together with the appropriate deletion mutant standard are then amplified in a radiolabelled PCR. As the quantity of external standard is known the amount of endogenous mRNA encoding either α1(I) or α1(III) procollagen may be estimated. The determination of quantitative data will for the first time allow meaningful quantitative studies of collagen gene expression in healing human wounds of differing aetiologies to be performed.

RZ 110 REGULATION OF FIBROBLAST COLLAGENASE EXPRESSION BY CYCLOSPORIN-A . Jorma Keski-Oja[1,2], Jouko Lohi[1] and Veli-Matti Kähäri[3], Departments of [1]Virology and of [2]Dermatology and Venereology, University of Helsinki, SF-00290 Helsinki, and [3]Department of Dermatology and Venereology, University of Turku, FINLAND.

Human dermal fibroblasts and HT-1080 fibrosarcoma cells were used as models to study the effects of cyclosporin A (CsA) on their metalloendoproteinase expression and activity. Fibroblast collagenase can be induced by the phorbol ester PMA, as well as by IL-1β and TNFα at nM concentrations. Incubation of fibroblasts with 10^{-5} - 10^{-6} M CsA together with PMA enhanced collagenase expression several fold as estimated by Northern hybridization analyses while no effect was observed with CsA alone. The superinduction was dependent on protein synthesis as shown by cycloheximide treatment. The PMA-induction of stromelysin or 92-kDa/type IV collagenase was not affected by CsA. Calcium ionophore A23187 is a known inducer of collagenase expression. However, it caused a decrease in the levels of secreted 72 kDa gelatinase in fibroblasts and HT-1080 cells and in 92 kDa gelatinase in HT-1080 cells as detected by gelatin zymographs. Similar inhibitory effects, although of lower amplitude, were observed also with ocadaic acid and CsA, which are inhibitors/modulators of protein phosphatase 2A and calcium/calmodulin regulated protein phosphatase 2B/calcineurin, respectively. Treatment of HT-1080 cells with PMA resulted in the appearance of 62 and 59 kDa gelatinolytic proteins, presumably cleavage products of 72 kDa gelatinase. Co-treatment with PMA and A23187 led to disappearance of these gelatinolytic proteins, and co-treatment with PMA and CsA or okadaic acid significantly decreased their amount. Our results indicate that the immunosuppressive drug cyclosporin A has modulatory effects on fibroblast collagenase expression. The results also show that disturbances of intracellular calcium levels and inhibition of certain serine/threonine specific protein phosphatases affect the expression of 92-kDa and 72-kDa type IV collagenases and possibly their activation.

RZ 111 CALCIUM REGULATES ADHESION OF ISOLATED NATIVE INTESTINAL EPITHELIAL CELLS TO TYPE IV COLLAGEN, R.P. Moore, J.L. Madara, R.J. MacLeod, Departments of Pathology, Tufts School of Veterinary Medicine and Brigham and Womens Hospital, Boston and Department of Pediatrics, McGill University Childrens Hospital Research Institute, Montreal

Minor defects in epithelial sheets characterized by focal loss of contiguous epithelial cells are a consequence of superficial intestinal injury in natural intestinal disease states. Such defects are restored by migration of epithelial cells shouldering the defect over the underlying matrix. We have previously shown in an *in vitro* guinea pig model that restitution of these defects is facilitated by type IV collagen and in a reductionistic approach using isolated native intestinal epithelial cells, further demonstrated the enhanced attachment of these cells to type IV collagen. Here we use this approach to characterize the role of extracellular divalent cations and intracellular calcium (Ca) in adhesion of native epithelial cells to type IV collagen. Intestinal epithelial cells were isolated by mechanical vibration and shown to be functionally viable by studies of 1) phloridzin sensitive ^{14}[C] alpha-methyl-glucoside uptake studies and 2) volume regulation in hypertonic media. Isolated cells were first incubated in the presence of 50μg/ml cycloheximide followed by incubation on type IV collagen for 30 min. at 37°C. Cell attachment was determined in the presence of medium with the following Ca/magnesium (Mg) concentrations: 1) normal extracellular Mg (1.25mM) and Ca ranging from Ca-free (1-2mM EDTA) to 5mM Ca, and 2) low Mg (μM) and Ca concentrations up to 2.5mM. In addition, cell attachment was determined in the presence of the cell-permeant intracellular Ca chelator, BAPTA (10, 50 and 100μg/ml). Attachment of epithelial cells to type IV collagen was greatest in Ca-free medium in the presence of 1.25mM Mg and least in the presence of Ca concentrations 1.25 mM or greater (300%+/- 47; 34+/-4 and 20 +/-3,expressed as percent of cells adhered compared to cells adhered at baseline concentration of 1.25mM Ca/1.25mMMg; $p<.005$ for each comparison). In the presence of BAPTA, cell attachment was diminished by more than 50% compared to control (43% +/-3, 38% +/-4 and 37% +/-3; 10, 50 and 100μg BAPTA/ml, respectively; $p<.001$ for each). In conclusion, these data suggest 1) physiologic extracellular Ca concentrations regulate attachment of native intestinal epithelial cells to type IV collagen and 2) attachment of native epithelial cells to type IV collagen is mediated by intracellular Ca.

RZ 112INDUCTION OF TISSUE-TYPE PLASMINOGEN ACTIVATOR AND 72KDa TYPE IV COLLAGENASE BY IONIZING RADIATION IN RAT ASTRO-CYTES, Jasti S. Rao[1], Philip J. Tofilon[2], S. Mohanam[1] and R. Sawaya[1], [1]Dpartment of Neurosurgery, [2]Department of Experimental Radiotherapy, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030

The pathophysiology of radiation induced damage in the CNS is poorly understood. The target cells of radiation are considered to be glial and/or endothelial cells. In this study, rat astrocytes were irradiated with single doses of x-rays and the levels of tissue plasminogen activator (tPA) and collagenase in serum-free media and cell extracts at different time intervals were estimated. ELISA and fibrin zymography revealed that increased levels of intracellular tPA activity at 12 hrs after irradiation. Gelatin zymography and ELISA showed continuous increased levels of extracellular 72KDa type IV collagenase. Quantitative analysis by densitometry showed 3 to 4 fold elevated levels of intracellular tPA at 12 hrs and 5 to 6 fold increased levels of extracellular 72KDa type IV collagenase at 72 hrs. This study adds considerable weight to the proposed role of plasminogen activators and type IV collagenases in the development of CNS damage after radiotherapy of brain tumors.

RZ 113REGULATION OF THE α1(I) COLLAGEN PROMOTER BY A TGF-β ACTIVATION ELEMENT

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A transforming growth factor-β (TGF-β) activating element (TAE), with a nuclear factor-1 (NF-1) like sequence, was previously located 1.6 kb upstream from the transcription start site in the α1(I) collagen promoter (Ritzenthaler, J.D., Goldstein, R.H., Fine, A., Lichtler, A., Rowe, D.W., and Smith, B.D.,(1991) *Biochem. J.* 280, 157-162.). Double stranded TAE, but not NF-1 consensus sequences, abrogated TGF-β stimulation of co-transfected collagen promoter-CAT constructs. However, TAE sequences failed to bind in vitro expressed NF-1 protein, to compete for NF-1 binding proteins and to bind with protein which react with antibodies to NF-1 family of proteins. Mutations in non-NF-1 binding sites, located by methylation interference, eliminated activity of the TAE oligonucleotide. Within the TAE there was an activator protein 2 (AP-2) binding site. Although AP-2 protein bound to TAE, AP-2 complexes migrated on gel electrophoresis at a different position than nuclear protein complexes, and antibodies to AP-2 did not react with nuclear protein TAE complexes. TAE bound to a 32,000 Da protein on southwestern analysis. However, the UV-cross-linked TAE nuclear protein complex was 83,000 Da.. Finally, a dose response study demonstrated that TGF-β increased TAE nuclear binding proteins at lower doses with a different response curve than NF-1 nuclear binding proteins. Taken together these data demonstrated that TGF-β functions in human lung fibroblasts to activate collagen transcription through TAE sites by protein complexes independent of NF-1 or AP-2 protein.

RZ 114 TRANSCRIPTIONAL ACTIVATION OF COLLAGEN GENE EXPRESSION IN TSK MOUSE FIBROBLASTS: INSENSITIVITY TO TGF β, Takeshi Tamaki, Eric Everett, E.Carwile LeRoy and Maria Trojanowska. Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC 29425

The tight-skin mouse (TSK) mutant, shows connective tissue abnormalities that are similar to those present in the skin of patients with scleroderma and is considered a model for the fibrosis of this disease. When propagated *in vitro*, both scleroderma and TSK fibroblasts express increased steady-state mRNA levels of collagen type I and III as well as fibronectin.

We have recently demonstrated that in scleroderma fibroblasts transcriptional activity of collagen α2(I) chain (COL1A2) is increased and insensitive to further stimulation by TGF β1 (BBRC,187,p45-50,1992). In this study we asked whether a similar mechanism is involved in collagen expression in TSK mice. Using CAT constructs (COL1A2/CAT) containing the human promoter for the α2(I) collagen gene in transient transfection assays with TSK/+ and control +/+ fibroblasts, we observed higher transcriptional activity of the COL1A2 gene in TSK/+ fibroblasts compared with controls. In contrast to +/+ fibroblasts, TSK/+ fibroblasts showed no further stimulation in the presence of TGF β1. Thus, a similar transcriptional abnormality may be responsible for increased collagen expression in both human scleroderma and TSK mouse.

RZ 115 CHARACTERIZATION OF COLLAGEN SYNTHESIZED BY IRC CELLS, Julia Thom Oxford and Nicholas P. Morris, Shriners Hospital for Crippled Children, Portland, OR 97201

Type XI collagen is a minor interstitial collagen of cartilage. However, in the case of a particular line of transformed rat chondrocytes (IRC, immortalized rat chondrocytes) type XI is the major collagenous product synthesized at the protein level. Northern blot analysis indicates that the mRNA level for type II is severly reduced with respect to nontransformed cells, but is still in large excess when compared to the amount of mRNA encoding the α1 and α2 chains of type XI. Normally, type XI copolymerizes with type II to form the heterotypic fibers of cartilage. Electron micrographs show that the IRC cells synthesize a fibrillar component of the extracellular matrix even in the absence of type II.

Type XI, a heterotrimer of three different alpha chains, shares a common alpha chain with type II, a homotrimer. Even though the α1(II) or α3(XI) chain is present within the cell, it is incorporated into type XI molecules rather than forming type II molecules. The mechanism for the incorporation of α1(II) chains into type XI molecules is unknown. Chain selection is thought to occur via the carboxyl propeptide domain. The possibility remained that α3(XI) might differ from α1(II) in this domain. Sequencing of cDNAs from IRC showed this domain to be the same as that synthesized by rat chondrosarcoma. The level of α1(II) protein can be modulated to some extent in these cells, and at elevated levels, homotrimers can be formed.

RZ 116 THE EFFECTS OF SHEAR STRESS ON THE EXTRACELLULAR MATRIX OF CULTURED BOVINE AORTIC ENDOTHELIAL CELLS,

Olivier Thoumine, Peggy R. Girard, and Robert M. Nerem, Bioengineering Center, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0405.

In blood vessels, the basement membrane (BM) underlying the endothelium supports endothelial cell (EC) attachment, spreading, migration, and proliferation. BM structure and composition may be modulated by hemodynamic shear stress, which may play a role in the pathogenesis of vascular diseases such as atherosclerosis. In this study, the *in vitro* effects of fluid shear stress on the extracellular matrix (ECM) of ECs were investigated. Cultured bovine aortic ECs (BAECs) were exposed to a steady laminar shear stress of 30 dyn/cm^2 from 3 to 48 hr. Parallel control cultures were maintained under static conditions. Immunofluorescent staining of fibronectin (FN), laminin (LN), and collagen type IV (CIV), revealed both a granular and a fibrillar pattern. Optical sectioning under a confocal scanning microscope revealed that the granular pattern was specifically found in the cellular layer, in a perinuclear location, and that the fibrillar pattern was localized underneath the EC layer. In contrast, vitronectin (VN) seemed to exhibit only a cytosolic granular pattern. In BAEC static cultures, FN was organized into a dense matrix of randomly oriented fibrils. These fibrils progressively (12-48 hr) aligned with the direction of flow, and/or grouped into thicker fibers. Extracellular LN and CIV formed a diffuse network of fibrils in static cultures. Exposure to shear stress generated a progressive (12-48 hr) grouping of LN and CIV into thick fibers, which, in contrast to FN, were randomly oriented. In order to examine the influence of shear stress on the protein composition of the ECM, the material remaining on the plastic substratum after removal of the BAECs by deoxycholate was analyzed by two-dimensional gel electrophoresis. Consistent increases in the levels of four acidic proteins (Mr/isoelectric pH = 52/4.9, 70/4.7, 70/5.5, and 110/4.4) were observed after 3-6 hr of exposure to flow. The relative levels of FN, LN, and VN, in the ECM fraction of sheared versus static BAECs, were determined by one-dimensional immunoblotting and scanning laser densitometry. The level of FN appeared to decrease after 12 hr of BAEC exposure to flow, and then increased after 24 and 48 hr. The level of LN exhibited an increase after 24 and 48 hr of flow, while the level of VN was not altered by shear stress. These results indicate that specific qualitative and quantitative changes occur in the ECM of BAECs exposed to shear stress. These changes, possibly linked to a reorganization of cytoskeletal elements, may support cell elongation and orientation in response to laminar steady flow.

RZ 117 CROSS-VALIDATION TO DETERMINE THE BEST CYANOGEN BROMIDE PEPTIDE RATIOS TO MEASURE THE PROPORTION OF TYPE II TO TYPE I COLLAGEN IN EQUINE CARTILAGE REPAIR TISSUE, R.J. Todhunter, J.A.M. Wootton, N.S. Altman, G. Lust, and R.R. Minor, Cornell University, Ithaca, NY 14853.

Collagen types I and II were purified from equine flexor tendon and articular cartilage respectively. Equine type I collagen lacked a methionine between α1(I)CB7 and CB6. Equal amounts of collagen types I and II were cleaved with cyanogen bromide (CB), and 11 different mixtures of peptides, containing increasing proportions of type II collagen were separated with SDS PAGE. The density of bands was measured in Coomassie-stained wet gels and the peak areas were used to form 6 different ratios of CB-cleaved peptides which had polynomial relationships with the proportion of type II collagen in the mixtures. Calibration curves for determining the proportion of type II collagen in the mixtures were constructed using ratios and combinations of ratios of peak areas. Cross-validation was used to identify the calibration curve(s) with the smallest difference between the calculated and actual proportion of type II relative to type I collagen (squared prediction error). Ratios of peak areas of each of 7 gels were treated, in turn, as the "unknown", and prediction was carried out using these unknowns and the ratios from the other 6 gels for calibration. The 2 ratios, ($\log_{10}$ [α1(II)CB10+α1(I)CB4-5-8/α2(I)CB4 +α2(I)CB4-2+α1(I)CB5-8] and [α1(II)CB10+α1(I)CB4-5-8/α1(I)CB7,6 +α1(II)CB11-8)], had the smallest squared average prediction error and calibration curves were computed for these 2 ratios with all 7 gels. These curves were used to calculate the proportion of type II to type I collagen in pepsin digests of articular cartilage, inner meniscus, outer meniscus, and cartilage repair tissue. Cross-validation enabled the selection of the best CB-peptide ratios for developing calibration curves for estimating the proportion of type II collagen in tissue. This technique can be applied to CB-peptides from pepsin-digested or non-digested (whole) tissue preparations. However, our studies showed that CB-peptide mapping with collagens purified from pepsin-digested tissues was preferable to the use of CB-peptides from whole tissues (particularly if C- and N-terminal peptides are included in the selection of optimal ratios), because the latter peptides may be involved in crosslinks and under-represented in the gels.

RZ 118 EXTRACELLULAR MATRIX PRODUCED BY CORNEAL ENDOTHELIAL CELLS CONTAINS THE 72 Kd GELATINASE / TYPE IV COLLAGENASE, Israel Vlodavsky[1], Dan Komarek[2], Matti Hoyhtya[2], Miao Huaquan[1], and Rafael Fridman[3], [1]Department of Oncology Hadassah-Hebrew University Hospital, Jerusalem 91120, Israel; [2]Molecular Oncology Inc. Gaithersburg, MD; and [3]Department of Pathology, Wayne State University, School of Medicine Detroit, Michigan 48201

The extracellular matrix (ECM) is a supramolecular complex of glycoproteins, collagens and proteoglycans that actively modulates cell behavior in both normal and pathological conditions. Previous studies have shown that some of the biological activities of the ECM are mediated by growth factors (bFGF, TGF-β, Osteogenin) and enzymes (t-PA, u-PA, thrombin) sequestered by ECM components such as heparan sulfate proteoglycans and collagens. Type IV collagenases play major roles in degradation and remodeling of ECM in normal and malignant processes involving cell invasion and migration. These enzymes are secreted into the extracellular space in a latent form which has been shown to be associated with tissue inhibitors of metalloproteinases (TIMPs). In the present study we report that ECM produced by bovine corneal endothelial cells contains the 72 kD gelatinase/type IV collagenase, as determined by gelatin zymography and "Western" blot analysis of ECM extracts. The ECM-bound enzyme is found primarily in the latent form and appears to be free of its specific inhibitor TIMP-2. In contrast, zymograms of ECM extracts did not show detectable activity of the 92 kD gelatinase/type IV collagenase. Moreover, incubation of ECM with exogenous recombinant 72 kD or 92 kD enzymes, resulted in specific binding of only the 72 kD enzyme. This suggests that association of the 72 kD gelatinase with ECM may be mediated, in part, by a domain different from the gelatin binding domain which is present in both type IV collagenases. Sequestration of collagenase by the subendothelial ECM may provide a readily available enzyme that is utilized by invasive cells in processes such as tumor metastasis, angiogenesis, inflammation and wound healing.

Proteoglycans, Growth Factors & Bone

RZ 200 *Abstract Withdrawn*

RZ 201 EXTRACELLULAR MATRIX COMPONENTS INDUCE ENDOCRINE DIFFERENTIATION IN NCI H716 CELLS

Fred T. Bosman, Winand N.M. Dinjens, Anthony F.P.M. de Goey, Dept. of Pathology, University of Limburg and Erasmus University, The Netherlands.

In the colon, tumors with endocrine characteristics are encountered in different forms. The most classical form is the carcinoid tumor, which consists almost exclusively of endocrine cells. A significant proportion of colon adenocarcinomas, however, also contains neuroendocrine cells. This finding is of interest because these adenocarcinomas appear to behave more aggressively than the carcinomas without this feature.

We studied endocrine differentiation in the NCI H716 human colon carcinoma cell line. Under standard conditions of tissue culture this cell line grows in suspension, in the form of floating aggregates of cells. A small proportion of these cells displays endocrine features. When xenografted into nude mice an undifferentiated carcinoma develops, which displays extensive neuroendocrine differentiation as reflected in dense core granules and chromogranin A immunoreactivity. This in vitro - in vivo difference suggests that stromal factors play a role in the induction of endocrine differentiation.

We next studied the effect of culturing conditions on the level of expression of chromogranin A in vitro. NCI H716 cells adhered to type IV collagen, with or without additional extracellular matrix components as well as to native extracellular matrices, including lamina propria of colon and amnion basement membrane. The cells also adhered to fibroblasts. On type IV collagen/heparan sulphate protoglycan, colon lamina propria, amnion basement membrane and various fibroblasts induction of endocrine differentiation was observed. In an attempt to identify individual factors responsible for this result, ßFGF and TGFß were added to NCI H716 cell cultures. ßFGF but not TGFß induced endocrine differentiation, as observed by immunostaining for chromogranin A as well as by Northern blotting for chromogranin A mRNA. These results indicate that stromal factors (including growth factors) play a role in the induction of endocrine differentiation.

RZ 202 GLYCOPROTEIN 88 (CD36) BINDS TUBULIN AND ENDOGENOUS THROMBOSPONDIN, Gordon F. Burns, Paul A. Tooney and Cliff J. Meldrum, Cancer Research Unit, University of Newcastle, N.S.W. 2300, Australia.

Glycoprotein 88 (also known as CD36 on leukocytes) has been described as a cell surface receptor for thrombospondin (TSP) although it has also been reported that COS cells transfected with cDNA did not bind added TSP. We show here by immunofluorescent staining of fixed cells permeated with 0.5% Triton that gp88 aligns with cytoskeletal elements in human melanoma cells and fibroblasts. This pattern is disrupted by treatment of the cells with the microtubule disrupting agent, nocodazol. Immunoprecipitation analysis of ^{35}S labeled cells showed some of the gp88 from melanoma cells resisted extraction with PHEM-Triton buffer but was extracted, along with tubulin, with the same buffer with 5mM Ca^{++}. Surface TSP in these cells co-localised with surface gp88 in double staining experiments. When transfected into rabbit kidney cells (RK13), gp88 aligned along cell-cell junctions and was resistant to PHEM-Triton extraction; again, 5mM Ca^{++} in PHEM-Triton extracted most of the gp88 along with tubulin. We predicted that the RSK sequence in the carboxyterminal region of gp88 may be involved in tubulin binding and carried out site-directed mutagenesis of the basic residues. The lysine ---> alanine (K469A) mutant no longer located to cell-cell junctions when transfected into RK13 cells. Most striking was the distribution of TSP in these permanently transfected cell lines: in the wild-type transfectants TSP co-localised along cell-cell junctions whereas in the K469A-transfected cells the endogenous TSP was diffusely distributed on the cell surface and showed no junctional staining.

RZ 203 DIFFERENTIAL EXPRESSION OF ACIDIC FIBROBLAST GROWTH FACTOR mRNA: GENE STRUCTURE AND DISTRIBUTION OF FOUR DIFFERENT aFGF TRANSCRIPTS

Ing-Ming Chiu, René L. Myers, Robert A. Payson and Maqsood A. Chotani, Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH, 43210

We have isolated four cDNA clones coding for human acidic fibroblast growth factor (aFGF) containing alternative 5' untranslated exons. Using RNase protection analyses, we demonstrated the presence of at least four upstream, untranslated exons which are alternatively spliced to the first protein-coding exon. We designate these four untranslated exons, -1A, -1B, -1C and -1D. Splicing of these exons to the first coding exon will generate mRNA 1.A, 1.B, 1.C and 1.D, respectively. Expression of these transcripts is regulated in a tissue specific manner, as the major aFGF transcript in human brain frontal cortex differs from that in kidney. Furthermore, the pattern of aFGF transcripts in several glioblastoma cell lines tested is different from that in normal brain tissue. We isolated nine overlapping genomic DNA clones containing these four upstream, untranslated exons. The four exons were localized on our clones by Southern hybridization and nucleotide sequence analysis. The overlapping clones are contiguous with our previously isolated genomic clones that contain the three aFGF coding exons. The sizes of the four introns are 82.9, 71.1, 29.3 and 6.9 kbp. The transcriptional start sites of each of the aFGF transcripts (1.A, 1.B, 1.C and 1.D) have been mapped using RNase protection and primer extension analyses. The sequences upstream of the start sites for aFGF 1.B, 1.C and 1.D mRNAs do not contain a consensus TATA box. In contrast, the canonical CCAAT and TATA sequences are located at the proper distances from the transcription start site of aFGF 1.A mRNA. Since aFGF has high affinity to heparin and is associated with extracellular matrix, characterization of aFGF gene expression may provide clues to the role of aFGF/extracellular matrix complex in development and disease.

RZ 204 EXPRESSION OF THROMBOSPONDIN (TSP1) AND ITS RECEPTORS (CD36, CD51) IN NORMAL, HYPERPLASTIC AND NEOPLASTIC HUMAN BREAST, Philippe CLEZARDIN, Lucien FRAPPART, Magali CLERGET, Christine PECHOUX, and Pierre D. DELMAS, INSERM U234 and Dept of Pathology, E. Herriot Hospital, Lyon, France.

Thrombospondin (TSP) is a large, trimeric glycoprotein synthesized and secreted by a wide range of normal and transformed cells. TSPs (TSP1, TSP2 and TSP3) are encoded by three related but not identical genes. We observed that TSP1 was present in normal breast secretions, and high levels of TSP1 were observed in malignant breast secretions and cytosols. Using immunohistochemistry and *in situ* hybridization, we now report on the distribution of TSP1 and its receptors (CD36,CD51) in normal, hyperplastic and neoplastic breast. In nonlactating ducts of normal and hyperplastic breast, TSP1 and CD51 are expressed in the basement membrane and in the basal surface of myoepithelial cells, respectively. In lactating adenomas, both TSP1 and CD51 disappear from the myoepithelial-stromal junction of ducts. However, TSP1 become selectively expressed at the apices of secretory epithelial cells of lactating ducts together with CD36, suggesting that the distribution of TSP1 and the appearance of its receptors are dependent on the secretory activity of human mammary ducts. In neoplastic breast, excessive TSP1 deposits are surrounding *in situ* carcinomas (preinvasive cancer) (n=27). Most of the invasive tumor cells (90%) in poorly differentiated ductal carcinomas (n=20) neither express TSP1 nor CD36. By contrast, in well differentiated lobular carcinomas (n=7), TSP1 is coexpressed with CD36 and CD51 in 40-80% of invasive tumor cells. The coexpression of TSP1 and CD36 by invasive lobular carcinoma cells is related to the secretory activity of these cells as previously observed for lactating adenomas. These findings suggest that, in neoplastic breast, the overexpression of TSP is associated with the differentiation of invasive tumor cells.

RZ 205 IDENTIFICATION OF OSTEOGENESIS RELATED GENES IN ORGAN CULTURES OF MOUSE MANDIBULAR CONDYLES. Willy Deleersnijder*, Przemko Tylzanowski, Rita Cortvrindt#, Karen Pittois, Guizhu Hong , Erick Van Marck#, André Van de Voorde* and Jozef Merregaert.
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The progenitor cells of mouse mandibular condyle (MMC) chondrocytes switch their differentiation pathway from chondro- to osteogenesis when the MMC is cultured *in vitro*.

We have used *in vitro* explanted MMC as a model system to identify novel genes involved in osteogenesis via subtractive hybridization and differential screening. Directional cDNA libraries were constructed from MMC, removed from mice at day 18 of embryonic development (= minus library) or from day 18 MMC that were subsequently cultured in vitro for 18-24 hrs (= plus library). Driver cRNA for the subtractive hybridizations was prepared by *in vitro* transcription (IVT) of the minus library. Target cDNA was obtained by IVT of the plus library followed by reverse transcription. Colony lifts of the plus library were differentially screened with subtracted and mock-subtracted probes. Out of 10,000 colonies screened, 20 clones showed an increased hybridization signal with the subtracted probe as compared to the mock-subtracted probe. Partial sequence analysis of those clones showed that the upregulated genes were unknown with the exception of clones B2 and B7 which encoded (parts of) a non-histone chromosomal protein HMG and a mouse retrotransposon (VL 30) respectively.

A number of colonies for which the hybridization signal was decreased with the subtracted probe were also partially sequenced. Most of these genes were known such as the embryogenesis related H19 gene, mitochondrial cyt c oxidase subunit, elongation factor 1-α, ferritin light chain, hsp 70, α1(I) and α1(II) procollagen and bone sialoprotein.

Further analysis of the upregulated genes via Northern blot analysis and in situ hybridization is in progress.

RZ 206 STRUCTURE OF FETAL RIB GROWTH PLATE AGGRECAN CHONDROITIN SULFATE. [1]Angela Deutsch, [2]Ron Midura and [1]Anna Plaas, [1]Shriner's Hospital for Crippled Children, 12502 N Pine Dr, Tampa, FL 33612 & [2]Dept Orthopaedic Surgery, Uni Iowa, Iowa City, IA.

During the normal functioning of growth plate cartilages, to prepare for calcification and bone formation, the resident chondrocytes undergo a transition from resting to proliferating to a hypertrophic phenotype. These changes in the cellular 'differentiation' state are accompanied by alterations in the biosynthetic pattern of the chondrocytes, in that the extracellular matrix surrounding the chondrocytes after hypertrophy becomes rich in aggrecan but deficient in fibrillar collagens and small proteoglycans (1). Aggrecan monomers synthesized and deposited by hypertrophying chondrocytes carry unusually long chondroitin sulfate (CS) chains. To determine the possible functional significance of these alterations in the CS chain structure, we have now extended our studies to investigate the sulfation pattern of aggrecan CS chains in growth plate cartilages. Seven 1mm transverse sections were prepared from 3rd trimester bovine fetal rib growth plates to seperate resting, proliferative and hypertrophic cartilage. These were extracted in 4M GdnHCl and aggrecan purified into the D1 of CsCl gradients. CS chains were digested with chondroitin ACII lyase and the resulting disaccharides (Di-S) separated by high pH anion exchange HPLC on a CarboPac PA1 column. Di-S were quantitated following their detection by either A232nm or integrated pulsed amperometry. The system was calibrated with known amounts of 0-, mono, di and trisulfated Di-S.
When ACII lyase digests from aggrecan extracted from all zones of the growth plate were analysed, both methods of detection consistently showed that Di-0S, Di-4S and Di-6S made up >99% of the digestible CS chains, with only trace amounts of disulfated Di-S detectable. All aggrecan populations contained 16 ±2% (n=9) Di-0S but there was a significant change in the relative amounts of Di-4S and Di-6S from different zones. The ratio of Di-4S:Di-6S was 1.05 ±0.5:1 in the resting, 1.34 ±0.1:1 in the proliferative and 0.62 ±0.1:1 in the hypertrophic zone (n=3 each zone).
Thus, during the transition of growth plate chondrocytes to the hypertrophic state, the cells secrete aggrecan monomers substituted with CS chains of increased size and 6-sulfation. Current studies are directed at investigating a possible spatial organization of the 6-sulfated disaccharides with regard to their location within the CS chains, and relative abundance on distinct regions of the aggrecan core protein. The elucidation of a possible spatial organization of the 6S rich CS chains on hypertrophic cartilage aggrecan might lead to an understanding of the possible significance of these changes in CS structure in the functional properties of a hypertrophic cartilage matrix.

(1) Plaas, AHK and Sandy, JD, Matrix (in press).

RZ 207 INTERACTIONS OF CTB PROTEOGLYCAN WITH THE EXTRACELLULAR MATRIX AND THE CELL SURFACE. Heidemarie Ernst, Mary K. Boackle, David G. Everman, Sandra L. Dutton and Stanley Hoffman. Department of Medicine, Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC 29425.

Cytotactin-binding proteoglycan (CTB PG) was originally isolated as a component of cytotactin (tenascin) preparations from embryonic chicken brains. CTB PG is a large chondroitin sulfate proteoglycan with an M_r 280,000 core protein. We present evidence that CTB PG and another large chondroitin sulfate proteoglycan, aggrecan, affect cell behavior by activating their own cell surface receptors: 1) In gravity cell adhesion assays where cells are exposed to extracellular matrix molecules for one hour, CTB PG and aggrecan strongly inhibit the adhesion of fibroblasts to fibronectin (FN) and vitronectin (VN). CTB PG, however, does not inhibit the binding of fibroblasts to FN or VN in an alternate adhesion assay where the interaction takes place during a two-minute centrifugation. 2) Wheras CTB PG binds strongly to cytotactin, it does not interact with other extracellular matrix molecules like FN, laminin, collagen I and collagen IV. 3) A proteolytic fragment of CTB PG has been isolated that binds to fibroblasts. Recently, the sequences of the chondroitin sulfate proteoglycans aggrecan, versican and neurocan have been determined. All of these molecules contain common domains (e.g. Ig-like, EGF-like, lectin-like, hyaluroninc acid binding, complement-regulatory-like) as well as unique regions. So far we have shown that CTB PG binds to hyaluronic acid. Further experiments are underway to determine whether CTB PG falls within the same family as aggrecan, versican and neurocan.

RZ 208 DIFFERENTIAL INDUCTION BY RETINOIC ACID OF OSTEOPONTIN AND ALKALINE PHOSPHATASE WHEN OSTEOBLASTS ARE GROWN ON COLLAGEN, David M. Findlay, Kong Wah Ng and Kathy Traianedes, St Vincent's Institute of Medical Research, Fitzroy 3065, Victoria, Australia

Extracellular matrix proteins (ECM) promote the attachment, proliferation, migration and differentiated function of many cell types *in vivo* and *in vitro*. The ECM of developing bone comprises predominantly type I collagen as well as non collagenous proteins which likely define the uniqueness of bone as a mineralizing tissue. The experiments reported here are part of a study to determine the role of the ECM of bone to regulate the differentiation and function of cells of the osteoblast lineage.

The rat UMR 201 cells, phenotypically preosteoblast, differentiate in response to retinoic acid (RA) in that the expression of a number of mRNA species is induced or enhanced (Zhou et al, J. Bone & Min. Res., **6**, 767, 1991). These include alkaline phosphatase (ALP) and osteopontin (OP), whose expression is associated with more mature osteoblasts. UMR 201 cells were plated onto plastic tissue culture dishes or dishes coated with gelled type I collagen. The initial interaction of cells with the substrates was determined by acute cell attachment asays. Cells adhered more avidly to collagen than to plastic although when assayed in the presence of RA, attachment to plastic was increased significantly while attachment to collagen was unaffected. Proliferation rates were similar on both substrates and RA treatment inhibited growth of cells to a similar extent in both cases. Striking differences were seen in the gene expression of the cells in either situation; when cells plated on collagen were treated with 10^{-6}M RA, it was found that induction of mRNA for ALP as well as ALP enzyme activity was weaker compared with cells grown on plastic. In contrast, treatment with RA induced much stronger OP expression in cells plated on collagen compared with plastic. When ascorbate was included in the medium by daily supplementation, cell proliferation was stimulated only with cells grown on collagen. In ascorbate treated cells RA also induced expression of ALP and OP mRNA although the latter was increased 2-3 fold compared to cells without ascorbate in the medium.

These results indicate that ECM components potentially regulate the function of, and are capable of modulating cytokine induced differentiation of, cells of the osteoblastic lineage.

RZ 209 CHARACTERIZATION OF A NEWLY ISOLATED 220 kDa DROSOPHILA EXTRACELLULAR MATRIX PROTEIN, Frances J. Fogerty, Liselotte I. Fessler, and John H. Fessler, Department of Biology, UCLA, Los Angeles, CA 90024.

A new Drosophila 220 kDa extracellular matrix protein provisionally called protein Y was identified. Protein Y, which is synthesized and secreted by a Kc-derived Drosophila cell line, was purified from conditioned media. Pure protein Y was used to generate mouse polyclonal antibodies and obtain an N-terminal amino acid sequence. Antibody staining of whole mount Drosophila embryos showed Y protein localization at muscle insertion sites. In sections of adult thoracic muscle Y protein colocalized with integrins at Z bands. These results implicate a role for Y protein in muscle development. Affinity-purified anti-Y IgGs were used to screen a cDNA expression library prepared from these cells. A 2.2 kb Y cDNA clone encoding the poly-A containing 3' end of the message was isolated. A radiolabeled nucleotide probe prepared from the most 5' end of this clone was used to screen a 12-24 h Drosophila embryo cDNA library and a 7.0 kb clone was isolated. This cDNA contains the entire protein coding sequence including an 18 amino acid signal sequence and the seven N-terminal amino acids originally identified in the purified protein. In situ hybridization to salivary gland polytene chromosomes placed the Y gene in region 26C on chromosome 2. Northern blotting revealed a prominent 7.5 kb Y transcript in the cultured cells and the embryos. In situ hybridizations to whole mount embryos showed Y transcripts present in the fat body and in hemocytes.

RZ 210ORIGIN AND ROLE OF THROMBOSPONDIN IN THE EARLY DEVELOPMENT OF THE HUMAN MAMMARY GLAND

Lucien Frappart*, Philippe Clezardin**, Robert Dante***, Claire-Marie Serre**, Magali Clerget**, Christine Péchoux*, *Laboratoire d'Anatomie Pathologique, Bât 10 - **INSERM U 234, Pavillon F, Hôpital Edouard Herriot, F 69437 LYON Cedex 3 - ***INSERM U 218, 28 rue Laennec, F 69373 LYON Cedex 03 - *Unité CNRS UPR-412, Passage du Vercors, F 69367 Lyon Cedex 07.

The patterns of deposition of the extracellular matrix protein thrombospondin (TSP), a homotrimeric adhesive multifunctional glycoprotein, were determined for the early stages of development of the human mammary gland, and its origin and role were examined using immunohistochemistry, PCR mRNA assay and *in situ* hybridization. Immunohistochemical analysis in light microscopy with monoclonal antibodies P10, MA I, MA II, directed against TSP, in normal fetal mammary gland showed positive staining only around the mammary bud and glands, where it was densely deposited. Using immunoelectron microscopy, monoclonal antibodies P10 and MA I produced positive staining of the cytoplasmic membrane of the basal cells at the interface between the epithelium and the connective tissue. There was also labeling at the apical membrane of the epithelial cells in the glands. TSP was codistributed with collagen type I in the extracellular matrix(ECM). The presence of TSP transcripts in nascent breast was therefore confirmed using a two-step PCR method. *In situ* hybridization confirmed that localization of TSP transcripts was restricted to the epidermis of the mammary bud, and the underlying glands. Monoclonal antibodies FA6-152 and OKM5, which are directed against CD36, revealed positive staining at the apical membrane of the epithelial cells of the glands and in the cytoplasm of the adipocytes in the fat pad. With monoclonal antibody LM142 specific for CD51, (the integrin subunit αv), there was a positive labeling of the basal cells in the mammary bud, and in the glands. The presence of TSP and its receptors in the ECM at the end of the bud may therefore facilitate the growth of epithelial cells, particularly the cap cells. The TSP-associated integrins may also be involved in regulating the growth of epithelial buds along the pathway where their ligands are expressed. Our data suggest an intimate relationship between TSP and the growth and differentiation of the human mammary gland.

RZ 211 EFFECTS OF MECHANICAL STRETCHING ON THE ULTRASTRUCTURE OF COLLAGEN LATTICE POPULATED BY FIBROBLASTS, Jacques M. Frey and Annette M. Chamson, Department of Biochemistry, Medical School, 42023 Saint-Etienne, France.

When fibroblasts are cultivated into a collagen gel, they are able to retract the gel and organize collagen microfibrils, then collagen bundles. This retraction takes place in three dimensional space : the collagen bundles have a randomized orientation. A mechanical device was able to block unidirectionally the retraction, and mechanical stretching occurred with a force equal to the contraction force of the fibroblasts. We used three different conditions for stretching, so that retraction was free, partially or totally blocked. Under these three conditions, the ultra-structure of the collagen lattices populated by fibroblasts was observed by transmission electron microscopy (TEM).

In the case of free retraction, we confirmed that the acid soluble collagen was organized by the fibroblasts to form typical collagen fibrils whose orientation was totally randomized. The stretching resulted in a unidirectional fibril orientation. However the collagen fibrils were partly oriented when the retraction was partially blocked.

The stretching also resulted in intracellular organelle modifications. In the free retracted lattices, the endoplasmic reticulum was plentiful and presented ergastoplasmic cisternae which demonstrated a biosynthetic activity oriented to protein production. In the case of lattices under tension, the endoplasmic reticulum was rare, but lysosomes and lamellar bodies were largely represented. The lamellar bodies are known as the transport organelles of cholesterol-sphingolipid complexes used for membrane organization.

This culture model under tension could be useful for studying the biological phenomena involved in connective tissues submitted to mechanical forces, as in tendons.

RZ 212 **Induction of c-ets-1 expression by TNFα and bFGF in human fibroblasts** F. Gilles, V. Fafeur, D. Stéhelin and B. Vandenbunder CNRS URA 1160. Laboratory of Molecular Oncology. Pasteur Institute of Lille. Lille. France. The proto-oncogene c-ets-1 encodes a transcription factor, of which the biological role is largely unknown. We previously reported that in human carcinomas, c-ets-1 expression was detected in stromal fibroblasts and endothelial cells, whereas tumor cells themselves were always negative (Wernert et al., Am. J. Pathol., 140:119-127, 1992). We have now investigated the possibility that growth factors can regulate the expression of c-ets-1 using human foreskin fibroblasts in culture. By Northern blot analysis, we show that both tumor necrosis factor-alpha (TNFα) and basic fibroblast growth factor (bFGF) increase c-ets-1 mRNA expression, whereas transforming growth factor-beta type 1 and platelet derived growth factor-type BB were ineffective. The increase of c-ets-1 transcription by TNFα occurs rapidly (within few hours) and in the absence of *de novo* protein synthesis, suggesting that c-ets-1 is an early TNFα-responsive gene. Previous *in vitro* studies have shown that Ets-1 can transactivate the promoters of stromelysin I and collagenase I, two members of the matrix-degrading metalloprotease family, which play an important role in the local degradation of the extra-cellular matrix. We found that the response of c-ets-1 to the growth factors tested correlates with an increased expression of the stromelysin I and collagenase I genes. These results suggest the involvement of c-ets-1 in the signal transduction pathways of TNFα and bFGF leading to increased production of stromelysin I and collagenase I. Interestingly, the same correlation between the expression of c-ets-1, stromelysin I and collagenase I genes has been established *in vivo* in stromal fibroblasts of some invasive carcinomas. We propose that an important role for c-ets-1 proto-oncogene products is to participate in the invasive process associated with tumor development.

RZ 213 ISOLATION OF cDNAs ENCODING CHICK EMBRYO HYALURONAN-BINDING PROTEINS, Nicholas Grammatikakis, Masahiko Yoneda, Aliki Grammatikakis, Shib D. Banerjee and Bryan P. Toole, Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111

We have recently identified a novel group of hyaluronan-binding proteins involved in pericellular matrix assembly (Q. Yu, S. Banerjee & B. Toole, Dev. Dynamics **193**: 145-151, 1992) and tissue morphogenesis (S. Banerjee & B. Toole, J. Cell Biol. **119**: 643-652, 1992). Using a monoclonal antibody (mAb IVd4) that recognizes these proteins, we have screened a library prepared from chick embryo heart mRNA and identified several positive cDNA clones; the cDNA inserts range in size from 0.9 to 2.3 kb. These cDNAs have been characterized by Southern and Northern hybridizations, and completely sequenced. Comparison with other sequences within available databases has not revealed any extensive homologies with previously characterized proteins, including hyaluronan-binding proteins such as CD44, RHAMM and link protein. The peptides encoded by the cDNAs have been expressed as fusion peptides in various prokaryotic vectors, including pGEX-2T, and shown to react with mAb IVd4 and to bind hyaluronan. In addition, overlapping deletion constructs have been made and expressed; these are currently being analyzed in order to determine which peptide domains contain the mAb IVd4 epitope and the hyaluronan-binding motif.

RZ 214 SELECTIVE IMPAIRMENT OF THE SYNTHESIS OF BASIC FIBROBLAST GROWTH FACTOR BINDING DOMAINS OF HEPARAN SULFATE IN A COS CELL MUTANT DEFECTIVE IN N-SULFOTRANSFERASE. Masayuki Ishihara, Yuchuan Guo, and Stuart J.Swiedler, Glycomed Inc., Alameda, CA 94501

N-sulfation is a key step in the overall sulfation of heparan sulfate. We have isolated a COS cell-derived mutant,CM-15,that is impaired in its ability to bind to basic fibroblast growth factor (bFGF) and has a 2-3-fold reduction in N-sulfotransferase activity. We now provide structural evidence that CM-15 is selectively impaired in the synthesis of highly sulfated regions or "blocks" that display high affinity binding to bFGF; these are completely N-sulfated blocks of decasaccharide or greater length that are enriched in O-sulfate groups. Transfection of CM-15 with a plasmid containing cDNA of N-heparan sulfate sulfotransferase recover the binding ability to bFGF and the synthesis of the highly sulfated blocks with high affinity for bFGF. The synthesis of sulfated blocks that did not show high affinity to the growth factor was relatively unimpaired in the mutant cells; this included fully N-sulfated octamer (or smaller) blocks,and,unexpectedly,decasaccharide or larger blocks that were poorly O-sulfated. In agreement with other studies,disaccharide analysis of the wild-type-derived sulfated blocks suggested that 2-O-sulfation of iduronate residues in the polymer was a necessary element to produce a high affinity binding sequence once N-sulfation was completed in the decasaccharide or larger fraction. These results suggest that a selective reduction in both N- and O-sufation in the larger blocks produced by CM-15 cells is a consequence of the reduction of N-sulfotransferase activity.

RZ 215 HIGH MEDIUM GLUCOSE CAUSES PROFOUND CHANGES IN GLOMERULAR EPITHELIAL CELL (GEC) PROTEOGLYCAN (PG) METABOLISM, B.S. Kasinath, Department of Medicine, Division of Nephrology, University of Texas Health Science Center, San Antonio, Texas 78284

In diabetic kidney disease, the glomerular basement membrane (GBM) content of heparan sulfate proteoglycan (HSPG) is reduced. As GEC synthesize GBM HSPG, we studied the effects of 8-day incubation with high medium glucose with no added insulin (30mMG) on GEC metabolism of PGs which were labeled with $^{35}SO_4$ for the last 24 hours of the incubation period. Compared to control, 30mMG induced a nearly 2-fold increase in the synthesis of labeled macromolecules in the cell layer and media compartments. Equimolar mannitol had a similar effect on ^{35}S-incorporation. On ion exchange chromatography, 30mMG and mannitol did not alter the anionic charge density of medium and cell layer PGs. The proportion of labeled low anionic macromolecules in the 30mMG medium was greater (57.3±7.7% vs. 36.2±11.4%, $p<0.01$) and that of PGs lower compared to control and mannitol. On Sepharose CL-4B chromatography control, mannitol and 30mMG media PGs eluted in 2 peaks of similar relative proportions and size (Peak I Kav 0.15, control vs. 0.19, 30mMG; Peak II Kav 0.58 control vs. 0.59, 30mMG). Mannitol did not affect the hydrodynamic sizes or the proportions of medium PG peaks. HSPG was the major PG secreted into the media by control, mannitol and 30 mMG treated cells. Cell layer PGs resolved into 2 peaks (Peak I Kav 0.38 control vs. 0.31, 30 mMG; Peak II Kav 0.72 control vs. 0.65, 30mMG), both peaks consisting mostly of HSPG. HSPG of Kav 0.38 approximates the size of GBM HSPG. The relative proportion of cell layer peak I was severely reduced by 30mMG [9.2±3.4%, 30mMG vs. 41.3±4.1% control; $p<0.01$]. A 58% reduction in peak I of 30mMG cell layer HSPG was seen when data was expressed in absolute amounts as cpm per mg cell protein . Mannitol did not cause reduction in cell layer peak I HSPG. We conclude that 30mMG induces (a) an increase in the medium content of ^{35}S-labeled low anionic macromolecules eg. glycoproteins, (b) no change in the anionic charge of PGs in the medium and cell layer, (c) severe reduction in the cell layer HSPG species that has the hydrodynamic size of GBM HSPG. Some of the effects of 30mMG, eg. increase in ^{35}S-incorporation, may be related to its osmotic effects, whereas the effects on PGs, eg. reduction in cell layer peak I HSPG, may be caused by dysregulation of proteoglycan metabolism. These changes may form the biochemical basis of glycopeptide and HSPG abnormalities of GBM seen in diabetic kidney disease.

RZ 216 CHARACTERIZATION OF THE HUMAN BONE SIALOPROTEIN GENE: IDENTIFICATION OF INVERTED "TATA" AND "CCAAT" BOXES IN THE GENE PROMOTER, Richard H. Kim, Howard S. Shapiro, Jack J. Li, Jeffrey L. Wrana, and Jaro Sodek, MRC Gp. in Perio. Phys., and Dept. of Biochemistry, U. OF TORONTO, Toronto, Ont. CANADA, M5S 1A8.

Bone sialoprotein (BSP) is a major structural protein of the bone matrix that is specifically expressed by fully-differentiated osteoblasts. To characterize the gene and to study the tissue- and differentiation stage-specific regulation of BSP gene transcription we have isolated and partially sequenced two overlapping genomic fragments which span the complete BSP gene and its promoter region. The ~15 kb gene comprises seven exons of 81 bp, 68 bp, 51 bp, 78 bp, 63 bp, 159 bp, and 2.5 kb (1 - 7, respectively), separated by six introns of ~3 kb, 92 bp, 95 bp, ~3 kb, ~0.5 kb and ~4.5 kb. All of the intron-exon boundaries defining the splice sites conform to the consensus sequence of: AG at the 3' splice site; and GT at the 5' splice site. The first exon encodes the 5'-UTR, the second exon the signal sequence and the first two amino acids, exons 3 and 4 the Tyr- and Phe-rich amino terminus, and exon 5 the first segment of polyglutamic acid. Exon 7 encodes over half of the protein including a second polyglutamic acid segment, the RGD cell attachment motif, the sulphated tyrosine-rich C-terminus and the 3'-UTR. The promoter region is characterized by an inverted TATA box (TTTATA), nts -29 to -24 from the transcriptional start site (+1), and an inverted "CCAAT" box (ATTGG) at -55 to -51. Analysis of chimeric constructs fused to a CAT reporter gene indicate that the presence of both the TATA and CCAAT elements are required for basal promoter activity. Comparisons with the rat BSP promoter reveal that the nature and position of the TATA and CCAAT boxes together with an AP1 (-149 to -143), CRE (-123 to -117) and a homeobox-binding protein site (-201 to -192) have been conserved. A putative Glucocorticoid Response Unit (GRU) consisting of a Glucocorticoid Response Element (GRE) and an overlapping direct repeat (DR5) similar to the retinoic acid receptor element (RARE) is present at -1039 to -1023. These studies have defined the structure of the human BSP gene and have identified novel transcriptional elements in the promoter that may be involved in the developmentally regulated expression of this gene.

RZ 217 OSTEOREGENERATION USING A FIBRIN SEALANT DELIVERY VEHICLE FOR DEMINERALIZED BONE MATRIX, Carlos Lasa, Jr., M.D., Jeffrey Hollinger, D.D.S., Ph.D.*, Reginald Kidd III, B.S., Hernan Nunez, Ph.D., Christian Haudenschild, M.D., William Drohan, Ph.D., and Martin MacPhee, Ph.D., Holland Laboratory, American Red Cross, Rockville, MD 20855, and *U.S. Army Institute of Dental Research, Washington, D.C. 20307.

A naturally biodegradable fibrin sealant (FS) produced by mixing virally-inactivated concentrated human fibrinogen and thrombin solutions was evaluated as a delivery vehicle for demineralized bone matrix (DBM). FS produced by the Red Cross differed from FS products licensed in other countries in that it was formulated with human instead of bovine thrombin, and it did not contain aprotinin, a bovine-derived protease inhibitor. Rat DBM powder (25 mg), alone or in a FS matrix (4, 8, 15 or 45 mg/ml fibrinogen concentration) of various defined shapes was bioassayed for bone induction by intramuscular implantation. Implants were retrieved after 4 weeks, x-rayed and prepared for histology. Results showed a positive bioassay as evidenced by radio-opacity and ossicle formation. The original shapes of the implants were generally retained. Subsequently, DBM (25 mg) alone or in a FS matrix (15, 30, or 45 mg/ml) was implanted into 8 mm rat craniotomy defects and retrieved after 28 days. X-rays showed DBM implants in a FS matrix to be generally more radio-opaque than DBM implants alone or untreated controls. Histology of DBM implants in a FS matrix showed osteoid matrix and trabeculae, more cellularity, denser connective tissue and greater neovascularization than DBM implants alone or untreated controls. Osteoregeneration was evident at all concentrations of FS tested. As a delivery vehicle for bone inductive powders such as DBM, FS facilitated the shaping of DBM into the desired form to fill the bony defect, maintained DBM within the defect, and may have been synergistic with DBM. Furthermore, soft tissue prolapse did not occur and bony contour was maintained. FS appears to possess an appropriate micro-architecture, biodegradation profile and release kinetics to support osteoregeneration.

RZ 218 EUKARYOTIC EXPRESSION OF CHONDROITIN SULFATE-SUBSTITUTED RECOMBINANT BIGLYCAN

David J. McQuillan and Anne M. Hocking, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville 3052, Australia

Biglycan (BGN) is a small interstitial proteoglycan (PG) associated with the cell surface and pericellular domain of many connective tissues. Despite sharing significant homology with decorin (DCN) and fibromodulin, the functional role of BGN is poorly understood. Detailed study of BGN requires effective isolation and purification, preferably in the absence of denaturing or reducing solvents. We have used a recombinant vaccinia virus/T7 phage expression system in conjunction with a novel eukaryotic fusion protein expression vector to generate recombinant BGN. The expression vector, pAH1, was constructed by subcloning a fusion protein cassette into a T7 promoter-driven expression vector, pTM1. The fusion protein cassette comprised an insulin signal sequence domain upstream of a rat hepatic lectin galactose recognition domain (GRD). The BGN construct was subcloned into the polylinker of pAH1, separated from the GRD by a factor Xa protease-sensitive peptide, resulting in the vector pAH1-BGN. The BGN construct was derived from a full length cDNA in which both the leader sequence and propeptide domains were removed prior to subcloning into pAH1. Cell lines were infected with a recombinant vaccinia virus (vTF7-3) encoding bacteriophage T7 RNA polymerase under control of the vaccinia promoter. vTF7-3 infected cells were transfected with pAH1-BGN and at 24 h post-infection cells were biosynthetically labeled by incubation with [^{35}S]-Met or [^{35}S]-SO_4. Secretion of both BGN core protein and BGN substituted with chondroitin sulfate chains was demonstrated by immunoprecipitation of purified BGN from both the cell layer and medium fractions and visualisation by SDS-PAGE. Generation of recombinant PG substituted with glycosaminoglycans will facilitate the definition of structural and functional domains by production of chemical amounts of native and mutant molecules.

RZ 219 **RETINOIC ACID EFFECTS ON PRIMARY HUMAN OSTEOBLASTIC BONE MARROW STROMAL CELLS ARE DOSE AND TIME DEPENDENT**, George Muschler, Vladimir Scerbin, Robin Hart, Section of Musculoskeletal Biology, Dept. of Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Introduction. Osteogenic cells isolated from bone marrow are induced to proliferate and differentiate by various regulatory factors. Retinoic acid (RA) has pleiotropic effects on osteoblastic cell lines ***in vitro***. Using primary human bone marrow stromal cells (PHBMS) harvested by aspiration, we examined the effect of transRA dose and time of exposure on proliferation (DNA) and alkaline phosphatase activity (AP).

Figure 1. DNA at day 9

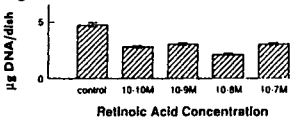
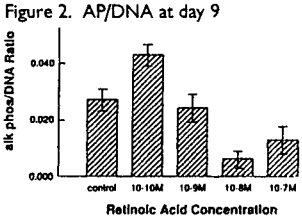


Figure 2. AP/DNA at day 9



Methods. Nucleated cells isolated from human iliac crest aspirates were plated at 2.6×10^5 cells/cm^2 and cultured in α-MEM, 10% FBS, 50 µg/ml ascorbate, 10^{-8}M dexamethasone, 10 mM β-glycerophosphate. Dose response was evaluated from 10^{-10}M to 10^{-7}M. Time dependent effects were evaluated at 9 and 16 days using 10^{-8}M RA given days 0-5, 0-9, 5-9 or 9-16.

Results. RA at any dose day 0-9 reduced cell numbers at both time points (Fig. 1). RA day 0-9 modified AP/DNA dose responsively. 10^{-10}M RA day 0-9 increased AP/DNA at day 9. (Fig. 2). By day 16, AP and DNA were reduced compared to controls at all doses. RA given day 9-16 had no effect at any dose.

Conclusion. RA effects on primary human bone marrow stromal cells are dose and time dependent in early osteoblastic differentiation.

RZ 220 A SHARK CARTILAGE-DERIVED PROTEIN RELATED TO MAMMALIAN OSTEOINDUCTIVE FACTOR AND AVIAN PG-Lb. Peter J. Neame, Shriners Hospital for Crippled Children and Department of Biochemistry and Molecular Biology, University of South Florida, Tampa FL 33612.

Shark cartilage is a useful source of skeletal extracellular matrix proteins which have diverged significantly from their mammalian counterparts. Analysis of conserved sequence motifs is helpful in defining structurally and functionally important domains. Shark cartilage is unusually acellular and is a very hard, glassy, tissue. It is unlikely that much diffusion of high molecular weight components occurs. It may also, therefore, act as a reservoir of proteins involved in production of the matrix. In the course of isolation of aggrecan and link protein from shark spinal cartilage, we have consistently found evidence for a protein which is not related to known structures in either of these molecules. Proteoglycan (PG) aggregates were isolated in conventional fashion, using dissociative extraction and sequential associative and dissociative CsCl gradient ultracentrifugation. Products from proteolytic digestion of aggrecan were separated by gel filtration followed by reversed-phase HPLC. Peptides which were not related to known sequences of aggrecan were consistently found. On alignment of several of these peptides, a 154 amino acid-sequence was found that was related to the family of leucine-rich PGs. The highest degree of similarity was found with chick PG-Lb (Shinomura and Kimata, JBC, 267:1265, 1992) (51% identity) and with bovine osteoinductive factor (Madisen et al., DNA Cell Biol., 9:303, 1990) (40% identity). PG-Lb and OIF are 48% identical. Other leucine-rich PGs, while sharing many of the same sequence domains, are dissimilar at the level of individual amino acids. A significant difference between OIF and PG-Lb is that OIF has three potential N-linked oligosaccharide sites in the C-terminal 150 amino acids, whereas PG-Lb has one. The single potential N-linkage site of PG-Lb is mirrored in the shark protein sequence. No evidence of an N-linked oligosaccharide was found. As the region where the N-X-S site is found is highly conserved in all three proteins, as opposed to the leucine-rich domain, it seems likely that this region, the C-terminal loop, is functionally important.

Funded by NIH AR 35322 and the Shriners of North America.

RZ 221 ROLE OF OSTEOGENIN AND RELATED BMPs IN CRANIOFACIAL REGENERATION IN BABOONS, Ugo Ripamonti, Medical Research Council, DRI, University of the Witwatersrand, Johannesburg, South Africa.

The recently isolated and characterized bone morphogenetic proteins (BMPs) induce endochondral bone differentiation in vivo, and may play multiple roles in embryonic development and organogenesis. This may provide the potential for controlled initiation of bone repair and regeneration in man. While substantial progress is being made to elucidate the molecular and cellular mechanisms involved in BMP-induced bone differentiation, the morphogenetic potential of BMPs, including osteogenin (BMP-3), is predominantly based on work in rodent or canine models. Information concerning the bone inductive potential of osteogenin and related BMPs in nonhuman primates is a prerequisite for the ultimate clinical application in man. To determine the efficacy of osteogenin (BMP-3) as potential therapeutic initiator of osteogenesis, we have developed an orthotopic model in which large osseous defects (2.5 cm in diameter) were surgically created in the calvaria of adult male baboons (Papio ursinus). Osteogenin was isolated from baboon bone matrix and purified by chromatography on heparin-Sepharose, hydroxyapatite and Sephacryl S-200. Final purification to homogeneity was obtained by electroendosmotic elution from a preparative SDS-polyacrylamide gel, resulting in a single band on a SDS-PAGE with an apparent molecular mass of 30-34 kDa. Baboon osteogenin fractions in conjunction with insoluble collagenous bone matrix induced complete regeneration of the calvarial defects. Osteogenin induced bone formation also when adsorbed onto porous inorganic hydroxyapatite substrata implanted in extraskeletal sites as well as calvarial defects of adult baboons. The finding that the biological activity of osteogenin can be expressed by both organic and inorganic substrata to obtain predictable phenotypes in primates, may help tissue engineers to design appropriate delivery systems for recombinant human BMPs that are amenable to contouring of form for the therapeutic initiation of osteogenesis.

RZ 222 REGULATION OF BASIC FIBROBLAST GROWTH FACTOR EXPRESSION IN CARTILAGE: SIMULTANEOUS EXPRESSION OF BASIC FGF AND OF A NATURAL ANTISENSE FGF TRANSCRIPT IN ARTICULAR CARTILAGE. Joachim Sasse, David Pearson, Prasanthi Govindraj and Georgeann Smale. Shriners Hospital for Crippled Children, Tampa, FL 33612.

Basic fibroblast growth factor (bFGF) is implicated in the processes of cell growth, development and angiogenesis as well as the synthesis and deposition of the extracellular matrix. By using bioactivity measurements, radioimmunoassays and an RNase protection assay we demonstrate that bFGF is expressed in the different zones of the fetal bovine cartilage growth plate and in articular chondrocytes. The detection of relatively large amounts of bFGF mRNA in the resting zone of the growth plate and in articular cartilage is consistent with a "housekeeping" role of bFGF in chondrocytes, possibly by contributing to the maintenance of the cartilage phenotype. The result that bFGF mRNA is prominently expressed in resting chondrocytes prompted us to investigate if bFGF expression at the protein level is regulated by a natural antisense bFGF transcript, recently described in the *Xenopus laevis* oocyte system (Kimelman, D. and Kirschner, M.W. 1989, Cell 51: 687-696; Volk, R., et al.,1989, Embo J. 8: 2983-2988.) By using an RNase protection assay, employing a probe specific for the bFGF antisense message, we demonstrate its expression in bovine articular chondrocytes while it is not detected in bovine capillary endothelial cells that are expressing high levels of the bFGF sense transcript only. We speculate that the generation of antisense bFGF transcripts may influence the expression of the bFGF gene post-transcriptionally. Alternatively, the protein encoded by the antisense transcript itself may have a biological function in articular cartilage.

These findings might have implications for the maintenance of the cellular phenotype and the regulation of extracellular matrix deposition of developing connective tissue cells, in general.

RZ 223 MECHANISMS OF SMOOTH MUSCLE CELL MIGRATION: THE ROLE OF HYALURONAN (HA) AND THE HA RECEPTOR RHAMM Rashmin C. Savani, Chao Wang, Baihua Yang, Michael Kinsella, Thomas N. Wight, Robert Stern and Eva A. Turley, Department of Pediatrics and Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada R3E 0V9.

Although little is known about its regulation, the migration of smooth muscle cells plays an important role in physiologic and pathologic processes. We have investigated the role of Hyaluronan (HA) and RHAMM (Receptor for HA-Mediated Motility), an HA receptor that mediates growth factor and oncogenic ras-mediated locomotion, in wound repair of adult bovine aortic smooth muscle cells (BASMC). Hyaluronan stimulated the random locomotion of BASMC and production of this glycosaminoglycan was increased following wounding injury. Wounding of BASMC monolayers resulted in a highly directional migration of cells into the wound between 4 and 18 hours after injury. Immunofluorescence of injured cells showed an increase in expression of RHAMM and a change in fluorescence from the cytoplasm to the cell membrane. Membrane-associated fluorescence closely coincided with the increased rate of cell locomotion observed after wounding. Immunofluorescence studies also showed that RHAMM was expressed throughout the monolayer, but staining was particularly intense on the lamellae of cells migrating at the wound edge. Immunoblot analysis of the injured monolayers confirmed an increase in RHAMM protein content 4-18 hours after wounding. RHAMM was also released from injured monolayers and increased amounts of soluble RHAMM in the medium coincided with decreasing migration and decreased expression of cellular RHAMM. Evidence of the crucial role of RHAMM in wounding was provided by the ability of a polyclonal antibody that is monospecific to RHAMM to both abolish migration into the wound and reduce the rate of cell locomotion after injury. These data show that RHAMM is critical for the migration of BASMC into wounds and that it is temporally and spatially regulated in an autocrine manner following injury of the BASMC monolayers.

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RZ 224 LATERAL PATTERNS OF PROTEIN MOLECULES IN NEURON PLASMIC MEMBRANES AS SELF-ORGANIZED DISSIPATIVE STRUCTURES STORING INFORMATION.

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Statistical analysis of immunocytochemical data showed the presence of regular lateral patterns in the surface topography of proteins on the process membrane of cultured nerve cells. To reveal biophysical mechanisms underlying such patterning, a mathematical model was suggested that predicted creation of similar self-organized space dissipative structures involving physiological variables of the neuron membrane. In the model, consideration of the ion channel diffusion has withdrawn contradictions in previously described models. Comparison of the experimental data and model predictions allowed discussion of mechanisms connecting intrinsic neuron properties and development of space temporal dissipative structures in neuron systems.

RZ 225 ACTIVATION OF LATENT TGF-ß BY THROMBOSPONDIN, S. Schultz-Cherry[#], D.R. Twardzik [*], J.E. Ranchalis[*], and J.E. Murphy-Ullrich [#]. Department of Molecular and Cellular Pathology [#], Birmingham, AL 35294, Bristol-Myers Squibb Pharmaceutical Research Institute[*] Seattle, WA 98121

TGF-ß is a potent growth regulatory protein normally secreted by cells in a latent form. Primary regulation of TGF-ß activity occurs through factors which control the processing of the latent to the biologically active form of the molecule. Thrombospondin (TSP), a platelet alpha granule and extracellular matrix protein, forms specific complexes with active TGF-ß in platelet releasates. TSP can activate endogenous latent TGF-ß secreted by endothelial cells. This activation is specific for TSP and is inhibitable with antibodies to TGF-ß or TSP. TSP also forms complexes with a recombinantly expressed form of latent TGF-ß. The interaction between TSP and latent TGF-ß results in the generation of biologically active TGF-ß, as assayed by the ability of NRK-49F cells to form colonies in soft agar and by the inhibition of 3H-thymidine incorporation by MvlLu cells. Activation of latent TGF-ß by TSP is time and concentration dependent. A maximal response is observed when 3 ug/ml (6 nM) TSP is incubated with 100 ng/ml (0.9 nM) latent TGF-ß for 2 hr at 37°C. Activation is inhibited by antibodies to TGF-ß or TSP. Activation of latent TGF-ß is not observed when incubated with either fibronectin, SPARC, or BSA. The mechanism whereby TSP activates latent TGF-ß is unclear, although TSP-mediated activation of latent TGF-ß was not sensitive to serine protease inhibitors, suggesting that the mechanism is independent of plasmin. The ability of TSP to convert latent TGF-ß to biologically active TGF-ß suggests that TSP may be a physiologic regulator of the activation of latent TGF-ß. TSP-TGF-ß interactions have potential therapeutic applications.

RZ 226 CELL ADHESIVE ACTIVITY OF A HUMAN BASEMENT MEMBRANE HEPARAN SULFATE PROTEOGLYCAN (HSPG) DEMONSTRATED BY DIGITAL VIDEOGRAPHY. Louis C. Sellett and Barry P. Peters, Department of Anatomy, Physiological Sciences and Radiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC, 27606.

An HSPG isolated from conditioned medium of cultured human bladder carcinoma cells (UM-UC-9, obtained from Dr. H.B. Grossman, University of Michigan Medical School) promoted the adhesion and spreading of UM-UC-9 cells on HSPG-coated plastic tissue culture dishes. Morphometric analysis of digitized phase-contrast videographs taken 7 hr after plating showed that cells seeded on HSPG spread more rapidly and to a significantly greater extent ($p < .005$) than cells on plastic, covering 307% ± 12% more surface area. In addition, the HSPG-plated cells were elongated in shape, exhibiting axial ratios 3-fold greater than cells on plastic. Time-lapse videography revealed that attachment to substratum is an active process during which cells repeatedly extend and retract filopodial projections, change positions relative to neighboring cells, and undergo intricate patterns of movement prior to spreading on the HSPG-coated surface.

The HSPG employed in these studies exhibited an apparent Mr=850,000 on silver-stained SDS gels. Heparitinase digestion produced an HSPG core protein preparation of 360 kDa whereas chondroitinase ABC had no effect on the HSPG. Antibodies raised against the purified HSPG also reacted with a similarly-sized HSPG that we previously reported in cultures of human choriocarcinoma (JAR) cells (Frenette, et al. J. Biol. Chem. (1989) 264 3078-3088). In addition, the UM-UC-9 HSPG was immunoprecipitated from conditioned culture medium with an antibody (provided by Dr. J. Stow, Renal Unit, Massachusetts General Hospital) against rat glomerular basement membrane HSPG.

Our observations suggest that the HSPG secreted by the UM-UC-9 human bladder carcinoma cells is a basement membrane component with cell-adhesive activity.

(Supported by USPHS Grant CA41359)

RZ 227 ANGIOTENSIN II REGULATES PROTEOGLYCAN SYNTHESIS IN SMOOTH MUSCLE CELLS, Behrooz G. Sharifi, Lee W. Bailey, David W LaFleur, James S. Forrester, and James A. Fagin, Division of Cardiology, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA 90048

Matrix production by vascular smooth muscle cells (VSMC) appears to play a major role in intimal thickening after injury. Proteoglycans (PG) are the predominate extracellular matrix component of early restenotic lesions. As angiotensin II (A II) appears to promote restenosis, we hypothesized that A II may affect PG synthesis by VSMC. Angiotensin II (1-100 nM) evoked a dose-dependent increase in both cell and media associated PG production. SMC constitutively synthesized small amount of PG with molecular mass 173-248 kDa. After treatment with A II, both the intensity and the molecular mass of the newly synthesized PG were increased to 213-297 kDa. Selective degradation by chondroitinases and heparinase identified chondroitin and dermatan sulfate PG as the predominate PG being induced. Exposure to either actinomycin-D or cycloheximide eliminated the response of SMC to A II. To study the effect of A II on SMC-matrix interaction, we study regulation of syndecan expression. Syndecan (a non-integrin extracellular matrix receptor) mRNA was not detectable under basal condition, however, 2 h after addition of A II syndecan mRNA was detectable, reached maximal levels (5 fold) after 4 h, and decreased to control levels 18 h after addition of 100 nM A II. Similar results were obtained when SMC were exposed to serum. Both actinomycin D and cycloheximide blocked A II effect suggesting that new RNA and protein synthesis are required for the induction of syndecan. These data suggest that the pathophysiological activity of A II in the vessel wall, at least in part, may be mediated by altering the composition of the extracellular matrix through stimulation of PG synthesis.

RZ 228 SENSE AND ANTI-SENSE cDNA TRANSFECTION OF CD36 (GLYCOPROTEIN IV) IN MELANOMA CELLS: ROLE OF CD36 AS A THROMBOSPONDIN RECEPTOR. R.L. Silverstein, M. Baird, and L. Yesner. Department of Medicine (Hematology/Oncology), Cornell University Medical College, New York, NY.

Thrombospondin (TSP) is a multifunctional adhesive glycoprotein found in platelet α-granules and extracellular matrix. TSP interacts with cell surfaces and may play a role in mediating cell adhesion, platelet aggregation, platelet-monocyte interactions, cell proliferation, angiogenesis, tumor metastasis, and protease generation. The molecular identity of the cellular receptor(s) for TSP is controversial. To clarify and confirm the function of CD36 (glycoprotein IV) as a TSP receptor, we have developed a stable transfected cell model using human melanoma cells genetically manipulated by sense or antisense cDNA transfection to express either high or near zero levels of CD36. Surface expression was confirmed by immunoprecipitation and by flow cytometry with monoclonal anti-CD36 IgG, and quantified by measuring radiolabeled antibody binding. Bowes melanoma cells, which in their wild type did not express CD36 and did not bind radiolabeled TSP, when transfected with the sense construct bound TSP in a specific, concentration-dependent manner. Binding was calcium-dependent and saturated at $\approx$ 200nM. At saturation, a 1:1 stoichiometric ratio of TSP binding and CD36 expression was observed. Conversely, wild type C32 melanoma cells expressed high levels of CD36 and bound radiolabeled TSP in a calcium-dependent manner. At saturation, the stoichiometry of TSP binding and CD36 expression was 1:1. These cells when transfected with an anti-sense CD36 cDNA did not express CD36 and did not bind TSP. In addition, we have developed an adhesion assay that quantifies the interaction of tumor cells to activated platelets. We found that transfected Bowes cells and wild type C32 cells, unlike wild type Bowes cells adhered to activated platelets in a TSP-dependent manner. These data; i.e. the gain of function with sense cDNA transfection and loss of function with antisense transfection, strongly support the TSP receptor function of CD36. The distribution of CD36 in vascular cells and tissues, and observations that it may participate in signal transduction events, suggests that TSP-CD36 interactions may play a role in mediating some of the pathophysiological processes associated with TSP.

RZ 229 STROMAL CELLS FORMED IN RESPONSE TO SUBCUTANEOUS IMPLANTATION OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN 2 (rhBMP-2) SUSTAIN BOTH HEMOPOIETIC STEM CELLS AND COMMITTED PRECURSORS, A.K. Sullivan, J. An, and L-G St. Marie, McGill University Hematology Program - Royal Victoria Hospital Unit, and St. Luc Hospital, Montreal, Canada, H3G 1Y6.

In 1974, Friedenstein demonstrated that blood cells would develop at extra-medullary sites (e.g. renal subcapsule) where marrow stromal cells had been implanted, and in 1975, Reddi and Huggins (1975) showed that subcutaneous bony nodules, formed after injection of bone matrix proteins, contained marrow-like tissue. The experiments reported here tested whether true hemopoiesis occurred within the bony nodules that had been formed in response to a single factor, recombinant human bone morphogenetic protein 2 (rhBMP-2). After implantation of a gelatin capsule containing rhBMP-2 into the subcutaneous space of a 1-2 month old rat, a reproducible sequence occurs. At 3 wk, the nodules contained mostly lymphoid and mast cells. By 4-5 wk, a typical marrow architecture was evident with remodeling bony spicules, and developing granulocytes, erythrocytes, and megakaryocytes. By 2 wk, CFU-M and CFU-GM were detected at 10-15% the frequency found in an equal number of control bone marrow cells, and by 5-6 wk, they had reached 100%. Similarly, CFU-E were detected at 3 wk (20-30% of marrow) and by 4-5 wk had reached >95%. Cells recovered from nodules 5 wk after implantation were infused into lethally-irradiated rats, some of which have survived longer than 8 weeks. In conclusion, the stroma induced in vivo by rhBMP-2 supports the growth of transplantable hemopoietic stem cells, myeloid and erythroid precursors, and three lineages of mature blood elements.

RZ 230 HA PRODUCTION IS INHIBITED BY L-LACTIC ACID. Gregory W. Tapper, Thomas K. Hunt, Heinz Scheuenstuhl, Daniel Schwartz, and Robert Stern, Departments of Surgery, Opthalmology, and Pathology, Universtiy of California, San Francisco, CA.

In the course of wound healing hyaluronan (HA) increases, reaches a maximum at 3d, and then falls rapidly (*Annals Surg.* 213:292, 1991). Lactate levels increase and PO_2 levels fall during this period. We wished to simulate these conditions in cultured fibroblasts to determine whether a cause and effect relationship exists between lactate, oxygen, and HA levels. Confluent human fibroblasts were cultured in gas-permeable chambers at 37°C, in 7.5% fetal bovine serum (FBS). Hyaluronan production increases as a function of time in human dermal fibroblasts, in primary culture. The level of HA was 2.5ug/cc, after 120h. The levels of HA were strongly inhibited by lactate. In 5mM lactate, HA levels decreased by 80%. After 120h, HA levels were 0.6ug/cc. The effects of oxygen were also determined. HA levels were assayed in cells exposed to 2%, 5%, and 10% O_2. Differences in oxygen concentration had no effect on HA levels. In summary, our evidence suggests that the lactate of wound fluid, which reaches a maximum at 3 days (*Surgery*, 107:187, 1990) underlies in part the fall in HA levels occuring at that time. Supported by the Chartrand Foundation and by DHHS-NIH grant CA-44768.

RZ 231 **DECORIN AND BIGLYCAN mRNA AND PROTEINS IN DISEASED MEDIA AND PROLIFERATIVE INTIMA OF CORONARY ARTERIES FROM HUMAN HEART ALLOGRAFTS,** Sheldon L. Thieszen, Janet E. Wilson, Maria Rosa Costanzo-Nordin, Bruce M. McManus, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-6495

Transplant arteriopathy (TA) is a rapidly proliferative and occlusive idiopathic disease of the coronary arteries in human heart allografts and a major cause of late cardiac graft failure, cardiac retransplantation and recipient death. Two striking histological features are common to the coronary lesions of TA: large intra- and extracellular deposits of cholesterol and other lipids, and an apparent accretion of proteoglycans (PG) as suggested by alcian blue-containing connective tissue stains. Histologically, the aberrant accumulation of lipids and PG was geographically coupled and specific lipid content ($\mu g/cm^2$) and concentration ($\mu g/mg$) were highly correlated with planimetrically digitized luminal narrowing in allograft coronary arteries. To further examine the role of PG in TA, we conducted immunohistochemical and in situ hybridization studies using polyclonal antibodies (LF-15 and LF-30) and cDNA probes (P16 and P2) for biglycan and decorin, respectively. Antibodies and cDNA sequences were obtained from Larry Fisher, PhD, NIH. Examination of coronary artery cross-sections from cardiac allografts by immunoperoxidase labeling revealed distinctive staining for both biglycan and decorin proteins in the proliferative intima, subjacent to lipid aggregates and within the morphologically irregular tunica media. The pattern of staining intensity was concordant to alcian blue histochemistry. *In situ* hybridization with ^{35}S- and digoxigenin-labeled probes revealed similar geographical localization of biglycan and decorin mRNA expression. Morphological smooth muscle cells of the neointima and media were multifocally positive for gene expression. *In vitro* evaluation of allogeneic modulation of smooth muscle cell PG expression and lipid uptake is in progress.

RZ 232 BONE MORPHOGENETIC PROTEINS INCREASE TYPE X COLLAGEN SYNTHESIS IN VIVO. R. Topping, R. Stone*, G. Lovell*, L. Prater*, D. Toerner*, P. Green* and Gary Balian, Univ. of Virginia School of Medicine, Charlottesville, VA 22908 and *Procter and Gamble Company, Miami Valley Labs, Cincinnati, OH 45239.

Bone morphogenetic proteins can augment bone healing in prosthetic joint surgery and in fracture nonunions. The synthesis of type X collagen by hypertrophic chondrocytes was evaluated to establish the effects of bone morphogenetic proteins (BMP) on bone induction. BMP was used in conjunction with demineralized bone matrix (DBM) powder, implanted subcutaneously in rats and removed after 6, 9, 12, 15 or 18 days. Type X collagen was immunolocalized in frozen sections using a polyclonal antibody to a synthetic peptide. For biochemical analysis the freshly removed implants were incubated in organ culture with media containing ^{14}C-proline, the proteins were extracted, treated with pepsin and analyzed by SDS-PAGE and fluorography. The initial fibrous material that developed within the implants changed to a cartilaginous matrix in which the chondrocytes began to hypertrophy, the matrix calcified and bone was formed. Immunostaining of type X was detected at day 9 within the DBM implants. By contrast, appearance of type X was delayed until day 12 in implants that were created using DBM which had been extracted with guanidine hydrochloride. Augmentation of DBM and of extracted DBM with BMP increased the intensity of staining for type X collagen. Similarly, greater amounts of type X collagen were detected in the metabolically labelled implants that had been augmented with BMP compared with the implants with DBM alone. All the implants exhibited an increase in type X collagen synthesis at day 6 with a second peak at day 18. BMP - augmented DBM stimulates increased synthesis of type X collagen. An analysis of molecular components within this bone induction system could help to elucidate the effects of various recombinant bone morphogenetic proteins on different stages of bone development.

RZ 233 THE IMPLICATIONS OF INTRACELLULAR TRAFFICKING AND PROCESSING EVENTS FOR EXTRACELLULAR MATRIX ASSEMBLY AND FUNCTION IN CARTILAGE. Barbara M. Vertel, Linda M. Walters, Bonnie Grier, David K. Mills, Nancy B. Schwartz*, Ann E. Kearns*, and Paul F. Goetinck**. Department of Cell Biology and Anatomy, UHS/The Chicago Medical School, No. Chicago, IL 60064; *Departments of Pediatrics and Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637; **Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129.

Cartilage tissue function reflects the structure of an extracellular matrix (ECM) composed primarily of large, link stabilized aggregates of hyaluronic acid and chondroitin sulfate proteoglycan (aggrecan) restrained within a network of type II collagen fibers. Sequential processing events which occur during the movement of these complex macromolecules through specific subcellular compartments of the constituitive secretory pathway are critically important for normal maturation and function of the ECM. Utilizing a combination of biochemical and morphological approaches and several inhibitors, we investigated the processing and intracellular trafficking of aggrecan in cultured chondrocytes. During the course of biosynthesis, aggrecan precursors are noted in a smooth membrane-limited, late endoplasmic reticulum (ER) subcompartment. Experiments to be presented suggest that separate phases of glycosaminoglycan chain elongation and sulfation may occur in the cis/medial/trans and trans/trans Golgi network compartments, while xylosylation is a function of an earlier compartment. Skeletal abnormalities may arise if these processing and translocation events fail to occur. In chickens, the lethal genetic mutation *nanomelia* is characterized by shortened and malformed limbs and greatly reduced amounts of aggrecan. Cultured nanomelic chondrocytes assemble an ECM that contains type II collagen, but lacks aggrecan. Notably, they synthesize a truncated aggrecan core protein precursor that disappears with time and is neither processed by the Golgi nor secreted. The defective precursor accumulates within smooth membrane-limited extensions of the ER. At no time are precursor molecules observed in the Golgi. Experimental manipulations employing temperature shifts or inhibitor treatments were unsuccessful in routing the defective aggrecan precursor out of the ER to the Golgi or further along the secretory pathway. Our results lead to the conclusion that nanomelic chondrocytes synthesize a defective aggrecan precursor that is partially modified in the ER, and subsequently degraded by a non-lysosomal mechanism, perhaps within the ER. The formation of a normal ECM requires compartmentalization and sequential synthetic, processing, and translocation events. Errors in these processes can lead to the construction of aberrant ECMs and diseases of connective tissues such as chondrodystrophies. Supported by NIH grants DK28433 (BMV), HD22016 (PFG), HD 17332 (NBS), HD 09402 (NBS), AR 19266 (NBS).

RZ 234 Characterization of the Binding of a Cyclic Arg-Gly-Asp (RGD) Peptide, [^{3}H]-SK&F-107260, to Purified Human Vitronectin Receptor ($\alpha_v\beta_3$), A. Wong, S. M. Hwang, J. M. Stadel, D. A. Powers, P. McDevitt, R. Matico and K. Johanson. SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406.

$\alpha_v\beta_3$ is a member of the integrin superfamily that mediates cellular attachment to RGD-containing adhesive proteins. A solid phase microtiter assay was developed to investigate the binding properties of $\alpha_v\beta_3$, using tritiated cyclo(S,S)-N$^\alpha$-2-mercaptobenzoyl-N$^\alpha$-methylarginyl-glycyl-aspartyl-mercaptophenyl-amide ([^{3}H]-SK&F-107260) as ligand. $\alpha_v\beta_3$, purified from human platelets or from human placenta, bound [^{3}H]-SK&F-107260 in a saturable, reversible and divalent cation-dependent manner. A single class of high affinity binding sites (K_d = 1.85 nM, B_{max} = 0.77 µmol [^{3}H]-SK&F-107260/gm $\alpha_v\beta_3$) was detected. [^{3}H]-SK&F-107260 binding to $\alpha_v\beta_3$ was inhibited by soluble vitronectin, fibrinogen, fibronectin, echistatin and small RGD-containing peptides. A monoclonal antibody LM609 that recognized a ligand binding site on $\alpha_v\beta_3$ also inhibited [^{3}H]-SK&F-107260 binding. The Arg-Gly-Glu-containing peptides (RGE) and a potent peptidomimetic fibrinogen receptor ($\alpha_{IIb}\beta_3$) antagonist N-[m-(p-amidinobenzamido)]-benzoyl-β-Ala-HCl had no effect. The relative potencies of the adhesive proteins and small RGD peptides in inhibiting [^{3}H]-SK&F-107260 binding to $\alpha_v\beta_3$ were the same as their relative potencies in inhibiting biotinylated-fibrinogen and biotinylated-vitronectin binding. $\alpha_v\beta_3$ exhibited similar binding characteristics when immobilized on a plastic surface or incorporated into mixed phosphatidylserine-phosphatidylcholine liposomes, suggesting that immobilization of the receptor did not alter the ligand binding characteristics. These data suggest that the solid phase [^{3}H]-SK&F-107260 binding assay is useful for the biochemical and pharmacological characterization of $\alpha_v\beta_3$.

RZ 235 A HYALURONAN RECEPTOR TERMED RHAMM CONTAINS HYALURONAN AND HEPARIN BINDING MOTIFS. Baihua Yang, Bing Luo Yang, Christine Hall, Rashmin Savani and Eva Ann Turley. Manitoba Institute of Cell Biology, and Dept. of Pediatrics, The University of Manitoba, 100 Olivia Street, Winnipeg, MB, Canada, R3E 0V9

Glycosaminoglycans are negatively charged polysaccharides that have been implicated in growth control, cyto-differentiation and cell locomotion. Heparin (HE) and hyaluronan (HA) have both been shown to affect these activities by binding to specific receptors. One receptor that has been shown to mediate the locomotion-promoting effects of HA is RHAMM (Receptor for Hyaluronic Acid Mediated Motility). We have previously identified two HA binding motifs in RHAMM (Yang et al. 1992. Mol. Biol. Cell 3:73a). We show here that these two motifs also bind HE specifically but do not interact with chondroitin sulfate and dermatin sulfate. Biotin-labelled HE, like HA, binds to RHAMM-GST fusion protein and to native RHAMM in cell lysates as determined by Western blot assays. HE and HA compete with each other for binding to RHAMM. In a truncation assay, biotin-labelled HE binds to the same 35 amino acid region as HA. Further, synthetic peptides mimicking the two HA binding motifs within this 35 amino acid region also bind to HE-Sepharose. Other peptides encoded in different regions of RHAMM and random sequences of these peptides do not bind to HE. Oligonucleotides encoding either of the two HE/HA binding peptides are linked to a cDNA encoding the amino terminus of RHAMM that does not bind to either glycosaminoglycan. Fusion proteins containing either of the genetically engineered peptides now exhibit HE binding activity in a Western blot assay. These results indicate that the 2 domains within RHAMM that have previously been identified as HA binding domains also bind to HE. HA has most frequently been linked to stimulation of cell locomotion while HE appears to often inhibit locomotion. The presence of common binding sites in RHAMM for these glycosaminoglycans predict a novel, competitive mechanism for the regulation of cell locomotion.

Fibronectin & Laminin

RZ 300 ALTERED EXPRESSION OF CELL SURFACE GALACTOSYLTRANSFERASE INCREASES PC12 CELL NEURITE OUTGROWTH ON LAMININ, Paul C. Begovac and Qiuling Huang, Department of Zoology, University of Oklahoma, Norman, OK 73019

Laminin is a potent promoter of neurite outgrowth and recent studies have shown that cell surface galactosyltransferase (GalTase) functions as a laminin receptor during neurite outgrowth by binding to the E8 domain of laminin (J. Cell Biol. 110:461, 1990; J. Cell Biol. 113:637, 1991.) To further understand the receptor function of surface GalTase during neurite outgrowth, PC12 cells were transfected with GalTase cDNAs to increase cell surface GalTase expression levels so the consequences during neurite outgrowth on laminin could be assessed. Transfected colonies were isolated by G418 antibiotic-resistance and these colonies were screened by Northern blot analysis and cell morphology. Northern blot analysis identified one colony expressing mRNA for the inserted GalTase gene and this colony was used for further analysis. Cell surface GalTase enzyme assays were used to assess protein expression and these results demonstrated approximately a four- to five-fold elevation in cell surface GalTase activity compared to control cells. More importantly, transfected cells having elevated levels of cell surface GalTase showed an enhanced ability for neurite outgrowth. When plated on laminin substrates, neurite formation by transfected cells was several-fold higher than the neurite outgrowth observed for control cells after 4 hours of culture. These results suggest that the levels of cell surface GalTase are related to the ability of PC12 cells to carry out neurite outgrowth and that the regulation of cell surface GalTase expression may be important for neurite outgrowth. Studies are underway to further characterize these results in light of integrin function during neurite outgrowth on laminin as well.

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RZ 301 THE EFFECT OF OVEREXPRESSION OF A RHAMM-B-ACTIN-NEOMYCIN HYBRID GENE ON PROPERTIES RELATING TO CELL LOCOMOTION. E.A. Turley, B.Yang, B.S. Kornovski, and G.D. Curpen. Manitoba Institute of Cell Biology and Depts. of Pediatrics and Physiology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0V9.

Identifying the molecular mechanisms underlying cell migration is critical to understanding physiological processes occurring in development wound repair, and such disease states as tumorigenesis. A novel hyaluronan (HA) receptor that has been recently cloned (Hardwick et al., 1992, J. Cell Biol. **117**:1343) and is referred to by the acronym RHAMM for Receptor for HA Mediated Motility, has been shown to be important in the motile behavior of *ras*-transformed cells (Turley *et al.*, 1991, J. Cell Biol. **112**:1041). Based on evidence for RHAMM's involvement in cell locomotion, it was predicted that the overexpression of this protein will affect some aspect of cell motile behavior. Therefore, a lipofection technique was used to transfect mammalian 10T1\2 cells, which show strong contact inhibited behavior and are poorly invasive, with the complete RHAMM cDNA in an expression vector containing the B-actin promoter and a neomycin construct. Stable transfectants were selected by drug resistance, and three clones overexpressing the RHAMM protein were identified by Western analysis and subjected to further analysis. Cells with elevated levels of the RHAMM protein were more invasive in collagen gels than controls. Furthermore, these cells resembled virally transformed cells in their loss of contact inhibition, as quantitated with a nuclear overlap ratio, and their ability to form foci in monolayer cultures. As well, contrary to control vector transfected cells and the 10T1\2 parent line, the transfected cells did not form focal contacts. These results indicate that RHAMM is involved in disrupting normal cell substratum contacts and conferring a transformed phenotype.

RZ 302 3T3 L1 ADIPOCYTES STIMULATE ANCHORAGE-INDEPENDENT GROWTH OF A MURINE MAMMARY CARCINOMA: POSSIBLE ROLE OF FIBRONECTIN, B.E. Elliott[1], B. Bhardwaj[1], R. Lall[1], D. Leopold[1], L. Maxwell[1], R. Saulnier[1], E. Tremblay[1] and K. Rubin[2]. [1]Cancer Res. Lab. Queen's U., Kingston, ON, CANADA, K7L 3N6; and [2]Dept. Med. Physiol. Chem., BMC, U. Uppsala, S-751 23, SWEDEN.

We have previously demonstrated the capacity of adipose tissue to support *in vivo* growth and metastasis of a murine mammary carcinoma SP1 (Elliott, *et al.* I.J. Cancer 51:416-424, 1992). Adipocytes are a dominant cell type in mammary stroma; we therefore examined the capacity of an adipocyte cell line (3T3 L1) to stimulate growth of SP1 cells *in vitro*. Proliferation of SP1 cells occurred in the presence of adipocytes (induced 3T3 L1 cells), but not preadipocytes (uninduced 3T3 L1 cells) or fibroblasts. Growth of SP1 cells on plastic was stimulated by substrata from adipocytes or preadipocytes and conditioned media from 3T3 L1 adipocytes. The stimulatory effect was retained by dialysis membranes of pore size less than 12-14 kD, and was abolished by treatment at low pH (2.8, 3 min), or by heat (99°C, 3 min). To determine whether the above adipocyte interactions contribute to the tumorigenic phenotype, we examined anchorage-independent growth of SP1 cells separated from feeder cells by 0.6 % striated agar. If no feeder cells were present, no growth of SP1 cells occurred at FCS concentrations between 0.01% and 1%; under these conditions anchorage-dependent growth of SP1 cells on plastic was observed. Monolayers of 3T3 L1 adipocytes stimulated anchorage-independent growth of SP1 cells more effectively than uninduced 3T3 L1 preadipocytes or fibroblasts. We have previously shown that fibronectin, but not collagen type I, can promote a growth factor-dependent proliferative response of SP1 cells *in vitro* (Elliott *et al.* J. Cell Physiol. 152:292-301, 1992). Interestingly, increased presence of extracellular fibronectin, detected by immunofluorescence and confocal microscopy, was demonstrated in 3-dimensional SP1 colonies in agar compared to 2-dimensional SP1 cultures on cover slips. In SP1 colonies, fibronectin appeared to be deposited extracellularly in the form of microfibrils; in SP1 cells on coverslips fibronectin was expressed intracellularly with no detectable fibril formation. These findings suggest that augmentation of the fibronectin adhesion system, in particular fibronectin deposition, may be one important regulatory event in adipocyte-mediated anchorage-independent growth of SP1 cells. (Supported by grants from NCI(C) and MRC).

RZ 303 NIDOGEN, THE LINKAGE PROTEIN OF THE BASAL LAMINA, IS OF MESENCHYMAL ORIGIN, Raul Fleischmajer, E. Douglas MacDonald II, Te-Cheng Pan, Rupert Timpl, Mon-Li Chu, Department of Dermatology, Mt Sinai School of Medicine, New York, NY 10029, Department of Biochemistry and Molecular Biology and Dermatology, Thomas Jefferson University, Philadelphia, PA 19107, Max-Planck Institut fur Biochemie, D-8033, Martinsried, Germany

It has recently been shown that nidogen binds to laminin, type IV collagen and heparan sulfate and appears to be the linkage protein for the formation of the basal lamina. It is not known whether nidogen is of epithelial or mesenchymal origin or both. To elucidate this question, two culture models were used in this study (a) preputial fibroblasts grown for 4 weeks in a nylon mesh (Dermal Model or DM) and (b) the above model recombined with preputial keratinocytes for additional 4 weeks growth (Dermal Keratinocyte Model or DKM)(Advanced Tissue Sciences, La Jolla, CA). Electron microscopy of the DM revealed active fibroblasts embedded in a rich extracellular matrix containing collagen fibrils and elastin-associated microfibrils. No evidence of basal lamina structures could be detected in this specimens. Immunochemistry of the DM revealed large amounts of nidogen in a random distribution. Electron microscopy of the DKM revealed a well differentiated epidermis, a basal lamina, an anchoring zone with anchoring fibrils and large amounts of elastin-associated microfibrils and collagen fibrils. Immunochemistry of the DKM revealed small amounts of nidogen, usually restricted to fibroblasts and the basal lamina. No distinct staining of the epidermis was noted. Following extraction procedures, the DM and the DKM were subjected to Northern hybridization with a human nidogen probe. Most of the mRNA nidogen was found in the DM with very small amounts present in the DKM. This study suggests that in the DM nidogen is mostly synthesized by fibroblasts but in a random non-purpose distribution. On the other hand, the epidermis appears to exert a negative feed-back mechanism in nidogen production by fibroblasts and may also regulate its polymerization with other components of the basal lamina.

RZ 304 CHARACTERIZATION OF LYMPHOCYTE FIBRONECTIN; EVIDENCE FOR ALTERNATIVE SPLICING WITHIN THE III CS REGION.

Dan Hauzenberger°, Nancy Martin*, Staffan Johansson*, Eva Bergdahl° and Karl-Gösta Sundqvist°.
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In vitro cultured "activated" peripheral blood lymphocytes and certain T cell lines synthesized a high molecular weight gelatin binding molecule (MW 660 kD) whereas resting lymphocytes showed poor or negligible synthesis of the same component. Concanavalin A-mediated anchorage of the lymphocytes to a substratum potentiated synthesis of the high molecular weight molecule.The magnitude of this synthesis was proportional to the number of cells showing attachment to substratum and cytoplasmic spreading, suggesting that attachment and spreading may constitute an activating signal or enhance synthesis of the high molecular weight component. Noteworthy, the high molecular weight gelatin-binding molecule preferentially was cell associated with no or relatively little presence in the culture medium. In contrast, control cultures of human umbilical vein endothelial cells released fibronectin which was detectable in the medium. Western blotting of the gelatin-binding lymphocyte molecule demonstrated reactivity with anti-fibronectin antibodies. Furthermore, the use of antibodies specific for human fibronectin excluded that the fibronectin detected by Western blotting originated from the bovine serum in which the cells were cultured. Peptide mapping demonstrated that the purified lymphocyte derived fibronectin and plasma fibronectin had very similar albeit not identical digestion patterns. RT-PCR of total RNA from CD 4+ T cells showed that the most abundant species of fibronectin mRNA lacked the entire III CS exon encoding the $\alpha_4\beta_1$ binding region LDV. Amplification of the III CS region from the Jurkat T- cell line demonstrated that these cells expressed several fibronectin pre mRNA isoforms most of which were lacking the LDV coding sequence. This lymphocytic overrepresentation of isoforms with the spliced out III CS was in contrast to the splicing pattern of the adherent epithelial cell line Hep II which mainly showed exon subdivision at the III CS.

RZ 305 CALCIUM-INDUCED STRATIFICATION OF HUMAN KERATINOCYTES: EVIDENCE OF INTEGRIN-CADHERIN INTERACTIONS, Kairbaan J. Hodivala and Fiona M. Watt. Imperial Cancer Research Fund, London, U.K.

When grown in medium containing a standard concentration of calcium ions (1.8mM) human keratinocytes form stratified sheets in which proliferation is confined to the basal layer and terminal differentiation occurs in the suprabasal layers. When the calcium concentration is reduced to 0.1mM, keratinocytes still proliferate and undergo terminal differentiation, but are prevented from stratifying because they cannot assemble functional desmosomes and other calcium-dependent cell-cell adhesion complexes. When the level of calcium ions is raised to 1.8mM, stratification occurs within 24 hours with selective migration of the terminally differentiating keratinocytes out of the basal layer.

In standard calcium medium integrin expression is confined to the basal layer and suprabasal, involucrin-positive keratinocytes lack integrins. In contrast, involucrin-positive cells in low calcium medium contain integrin mRNA and synthesise functional β1 integrins that have the same half life as in standard medium. Within 24 hours of raising the levels of calcium ions to 1.8mM, involucrin-positive cells no longer contain integrin mRNA and lack surface integrins. The loss of integrins is not a direct response to increased extracellular calcium, and can be prevented by anti- P and E-cadherin antibodies. We propose that cadherins play some role in the loss of integrins associated with keratinocyte terminal-differentiation.

RZ 306 MODULATION OF THE ADHESIVE PROPERTIES OF BREAST TUMOR CELL LINES BY FIBRONECTIN AND THROMBOSPONDIN. Francesca Incardona, Fabien Calvo, Françoise Lafève, Yves Legrand and Chantal Legrand. U 353 INSERM and IGM, Hôpital St Louis, Paris, France.

The attachment of cancer cells to adhesive molecules in the extracellular matrix plays a critical role in tumor invasion and metastasis. In this study, we have compared the capacity of several breast adenocarcinoma cell lines (MDA-MB-231, ZR75-1, T47D, MCF7) to synthesize and attach to fibronectin (FN) and thrombospondin (TSP). The levels of FN and TSP in the conditioned medium and in the cells were determined by ELISA. MDA-MB-231 cells were shown to produce higher level of TSP than the other cell lines and were therefore used in subsequent studies. As compared to FN that was essentially recovered in the medium, the bulk of the TSP synthesized was retained with the cells. Flow cytometric analysis indicated the presence of both occupied and unoccupied sites for TSP on the cell surface. ^{125}I-TSP bound saturably to 1.2 x 10^6 sites/cell with a kd of 20 nM. The binding was inhibited by an excess of TSP and by heparin, suggesting that the receptor could be a heparan sulfate proteoglycan or a sulfatide. The cells attached to a TSP and spread on a FN coated substratum. These results indicate that TSP may act as a substratum for breast tumor cell attachment to extracellular matrix.

RZ 307 TUMOR SUPPRESSOR ACTIVITY *IN VIVO*, NOT *IN VITRO*, CAUSED BY A NOVEL *PER* REPEAT SEQUENCE: THE RELATION BETWEEN FIBRONECTIN ELEVATION AND TUMOR SUPPRESSION.

Norio Ishida[1,2], Shin-ichiro Nishimatsu[1], Masaaki Kondo[1], Kimiko Amanuma[1], Erkki Ruoslahti[2] and Youji Mitsui[1]

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Per repeat sequence is rodent DNA fragments which are homologous to the repetitive sequence of the Drosophila clock gene "*period*". A mouse spleen cDNA containing a *per* repeat sequence was cloned and sequenced. The open reading frame including *per* repeat sequence encodes a 133 amino-acids protein named sp41 which has no similarities with known proteins in EMBL database. To elucidate the function of sp41 protein, the sp41 was transfected into a transformed human endothelial cell line (tHUE-2). Stable sp41 transformants morphologically resembled retracted endothelial cells (The transtion state to form a cappillary structure) and displayed reduced migratory activity *in vitro*. Then the level of fibronectin expression was increased in all sp41 transfected-tHUE-2 cells. Thus, the morphological changes and inhibition of cell migration of sp41 transformants could be explained by the elevated expression of fibronectin in these cells. The *in vitro* growth rate of sp41 transformants and their ability to grow in soft agar was not significantly altered, but their tumorigenecity was greatly reduced in nude mice. The results suggest the sp41 cDNA encodes a novel tumor suppressor molecule associated with the fibronectin elevation that reduces tumorigenicity *in vivo* but does not substantially affect *in vitro* growth properties of cells.

RZ 308 SEPARATE MATRICES FORMED BY BASEMENT MEMBRANE AND INTERSTITIAL MATRIX COMPONENTS IN HETEROKARYONS OF EPITHELIAL PYS-2 CELLS AND FIBROBLASTS, Pekka Laurila and Ilmo Leivo, Department of Pathology, University of Helsinki, SF-00290 Helsinki, Finland.
To obtain further understanding of the spatial organization of interstitial and basement membrane matrices, we have here studied the expression of the interstitial matrix protein, fibronectin and the basement membrane protein, laminin in heterokaryons formed by normal fibroblasts and teratocarcinoma-derived, epithelial PYS-2 cells. Cell fusion was induced either by Sendai virus or polyethylene glycol. The resulting multinucleated cells were identified by cytoplasmic labeling with polystyrene particles of two different sizes. The expression of fibronectin and laminin was studied by indirect immunofluorescence microscopy. The heterokaryons showed varying distributions of the matrix proteins depending on the proportions of the different parental cell nuclei within the cytoplasm of the cell. Heterokaryons, containing either equal numbers of fibroblast and PYS-2 cell nuclei or an excess of the epithelial cell nuclei, showed an abundant laminin matrix subcellularly and only minor amounts of fibronectin matrix at the periphery of the cells. On the other hand, in heterokaryons containing an excess of fibroblast nuclei, the laminin matrix was reduced and a fibrillar fibronectin matrix was seen also on top of the cell body. Furthermore, extracellular laminin and fibronectin matrices did not codistribute around the heterokaryons but the two proteins were assembled into separate structures. The results suggest a gene-dosage type of effect on the expression of these matrix proteins in our heterokaryons. Furthermore, the lack of codistribution of fibronectin and laminin matrices in the heterokaryons suggests that the molecular interactions, which determine the assembly of basement membrane and interstitial matrices in these cells are highly type-specific. Similar mechanisms may also operate in the assembly of extracellular matrices in vivo.

RZ 309 TISSUE-SPECIFICITY OF THE DISTRIBUTION AND MOLECULAR STRUCTURE OF MEROSIN, A LAMININ-RELATED BASEMENT MEMBRANE PROTEIN, Ilmo Leivo, Ulla Karvonen, Pekka Laurila and Eva Engvall, Department of Pathology, University of Helsinki, SF-00290 Helsinki, Finland and La Jolla Cancer Research Foundation, La Jolla, CA 92038.

Merosin has previously been found in some basement membranes of the placenta, striated muscle and peripheral nerves. We now show the presence of the heavy M-chain of merosin in the walls of many brain capillaries and in the capillaries of some endocrine organs such as the adrenal cortex, the adenohypophysis and the parathyroid gland. The M-chain is also seen in the epithelial basement membranes of serous glands in the exocrine pancreas as well as in gastric glands. Yet it is absent from the ductal basement membranes of these glands. The M-chain is also seen in some glandular basement membranes of mucinous glands, e.g. the duodenal Brunner glands, while no M-chain is detected in the basement membranes of the non-lactating mammary gland. In endocrine glands, such as the adenohypophysis, the adrenal cortex and the parathyroid glands, merosin outlines groups of glandular cells. The M-chain is also found in the walls of the seminiferous tubules of the testis, in the pia mater and in glomerular mesangial cells. In immunoblotting experiments, antibodies to a merosin M-chain peptide detect a 300 kDa polypeptide in human placenta, sciatic nerve and heart extracts while a predominant 100 kDa polypeptide is seen in human and mouse skeletal muscles. In the term mouse placenta which has a structure different from the human counterpart, no M-chain was detected. The results demonstrate that, in addition to the basement membranes of the trophoblast, striated muscle and Schwann cells, merosin is also present in some specialized capillaries, in many secretory glandular organs and in the kidney mesangium. The immunoblotting results suggest that there is tissue-specific heterogeneity in the structure of the M-chain.

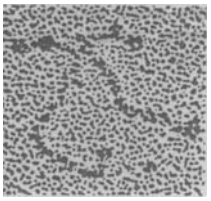
RZ 310 **NATIVE LAMININ ISOFORMS FROM BOVINE KIDNEY AND HEART AND FROM HUMAN PLACENTA-ISOLATION AND CHARACTERIZATION**
Anders Lindblom & Mats Paulsson, M.E.Müller Institute for Biomechanics, University of Bern, CH-3010 Bern, Switzerland

Laminin from murine tumor tissue (EHS) has been shown to possess a range of biological properties, such as binding of cells via integrin receptors, network formation through self-interaction, binding of nidogen, collagen type IV and heparin. In recent years, new laminin subunits have been discovered that are genetically different from those of mouse-laminin. Laminin from the murine EHS-tumor consists of a 400 kD subunit called Ae, and two distinct 200 kD subunits called B1e and B2e, respectively. Merosin, a new kind of A-chain (Am) with Mr approx. 300 kD, was found in extracts of placenta and heart tissue, and S-laminin was identified as a laminin subunit replacing the B1-chain (B1s) in neuromuscular junctions. Still other laminin forms, both A- and B-chain equivalents, have been isolated from skin and keratinocyte cultures.
It thus appears as if laminin is to be regarded as a family of related proteins, and hence different laminins might provide some of the functional and morphological heterogeneity of basement membranes. In order to compare the functional properties of these various laminin isoforms, a supply of the native proteins is needed. We have elaborated a procedure for efficient extraction and purification of laminin from bovine kidney and heart, as well as from human placenta. From the kidney extract we purify a laminin resembling the EHS-tumor laminin, which after reduction resolves into a 400 kDa A-chain and two components around 200 kD. This laminin also seems to contain a stoichiometric amount of nidogen. The extracts from heart and placenta tissue yield laminin containing the merosin chain, Am. Heart laminin apparently contains significantly more nidogen than placenta laminin. Both the latter preparations contain an Mr 80 kDa-component, probably a proteolytically severed part of the Am-chain and which apparently remains non-covalently associated with the laminin complex.These preparations are now being characterized for structural features and biological activity.

RZ 311 KALININ AND K-LAMININ ASSEMBLE INTO A COVALENTLY ASSOCIATED COMPLEX IN TISSUE.
Gregory P. Lunstrum, M. Peter Marinkovich, Patricia Rousselle, Douglas R. Keene and Robert E. Burgeson, Shriners Hospital and Oregon Health Sciences University, Portland, OR, and Cutaneous Biology Research Center, MGH/Harvard Medical School, Boston, MA

Previously, we have identified two novel basement membrane components in human keratinocyte and squamous cell carcinoma cultures. Kalinin is both a component of anchoring filaments in vivo and the major attachment factor for keratinocytes in vitro (JCB 114, 567). Three heterotrimeric assemblies of kalinin have been identified; the cell associated form (KC) and two media forms (KM1 and KM2) are related through the protyeolytic processing of two separate subunits (JBC 267, 17900). Additionally, a novel laminin variant, K-laminin, comprised of B1, B2, and a smaller (190kDa) subunit which substitutes for the A chain has been characterized (JCB 119, 695).

In order to understand the significance of the kalinin processing, an analysis of the tissue form of kalinin was initiated. Affinity purification of kalinin from collagenase extracts of amniotic membrane was performed with kalinin specific monocional antibodies. The isolated material was complex, containing both kalinin subunits and the K-laminin components described above. The kalinin subunits are identical to those found in KM2. Subsequent ion exchange FPLC on Mono-Q separated kalinin from high MW assemblies containing both kalinin and K-laminin subunits as determined by reduced SDS-PAGE. Without prior reduction this complex exhibited an electrophoretic migration slower than individual kalinin or K-laminin assemblies. Images obtained following rotary shadow analysis indicate this assembly is composed of two short and two long arm domains (shown). The distribution of long arm lengths is bimodal with peaks at 81 and 103 nm. Further, under mild reducing conditions the complex dissociates into kalinin and K-laminin. From these results we conclude that kalinin and K-laminin are covalently asssociated in tissue.



RZ 312 TYPE VI COLLAGEN BINDS TO THE CELL-BINDING DOMAIN OF FIBRONECTIN, Cahir A. McDevitt and Jose Marcelino, Section of Musculoskeletal Biology, Department of Biomedical Engineering, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195

Electron microscopic studies suggest that type VI collagen is an adhesion protein that binds to other extracellular matrix macromolecules. The mechanism by which type VI collagen binds to other matrix molecules is critical to understanding the role this adhesion protein plays in the tissue. In this study, we report that type VI collagen binds to the central, cell-binding region of fibronectin.

Type VI collagen was isolated from bovine menisci by extraction with 6 M GuHCl and PEG precipitation. Binding of type VI collagen to plasma fibronectin or to specific fragments of the molecule was explored in solid phase ELISA assays with an anti-type VI collagen monoclonal antibody and an alkaline phosphatase conjugated secondary antibody.

Fibronectin bound to micro wells coated with type VI collagen in a dose dependent fashion. The interaction was insensitive to NaCl (0.15 M - 2.0 M), but was abolished by 0.5 M GuHCl. Competitive inhibition experiments demonstrated that type VI collagen and fibronectin bound to each other in liquid phase, confirming the validity of the solid phase assays.

Interaction studies with fragments of fibronectin demonstrated that the central 120 K cell-binding fragment, but not the 45 K gelatin binding or the 60 K heparin-binding fragment, bound to type VI collagen.

In summary, intact type VI collagen binds to the cell binding domain of fibronectin. As type VI collagen also binds to hyaluronan, it appears to be a pivotal adhesion protein in the macromolecular organization of cartilaginous tissues.

RZ 313 INTERACTION OF FIBRONECTIN WITH HUMAN PLASMINOGEN AND TISSUE-TYPE PLASMINOGEN ACTIVATOR, Tammy L. Moser, Salvatore V. Pizzo and M. Sharon Stack, Department of Pathology, Duke University Medical Center, Durham, NC 27710. Fibronectin (FN) participates in complex interactions with other macromolecules to influence a variety of cellular properties and biological activities. The present study describes the binding of both human plasminogen (Pg) and tissue-type plasminogen activator (t-PA) to FN. Pg binds specifically and saturably to intact FN with a $K_{d,app}$ value of 0.12 uM. Limited proteolysis coupled with ligand blotting analysis shows that Pg and t-PA preferentially bind to the identical 55 kDa FN fragment. Amino terminal sequence analysis demonstrated that this fragment is comprised of the NH_2-terminal heparin binding domain as well as a portion of the gelatin binding domain of FN. The reverse ligand blotting experiments using isolated Pg domains demonstrate that FN binding to Pg is mediated primarily via lysine binding site-dependent interactions with Pg kringles 1-4. Furthermore, although intact FN does not enhance the rate of t-PA-catalyzed Pg activation, proteolytically degraded FN stimulates activation. Based on this observation, a variety of peptides derived from the Pg/t-PA binding region of FN were synthesized and assayed for their effect on the initial rate kinetics of Pg activation by t-PA. Kinetic studies indicated that a specific octapeptide, SRNRCNDQ-NH_2, consisting of residues 196-203 of the FN molecule, is a potent stimulator of t-PA-catalyzed Pg activation, resulting in a 20-fold increase in the k_{cat}/K_m of the activation reaction. These studies provide further evidence that interaction of Pg and t-PA with extracellular matrix components such as FN may provide a mechanism for localized regulation of plasmin generation within the extracellular matrix.

RZ 314 A NOVEL PROTEIN WHICH CO-LOCALIZES WITH LAMININ DURING DROSOPHILA EMBRYOGENESIS, Carol Graham Parker, Liselotte I. Fessler, and John H. Fessler, Department of Biology, UCLA, Los Angeles, CA 90024.

We have investigated several extracellular matrix proteins of Drosophila to learn how basement membranes influence development. One of these proteins, that we are calling Z, was isolated as a 160 kd apparent molecular weight protein from conditioned cell culture media of the Drosophila cell line Kc. Antisera raised against this protein was used to immunostain whole embryos. Protein Z co-localizes with laminin, first in the region of the developing nerve cord, then later to the newly formed basement membrane around peripheral sense organs.

The antisera were used to isolate clones for protein Z from a Drosophila cell culture cDNA lambda ZAP expression library. Corresponding nucleic acid probes identified a 5.5 kb message in cell culture and embryo mRNA. This message is expressed early in the embryo and peaks at 10-12 hours of development. A 5.3 kb clone was isolated from a Drosophila embryo cDNA library. A major portion of the sequence has been determined and shows this to be a novel gene, with significant amino acid identity to a protein of unknown function in C. elegans.

The gene for protein Z was located to the Drosophila chromosome position 75 D,E by in situ hybridization. A Drosophila strain that has substantial chromosomal deletion at this site exists and may provide insight into the effects of eliminating protein Z on basement membrane formation in embryos.

RZ 315 RECOMBINANT BACTERIAL FUSION PROTEINS DEFINE A THROMBOSPONDIN BINDING DOMAIN ON CD36, S. Frieda A. Pearce, Jun Wu and Roy L. Silverstein, Department of Medicine (Hematology/Oncology), Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

CD36, also known as platelet GPIV or GPIIIB, acts as a surface receptor for the complex adhesive protein Thrombospondin (TSP), binding with micromolar affinity. This interaction may mediate platelet-monocyte and platelet-tumor cell adhesion during thrombosis, atherosclerosis and tumor metastasis. To determine the putative TSP binding site on CD36, recombinant fusion proteins were synthesized and purified using fragments of the cDNA cloned into the bacterial expression plasmid, pGEX. These glutathione-S-transferase-CD36 fusion proteins were examined for TSP binding using a solid phase assay. A peptide containing the CD36 amino acid sequence 93-298 bound TSP with a similar dissociation constant as purified CD36. Peptides containing overlapping sequences 93-157 also bound TSP although with lower affinity (100nm-5μM) than native CD36. Peptides with the sequence of amino acids from 298 to 494 did not bind TSP. The fusion proteins containing amino acids 93-157 also inhibited binding of TSP to purified CD36 with an IC_{50} 50-100nm. The binding of TSP to fusion proteins was saturable and specific as it was inhibited by anti-CD36 antibodies as well as with excess cold fusion protein. From these studies we conclude that the region (93-157) contains a putative binding site for TSP. Peptides synthesized from the 93-157 region of CD36 may more clearly define the role of these amino acids in TSP binding and platelet adhesion.

RZ 316 THE BIOSYNTHESIS AND SECRETION OF LAMININ AND S-LAMININ BY HUMAN PROSTATE CARCINOMA CELL LINES, Isaac Rabinovitz, Ray B. Nagle and Anne E. Cress, Departments of Radiation Oncology [I.R. and A.E.C.] and Pathology [R.B.N.], University of Arizona Cancer Center, Tucson, AZ 85724

Laminin, an extracellular matrix glycoprotein has been shown in previous studies to participate in the process of tumor invasion and metastasis. The formation of tumors by human prostate cell lines ca n be influenced by the exogenous addition of laminin. Using the human prostate tumor cell lines DU-145 and LnCAP, we have found that these cell lines produce a variant form of the B1 chain of laminin, known as S-laminin. The DU145 cells produce laminin containing a ratio of A:B1:B2:S of (1.8):(1.0):(2.5):(1.0) respectively compared to the non-tumorgenic prostate tumor cell line, LnCAP, that had a ratio of A:B1:B2:S of (1.0):(0):(10):(2.5). The LNCaP cells retained most of its laminin production and secreted only immature laminin forms characterized by decreased levels of n-acetyl glucosamine and sialic acid, in contrast to DU145 that secreted most of its laminin production in mature forms similar to other well characterized human cell lines. The LNCaP cells bound very little laminin to their surface. These features in LNCaP cells could contribute to their documented low adhesivity in vitro and its non-tumorigenic phenotype. The fact that S-laminin was found in these human prostate carcinoma cell lines including the PC-3 cells, suggests an important role of S-Laminin in prostate cancer.

RZ 317 THROMBOSPONDIN 1, 2, AND 3 ARE DIFFERENTIALLY EXPRESSED BY ENDOTHELIAL CELLS *IN VIVO* AND *IN VITRO*. May J. Reed*, Luisa Iruela-Arispe+, Paul Bornstein* ^, and E. Helene Sage+, Departments of Medicine*, Biological Structure+, and Biochemistry^, Univ of WA, Seattle, WA 98195

The thrombospondins (TSPs) are a family of proteins with related gene sequences but diverse tissue distribution and function. TSP1 is anti-angiogenic for endothelial cells *in vitro*, whereas the roles of TSP2 and TSP3 are unknown. We have begun to investigate the function of TSP1, 2, and 3 in endothelial cells through a study of their differential expression during angiogenesis *in vivo* and *in vitro*.

The expression of TSP1, 2, and 3 *in vivo* was studied by *in situ* hybridization of developing blood vessels in Swiss-Webster mice from embryonic day 10 to postnatal day 2. Angiogenesis *in vitro* was studied by Northern blot analysis and *in situ* hybridization for TSP1, 2, and 3 in a well-characterized model of bovine aortic endothelial cells that spontaneously form cords and tube-like structures with patent lumina.

In vivo, expression of TSP2 occurred in the dorsal aorta as early as embryonic day 10. At day 13 expression in small blood vessels was also apparent. This pattern persisted to postnatal day 2. In contrast, TSP1 was not detected in endothelial cells until day 18, when it was noted in small, medium and large blood vessels. TSP3 was not detected in blood vessels at any time. *In vitro*, the expression of TSP1 decreased as endothelial cells progressed from subconfluence to cultures that contained cords and tubes. In contrast, TSP2 increased, and there was no detectable change in the low level of expression of TSP3.

In summary, the differential expression of TSP1 and 2 supports contrasting roles for these proteins in endothelial cells. The late expression of TSP1 *in vivo* and its decrease during angiogenesis *in vitro* is consistent with its known role as an anti-angiogenic protein. In contrast, the early expression of TSP2 *in vivo* and its increase during angiogenesis *in vitro* is indicative of a different role in the angiogenic process.

RZ 318 TISSUE-SPECIFIC EXPRESSION OF TENASCIN SPLICE VARIANTS DURING DEVELOPMENT, Richard P. Tucker, Paige M. Poss and Ruth Chiquet-Ehrismann*, Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157, and *Friedrich Miescher Institute, P.O. Box 2543, Basel 4002, Switzerland

Tenascin is a six-armed glycoprotein found in the embryonic extracellular matrix in regions of epithelial-mesenchymal interactions, along the migratory pathways of invasive cells, as well as in the central nervous system (CNS) and connective tissue. In the chick there are 4 major splice variants of tenascin with apparent molecular weights of 240, 230, 200 and 190 kDa. These variants differ from one another in the number of FN-type III repeats found in the carboxyl-half of each tenascin "arm". In order to learn more about the potential roles of tenascin splice variants during development, we have used reverse transcriptase PCR to generate splice variant-specific cDNA probes. These probes were then used to localize the transcripts encoding the different tenascin forms by in situ hybridization. cTNC, a probe that is specific for the 240 kDa form of tenascin, hybridized in mesenchyme at the growing tips of lung bronchioles and at the base of growing feather buds. A second probe (cTN230) that recognizes the transcripts encoding the 240 kDa and 230 kDa forms of tenascin hybridized in the same regions as cTNC as well as in proliferating and migrating glia in the developing CNS. cTN8, a cDNA probe that recognizes all 4 splice variants, hybridizes in the above regions as well as in areas of chondrogenesis. We have shown previously that neural crest cells make tenascin as they migrate. By in situ hybridization with cTN230 and reverse transcriptase PCR using trunk neural crest cell poly-A RNA we have learned that these cells are making the 230 kDa form of tenascin. Thus, the expression of the largest forms of tenascin (240 kDa and 230 kDa) is associated with active tissue modeling and cell motility. In contrast, only the smaller forms of tenascin (200 kDa and 190 kDa) are expressed at detectable levels in areas of cartilage morphogenesis. These distinctive patterns of expression indicate that tenascin splice variants may have different functions during development.

RZ 319 MODULATION OF ENDOTHELIAL CELL FUNCTIONS BY RECOMBINANT HEPARIN-BINDING DOMAIN OF THROMBOSPONDIN AND PEPTIDES FROM THE TYPE I REPEATS CONTAINING Trp-Ser-Pro-Trp.

T. Vogel, N-h Guo, H.C. Krutzsch, D.A. Blake, J.R. Hartman, S. Mendelovitz, A. Panet and D.D. Roberts.

BioTechnology General, Ltd., Rehovot, Israel; Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and Department of Biochemistry, Meharry Medical College, Nashville, TN 37208.

Thrombospondin is an inhibitor of angiogenesis that modulates endothelial cell adhesion, proliferation, and motility. Recombinant heparin binding fragment from the amino-terminus of thrombospondin and peptides from the second type I repeat of human thrombospondin containing the consensus sequence - Trp-Ser-Pro-Trp- mimic several of the activities of the intact protein. The peptides and heparin-binding domain promote endothelial cell adhesion, inhibit endothelial cell chemotaxis to basic fibroblast growth factor (bFGF), and inhibit mitogenesis and proliferation of aortic and corneal endothelial cells. Inhibition of proliferation by the peptide is time-dependent and reversible. The peptides also inhibit heparin-dependent binding of bFGF to corneal endothelial cells. The antiproliferative activities of the peptides appears to correlate with their ability to bind to heparin and to inhibit bFGF binding to heparin. Peptides containing amino acid substitutions that eliminate heparin-binding do not alter chemotaxis or proliferation of endothelial cells. Thus, the anti-proliferative activities of the thrombospondin peptides and recombinant heparin-binding domain result at least in part from competition with heparin-dependent growth factors for binding to endothelial cell proteoglycans. These results suggest that both the Trp-Ser-Xaa-Trp sequences in the type I repeats and the amino-terminal domain play roles in the anti-proliferative activity of thrombospondin.

Development & Diseases

RZ 400 TRANSGLUTAMINASE ACTIVITY IS PRESENT IN THE PERICELLULAR MATRIX OF CHONDROCYTES IN MINERALIZING AREAS OF CULTURED TRACHEAS, Daniel Aeschlimann and Mats Paulsson, M. E. Müller-Institute for Biomechanics, University of Bern, CH-3010 Bern, Switzerland

We previously reported that tissue transglutaminase is involved in cross-linking of extracellular matrix components and that its expression in skeletal tissues is strictly regulated and correlates with chondrocyte differentiation and cartilage calcification in endochondral bone formation and in maturation of tracheal cartilage. Rat trachea was labeled in culture for 24 h with the transglutaminase substrate monodansylcadaverine (DNSamidopentylamin) or an unreactive substrate analogue (DNSamidopentanol). Incorporation of label was subsequently detected with anti-dansyl antibodies either on tissue sections or in cartilage extracts. Covalent attachment of the dansyl-compound could be detected using the transglutaminase substrate and not with the control derivative. The label was found in the pericellular matrix of chondrocytes in the calcifying areas in the center of the cartilage rings and could be assigned to distinct protein bands by immunoblotting. Viability of the cells in culture after labeling with either dansyl-compound was assessed by pulse-chase labeling with ^{35}S-Met and autoradiography. Consequently, a transglutaminase, most likely tissue transglutaminase, is secreted by the chondrocytes and may function by cross-linking matrix components, *e.g.* osteonectin, prior to mineralization of the tissue.

RZ 401 STRUCTURE-FUNCTION ANALYSIS OF COLLAGEN I PRO α1(I) C-PROPEPTIDE BY SITE-DIRECTED MUTAGENESIS, John Bateman, Shireen Lamande and Simon Fenton, Department of Paediatrics, Univ. of Melbourne, Victoria 3052, Australia

Mutations have been produced in the type I collagen proα1(I) gene in domains coding for the C-propeptide domain, a region thought to play a critical role in procollagen, assembly, helix formation and extracellular fibril formation. These genes, which were engineered to carry a silent Met-Ile^{822} change, were stably transfected into Mov-13 cells, which express no endogenous α1(I), or mouse 3T6 cells to allow analysis of the consequences of co-expression of mutant and normal α1(I). The first mutation produced was a 2bp deletion causing a codon frameshift and producing an elongated proα-chain with an anomalous sequence covering the most C-terminal 109 amino acids. Transfected cells produced the mutant mRNA but only minute amounts of pepsin-stable mutant protein was detected. The failure of the mutant chains to efficiently associate to produce stable procollagen targeted them for rapid degradation in the ER by a mechanism distinct to that used for the degradation of excess normal chains. The second mutation removed the high mannose N-linked oligosaccharide attachment site (Asn^{1187}) to test the proposal that this moiety is important in procollagen biosynthesis and secretion. Mutant proα1(I) chains assembled with proα2(I) chains correctly and detailed kinetic studies showed no impairment of secretion of the mutant chains. *In vitro* studies of matrix formation also indicated that the mutant procollagen was processed and efficiently deposited in the matrix. These data suggest that the mannose moiety is not essential for the correct biosynthetic handling of procollagen by the cell. To study the consequences of the blockage of C-propeptide cleavage during extracellular procollagen maturation, mutations were introduced into the putative C-proteinase cleavage site. Alteration of the site (Ala-Asp) to Met-Phe did not prevent propeptide cleavage *in vitro* or in transgenic mice suggesting that the C-proteinase (or proteinases) may be able to cleave procollagen at sites other than Ala-Asp. A further mutation was produced that deleted the Ala-Asp and other more C-terminal sequences that could be proteolytically sensitive. The mutant was unable to be processed in long-term cultures where the endogenous normal procollagen product was efficiently processed and deposited into the matrix. The uncleaved mutant was accumulated in the medium fraction directly demonstrating that the efficient cleavage of the C-propeptide is an essential step for incorporation of the collagen into the extracellular matrix. Mutations that prevent this cleavage will thus prevent any type I collagen deposition and are likely to be embryo lethal.

RZ 402 IMMUNOLOGICAL APPROACH TO ESTIMATION OF THE ELASTIN TURNOVER ACTIVITY UNDER PHYSIOLOGICAL CONDITIONS IN AGING AND DEVELOPMENT. Stephan Baydanoff, Chavdar Alexiev, Georgi Nicoloff. Department of Biology and Immunology, University School of Medicine, 5800 Pleven, Bulgaria

Though the elastin macromolecule is exceptionally stable, there is enough evidence nowadays that the physiological turnover of elastin exists throughout the whole human life. The aim of our study was to estimate the activity of this turnover via determination of circulating elastin-derived peptides (CEDP), anti-elastin antibodies (AEAB) and elastin-antielastin circulating immune complexes (EAECIC) by immunological techniques based on ELISA. We tested clinically healthy subjects within the age range between 1 and 70 years. The different age groups showed different characteristic constellations of the indices studied. Among children (1-7 years old) we found relatively high level of CEDP and relatively low level of AEAB. The levels of CEDP and AEAB were most stable at the age between 18 and 50 as the turnover of elastin at this age is minimal. After 50 the level of AEAB remained the same, though the level of CEDP significantly increased. Another constellation was found in sera from subjects over 60 - high level of CEDP and relatively low level of AEAB. We found EAECIC only in this group. However, these complexes were with big size and low elastin content.

Finally we may conclude that the parameters used in this study may become a valuable tool for characterizing the activity of **elastin degradation** *in vivo*.

RZ 403 REGULATION OF COLLAGEN METABOLISM BY GLYCYL-L-PROLINE, A DIPEPTIDE INCREASED IN PROLIDASE DEFICIENCY, Annette M. Chamson and Jacques M. Frey, Department of Biochemistry, Medical School, 42023 Saint-Etienne, France. Prolidase deficiency is a rare hereditary disease characterized by an iminodipeptiduria specifically constituted by Glycyl-L-Proline (Gly-L-Pro) which is not further degraded. We showed previously (1) that the collagen metabolism of fibroblasts from prolidase deficient patients was disturbed. Further, we demonstrated an increase in the rapidly degraded collagen and a decrease in the proline pool. These effects could be mimicked by addition of Gly-L-Pro to normal fibroblasts. In order to understand this mechanism, experiments were made with Gly-L-^{14}C Pro and an HPLC method for Gly-L-Pro characterization in fibroblast cultures was developed. After 24 h incubation with normal fibroblasts, Gly-L-Pro was 93 % degraded. Taking this degradation into account, it was calculated that 0.18 umol/l of Gly-L-Pro caused a 200 % increase in the rapidly degraded collagen and a 40 % decrease in the proline pool. The effect of so small an amount showed Gly-L-Pro to be a powerful regulator of the collagen metabolism.

The proline from Gly-L-Pro was used for the protein synthesis as demonstrated by the correlation between Gly-L-^{14}C Pro and the ^{14}C aminoacyl t RNA (r = 0.91) and between Gly-L-^{14}C Pro and the ^{14}C proteins (r = 0.97). Our hypothesis is for the existence of competition between the Gly-L-Pro degradation and the collagen degradation. Thus in the case of prolidase deficiency, which is characterized by an inhibition of Gly-L-Pro degradation, the collagen degradation must compensate for the proline requirement. Moreover the Gly-L-Pro accumulated in this pathology could stimulate the collagen degradation.

1. A. Chamson, V. Voigtländer, I. Myara, J. Frey. Clin. Physiol. Biochem. 1989, 7:128-136.

RZ 404 **THE ROLE OF RETINOIC ACID (RA) AND FIBRONECTIN (FN) IN THE COMMITMENT OF CHONDROCYTE DIFFERENTIATION** in vitro. J. Chimal-Monroy, L. Hernández-Lagunas and L. Díaz de León, Connective Tissue Laboratory, Dept. of Develop. Biol, Inst. Invest. Biomédicas., UNAM, A.P. 70228 Mexico, City, Mexico, 04510.

Cartilage differentiation is initiated by mesenchymal tissue condensation to originate chondrogenic blastema. From this structure cartilage differentiation proceeds. It is known that certain molecules, such as retinoic acid (RA) and fibronectin (FN), inhibit cartilage differentiation by blocking the expression of type II collagen and inducing the synthesis of type I collagen. Also, it has been reported that both RA and FN cause morphological changes in chondrocytic tissues. In this work we examined whether this inhibition occurs before or after the cellular commitment for differentiation. The results obtained showed that when chondrocytes are cultured in the presence of RA (10 μM), FN (75 μg/assay) or both at a density of 1 X 10^4 cells/cm^2, the expression of type I collagen is induced. In order to determine the timing of this inhibition, chondrocytes were cultured in micromass conditions in the presence of RA. It is known that this experimental model favours chondrocytic differentiation per se. So, if chondrocytes treated with RA, FN or both are in a stage prior to this commitment, in micromass cultures treated with cytochalasin B (CB) and devoid of fetal bovine serum (FBS), no induction of chondrogenesis is expected. The results obtained in this study suggest that RA, FN or both inhibit chondrocyte differentiation at a stage after the cellular commitment.

RZ 405 COLLAGENASE EXPRESSION IN THE LUNGS OF TRANSGENIC MICE CAUSES PULMONARY EMPHYSEMA, Jeanine D'Armiento, Seema Dalal, Yasunori Okada and Kiran Chada, Dept. of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ08854

The overall pattern of the ECM is maintained via an intricate balance between the synthesis and degradation of its structural components. The appearance of the breakdown in collagen fibres is a key irreversible step in tissue destruction in many disease states. Therefore, to investigate the role of collagenase in liver disease, nine independent transgenic mouse lines were established. They harbored a transgene consisting of the haptoglobin promoter linked to the human interstitial collagenase gene. RNA was isolated from various tissues of representative hemizygous transgenic mice from each line and analyzed by RNAse protection. Surprisingly, the predominant site of expression of the transgene was observed, not in the liver, but in the lungs.

The phenotypic consequences of collagenase overexpression in the lung were then examined. Histological analysis demonstrated destruction of the alveolar walls and coalescence of the alveolar spaces with no evidence of fibrosis or inflammation. In general, the disrupted areas were focal and interspersed within normal alveolar architecture. However, when the level of transgene expression was higher, the changes were uniformly distributed throughout the lungs. This pathology is strikingly similar to the morphological changes observed in human emphysema and therefore implicates interstitial collagenase as a possible etiological agent in this disease process. Although elastase has been proposed as the primary enzyme responsible for emphysematous lung damage, this study provides evidence that other ECM proteinases could play a role in emphysema. Also, these transgenic mice are a defined genetic model system to study the pathogenesis of emphysema.

RZ 406 THE ROLE OF EXTRACELLULAR MATRIX (ECM) IN AIRWAY EPITHELIAL CELL DIFFERENTIATION, Elizabeth A. Davenport, Veronica B. Godfrey, Scott H. Randell, Paul Nettesheim, Lab of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC.

We are examining the effect of substratum on rat tracheal epithelial (RTE) cell differentiation and ECM gene expression. The tracheobronchial tree is lined with a pseudostratified epithelium composed mainly of basal, mucous, and ciliated cells. Based on morphology, cytochemical markers, and apical secretion of mucous-like glycoproteins, RTE cells propagated on a plastic substratum did not maintain mucociliary differentiation. On collagen I gel-coated inserts in medium supplemented with retinoic acid, RTE cells in logarithmic phase were poorly differentiated while plateau cultures displayed a pseudostratified mucociliary phenotype both morphologically and cytochemically. Northern blot hybridization of RNA isolated from RTE cells propagated on plastic or on collagen I gel-coated inserts to various ECM gene probes demonstrated that expression of α-1 collagen IV, fibronectin (FN), and thrombospondin (TSP) were significantly lower on collagen I gel-coated inserts compared to plastic. On collagen I gel-coated inserts, both FN and TSP expression were lower in mucociliary differentiated RTE cells than in poorly differentiated cells. Laminin B1 expression was unaltered by substratum or differentiation state and collagen I expression was not detected. These data suggest that expression of some ECM genes is down-regulated on a substratum that promotes RTE mucociliary differentiation.

RZ 407 TYPE I PROCOLLAGEN IS REDUCED IN CHRONICALLY PHOTODAMAGED HUMAN SKIN IN VIVO. GJ Fisher, HS Talwar, CEM Griffiths, and JJ Voorhees. University of Michigan, Department of Dermatology, Ann Arbor, MI 48109

Chronic exposure to ultraviolet irradiation from the sun results in a number of damaging effects to human skin. Accumulating evidence suggests that many of these effects are due, in large part, to alterations in composition and organization of the dermal extracellular matrix. To investigate the molecular basis of photodamage, we have determined the distribution and levels of type I procollagen in chronically sun-exposed and sun-protected human skin. Skin biopsies from extensor forearms (sun-exposed) and buttock or underarm (sun-protected) were stained with a monoclonal antibody raised to the amino-propeptide of type I procollagen. The extent of staining was assessed on a semi-quantitative 0-5 scale, with 0 being absent and 5 being maximum. Type I procollagen levels were determined by RIA, using polyclonal antibody raised to the carboxy-propeptide. Procollagen I staining in sun protected skin was observed extracellularly in a broad band in the upper dermis and diffusely in the lower dermis, and intracellularly within fibroblasts. Staining of extracellular procollagen within the upper dermis of photodamaged skin was reduced 56%, compared to sun-protected skin (1.2$\pm$0.2 vs. 2.7$\pm$0.3, respectively; N=26; $p<0.0001$). Extracellular staining within the deep dermis and intracellular fibroblast staining were not different between sun-exposed and sun-protected skin. Measurement of type I procollagen by RIA also revealed significantly less procollagen in sun-exposed, compared to sun-protected skin (83.5$\pm$9.2 vs. 159$\pm$19.7, respectively: N=24; $p<0.0001$). There was a significant correlation between the severity of photodamage and the reduction of type I procollagen, measured by either immunohistochemistry or RIA. These data indicate that synthesis of type I procollagen is reduced and/or its degradation is elevated in sundamaged human skin. Reduction of type I procollagen is likely to be a major factor in the decreased strength and resiliency of photodamaged human skin.

RZ 408 IN VITRO COLUMN FORMATION OF GROWTH PLATE CHONDROCYTES, J. Flechtenmacher, M.B. Aydelotte, H.J. Hauselmann, T. Schmid, A. Cole, J. Mollenhauer, K.E. Kuettner, E.T. Thonar, Department of Biochemistry, Rush University, Chicago, IL, 60612.

A characteristic feature of growth plate cartilage is its longitudinal growth. Chondrocytes from different topographic locations have been successfully cultured as monolayers or suspension cultures in liquid medium, as high density pellet cultures and in gels of agarose, collagens, methyl cellulose or alginate. In all of these culture systems, longitudinal growth could not be mimicked in vitro. We have cultured fetal bovine growth plate chondrocytes in alginate using the method of Hauselmann et al. (1992) with a single modification: the osmotic difference between alginate and the gel-inducing $CaCl_2$ was increased. Column formation first was observed on day 6 of culture (Fig). The average length as well as the number of columns increased with time.

Oriented growth is found in various biological systems. Certain types of algae rich in alginate show longitudinal growth and histologically resemble the columns of chondrocytes in cartilage of the growth plate. As the alginate is present at a lower osmolarity than the surrounding seawater it is possible that it is closely involved in cell wall formation and its longitudinal elongation. From this biological system and our own findings on the growth of chondrocytes as columns, we conclude that osmotic differences are involved in oriented gelling. One explanation for the failure of other researchers to effect longitudinal growth could be that they all worked with iso-osmolar solutions. We hypothesize that interactions between cells and the surrounding matrix are responsible for longitudinal growth, that a micro organization of the alginate matrix can result from differences in osmolarities during gelation, and that this micro structure does promote longitudinal growth of growth plate chondrocytes *in vitro*.

Ref 1: Hauselmann H. et al., Matrix 1992;12:116-129.

RZ 409 **EXPRESSION OF CHONDROITIN SULFATE-TYPE D COINCIDES WITH CARDIAC MESENCHYMAL CELL MIGRATION,**

Fiona M. Funderburg, J. Kevin Langford and Elizabeth A. Lunow, Department of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Alternating regions of the developing heart undergo an endothelial -mesenchymal transition whereupon the resultant mesenchymal cells migrate through an expansive extracellular matrix (ECM) toward the myocardium. They subsequently differentiate into fibroblasts serving as the anlagen for cardiac valves and connective tissue septa.

During their migration, cardiac mesenchymal (CM) cells secrete a chondroitin sulfate proteoglycan (CM-CSPG) whose glycosaminoglycan chains differ in their sulfation pattern from the myocardially derived CSPG. It has been suggested that CM-CSPG modulates cell migration by modifying the surrounding substratum to mask cell attachment sites.

We examined the distribution of CS in the embryonic chick heart temporally and regionally using monoclonal antibodies which recognize either CS-type D (MO-225) or CS-types C and D (CS-56). Immunohistochemical comparisons revealed two distinct patterns of expression in hearts ranging from d2.5 to d14 of development.

CS-D was detected in the CM-forming AV region *after* the endothelial-mesenchymal transition, was restricted to *post-migratory* ECM, and remained intense until fusion of the CM pads. Thereafter, the signal *diminished with time* and was barely detectable by d14. CS-D was not detected in the ECM of the non-CM-forming V at any stage.

In sharp contrast, was the staining pattern obtained with CS-56. Signal, presumably due to CS-C in pre-CM hearts, was ubiquitously distributed throughout the ECM, both in CM-forming and non-CM-forming regions at all stages. There was no distinction between pre- and post-migratory ECM in the AV region; in the V, the ECM was always positive.

These observations support the contention that there are at least two CS-PGs expressed during cardiac morphogenesis and that CM cells modify their substratum during migration. CM-CSPG contains CS-D and appears to be *developmentally regulated*.

RZ 410 MULTIPLE PHENOTYPIC RESPONSES OF EMBRYONIC PANCREATIC EPITHELIUM TO ENVIRONMENTAL SIGNALS, George K. Gittes, Philip E. Galante, Haile T. Debas, and William J. Rutter. Department of Surgery and the Hormone Research Institute, University of California San Francisco, San Francisco, CA, 94143-0660.

Organogenesis is dependent on a specific interaction between an embryonic epithelium and its surrounding mesenchyme. Pancreatic organogenesis involves the rapid growth and differentiation of precursor cells in the wall of the duodenal anlage into ductal, endocrine, and exocrine pancreatic cell lineages. Both the endocrine and exocrine cells are thought to derive from early ductal cells. We have developed a system by which pancreatic organogenesis can occur *in vitro*, and have used this system to study the nature of epithelial differentiation in the undifferentiated embryonic pancreas. The pancreas is removed from early post-implantation mouse embryos (11 days gestation) and the mesenchyme removed by gentle trypsinization and microdissection. The epithelium is then grown suspended at the air/media interface on a transparent Millipore filter insert. Alternatively, similar embryonic epithelia were placed under the renal capsule of syngeneic adult mice. Tissues were then assayed 10-14 days later immunohistochemically and morphologically for evidence of differentiation.

Without mesenchyme the epithelia *in vitro* did not grow and died after one week in culture. When EGF or other growth factors were added to the growth media, however, the epithelia survived longer and even grew to some extent, but showed no immunohistochemical evidence of differentiation. When these epithelia were grown embedded in a basement-membrane-rich matrix (Matrigel), they formed predominantly large differentiated ductal structures with little endocrine and no exocrine differentiation. When grown under the renal capsule these isolated embryonic epithelia developed into clusters of mature islets, devoid of ductal or exocrine cells/structures.

Based on these data we hypothesize that embryonic pancreatic epithelium has several potential patterns of growth and differentiation depending on environmental cues: 1) growth without differentiation, possibly induced by mitogenic factor(s) in the mesenchyme 2) duct-only differentiation, possibly induced by basement-membrane-like molecules in the mesenchyme allowing for the polarization necessary to form functional ducts and 3) islet-only differentiation, previously undescribed, but possibly signifying a default differentiation pathway for pancreatic epithelium devoid of mesenchyme or basement membrane.

RZ 411 PURIFICATION AND CHARACTERIZATION OF NATIVE CMP (CARTILAGE MATRIX PROTEIN) AND ITS TISSUE DISTRIBUTION IN MOUSE, Nik Hauser and Mats Paulsson, M.E. Müller Institute for Biomechanics, University of Bern, P.O. Box 30, 3010 Bern, Switzerland

Cartilage Matrix Protein (CMP) is a major component of non-articular cartilage. It is a noncollagenous glycoprotein with a molecular weight of 148 kDa consisting of three identical subunits of 52 kDa molecular weight. CMP appears to be restricted to cartilage and has so far been purified from guanidine HCl-extracts of bovine trachea and chicken xiphisternal cartilage. With the aim of performing a more comprehensive structural and biological characterization, we purified CMP with non-denaturing agents from Swarm rat chondrosarcoma (SRC) as well as from fetal bovine tracheal cartilage. The purification of the native protein involved selective extraction and several chromatography steps. We determined the aminoterminal sequence of the bovine and rat CMP and confirmed their identity by comparison with published cDNA sequences for chicken and human. To get structural information on this noncollagenous cartilage protein, we examined it by electron microscope using the negative staining and glycerol-spraying / rotary shadowing techniques. Polyclonal antibodies raised against bovine and rat CMP were used for immunoblots and immunohistochemical work. The crossreactivity of these antibodies was sufficient to allow their use in immunohistochemistry of mouse tissues. The expression pattern of CMP in mouse was compared to similar data from chicken and steer.

RZ 412 A CASE OF TYPE VI EHLERS-DANLOS SYNDROME CAUSED BY A HOMOZYGOUS DELETION OF TWO EXONS IN THE mRNA FOR LYSYL HYDROXYLASE, Timo Hautala*, James Hyland*, Jari Heikkinen*, Thomas Krieg#, Kari I. Kivirikko* and Raili Myllylä*, * Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University Of Oulu, Oulu, Finland. # Klinik und Poliklinik für Dermatologie und Venerologie der Universität zu Köln.

The Ehlers-Danlos syndrome is a connective tissue disorder characterized by joint hypermobility, skin fragility and hyperextensibility and kyphoscoliosis. Some patients also have ocular manifestations. The biochemical defect in most patients is a deficiency in the activity of lysyl hydroxylase (EC 1.14.11.14), the enzyme catalyzing the formation of hydroxylysine in collagens and other proteins with collagen-like amino acid sequences. We have recently reported on molecular cloning of the cDNA for lysyl hydroxylase and on two mutations leading to the type VI variant of Ehlers-Danlos syndrome. A large duplication rearrangement in the gene for lysyl hydroxylase was found in two siblings and a point mutation creating a premature stop codon in another two siblings. We now report on a mutation of the typical type VI Ehlers-Danlos syndrome in a patient with 10% lysyl hydroxylase activity. The parents are third-degree cousins and their dermis contained only half the normal amount of hydroxylysine (Krieg et al.: Hum. Genet.46,41-49, 1979). The mRNA for lysyl hydroxylase has the nucleotides from 1850 to 2102 deleted, corresponding to two exons. These exons are present in the genomic DNA of the patient, which suggests a defect in the splicing of the mRNA. Only one shortened type of mRNA, the size of which is in agreement with the deletion, exists in the fibroblasts of the patient, suggesting homozygosity.

RZ 413 PARTIAL SEQUENCE OF PORCINE LIVER HYALURONIDASE: IDENTIFICATION OF A HEMOPEXIN AND TWO PUTATIVE HA-BINDING DOMAINS. Thomas J. Hope, Li Zhu, Tristram G. Parslow, Baihua Yang, Michael Stern, Eva A. Turley, and Robert Stern. Departments of Pathology, and Oral Maxillofacial Surgery, U.C. San Francisco, USA, and Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada.

Hyaluronidase is the enzyme that degrades hyaluronan (HA), a glycosaminoglycan component of the extracellular matrix (ECM) that is prominent in embryogenesis, tumorigenesis, wound healing, and whenever rapid tissue turnover occurs. The hyaluronidases have not been well characterized previously owing to the lack of a simple assay -- a problem that has now been overcome with the development of a rapid ELISA-like procedure (Matrix, in press, 1992). Facilitated by this assay, hyaluronidase from pig liver was purified, and partial amino acid sequences were obtained from both the N terminus and internal CNBr peptides. Based on these sequences, a pair of degenerate oligonucleotide primers were prepared. These were used to isolate DNA complementary to hyaluronidase mRNA by reverse transcription and polymerase chain reaction using adult pig liver mRNA as a template. The cloned DNA sequence contains an uninterrupted open reading frame encoding a protein that is predicted to contain a hemopexin domain similar to those found in the metalloproteinases, a class of enzymes also involved in the catabolism of ECM components. In addition, the predicted sequence includes two regions homologous to the HA-binding motif observed in RHAMM, an HA receptor involved in HA-mediated cell motility. Northern blot analysis of pig liver polyA RNA, using the cloned cDNA as a probe, reveals a single mRNA of approximately 1.8 kb. The cloned sequence was expressed as a glutathione S-transferase fusion protein in bacteria and was tested for hyaluronidase activity. This represents the first molecular cloning of a vertebrate hyaluronidase.

RZ 414 THE ROLE OF TIMP-2 BOUND TO GELATINASE-A IN THE INHIBITION OF ACTIVATED GELATINASE-A-TIMP-2 COMPLEXES, Eric W. Howard, Elizabeth C. Bullen, and Michael J. Banda, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750

Cultured cells often secrete gelatinase-A (72-kDa gelatinase) as a 1:1 complex with the tissue inhibitor of metalloproteinase-2 (TIMP-2). The TIMP-2 binds to the carboxy terminus of the progelatinase, and the complex degrades substrate after activation. The TIMP-2 associated with gelatinase-A remains bound to activated gelatinase, and it participates in the inactivation of the complex. Inactivation is triggered by exogenous TIMP-1 or TIMP-2, and can occur in a catalytic fashion. While the exogenous TIMP is necessary for this inhibition of complexes, it does not become stably associated with the active site of the gelatinase. Instead, the TIMP-2 bound to the carboxy terminus of gelatinase-A is triggered to block the active site of gelatinase-A. Investigations of TIMP-1 and TIMP-2 binding to both free and TIMP-2-complexed gelatinase using immunoprecipitation and size-exclusion chromatography revealed that TIMP-1 can only stably bind to free, active gelatinase-A, not gelatinase-A-TIMP-2 complexes. In addition, exogenous TIMP-2 will only exchange with progelatinase-TIMP-2 complexes, and not with activated complexes. Interaction of free TIMP-2 with activated complexes results in triggered inhibition of gelatinase by carboxy-terminally bound TIMP-2. This represents a unique mechanism for the inactivation of a proteinase. It is possible that control of proteolysis by gelatinase-A is mediated by its already bound inhibitor, and that disruption of this complex may play a significant role in disease states.

RZ 415 Laminin, Collagen IV and Integrins Expression During Rat Parotid Gland Development. K.W. LAZOWSKI, P.M. MERTZ, R.S. REDMAN, E. KOUSVELARI (CIPCB, NIDR, NIH and VAMC, USA).

It is known for some time that epithelial-mesenchymal interactions can direct epithelial specialization and morphogenesis during development. The rat parotid gland is an excellent model to study such processes, since the major developmental events occur after birth. The aim of the present study was to examine the pattern of gene and protein expression of laminins, collagen IV and ß1, α6 receptors of the integrin family. Parotid glands from 0,7,14,21,42 and 90 days postnatal were used for RNA isolation and tissue sections. The expression of the laminin subunits B1, B2, collagen IV, ß1 and α6 integrin genes was studied by Northern blot hybridization using ^{32}P labeled cDNA probes. For the immunolocalization of their respective proteins rabbit antibodies against, laminin, collagen IV and ß1 integrin, were used. Laminin and collagen IV genes were highly expressed at day 0,7 and 14 and decline with age. The proteins were distributed around the acinar cell membranes. The expression of integrin ß1 and α6 genes followed the same pattern as laminins and collagen IV. Integrin ß1 was localized on the basal membrane of the forming acinar cell. The data indicate that integrin receptors may mediate acinar cell-extracellular matrix interactions during rat parotid gland development.

RZ 416 A MURINE MODEL FOR THE EOSINOPHILIA-MYALGIA SYNDROME (EMS), Anna Ludwicka, Takashi Ohba, Sarah Bingel, Russell Harley, Marta Hampton, John Maize, Melvyn Heyes, and Richard Silver, Medical University of South Carolina, Charleston, SC 29425 and National Institute of Mental Health, Bethesda, MD 20892.

The eosinophilia-myalgia syndrome (EMS) occurred in epidemic form in 1989, and was associated with the ingestion of L-tryptophan containing products (LTCP) contaminated with 1,1'-ethylidenebis (L-tryptophan) (EBT). EBT has been postulated to be the etiologic agent responsible for EMS.

We have developed an animal model to test purified EBT at concentrations found in contaminated LTCP for the ability to induce inflammation and fibrosis in vivo. C57BL/6 mice were administrated daily injections of EBT or L-tryptophan alone or in combination (intraperitoneal). Control mice received saline. Mice were sacrificed after 3 days and 1, 3 and 6 weeks and skin tissues were examined by hematoxylin and eosin and tridrome staining. Inflammatory and fibrotic changes appeared after one week and became severe after three weeks and persisted after six weeks of treatment. The most striking effects were seen in the skin and subcutaneous tissue, where EBT was administered and was associated with an intense mononuclear cell infiltrate extending from the dermis to the superficial and deep adipose layers accompanied by severe fibrosis. Tissue eosinophilia was not apparent. Results of sequential biopsies, as well as studies of L-tryptophan metabolism, will be presented.

The murine model indicates that EBT is capable of inducing lesions consistent with EMS, and it should facilitate study of the pathogenesis of EMS.

RZ 417 TARGETED DISRUPTION OF THE MURINE STROMELYSIN-1 MATRIX METALLOPROTEINASE GENE, John S. Mudgett, Nicole A. Chartrain, Howard Chen, Myrna Trumbauer, Michael Tocci, and Nancy I. Hutchinson. Department of Molecular Immunology, Merck Research Laboratories, Rahway, NJ 07065.

Murine stromelysin-1 (transin-1) is a member of the matrix metalloproteinase family implicated in tissue remodeling in development, repair, and in disease processes. We constructed cosmid libraries from mouse embryonic stem (ES) cell genomic DNA, isolated the murine stromelysin-1 (SLN-1) gene, and constructed replacement vectors to inactivate the catalytic domain of the stromelysin-1 gene in mouse ES cells. We used a dual approach to isolate SLN-1 targeted ES cells: 1) isolating individual transformants and characterizing their genomic DNAs, and 2) culturing small pools of about 15 transformants and analyzing the pools' DNA by PCR. All PCR positive ES cell lines were fully characterized by Southern hybridization to confirm targeted disruption of the stromelysin gene. Independent SLN-1 targeted ES cell lines were injected into mouse blastocysts, and progeny mice were screened for coat color chimerism. Male coat color chimeric mice were bred for determination of germline penetrance and generation of heterozygous $SLN\text{-}1^{-/+}$ offspring in both C57BL/6 and 129Sv mouse lines. We have generated and genotyped heterozygous $SLN^{+/-}$ transgenic mice, which were bred for the generation of homozygous stromelysin-1-deficient transgenic mice. We will characterize the resultant progeny mice to determine the viability of stromelysin-1-deficient homozygous transgenic mice, and the effects of stromelysin-1 deficiency on normal and disease processes.

RZ 418 THE ROLE OF THROMBIN IN SCLERODERMA LUNG DISEASE, Takashi Ohba, Anna Ludwicka, Ken McDonald, Richard M. Silver, Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC 29425

Thrombin, a serine protease, has a many activities as a paracoagulant monocyte chemoattractant, stimulator of granulocyte adherence and fibroblast mitogen. We have recently determined the level of thrombin by a fluorometric assay in bronchoalveolar lavage fluid (BAL). We have demonstrated increased levels of thrombin in BAL fluids of scleroderma (SSc) patients with varying degrees of pulmonary disease, as compared to healthy control subjects. The thrombin levels were increased 5-fold to 20-fold in SSc patients as compared to healthy controls. We have found that SSc and normal lung fibroblasts respond mitogenically to *in vitro* exposure of thrombin. The effect of thrombin on *in vitro* expression of fibronectin, PDGF-AA and PDGFα and PDGFß receptors on mRNA and protein level was determined. The increase of fibronectin and PDGF-AA, as well as PDGFα and ß receptors, was observed after thrombin pretreatment.

RZ 419 NOVEL KERATAN SULPHATE PROTEOGLYCAN OF HUMAN TERATOCARCINOMA CELLS, Martin F. Pera and Sue Cooper, CRC Growth Factors, Department of Zoology, Oxford University, South Parks Rd., Oxford, OX13PS U.K.

A search for markers expressed by multipotent human teratocarcinoma cells has led to the identification and characterization of a keratan sulphate proteoglycan whose expression is switched off during differentiation of the tumour stem cells in vitro. Originally defined as the molecule bearing an epitope recognized by the monoclonal antibody GCTM-2, the proteoglycan can be isolated from cultured human embryonal carcinoma cells either as a giant supramolecular aggregate which does not migrate out of SDS-PAGE stacking gels (the predominant form in extracelluar matrix), or as a 200 kda soluble form present in culture supernatant. In either case the glycosaminoglycan content of the proteoglycan consists solely of keratan sulphate and chondroitin sulphate, attached to a core protein of apparent molecular weight 55 kda. Immunocytochemical studies with a new monoclonal antibody, TG 34.3, raised against purified preparations of the secreted form, confirm earlier studies with antibody GCTM-2, which demonstrated that the proteoglycan is expressed during human foetal development in gut, other epithelia, and in muscle, as well as in nonseminomatous germ cell tumours and colorectal carcinomas. No expression is seen in cartilage or cornea. A clone of embryonal carcinoma cell line GCT 27 which was immunoselected for low levels of surface proteoglycan expression shows altered morphology and substrate interaction in vitro, suggesting that the proteoglycan may promote cell adhesion. Availability of n-terminal amino acid sequence and new monoclonal antibodies will facilitate molecular cloning of the novel proteoglycan core protein from cDNA expression libraries (work in progress). The modulation of proteoglycan expression observed during teratocarcinoma differentiation in vitro, and the patterns of tissue-specific expression seen in foetal specimens, indicate that the molecule may play a regulatory role in some developmental processes in man.

This work is supported by the Cancer Research Campaign.

RZ 420 EFFECTS OF COAL DUST EXPOSURE ON DEPOSITION OF EXTRACELLULAR MATRIX BY TYPE II PULMONARY EPITHELIAL CELLS IN PRIMARY CULTURE. D. Eugene Rannels and Yu-Chen Lee. Departments of Cellular & Molecular Physiology and Anesthesia, Penn State University College of Medicine, Hershey PA 17033.

The extracellular matrix (ECM) modulates differentiation of type II pulmonary epithelial cells (T2P) in a composition-dependent manner (Chest 96: 165, 1989). Recent data indicates that T2P also modify the matrix through synthesis and turnover of ECM components (Am J Physiol 262: L582, 1992). The extent of pulmonary epithelial cell differentiation and associated changes in the sub-epithelial basement membrane appear to be important to the pathobiology of fibrotic lung diseases (Am J Physiol 259: L159, 1990) such as those induced by respirable particulates. Direct effects of coal dust on ECM deposition by T2P were thus investigated in primary culture. Partitioning of newly-synthesized proteins into cellular and ECM fractions, the latter derived by extraction of the epithelial monolayer with NH_4OH and high salt, was measured. Dust samples were ground in a zirconium ball mill, sonicated in DMEM-D, and resuspended in DMEM-D containing 10% FCS. Both generic anthracite (#867) and bituminous (#1361 and #1451) coal dust samples of respirable size, as well as mine dust (MIT #3), modified radiolabeling of proteins in the cell and matrix fractions in a time- and dose-dependent (300 to 750 µg/ml) manner, without detected cytotoxicity and independent of dust SiO_2 content. Moreover within 6 hours, exposure of T2P to either 867 or 1451 increased the proportion of newly-synthesized proteins partitioned into the matrix fraction. MIT-3 had qualitatively similar effects, but only after a longer interval of exposure. ECM fibronectin content, quantitated from western blots, increased at rates more than 5-times the control between culture days 1 and 3 in cells treated with 867. This result suggests effects of the dust on turnover, as well as synthesis, of proteins in the ECM fraction. The data indicate that coal dust exposure modifies synthesis and deposition of extracellular matrix by pulmonary epithelial cells. Changes in matrix fibronectin content are known to alter pulmonary epithelial cell growth and differentiation. In this context, the present observations suggest that dust-induced changes in the sub-epithelial matrix may impact alveolar structure and function, and thus contribute to associated pulmonary disease. Supported by US Bureau of Mines Generic Mineral Technology Center for Respirable Dust G1105142 and by HL-31560 from the NHLBI.

RZ 421 MESANGIAL CELL (MC) STRETCH ENHANCES EXTRACELLULAR MATRIX (ECM) AND TGF-ß SYNTHESIS: POSSIBLE ROLE IN PROGRESSIVE RENAL DISEASE, Bruce L. Riser, Pedro Cortes and Robert G. Narins, Dept. of Medicine, Henry Ford Hospital, Detroit, Michigan.

Once a critical amount of kidney mass is lost, residual glomeruli develop scarring with progressive accumulation of ECM, regardless of the activity of the original stimulus. Damaged kidneys vasodilate and deliver a greater fraction of systemic blood pressure to glomeruli; this increased glomerular pressure being an intrinsic feature of progressive nephropathy. One central question that remains unanswered is how glomerular hypertension is translated into increased ECM accumulation. We hypothesized that increased capillary pressure distends the glomerulus and adjacent MCs. Stretching of MCs then stimulates ECM production, mediated or modified by TGF-ß, a potent modulator of ECM production by MCs in culture. By perfusing isolated glomeruli, we determined that the glomerulus is a pliant structure expanding as much as 61% under the force of intraglomerular pressure, therefore resulting in stretch of its MCs. To determine if MC stretch stimulates TGF-ß and ECM production we cultured MC on elastin-coated flexible bottom plates and subjected them to cyclic stretching at 19% maximum elongation. In both experimental and static control groups, collagen I, collagen IV and laminin secretion, determined by enzyme-linked immunosorbent assay (ELISA), was highest initially (3 days) and then declined rapidly. Fibronectin, undetectable in early cultures, demonstrated maximal accumulation after 4-5 days. At periods of maximal ECM formation, mechanical strain significantly increased the production of collagen types I (117%) and IV (79%), fibronectin (43%) and laminin (47%). Immunohistochemical staining, quantitated by computer-assisted image analysis, demonstrated a dose-response relationship between the intensity of mechanical strain and the production of ECM components. With similar techniques, using an antibody recognizing the active forms of TGF-ß1 and TGF-ß2, static control MCs showed little or no detectable staining. However, after only 6 h of stretch MCs demonstrated significant staining which increased in intensity after 2 days. Stretching of MC for 48 h also resulted in increased TGF-ß (35%) secreted into the culture medium, as determined by a mink lung cell biological assay. The mechanical environment is likely to be important in the normal regulation of ECM production. These findings suggest that mechanical strain imposed on MCs by glomerular distention resulting from hypertension, may be a critical pathogenetic factor in ECM accumulation, an effect which may be mediated by TGF-ß.

RZ 422 EXTRACELLULAR MATRIX PROTEOLYSIS BY OVARIAN CARCINOMA-ASSOCIATED PROTEINASES, M. Sharon Stack, Tammy L. Moser, Tim N. Young, Gustavo C. Rodriguez, and Salvatore V. Pizzo, Depts. of Pathology and Ob/Gyn, Duke University Medical Center, Durham, NC 27710.

Extracellular matrix proteolysis has been correlated with the invasion and metastasis of many tumor types. To investigate the role of extracellular matrix proteolysis in the pathology of ovarian carcinoma, we have characterized the proteolytic profiles of five established ovarian carcinoma (OVCA) cell lines (OVCA 420, OVCA 429, OVCA 432, OVCA 433 and DOV 13) compared to normal ovarian epithelial (NOE) cell cultures. Initial analysis of proteinase production by zymography demonstrated that all OVCA cell lines produce plasminogen activators (both tissue-type, or t-PA and urinary-type, or u-PA) as well as matrix metalloproteinases (MMP). Very little MMP or PA activity was detectable in the conditioned medium of NOE cells. The predominant OVCA-associated MMP, produced primarily by DOV 13 cells, was purified and identified as MMP-2 (72 kDA gelatinase/type IV collagenase). Quantitation of PA activity by immunocapture demonstrated differential production of u-PA and t-PA among OVCA cell lines. The predominant PA was u-PA, which was produced in the highest amounts by OVCA 429 and OVCA 433 cells. Furthermore, OVCA-produced PAs efficiently activate plasminogen to the proteinase plasmin which degrades extracellular matrix fibrin(ogen), laminin and fibronectin. Degradation of extracellular matrix proteins by OVCA-associated proteinases was confirmed using a Matrigel invasion assay. OVCA cell lines displayed marked heterogeneity of invasiveness which correlated with total PA levels. Together these data demonstrate increased proteinase production and extracellular matrix proteolysis by OVCA cells relative to NOE cells, suggesting a biochemical mechanism for the ob served invasive activity of OVCA cells in vivo.

RZ 423 A ROLE FOR NEUTROPHIL ELASTASE IN UVB IRRADIATED SKIN. Barry Starcher and Matt Conrad. Dept. Biochem., Univ. TX Health Center, Tyler TX, 78710.

One of the major affects of UVB irradiation of the skin is an accumulation of thickened and twisted elastin fibers in the dermis. It is not known whether solar elastosis is caused by a direct affect of the UV on the elastin fibers or whether there are indirect affects mediated by oxidants or proteolytic enzymes. In this study we looked at the affects of UVB irradiation on elastin metabolism in the skin of neutrophil elastase deficient beige mice. SKH Hairless mice (BBhh) were crossed with beige mice (bbHH) for several generations to develop a strain of hairless mice, deficient in neutrophil elastase (bbhh). The deficient mice and their normal littermates were exposed to.09J three times each wk for up to 20 wks. The animals were killed and the back skin removed for histology and measurements of breaking strength, elastin and collagen. Initial studies showed that a 16 wk exposer for 3.1 total joules gave maximum results. Daily exposer to accumulate the same total joules in a shorter time frame was not effective. On gross examination, the skin of the normal UVB mice contained many basal cell carcinomas while the skin of the deficient UVB mice was devoid of tumors. Histologically, the normal mice showed a large accumulation of dermal elastin following UVB exposer. Elastin in the deficient mice showed no evidence of an increase and remained as long thin fibers indistinguishable from the non exposed controls. Skin collagen content as well as the breaking strength was significantly reduced in all groups of mice exposed to UVB. Elastin content was elevated in the UVB normal mice but remained unchanged in the UVB elastase deficent mice.The experiments suggest that neutrophil elastase, and possibly mast cell elastase play an important role in the solar elastosis observed in UVB irradiated skin.

RZ 424 IMMUNOLOGICAL CHARACTERIZATION AND ISOLATION OF A 65 kD CHONDROCYTE MEMBRANE AUTOANTIGEN

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Chondrocyte membranes have been recognized as a target for both humoral and cellular autoimmune reactions in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). In T lymphocyte proliferation assays and in serum antibody tests distinct reactions were found against chondrocyte cell surface proteins. One of the dominant antigens was a 60-65 kD polypeptide (CH65) that was recognized by the majority of RA and OA sera. Ch65 is probably located on the cell membrane because of its co-enrichment with plasma membranes. It has been described that expression of the 60 kD heat shock protein (hsp60) is elevated and characteristic for synovial tissues in rheumatic joints and hsp60 is recognized by RA sera. Whether a relationship between CH65 and hsp60 exists, was investigated in Western blots with polyclonal and monoclonal antibodies raised against both proteins. Of the 7 mab raised against CH65, prepared from chicken sternum chondrocytes, only 2 showed some reactivity against the mycobacterial hsp60. Four established mab and one polyclonal directed against hsp60 did not react with CH65. In addition, patient sera exhibited a distinct reaction pattern to both hsp60 and CH65. Further characterization of CH65 showed that unreduced CH65 appears to have a molecular weight greater than 300kD in SDS-PAGE; only disulfide-reduced samples exibit the 65kD band. In contrast to the hsp60, the CH65 is glycosylated.

These data suggest significant immunological and biochemical differences between CH65 and hsp60. The CH65 seems to be directly exposed to the autoimmune attack in arthritis as a primarily recognized cellular antigen.

RZ 425 EXTRACELLULAR MATRIX PROTEOLYSIS IN OVARIAN CANCER: ROLES OF URINARY PLASMINOGEN ACTIVATOR AND TYPE IV COLLAGENASE EXPRESSION,

Timothy N. Young, Tammy L. Moser, Gustavo C. Rodriquez, Salvatore V. Pizzo, and M. Sharon Stack, Depts. of Pathology and Ob/Gyn, Duke University Medical Center, Durham, NC 27710.

Much evidence demonstrates that secretion of proteinases by tumor cells confers the ability to degrade extracellular matrix (ECM) and invade tissues; however, little information exists on proteinase secretion by ovarian cancer (OVCA) cells. To study the role of ECM proteolysis in OVCA, we examined ascites fluids from OVCA patients. Western blot analysis demonstrated significant amounts of degraded fibronectin and laminin. Also, Matrigel invasion assays employing established OVCA cell lines indicated the cancer cells themselves are capable of ECM proteolysis. We therefore characterized the cellular activities responsible for ECM proteolysis. Zymographic analysis demonstrated that 3 of 6 cell lines secrete a 66 kDa active metalloproteinase (MMP), while 1 of 6 secretes an 86 kDa active MMP. The 66 kDa MMP was purified from conditioned medium as a zymogen with molecular weight of 72 kDa and identified as MMP-2. Upon activation, the enzyme cleaves soluble native type IV, but not types I, III, or V collagen; however, all of the corresponding gelatins are efficiently cleaved. The 86 kDa MMP, currently under investigation, appears to be MMP-9, a reported type IV collagenase. Additionally, zymography and immunocapture assays demonstrate high levels of urinary-type plasminogen activator (u-PA) expression in all cell lines. By immunohistochemical staining, all cell lines prominently express u-PA receptor (uPAR), an important regulator of u-PA activity which concentrates plasmin generation at cell surfaces. Only trace amounts of MMP and u-PA are detected in conditioned media of normal ovarian epithelial cells and only light staining for uPAR is observed. Elevated expression of these molecules may explain the ability of these tumor cells to degrade and invade ECM and may be an important determinant of malignancy.

Late Abstracts

FUNCTIONAL ANALOGUES OF HEPARAN SULFATE AS BONE HEALING AGENTS

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We have studied in a model of skull trephine defect in rats the osteoinductive potential of one member of a family of derivatized dextrans.These derivatives, corresponding to chemically substituted dextrans by the addition of carboxymethyl, benzylamide and sulfonate groups, are named carboxymethyl-benzylamide-sulfonated dextrans (CMDBS). Several CMDBS, behaving as functional analogues of heparan sulfates, were selected for their affinity for fibroblast growth factors (FGFs) as well as for their capacity to protect these GFs against proteolytic and thermal denaturation and to potentiate their in vitro biological activities. We found that CMDBS added alone was able to induce an important bone regeneration (BR) in a dose dependant manner in calvarial defects. BR was approximately 80% in the groups treated with either 25 or 100 µg/ml CMDBS solutions and 56% with 400 µg/ml while controls were not repaired (BR were approximately 30% for these groups). For several CMDBS treated animals, a normal tissue appearance, including the presence of a sagittal suture, was observed. These results indicate a remarkable osteoinductive potential for CMDBS, which may result from the capacity of this product to protect the endogenous HBGFs from proteolysis and to modulate their biological activities, in a similar manner to that observed for FGFs and heparan sulfates. CMDBS represent a new form of bone healing agents, which have the advantage of being produced by a controlled chemical synthesis, and of avoiding the use of exogenous GFs because of their capacity to enhance the bone healing potential of the endogenous GFs.

EARLY CHANGES IN CTC-CONTAINING SITE BINDING COMPLEXES DURING THE RAPID ACTIVATION OF LAMININ B_2 PROMOTER IN CULTURED HEPATOCYTES.

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Laminin B_2 chain is present in all basement membranes and is expressed at high level in various diseases, such as hepatic fibrosis. We have investigated *cis* and *trans* acting elements involved in the regulation of this gene in hepatocytes and in hepatoma cell lines. Northern-blot analyses showed that laminin B_2 mRNAs were barely detectable in freshly isolated hepatocytes and expressed at high levels in both hepatocytes in primary culture for 4 h and hepatoma cells. Actinomycin D and cycloheximide treatment *in vivo* and *in vitro* indicated that laminin B_2 overexpression in cultured hepatocytes was under the control of transcriptional factors which were neosynthesized during liver dissociation. Transfection of deletion mutants of the 5' flanking region of the gene in hepatoma cells demonstrated that a -224 to -94 bp region was involved in gene regulation. This segment contained 3 GC boxes, a CRE-like motif, a sequence potentially interacting with Egr family, and a CCCTCCCATCT- containing motif which bound nuclear factors from both hepatoma cells and normal hepatocytes in foot-printing analysis. Gel shift retardation assays showed that this sequence formed two different complexes, X and Y, with nuclear factors from hepatoma cells and normal livers, respectively. Early appearence of the X complex occurred in hepatocytes during liver disruption in a protein-independent synthesis manner. X complex formation was also detected in fibrotic cholestatic rat livers, while normal adult tissues, namely brain, lung, spleen and kidney contained predominantly the Y complex. Southwestern analysis showed that the X complex contained a Mr = 110-90,000 polypeptide(s), while both southwestern and CCCTCCCATCT affinity chromatography demonstrated that a Mr = 60-50,000 polypetpide(s) formed the Y complex. These data indicate that rapid activation of laminin B_2 gene might require both synthesis of *trans* activators and activation of preexistent transcriptional factor(s) which recognize a CCCTCCCATCT-containing site. This motif is found in the 5' flanking region of various protooncogenes and extracellular matrix genes that are expressed at high levels in both hepatoma cells and early hepatocyte cultures.

EXPRESSION OF LAMININ ISOFORMS IN RABBIT ENDOMETRIUM IS HORMONALLY REGULATED

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Successful embryo-implantation requires proper hormonal stimulation of the uterus in the preimplantation phase. Electron microscopy had revealed a change of the ultrastructure of the epithelial basement membrane in the endometrium of rabbits during this preimplantation phase. Here we demonstrate that these ultrastructural changes might be due to a change in the composition of the laminin molecule. We investigated the expression of the laminin-chains A, B1, S and M by means of indirect immunofluorescence in uteri of non-pregnant, pregnant and of estrogen- and progesterone-treated rabbits. The results demonstrate that the laminin molecule of the epithelial basement membrane is composed of the A-, S-, (and presumably the B2-) chain in non-pregnant animals. Beginning on the second postovulatory day with increasing serum levels of estradiol and progesterone there is a pronounced expression of the M- and the B1-chain which further increases until the 4th postovulatory day and both chains remain detectable at a constant level until beyond the implantation period (day 7-9). At the same time there is some decrease of A- and S-chain expression. Simultaneous there is a marked increase in expression of the B1-chain in the endothelial basement membranes in the endometrium. Treatment with estradiol and progesterone but not with estradiol alone leads to the same expression pattern of the laminin subunits as observed in the preimplantation phase.
Here we could demonstrate for the first time that the composition of the laminin-molecule can be hormonally regulated. Whether this change in composition does influence the epithelal differentiation via a modulation of the adhesive interactions with cellular laminin-receptors will await further investigation.

REGULATION OF THROMBOSPONDIN EXPRESSION IN INFLAMMATION (RHEUMATOID ARTHRITIS AND ENDOMETRIOSIS), Marie-Josée Fournier, Monique La Fleur*, Christian Jobin, André Lemay and Christophe Kreis, Department of Obstetrics/Gynecology, Université Laval, Québec, Canada, G1L 3L5 and *Laboratory of Radiation Biology, UCSF, SF, Ca, 94143

Thrombospndin (TSP) may be an important inflammatory mediator which modulates leukocyte activity at sites of inflammation. Aberrant accumulation of TSP at sites of inflammation may significantly contribute to the development of chronic inflammation. In the short term, the goal of these studies is analyze the regulation of TSP expression in inflammation and the relevance to pathological states. Rheumatoid arthritis (synovial fluid) and endometriosis (peritoneal fluid) were chosen for these studies because chronic inflammation characterizes both disorders and the lesions are characterized by large numbers of neutrophils (95%; RA) and macrophages (80-90%, endometriotic peritoneal fluid). Neutrophils were isolated from the synovial fluid (SF) of patients with RA and matched peripheral blood. Macrophages were isolated from the peritoneal fluid (PF) of women with endometriosis and without endometriosis. Ex vivo, synovial fluid neutrophils and PF endometriosis macrophages accumulate TSP mRNA while PB neutrophils and normal PF macrophages do not. In culture (18 h), SF neutrophils and endometriosis macrophages synthesize TSP while PB cells and normal PF macrophages do not. These results indicate that TSP is controlled differently in RA and endometriosis than in endothelial cells where it is rapidly induced and has a rapid turnover rate. Sequence analysis of the TSP mRNA from neutrophils shows that it is shorter than endothelial TSP mRNA, contains a non-canonical poly A signal, the poly A tract has a length of 27 residues, it lacks the 3'-UTR segment which may be involved in the control of mRNA stability. This truncated transcript may have greater stability than the nontruncated transcript and may lead to aberrant protein expression. Further studies are aimed at understanding the relevance of this type of control to the function of TSP in RA and endometriosis.

REPERFUSION DURING ACUTE MYOCARDIAL INFARCTION INCREASES COLLAGEN DENSITY

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After acute myocardial infarction the ventricle undergoes extensive remodelling and collagen deposition, that may be altered by reperfusion. The present study assesses changes in ventricular collagen network. We studied 3 groups of 10 mongrel dogs each: A (sham operated controls), B (permanent occlusion of LAD) and C (90' occlusion of LAD+reperfusion). The dogs were sacrificed 5 days later. The hearts were arrested in diastole, perfusion fixed, and sliced. Sections from each slice were stained with hematoxylin and eosin or picrosirius red. Infarct size was analysed in relation to baseline variables including the anatomic area at risk, collateral blood flow and hemodynamic determinants of myocardial metabolic demand. Colagen analysis was performed using polarized light microscopy. Using a random sampling morphometric technique the relative (%) density of collagen (DC) in different left ventricular wall segments, for the Internal (I), Middle (M), and External (E) sections were calculated:

	Infarct zone			Transition zone			Normal zone		
	I	M	E	I	M	E	I	M	E
Group A							3.2±1.0	3.7±1.3	5.5±2.6
Group B	13.5±3.6	10.1±3.0	12.9±4.2	12.4±2.9	9.8±3.6	14.1±5.8	8.4±2.2	8.7±3.5	9.4±3.1
Group C	19.7±8.9	18.0±5.7	24.2±3.4	20.0±5.8	19.8±2.5	21.1±5.1	8.3±2.5	8.6±2.4	9.6±1.9
p value	0.049	0.001	0.001	0.002	0.0001	0.011			

No diference in (DC) was found between groups B and C in the normal zone (p= n.s.). However, there was an increase in (DC) in the normal zone in B and C when compared to A ($p<0.0001$). An increase in (DC) was found in the infarct zone and transition zone in group C when compared to B. The perimysial fiber disorganisation seen in group B, is substantially reduced in group C, in the infarct and transition zones.
In conclusion, reperfusion of ischemic myocardium preserves the organization of collagen network and promotes the increase of collagen content in the infarct, and transition zones. These changes are important in maintaining structural integrity after reperfusion.

THE RELATIONSHIP BETWEEN INTERLEUKIN-1 GENE EXPRESSION AND TYPES I AND III PROCOLLAGEN GENE EXPRESSION IN HEALING HUMAN ACUTE WOUNDS

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The integration of the biosynthesis of extracellular matrix molecules and the cellular response seen in the wound healing process is dependent on the coordinated activity of small regulatory peptides. We have examined the expression of the two Interleukin-1 isoforms, IL-1α and IL-1β and the α1(I) and α1(III) procollagens using the reverse transcriptase-polymerase chain reaction technique (RT-PCR) on 6 mm biopsies taken from human surgical excision wounds over a time course from surgery to closure. IL-1s are released from monocytic cells in the early phase of inflammation and are known to increase α1(I) and α1(III) procollagen gene expression in cultured cells via a transcriptionally regulated mechanism. The data generated by this work demonstrated that an increase in IL-1β expression occurs at or before 3 days following surgery with IL-1α expression being delayed and not so marked as that of the β isoform. The increased expression of the α1(III) procollagen seen rapidly after surgical excision seems to be temporally related to IL-1β expression. We are currently examining for IL-α and β expression throughout the time course of healing from surgery to complete clinical healing (8-10 weeks) and will correlate this with the expression of the α1(I) and α1(III) procollagen genes. Future studies will include quantitative analysis of IL-1α and β mRNA using RT-PCR in which deletion mutants of the mRNAs encoding the 2 isoforms are used as external standards.

EPITOPE MAPPING OF MONOCLONAL ANTIBODIES RECOGNIZING HYALURONIC ACID BINDING PROTEOGLYCANS AND LINK PROTEIN, C.E. Hughes[1], A. Calabro Jr[2], J. Stevens[2], V.C. Hascall[2] and B. Caterson[1]; [1]UNC at Chapel Hill, NC 27599, [2]N.I.H., Bethesda, MD 20892.

The specificity of several monoclonal antibodies (MAb) that recognize epitopes in the paired tandem repeat (PTR) domains of cartilage aggrecan and link protein (LP) have been described (1, 2). The antibodies were raised against purified aggrecan and LP isolated from Swarm rat chondrosarcoma (RC). We used PIN technology (Chiron Mimotopes, Australia) to synthesize a large number of *overlapping* 10 amino acid (10-mer) peptides that covered analogous and homologous amino acid sequences within the PTR domains of aggrecan and LP from human, bovine, porcine, rat, mouse, chicken and shark cartilage. These sequences were tested in direct ELISA to determine epitopes, epitope analogs and mimotopes (related cross-reacting sequences) for 8A4, 8A5 and 3B1 (anti LP) and 1C6 and 5C4 (anti G1 or G2 domain of aggrecan). Specificity, redundancy and cross-reactivity of different PIN-derived sequences was further established using ELISA inhibition assays with synthetic peptide homologs and analogs (5- to 10-mers) as competing antigens. All five MAbs had linear epitopes of 5-8 amino acids which were were conserved in cartilage aggrecan and LP from shark to humans. 8A4 and 8A5 recognized a LP specific sequence PISRP or PITKP present in the PTR of LPs. 3B1 recognized a nearby sequence with the link specific tripeptide sequence GSV. 5C4, that recognizes epitopes in both aggrecan and LP, included the amino acid sequences QTVR and RSVR that occur at the apices of the PTR loops of both the G1 and G2 domains of RC-aggrecan. Mimotopes of 5C4 included sequence variants occurring in the PTR loops of LP, rat neurocan and human versican. The epitopes and mimotopes of 5C4 appear to recognize all hyaluronan-binding proteoglycans and LPs. 1C6 recognized a linear 8-mer epitope (QAAYEAGY) in the G2 domain of RC-aggrecan. A low affinity mimotope (QAAYEDGF) was present in the G1 domain.

1. Calabro A., et al, Archiv. Biochem. Biophys, 298, 349 (1992)
2. Caterson B., et al, Articular Cartilage Biochemistry, pp 59-73, Raven Press (1985)

FIBRONECTIN INDUCES LEUKEMIA INHIBITORY FACTOR RECEPTORS ON NEUROBLASTOMA CELLS.

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Leukemia Inhibitory Factor (LIF) has been implicated in the survival and differentiation of neural precursor cells in vitro. We have performed ligand binding assays with radiolabelled LIF on a variety of human neuroblastoma cell lines. These studies did not demonstrate the presence of LIF receptors on neuroblastoma cells. These data were confirmed by RT-PCR for up to 60 cycles. Attachment of neuroblastoma cells on fibronectin coated flasks resulted in the induction of high affinity LIF receptors as determined by ligand binding studies, Northern analysis, RT-PCR and in situ hybridisation. The survival of neuroblastoma cells was decreased significantly for LIF treated microwell cultures coated with fibronectin compared to non LIF-treated fibronectin coated wells. Similar studies performed on primary neuroblastoma cells obtained from biopsy specimens revealed that LIF receptors were induced on these cells by fibronectin. LIF was able to increase differentiation and cell death of neuroblastoma cells in vitro exposed to fibronectin, compared to control cultures. These studies indicate an important role for extracellular matrix components in the differentiation of neuroblastoma cells, and in particular, that fibronectin may lead to upregulation of the LIF receptor protein on the surface of neuroblastoma cells.

EXPRESSION OF THE GENES ENCODING THE PRO-α CHAINS OF HUMAN TYPE XI COLLAGEN IS NOT RESTRICTED TO CHONDROGENIC TISSUES, Vincent C.H. Lui[1], Richard Y.C. Kong[1]*, John Nichols[2] and Kathryn S.E. Cheah[1] Depts of [1]Biochemistry and [2]Pathology, Hong Kong University, Sassoon Rd., Hong Kong.

Type XI collagen, a minor fibrillar collagen component of hyaline cartilage, is a heterotrimer composed of α1(XI), α2(XI) and α3(XI) chains. The human α1(XI) and the α2(XI) chains are encoded by separate genes but the α3(XI) chain is believed to be a variant of the α1 chain of type II collagen. Therefore, α1(II) collagen mRNAs detected in tissues may represent either type II or type XI collagen or a mixture of both. Using RNase protection assays, α1(XI), α2(XI) and α1(II)/α3(XI) mRNAs have been detected in human fetal chondrocytes as well as in non-chondrogenic tissues such as bone, kidney, tendon and the thalamus and caudate nucleus of the brain. However co-expression of mRNAs for all three α chains of type XI collagen was not found in fetal heart, muscle, lung, spleen and the parietal, parietal occipital, temporal and frontal lobes of the brain. In addition in some of these non-chondrogenic tissues co-expression of some or all three type XI collagen mRNAs with α2(V) and α1(I) collagen mRNAs was found. This indicates that the chain composition of type XI collagen molecules in non-chondrogenic tissues may be homotrimers and/or inter type heterotrimers other than α1(XI):α2(XI):α3(XI).

CHANGES IN EXPRESSION OF EXTRACELLULAR MATRIX AND BONE MORPHOGENETIC PROTEINS IN DENTAL PULP CELLS, Misako Nakashima[1], Y. Yamada[2], and A.H. Reddi[3], [1]Department of Conservative Dentistry, Faculty of Dentistry, Kyushu University, Fukuoka. [2]LDBA, NIDR, NIH, Bethesda, MD. [3]Johns Hopkins University School of Medicine, Baltimore, MD.

The potential of dental pulp cells to differentiate into odontoblasts is well known. The local and systemic signals that regulate pulp cell differentiation is not well understood. Previous work has demonstrated bone morphogenetic protein-2 (BMP-2) expression by in situ hybridization in dental papilla and odontoblasts. Further, demineralized tooth matrix extracts induce differentiation of dental pulp cells into an odontoblast-like phenotype in vivo and in vitro. We have investigated the gene expression of extracellular matrix components and BMPs during odontoblastic differentiation of adult bovine pulp cell cultures. The four stages of odontoblastic differentiation in vitro are: proliferation (1 wk), matrix maturation (2 wk), multi-layered appearance (3 wk) and initial mineralization (4 wk). Total RNA was isolated at the initial time point (0 wk) and at 1,2,3, and 4 weeks and the steady state levels of mRNA for collagens I, III, fibronectin, alkaline phosphatase, osteocalcin, BMP-2, BMP-4 and TGF-β_1 using ^{32}P labelled cDNA probes was examined by Northern blots. During proliferation (1 wk) expression of collagen III and fibronectin decreased while collagen I increased. The expression of collagen I, III, fibronectin and BMP-4 increased at 2 wks. By 3 wks (multi-layered stage) alkaline phosphatase was increased while TGF-β_1 expression declined. During mineralization (4 wk) BMP-2 expression and osteocalcin begin to increase. These results demonstrate the stage-specific control of gene expression during odontoblastic differentiation from pulp cells.

HUMAN FETAL INTESTINAL SMOOTH MUSCLE CELLS SECRETE TGF-ß AND SYNTHESIZE COLLAGEN WITHOUT CELL PROLIFERATION. Hilary A. Perr and Danna Johnson. Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of California, San Francisco, CA 94143 and the Department of Pathology, Medical College of Virginia, Richmond, VA, 23298.

Collagen production by intestinal muscle decreases with fetal age. Its regulation is better understood in the adult human whereTGF-ß selectively increases collagen synthesis, but serum enhances both cell proliferation and collagen production. Human intestinal smooth muscle cells were isolated at 11 to 20 weeks gestation and grown in tissue culture in 0%and 10% fetal calf serum (FCS). Collagen synthesis was assayed by measuring the uptake of 3H-proline into collagenase-digestible protein. Cell proliferation was determined by measuring DNA content fluorometrically. In 0% FCS, muscle cell collagen synthesis increased without changes in cell proliferation and was 50% greater than in 10%FCS. Dermal fibroblasts isolated from the same fetuses produced the same amounts of collagen in 0 and 10% FCS. Immunohistochemical methods were used to identify TGF-ß within the fetal intestinal wall and in isolated smooth muscle cells in vitro. The distribution of TGF-ß1 and 2 in muscle layers did not change with fetal age. Cultured muscle cells stained for TGF-ß, but primarily in subconfluent cells. TGF-ß biologic activity in muscle cell conditioned medium and cell homogenates was evaluated using the mink lung epithelial (CCL-64) cell growth inhibition assay. TGF-ß production by muscle cells decreased with fetal age. 340% more TGF-ß was produced at 11 weeks than at 20 weeks gestation. The majority of the TGF-ß was found in the medium and activated within 48 hours. Therefore, fetal muscle collagen synthesis differs from that of adult muscle and fetal fibroblasts in that it increases without added serum factors and without proliferation. These are the first studies to show that fetal human intestinal smooth muscle cells uniquely activate their own TGF-ß and that secretion is a function of subconfluent cells. Studies are in progress to evaluate how changes in endogenousTGF-ß regulate fetal muscle collagen synthesis in an age and cell type specific manner. NIH grant DK-01849.

VARIATION IN THE CONCENTRATION OF ANIONS IN Hymenolepis sp. (Rattus norvegicus) IN ITS MICROENVIRONMENT - AN IN-VITRO ANALYSIS. **Shabad Preet, A.K.Sinha, S.S.Srivastava & Soam Prakash, Environmental Parasitology Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra -282005, INDIA.**

In the present study, variations in fluoride, acetate, chloride, nitrate and sulphate ions were observed in the microenvironment developed in and around the cyst as well as in the parasite recovered from the developing cyst of Hymenolepis sp. obtained from the liver of Rattus norvegicus. Dionex Ion Chromatography technique was employed to analyse the anions contributing to these microenvironments of the parasites. Transport of anions from the cyst into the medium, and also from the worm into the same cystic fluid has been analysed. A relationship of cyst weight and the size of worm with that of anionic variation in concentration was established.