EXTRACELLULAR MATRIX: STRUCTURE AND FUNCTION

A. H. Reddi, Organizer

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Plenary Sessions

Poster Sessions

Collagen Structure — Chemistry

1532 **COLLAGEN CHEMISTRY: ANSWERS AND QUESTIONS**, Karl A. Piez, Connective Tissue Research Laboratories, Collagen Corporation. Palo Alto, CA 94303

Eight collagen types have been well enough defined in vertebrate species to be sure that they are genetically, structurally, and functionally distinct. Together they are composed of sixteen unique polypeptide chains. Some of the types have more than one form as the result of different distributions of chains within the triple-chain collagen molecules. This diversity is still not complete; more collagen types are yet to be characterized. The collagen triple-helix, common to all collagens of course, is well defined. The non-collagenous portions of types I, II, and III collagen have also been characterized. Much less is known about the non-collagenous portions of the other collagens.

The aggregate structure common to types I, II, and III collagen, the characteristic banded fibril, has received a lot of attention and is well understood, even though some details remain controversial. The aggregate forms of the other collagens are different and apparently all unique. Since the aggregate forms of the collagens are the functional forms, this information is important.

All of the collagens are, of course, extracellular and play predominantly structural roles. But there seems to be an overwhelming amount of information and a complexity that is hard for even the expert to grasp. There are more answers than questions. There is a need to look for simplifying concepts.

From a protein chemical point of view, there are two concepts that are useful. First, the triple-helix is the structural unit. The length of this unit varies among the eight known collagen types from about 100 nm in type VI collagen to about 450 nm in type VII. The interstitial collagens, types I, II, and III, and also type V collagen, are intermediate at 300 nm. Type IV shows another variation where the helix is about 380 nm long but is interrupted to give hinged or segmented structural units of varying length not yet well characterized. The smallest, at the N-terminal end, is about 60 nm long. Type VIII also seems to have a segmented helix as long as or longer than type IV.

The second general concept is that the nonhelical portions of the collagens are the sites of specific interactions that stabilize the native aggregate. This feature is best understood in the case of types I, II, and III collagen where parallel, staggered molecules form a periodic fibril which is stabilized first by interactions and then by covalent crosslinks originating in the nonhelical ends of the molecules. In type IV collagen, N-terminal ends associate in fours and C-terminal ends in twos to form a mat-like structure. The chemistry of the association is yet to be defined. Type VI collagen associates in a more complex way with molecules apparently both aligned and parallel and staggered and antiparellel, but again, nonhelical regions seem to regulate the necessary interactions. The native aggregates of types V, VII, and VIII collagens have yet to be identified. Type VIII likes to form overlapped and antiparallel dimers which could bear some relationship to the native aggregate.

Thus the collagens can be viewed as a family of molecules in which variations in the length of the triple helix and the chemistry of the nonhelical regions modulate the nature of the native aggregate. In this way, a variety of structural functions can be served.

1533 TYPE VIII COLLAGEN: DISTRIBUTION, CHARACTERISTICS, AND COMPARISON WITH OTHER MEMBERS OF THE COLLAGEN FAMILY. Helene Sage, Department of Biological Structure, University of Washington, Seattle, WA 98195

At least seven structurally distinct collagen types have been characterized and include the "interstitial" collagens (types I, II, and III), basement membrane collagen (type IV), and pericellular/stromal collagen (type V). Type VI (intima) collagen and type VII ("long-chain") collagen have been recently described (1,2), as well as endothelial collagen (EC), a pepsin-sensitive protein synthesized by endothelial (3,4), and human astrocytoma cells (5).

Examination of several cell strains derived from normal tissues revealed that EC was absent from human endothelial cells cultured from both large and small vessels, but was present in bovine cells, including those from capillaries. Human foreskin fibroblasts also secreted this protein in small amounts relative to interstitial procollagens, but it was not detected in two human epithelial cell strains. EC was observed in human cell lines derived from several carcinomas and comprised the major collagenous protein secreted by cells cultured from a Ewing's sarcoma. In contrast, malignant or transformed murine cells did not produce EC *in vitro*, and the protein was not apparent after metabolic labeling of human cells from an epidermoid carcinoma, a fibrosarcoma, and two Wilm's tumors.

Pulse-chase experiments with bovine aortic endothelial cells revealed two nondisulfide bonded collagens, of apparent chain M_r 177,000 and 125,000, with an estimated synthesis and secretion time of 75 min. Stepwise, quantitative processing to stable, lower molecular weight forms as described for type I procollagen was not observed. EC was secreted over a temperature range of 24°-37°C and, prior to heat denaturation, did not display affinity for a gelatin-binding fragment of fibronectin coupled to Sepharose. The presence of a pepsin-resistant domain (M_r 50,000) in both the soluble and cell layer-associated forms of this protein was shown by ion-exchange chromatography and SDS-PAGE. EC was cleaved by vertebrate collagenase into several peptides that differed in molecular weight from the characteristic α^A and α^B fragments generated from the interstitial collagens. Nontriple helical domains corresponding to the N- and C-terminal propeptides of other procollagen types were not found after incubation of endothelial collagen with bacterial collagenase.

In view of this unique set of structural characteristics, and a distribution that is not restricted to the endothelium, we have designated this protein as type VIII collagen.

1. Furthmayr *et al.* (1983) Biochem. J. 211:303. 2. Bentz *et al.* (1983) Proc. Natl. Acad. Sci. USA 80:3168. 3. Sage *et al.* (1980) Biochemistry 19:5747. 4. Benya, P. (1980) Renal Physiol. 3:30. 5. Alitalo *et al.* (1983) J. Biol. Chem. 258:2653.

1534 STRUCTURE AND BIOLOGICAL PROPERTIES OF BASEMENT MEMBRANE COLLAGEN (TYPE IV), Rupert Timpl, Helga von der Mark, Monique Aumailley, Sakuhei Fujiwara, Georg Wick, Sabine Weber, Jürgen Engel, Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG; University of Innsbruck, Institut of experimental Pathology, Austria, and Biozentrum, University of Basel, Switzerland.

Collagen type IV (m.w. 550,000, length 390 nm) is a protein unique to basement membranes. It consists of two larger non-collagenous domains (NC1, NC2), a major triple helix and a short triple helical segment (7S domain). The major triple helix is rather flexible due to several conformational interruptions. The protein associates into network-like structures using the terminal domains (7S, globule NC1) for interactions (1). The interacting globular domain is a hexameric structure which can be reversibly dissociated into monomeric and dimeric subunits. These interactions are initially established by weak non-covalent binding and are then stabilized by covalent bonds (disulfide bridges, non-reducible cross-links). The various domains possess in addition different biological and immunological properties. The terminal structures show weak binding activity for heparan sulfate proteoglycan. The central triple helical domain can interact with fibronectin and promotes the adhesion of cells. Cell binding to the triple helix does not depend on fibronectin or laminin. The antigenically most important regions are found in the 7S and globular domain. Previous indications (2) that the Goodpasture autoantigen is related to the globule NC1 could be confirmed with authentic hexameric structures. The autoantibodies react with all subunits but different tissues (kidney, lung, placenta, mouse EHS tumor) possess variable amounts of the autoantigenic structures. This points to a possible structural heterogeneity in the globular domain. Immunization of mice with the globule produces a distinct autoantibody response associated with the destruction of renal and alveolar basement membranes. Similar observations are made after injecting antibodies against the globule into mice. Thus the collagen IV globule allows to establish experimental models for autoimmune lung and kidney diseases.

1) Timpl, R., Wiedemann, H., van Delden, V., Furthmayr, H. and Kühn, K. (1981) Eur.J.Biochem. 120, 203-211.
2) Wieslander, J., Bygren, P. and Heinegard, D. (1983) Proc.Natl.Acad.Sci.USA in press.

1535 NEW COLLAGENS OF CARTILAGE, Richard Mayne, Michel van der Rest and Darrel C. Weaver, Departments of Anatomy and Biochemistry, University of Alabama in Birmingham, Birmingham, AL 35294, USA and Genetics Unit, Shriners Hospital for Crippled Children, Montreal H3G 1A6, Canada.

Several new collagens have recently been isolated from pepsin digests of hyaline cartilage and fractionated from type II collagen (reviewed in 1). The 1α and 2α chains appear to be the cartilage-equivalent of the α1(V) and α2(V) chains of type V collagen, whereas the 3α chain is very similar to the α1(II) chain (2,3). Recent experiments have shown that after growth of chick chondrocytes in BrdUrd, or growth of a clone of chondrocytes to senescence, the synthesis of 1α and 2α chains will switch to the synthesis of α1(V) and α2(V) chains (4). Two additional collagenous fragments, which possess interchain disulfide bridges, have been isolated from chicken sterna and called HMW and LMW (3). LMW, after reduction and alkylation of cysteine residues, was separated into four fractions by CM-cellulose chromatography. The fractions were designated peaks 1, 2A, 2B and 3. Amino acid compositions of each fraction and peptide mapping after cleavage with CNBr, trypsin and V8 protease showed that at a minimum three genetically distinct chains must be present in LMW. Peaks 2A and 2B were closely related, but some microheterogeneity was observed during the separation of tryptic peptides by reversed phase HPLC. Peaks 1, 2A+2B and 3 were consistently obtained in a 1:1:1 chain ratio, suggesting that the probable chain organization of LMW is (1)(2A+2B)(3). Previously, HMW was shown to contain at least two genetically distinct chains, one of which was cleaved during pepsin digestion, giving rise to a characteristic kink in molecules observed after rotary shadowing (5). Recent experiments involving cleavage of HMW at cysteine residues after S-cyanylation demonstrated cleavage only at the kink region, suggesting that this site may be stabilized by interchain disulfide bridges. The chain of HMW, previously designated C-2 (3), was further fractionated by reversed phase HPLC into two peaks designated C-2A and C-2B. Peptide mapping experiments indicated that these two peaks are derived from different chains, and that HMW also contains at least three chains. The present results suggest that HMW and LMW may both be pepsin-resistant fragments of a larger collagen molecule in which the collagenous domains are interspersed by short non-collagenous domains containing cysteine residues.

1. Mayne, R. and von der Mark, K. (1982) In: Cartilage (Hall, B.K., ed.) Vol. 1. pp. 181-214, Academic Press, New York. 2. Burgeson, R.E., Hebda, P.A., Morris, N.P. and Hollister, D.W. (1982) J. Biol. Chem. 257:7852-7856. 3. Reese, C.A. and Mayne, R. (1981) Biochemistry 20:5443-5448. 4. Mayne, R., Elrod, B.W., Mayne, P.M., Sanderson, R.D. and Linsenmayer, T.F. (1984) Exp. Cell Res. In press. 5. Reese, C.A., Wiedemann, H., Kühn, K. and Mayne, R. (1982) Biochemistry 21:826-830. Supported by N.I.H. AM 30481.

Collagen Structure — Biophysics

1536 STRUCTURAL STUDIES ON COLLAGEN FIBRILS, A. Miller, Department of Biochemistry, University of Edinburgh, UK: J. Bradshaw and E.Y. Jones, Laboratory of Molecular Biophysics, University of Oxford, UK: R.D.B. Fraser, T.P. Macrae and E. Suzuki, CSIRO Division of Protein Chemistry, Melbourne, Australia.

Recent work on the molecular structure of collagen fibrils will be described. X-ray diffraction patterns from treated tendons have been of adequately high quality to allow definition of the full three-dimensional unit cell. In addition the molecular arrangement of the ordered part of the fibril is revealed as quasi-hexagonally packed parallel molecules, tilted in a specific direction at about 4° to the fibril axis. The ordered parts are determined to be about 34 nm long and some information is obtained about the azimuthal packing of the helical molecules by analysis of the 9.5Å layer-line. Analysis of the 4.1Å layer-line and model building by computer graphics are now being used to investigate the precise axial register of the molecules.

Meridional X-ray diffraction patterns are also being used to compare the one-dimensional structures of normal and diseased collagen (Fibrogenesis Imperfecta Oseum; Osteogenesis Imperfecta) as well as the structures of fibrils containing collagens of different genetic types.

Brillouin scattering has been used to measure the microscopic elastic moduli of unmineralised and mineralised connective tissue with a view to relating the molecular structure to the mechanical (and hence biological) properties of these tissues.

Hulmes, D.J.A. and Miller, A. (1979) Nature 282, 878-880.
Fraser, R.D.B., Macrae, T.P., Miller, A. and Suzuki, E. (1983) J. Mol. Biol., 167, 497-521.
Cusack, S. and Miller, A. (1979) J. Mol. Biol., 135, 39-51.

1537 MOLECULAR STRUCTURE AND MODELS OF COLLAGEN IN VITRO FIBRIL ASSEMBLY, Arthur Veis, Department of Oral Biology, Northwestern University, Chicago, Illinois 60611

Most models of collagen self-assembly into fibrils treat the collagen molecule as a rigid rod with a fixed conformation and seek to pack these rigid structures into a highly ordered array. New evidence suggests that such models are no longer tenable. It is now evident that the interstitial collagens are multidomain structures and each domain has been shown to have a distinctive role in in vitro fibrillogenesis. Even in compact globular protein domains, individual backbone atoms move about their average positions in a fluidlike manner. These local motions can lead to large-scale changes in relative domain positions, and have a time constant typical of Brownian motion, picoseconds. In the collagen molecule, such behavior is present in the collagen helix region as well as within the telopeptide domains. Hydrodynamic measurements at pH 7.4, 0.15 ionic strength show that monomeric collagen cannot be modeled as a rigid rod. The molecule has an effective length of 220 ± 10nm, and can be depicted as a semiflexible rod with a persistence length of about 170nm. Although the viscoelastic data are difficult to interpret in terms of specific hinge regions, all the data taken together suggest that a few flexible regions might exist as also indicated by rotary shadowing data. The major regions proposed correspond to the sequences of residues 81-102 and 916-939 in the α1(I) chain. These are also the regions which are the binding receptors for the carboxyl-and amino-telopeptides, respectively.

Studies of the ^{1}H-NMR spectra of the tyrosyl residues in the telopeptides (α1(I)CB1, α1(I)CB6) in D_2O solutions show the COOH-telopeptide to have a stable structure in which the tyrosyl residues are buried or restricted even at 60°C. In contrast, the NH_2-telopeptide is conformationally labile. During interaction with its triple-helix-region receptor, the NH_2-terminal region structure becomes fixed and there is a corresponding decrease in the mobility of the ^{1}H-NMR resonance of the receptor region phenylalanine. Thus, the interaction which registers collagen molecules also stabilizes the helix and telopeptide regions in a cooperative fashion. This new model implies that collagen monomers have substantial local elastic motions in specific regions and large-scale flexural deviation from rigidity. While nascent and newly synthesized collagen chains may have considerable structural order immediately upon assembly into the three-chain unit in vivo, complete stability may not be achieved until either secretion or accumulation in secretory vesicles. Assembly into D D packages intracellularly may be one route to accomplish the perfection of the triple helix. Supported by NIH Grant AM 13921.

Biosynthesis of Collagen

1538 REGULATION OF COLLAGEN BIOSYNTHESIS. Paul Bornstein, Departments of Biochemistry and Medicine, University of Washington, Seattle, WA 98195

The regulation of collagen production by cells poses an interesting problem in cell biology. How do cells gauge an appropriate level of synthesis of a protein that is secreted, modified and rapidly insolubilized? How are rates of synthesis, intracellular degradation and extracellular turnover coordinated? How do these regulatory processes interface with mechanisms for differential expression of different collagen types by the same cell?

Since normal physiologic mechanisms have been difficult to discern, attention has focused on experimental manipulation of cells in culture and on studies of genetic disorders of collagen metabolism. Reduced levels of collagen messenger RNA have been demonstrated in Rous sarcoma virus transformation of fibroblasts (1,2) and chondrocytes (3) and following addition of phorbol esters to mouse epidermal cells (4). Since the transforming gene product of Rous sarcoma virus, pp60^{src}, is a protein kinase and phorbol esters stimulate protein kinase C, phosphoprotein(s) may mediate the inhibitory effect in the nucleus. Translational control of collagen synthesis by procollagen-derived fragments has been demonstrated both in cells (5) and in a cell-free translation system (6,7). If both effects are mediated in the same manner, a means must exist for transfer of these fragments across membranes and into the cytosol. Translational control of collagen synthesis has also been inferred from studies of rapidly growing and quiescent cells in culture (8). It is well known from a wide variety of studies that interference with co- and posttranslational modifications of collagen inhibits secretion of the protein and leads to increased intracellular degradation. Presumably, a stable triple helix is required for efficient secretion of some (but not all) collagens. A variety of genetic disorders that affect abnormal collagen structure appear to lead to intracellular retention and turnover of the collagens. Some of these disorders may affect regulatory steps primarily and these could be fruitful for study.

A number of other factors are known to influence the relative rate of synthesis of different collagens (collagen type-switching) by cells in culture. We have recently shown that rat smooth muscle cells can be induced to synthesize a low molecular weight collagen (60 K) by treatment with heparin (9). This finding provides additional evidence for the importance of cell surface interactions in determining the biosynthetic phenotype of cells.

1. Sandmeyer, S., et al., J. Biol. Chem. 256:5022 (1981). 2. Sobel, M. E., et al., Biochemistry 20:2678 (1981). 3. Adams, S. L., et al., Cell 30:373 (1982). 4. Sobel, M. E., et al., Mol. Cell. Biochem. 3:1527 (1983). 5. Wiestner, M., et al., J. Biol. Chem. 254: 7016 (1979). 6. Paglia, L., et al., Biochemistry 18:5030 (1979). 7. Hörlein, D., et al., Proc. Natl. Acad. Sci. USA 78:6163 (1981). 8. Tolstoshev, P., et al., J. Biol. Chem. 256: 3135 (1981). 9. Majack, R. A., and Bornstein, P., J. Cell Biol. 97:2a (1983).

1539 BASEMENT MEMBRANE COLLAGEN BIOSYNTHESIS, J.H. Fessler, B. Blumberg, K.J. Doege, J.P. Petschek, and L.I. Fessler, Molecular Biology Institute and Department of Biology, University of California, Los Angeles, CA 90024

Separately from a review of collagen synthesis at the protein level (1a,1b,2), the following is a summary of some work in this laboratory. Similarity of carboxyl propeptides of different proα chains (3,4) indicates some common functions, and they probably assist the initial selection and assembly of three chains into molecules. Reassembly of individual, unfolded carboxyl propeptides I into disulfide-linked trimers (5) reaffirms their ability for selective interaction (6,7). Surprisingly, however, the folded, native carboxyl propeptides do not bind strongly to each other, both in procollagen I and IV. As a hypothesis we suggest that interaction of the carboxyl propeptides with the endoplasmic reticulum facilitates selection and longitudinal alignment of proα chains. The carboxyl propeptides of basement membrane procollagen IV are not disulfide-linked to partners within a triplet (8) but link, extracellularly, to carboxyl propeptides from another molecule (9). The amino end region of procollagen IV is internally disulfide-linked, and the ends of four molecules associate *in vitro* as the 7S junctional complex (10). We have now obtained the equivalent disulfide-linked tetramer of procollagen IV molecules from *in vivo* labeled basement membranes and conclude that it is a physiological intermediate of assembly in several tissues. Basement membranes of Drosophila contain a similar procollagen, also disulfide linked within that end of the molecule, and also disulfide linked between these ends of molecules into dimers and tetramers. Basement membrane collagens and their interactions with other components have therefore been conserved evolutionarily (11). The onset of basement membrane procollagen synthesis during Drosophila development is at the time of organogenesis and is linked to the start of synthesis of Drosophila laminin and other basement membrane components. However, more than one cell type is probably involved in the synthesis of basement membranes. In an established tissue, mammalian liver, cell regeneration is coupled with the synthesis of procollagen IV. Therefore initiation of basement membrane collagen synthesis, both at first occurrence in embryogenesis and during repair, is linked to differentiation and replication of adjacent cells. (1a) Prockop, D.J. (1979) New Engl. J. Med 301, 13, 77; (1b) Cunningham, L.W. et al. (1982) Meth. Enzym. 82; (2) Grant, M.E. et al., (1981) Bioscience Reports 1, 819; (3) Yamada, Y. et al. (1983) Nuc. Acids Res. 11, 2733; (4) Bächinger, H.P. et al. (1982) J. Biol. Chem. 257, 14590; (5) Doege, K.J. et al. (1983) J. Cell. Biochem., Suppl. 7B, 377; (6) Fessler, L.I. et al. (1974) J. Biol. Chem. 249, 7637; (7) Bächinger, H.P. et al. (1981) J. Biol. Chem. 256, 13193; (8) Fessler, L.I. et al. (1982) J. Biol. Chem. 257, 9804; (9) Timpl, R. et al. (1981) Eur. J. Biochem. 120, 203; (10) Duncan, K.G. et al. (1983) J. Biol. Chem. 258, 5869; (11) Fessler, J.H. et al. (1983), in press, Natl. Meeting of Amer.

1540 REGULATED EXPRESSION OF COLLAGEN GENES IN NORMAL AND TRANSFORMED CELLS, Benoit de Crombrugghe, Azriel Schmidt, Catherine McKeon, Gene Liau and Ira Pastan, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, MD

One of the changes that is caused by the presence of active oncogenes in fibroblasts is a large decrease in type I collagen synthesis. This reduction is mediated by a transcriptional control mechanism. Indeed, isolated nuclei from transformed cells synthesize much less type I collagen RNA than untransformed cells.

The promoter of the α2(I) type I collagen gene shows typical features of other eucaryotic promoters. These include a TATA box, a CAT box and a discrete site that is very sensitive to nuclease S_1 when the DNA of the promoter is in supercoiled form. Furthermore, in chromatin of fibroblasts this promoter contains a site that is highly sensitive to DNase I. This hypersensitive site is not found in tissues which do not express the gene. However, in chick fibroblasts transformed by Rous Sarcoma virus the same hypersensitive site is present. Hence, transformation, which greatly reduces the rate of transcription of this gene, does not cause a change in the DNase hypersensitive site in the promoter.

We have constructed cell lines in which a hybrid transcription unit consisting of the α2(I) collagen promoter linked to the bacterial chloramphenicol acetyltransferase (CAT) gene is stably integrated in the genome of mouse fibroblasts. In these cells the CAT gene is transcribed from the α2(I) collagen promoter. The levels of CAT enzyme or CAT RNA decrease after these cells are transformed by mouse Moloney Sarcoma virus. This decrease is similar to the reduction in the levels of endogenous mouse α2(I) collagen RNA. This result suggests that the cloned α2(I) collagen promoter after it has been introduced in mouse cells responds to the same type of regulatory mechanisms as the endogenous α2(I) collagen promoter.

1541 POST-TRANSLATIONAL MODIFICATIONS, Kari I. Kivirikko, Collagen Reseach Unit, University of Oulu, Department of Medical Biochemistry, SF-90220 Oulu 22, Finland

Collagen biosynthesis involves a large number of post-translational modifications, many of which are unique to collagens and a few other proteins (1). The co-translational and post-translational events that occur within the cells include removal of the pre-protein sequences, hydroxylation of certain proline and lysine residues to 4-hydroxyproline, 3-hydroxyproline and hydroxylysine, glycosylation of appropriate hydroxylysine residues to galactosylhydroxylysine and glucosylgalactosylhydroxylysine, addition of a mannose-rich oligosaccharide to the C-terminal propeptides, association of the C-terminal propeptides through a process governed by the structure of these domains, formation of intra-chain and inter-chain disulphide bonds, and folding of the polypeptide chains into the triple-helix. The procollagen molecules are then secreted into the extracellular space and the subsequent events consist of the conversion of procollagen to collagen, catalyzed by separate procollagen N-terminal and C-terminal proteinases, self-assembly of the collagen into fibres, and oxidative deamination of certain lysine and hydroxylysine residues to aldehyde derivatives which form collagen cross-links. These modifications require at least eight specific enzymes and several non-specific enzymes, most of the enzymes and their corresponding reactions being now well characterized. One of the enzymes, procollagen N-terminal proteinase, has been shown to possess collagen type-specific isozymes, whereas most of the other enzymes appear to act on many different collagen types. The functions of many of the post-translational modifications have likewise been elucidated in great detail, and some information is available on the regulation of the rates and extents of the reactions. The latter data will form a basis for an understanding of the marked differences that are found in the extents of the modifications between the genetically distinct collagen types and even within the same collagen type in various physiological and pathological states.

1. Kivirikko, K.I. & Myllylä, R. (1982) in Collagen in Health and Disease (Weiss, J.B. & Jayson, M.I.V., eds.), pp. 101-120, Churchill Livingstone, Edinburgh.

Elastin

1542 BIOSYNTHESIS OF ELASTIN AND CHARACTERIZATION OF ELASTIN cDNA, Joel Rosenbloom, Kyonggeun Yoon, *Louise Oliver and *Charles Boyd, Center for Oral Health Research, Dental School, U. of Pa., Phila. PA 19104 and *MRC Unit for Molecular and Cellular Cardiology, U. of Stellenbosch Medical School, Tygerberg, 7505, South Africa

The 72,000 dalton polypeptide, tropoelastin, containing a signal sequence is the primary translation product of elastin mRNA and it is a soluble intermediate in the biosynthesis of the insoluble fiber (1). Immuno electron microscope studies have demonstrated that tropoelastin secretion follows the classical pathway via the Golgi apparatus and secretory vesicles. A chick elastin cDNA clone containing 3' non-translated sequences has been used to identify and to estimate the relative amounts of elastin mRNA in the developing chick aorta by blot hybridization. A single mRNA species of 3.5 kb hybridized to the cDNA probe and this species increased greatly between day 7 and day 14. When these levels were compared to functional elastin mRNA measured by translation in a rabbit reticulocyte lystate system and to the rate of elastin synthesis in freshly isolated aortas of various ages incubated in vitro, the results suggested that the changes in elastin synthesis seen during development are governed by the elastin mRNA content of the aorta (2). Similar results were found when the levels of functional elastin mRNA in the developing sheep lung and ligamentum nuchae were compared to the rates of elastin synthesis in the tissues (3). Since tropoelastin contains about 830 amino acid residues, approximately 1 kb of the 3.5 kb elatin mRNA are untranslated. We have recently isolated and sequenced a 1.2 kb sheep elastin cDNA which contains a 150 bp coding sequence at its 5' end including 48 bp which code for an amino acid sequence identical to a published sequence found in porcine tropoelastin. The GC content of the translated region was a remarkably high 72% and comparable to that of the collagen α chains. In contrast to the glycine codon usage found in collagen in which there is a strong preference for a pyrimidine in the third position, in the elastin translated sequence a purine was found preferentially.

1. Burnett, W., Eichner, R. and Rosenbloom, J. (1980) Biochemistry 19, 1106-1111.

2. Burnett, W., Finnigan-Bunick, A., Yoon, K. and Rosenbloom, J. (1982) J. Biol. Chem. 257, 1569-1572.

3. Davidson, J.M., Smith K., Shibahara, S., Tolstoshev, P. and Crystal, R. (1982) J. Biol. Chem. 257, 747-754.

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1543 EXTRACELLULAR MATRIX PROMOTES ELASTOGENIC DIFFERENTIATION AND CHEMOTACTIC RESPONSIVENESS TO ELASTIN IN LIGAMENT FIBROBLASTS, Robert P. Mecham and Robert M. Senior, Respiratory Disease and Critical Care Division, Department of Medicine, Jewish Hospital at Washington University Medical Center, St. Louis, MO. 63110.

Bovine ligament fibroblasts produce elastin and show chemotaxis to elastin-derived peptides. To assess the mechanisms that regulate elastin gene expression and chemotactic responsiveness to elastin during development, we studied developing bovine ligamentum nuchae fibroblasts. Undifferentiated (non-elastin producing) ligament cells from early gestation animals (95-140 days gestation) initiate elastin synthesis when grown on extracellular matrix prepared from intact ligament from a 270 days gestation fetus. Matrix-induced differentiation requires direct cell-matrix interaction, is dependent upon cell proliferation after cell-matrix contact, and can be blocked selectively by incorporation of bromodeoxyuridine (BrdU) into the DNA of undifferentiated cells prior to (but not after) contact with inducing matrix. Matrix-induced cells continue to synthesize elastin when removed from ECM substratum, suggesting that the young cells have undergone a stable phenotypic transition in response to the matrix and that elastogenic differentiation is guided by inductive interactions between the ligament cell and its own secreted extracellular matrix.

To determine if development of chemotactic responsiveness to elastin parallels expression of the elastin phenotype, we compared chemotactic activity in young ligament cells before and after differentiation was induced by ECM. Before induction, young cells demonstrate chemotaxis to two known chemoattractants for fibroblasts [fibronectin and platelet-derived growth factor (PDGF)] but do not show chemotaxis to elastin peptides. Following matrix-induced differentiation, however, young cells display a positive chemotactic response to elastin peptides that persists even after the cells are removed from the matrix substratum. Similar to induction of elastin synthesis by ECM, the onset of chemotactic responsiveness to elastin in response to ECM induction can be blocked selectively with BrdU if incorporated into cellular DNA before contact with inducing ECM. These results suggest that ligament ECM provides signals of differentiation for ligament fibroblasts and that expression of the elastin phenotype and acquisition of chemotactic responsiveness to elastin are affected by the same inducing elements or processes and are closely coupled in development.

Proteoglycans I

1544 PROTEOGLYCANS - AN OVERVIEW, Klaus E. Kuettner, Department of Biochemistry, Rush Medical College, Chicago IL 60612.

Proteoglycans are defined as macromolecules which contain one or more glycosaminoglcyan chains covalently bound to a core protein. This definition encompasses many structures serving a variety of functions. Different classes can vary in the nature and size and degree of sulfation of the glycosaminoglycan chains, and in the type(s) and number of oligosaccharides present on the core protein(s). Further, the final structure of a proteoglycan depends upon the coordination of extensive post translational steps resulting from most of the known biosynthetic pathways involved in oligosaccharide and glycosaminoglycan synthesis. Thus, regulation or modulation of any of these pathways can greatly influence the final structures and functions of these macromolecules.

Most proteoglycans are extracellular in location. A variety of classes are present in different tissues with certain connective tissues such as cartilage being particularly enriched. The chemical structures of proteoglycans are responsible for their distinct physical properties which influence the characteristics of the tissues in which they are found. The hydrophilic glycosaminoglycan chains are relatively stiff and extended in solution and therefore resist compressive load. With many glycosaminoglycan chains per core protein, proteoglycans are branched structures with high charge density, forming macromolecules that occupy large domains. Within the tissue, their domains are often partially compressed, excluding other macromolecules, but permeable to small molecules. In some connective tissues (e.g., cartilage) most proteoglycans exist in the form of aggregates of high molecular weight formed by the non-covalent interaction of monomers with a single molecule of hyaluronic acid stabilized by link protein(s). These macromolecular structures are considered to have biological functions such as involvement in matrix assembly, regulation of matrix synthesis, regulation of matrix breakdown, and possibly selective binding of metal ions (e.g. during calcification) and other positively charged small molecules. Other biologic functions have been ascribed to the heparan sulfate proteoglycans associated with or embedded within the plasma cell membranes and basement membranes such as regulation of cell attachment, cell-cell and cell-matrix interactions, and permselectivity in glomerular filtration. Future work will have to define to what extent these selective biologic functions can be ascribed to specific glycosaminoglycan chains or to the structure of the protein core and its associated oligosaccharides. More will be learned about the functions of the proteoglycans in tissues when we understand better such processes as osteoarthrosis, platelet aggregation and adhesion induced pathogenesis of arteriosclerosis, osteoarthrosis, or other diseases associated with abnormalities of proteoglycan metabolism. It has become clear, however, that proteoglycans play a major role in tissue maturation as well as histogenesis and organogenesis.

1545 STRUCTURE OF THE CORE PROTEIN OF THE RAT CHONDROSARCOMA PROTEOGLYCAN, Vincent Hascall and Jeff Stevens, Mineralized Tissue Research Branch, NIDR, NIH, Bethesda, MD 20205

Extensive clostripain digestion of rat chondrosarcoma proteoglycan aggregate yields a limit complex of hyaluronic acid-binding region (HA-BR-65) and link protein (LP-43) bound to hyaluronic acid. N-Terminal amino acids in purified complex were selectively 14-C-carbamylated. SDS-PAGE resolved derivatized HA-BR-65 (∿65,000) from derivatized LP-43 (∿43,000). These labeled bands were isolated, digested with trypsin and developed on 2-dimensional TLC sheets (1). Each yielded a distinct, major labeled spot. That from HA-BR was about 10,000 MW on SDS-PAGE. When 14-C-carbamylated complex was digested with chymotrypsin, HA-BR-65 was converted to HA-BR-55 (∿55,000) without loss of label, indicating that the peptide(s) removed was from the carboxy terminal region of HA-BR-65. Partial clostripain digests of aggregates were 14-C-carbamylated and analyzed on SDS-PAGE. Direct analysis revealed a prominent labeled, Coomassie blue stained band at about 109,000 MW (HA-BR-109). After chondroitinase digestion, additional labeled bands appeared at about 120,000 and 140,000 MW (HA-BR-120 and HA-BR-140). Each of these labeled intermediates yielded a prominent spot in tryptic digests which migrated to the same position as the N-terminal peptide in HA-BR-65. The different HA-BR fragments reacted with a mouse monoclonal antibody selective for a tryptic peptide in HA-BR-65 (2), with HA-BR-109 and HA-BR-65 staining most intensely in an electroblot immunoperoxidase assay. The results indicate that the N-terminus of the core protein is at the HA-BR end of the polypeptide and that the chondroitin sulfate chains are first present on the core between HA-BR-109 and HA-BR-120. Other experiments indicate that almost all of the N-linked oligosaccharides are on HA-BR-65 (3). The relative amount of the *O*-linked oligosaccharides in different regions of the core remains to be determined.

1. Oike, Y. et al. (1982) J. Biol. Chem. 257:9751
2. In collaboration with Bruce Caterson
3. In collaboration with Yasuteru Oike, James Kimura and Stefan Lohmander

1546 PROTEOGLYCANS AND PROTEINS OF SKELETAL TISSUES, Dick Heinegård, Ahnders Franzén, Mats Paulsson and Yngve Sommarin, Dept. of Physiological Chemistry, University of Lund, P.O. Box 750, Lund, Sweden.

Proteoglycans constitute major structural components of cartilage matrix. Most types of cartilage contain four structurally different populations of proteoglycans (for ref. see 1). Two predominating types can specifically bind to hyaluronic acid and form proteoglycan aggregates. They have different molecular weights of 3.5 million and 1.3 million respectively, and furthermore differ with respect to relative contents of keratan sulfate, chondroitin sulfate, oligosaccharides and protein. The remaining 10–15 % (by weight) of the proteoglycans form two populations which do not bind to hyaluronic acid. One group of molecules have a size similar to that of the aggregating proteoglycans, although having higher protein contents and containing a lower proportion of keratan sulfate side chains. The fourth group of proteoglycans has a very different character. These molecules have a molecular weight of about 80 000 and contain only two or three large chondroitin sulfate chains compared with the 50 to 100 such chains of the large proteoglycans. The core protein has a distinct amino acid composition and typically these proteoglycans are less polydisperse than are other types. Bone calcified matrix contains only a proteoglycan with molecular characteristics very similar to those of the small cartilage proteoglycans. Proteoglycans of similar structure can be identified in most connective tissues, but they differ with respect to contents of iduronic acid, i.e. dermatan sulfate. Also large proteoglycans of partially similar structure as those aggregating ones found in cartilage has been isolated from a number of connective tissues like aorta, sclera and tendon.

Cartilage matrix also contains substantial proportions of noncollagenous proteins. Tracheal cartilage contains an abundant protein (concentration varies with age to a maximum of 50 mg/g wet weight), made up of three subunits each with a molecular weight of 52 000, i.e. the 148 kDa protein (for ref. see 1). This protein appears to have the capacity to form fibrils in vitro. It is only found in cartilage, although in quite variable concentrations. Tracheal cartilage has the highest content, while the protein cannot be identified in articular cartilage. Nasal, sternal, ear and epiphyseal cartilage contain intermediate concentration of the protein. Another abundant protein, molecular weight 36 000, appears to be present in all cartilages. Its turnover is higher than that of the 148 kDa protein, which in turn has a slow turnover similar to that of proteoglycans (2).

References: 1. Heinegård, D., Paulsson, M. and Sommarin, Y. in Limb Development and Regeneration, Part B, eds. Kelley, Goetinck and MacCabe, Alan R. Liss Inc., 1983, pp. 35–43. 2. Paulsson, M., Sommarin, Y. and Heinegård, D., 1983, Biochem. J., 212, 659–667.

1547 BIOSYNTHESIS OF PROTEOGLYCANS, James H. Kimura, Depts of Orthopedic Surgery and Biochemistry, Rush Medical College, Chicago IL 60612.

Proteoglycan synthesis involves extensive postranslational modifications to a core protein precursor. The major proteoglycan of cartilage represents an extreme example of the modification process with over 90% of the molecular mass being added to the core protein precursor after translation. The major substituents are the glycosaminoglycan chairsn, chondroitin sulfate and usually keratan sulfate. In addition, about the same number of O-linked oligosaccharides and a smaller number of complex N-linked oligosaccharides also must be added to the core protein. The biosynthetic process then, can be viewed as one in which the post-translational modifications largely determine the nature of the completed macromolecule.

One approach to the study of this biosynthetic process has been the use of a model system consisting of primary cultures of chondrocytes from a transplantable rat chondrosarcoma. The results of these studies indicate that after a proteoglycan core protein precursor has been synthesized, ther is a 1.5 h intracellular half-time before the completed proteoglycan is secreted. The initial glycosylation of the core protein precursor occurs early in the synthesis with the addition of N-linked oligosaccharides, perhaps during polypeptide synthesis, yielding an intermediate about 370,000 in molecular weight. This intermediate exhibits a long half-life in the rough endoplasmic reticulum accounting for about 80% of the intracellular dwell time. Further modifications to the core protein precursor are not detected until it reaches the Golgi apparatus where virtually all of the polysaccharide and oligosaccharide chains are added. Kiietic labeling experiments indicate that O-oligosaccharides are added concurrently with the chondroitin sulfate chains. Since keratan sulfate chains are constructed on oligosaccharide primers, these results suggest that the keratan sulfate chains are also added in the Golgi. In addition, the N-linked oligosaccharies are processed from high mannose forms to the complex type found in the completed molecules. These glycosylation events occur quite rapidly relative to the intracellular synthetic time, exhibiting a half-time of about 15 min, compared to a 1.5 h intracellular half-time. These results suggest that at least for this model system, the synthesis of a proteoglycan molecule proceeds in two stages, the production of a single long lived intermediate consisting of a relatively unglycosylated core protein precursor followed by transport to the Golgi complex where almost all of glycosylation occurs in less than 20% of the intracellular transit time.

1548 THE ROLE OF PROTEOGLYCANS IN GROWTH AND METASTASIS OF HUMAN MELANOMA. R. A. Reisfeld, Scripps Clinic and Research Foundation, La Jolla, CA 92037

A combination of indirect immunoprecipitation analyses, pulse-chase biosynthetic studies, and enzymatic digestion experiments indicate that a monoclonal antibody (Mab 9.2.27) recognizes an antigenic determinant on an intact chondroitin sulfate proteoglycan (CSP) and its core glycoprotein (250 K) expressed by human melanoma cells. Topographical studies indicate a typical punctuated pericellular distribution fo CSP on the surface of human melanoma cells. Pulse-chase data demonstrate that addition of N-linked oligosaccharides is an early event in CSP core biosynthesis and that early antigens of M_r 210, 220, and 240K recognized by Mab 9.2.27 chase into an Endo H resistant glycoprotein and a high molecular weight CSP whose only appearance in immunoprecipitates coincides with the Endo-H resistance of the 250K core glycoprotein. Long term pulse-chase studies indicate that the 250K core glycoprotein has an extended half life within melanoma cells of 15.6 hours. Exposure of melanoma cells to monensin (2 hrs; 10^{-7}M) completely inhibits proteoglycan synthesis without affecting protein synthesis and also blocks or delays complete conversion of precursors to the 250K core glycoprotein. To assess whether proteoglycan formation is contingent upon core protein maturation, we examined the effect of NH_4Cl (15 mM) on these processes in pulse-chase studies. A marked inhibition of proteoglycan synthesis was observed while the kinetics of core glycoprotein synthesis and terminal processing were unaffected. Long term labeling of melanoma cells with ^{3}H-leucine in the presence of NH_4Cl resulted in a significant accumulation of the Endo-H resistant mature 250K core glycoprotein concomitant with inhibition of proteoglycan biosynthesis, suggesting that NH_4Cl-triggered inhibition of proteoglycan synthesis occurs at a biosynthetic step subsequent to that inhibited by monensin. Since NH_4Cl induces accumulation of an Endo-H resistant core protein that has apparently cleared the Golgi apparatus, it is apparent that glycosaminoglycan biosynthesis may involve either recycling of an intracellular receptor or low pH-dependent fusion of intracellular membranes. A functional role of chondroitin sulfate proteoglycans in human melanoma cells is suggested by the fact that Mab 9.2.27 blocks early events of melanoma cell spreading on endothelial basement membranes while only slightly inhibiting cell adhesion. These observations suggest that CSPs may play a role in cell substratum interactions in this in vitro model for metastatic invasion. In vivo experiments in athymic, nude mice bearing human melanoma tumors indicate that Mab 9.2.27 when conjugated to immune effector cells can completely inhibit tumor growth and destroy large, established tumors. These data strongly suggest a key functional role for CSP and its 250K core glycoprotein in the immunotherapy of malignant melanoma.

Proteoglycans II

1549 BASEMENT MEMBRANE PROTEOGLYCANS John R. Hassell, Steven Ledbetter, Webster C. Leyshon, Bernadette Tyree, Gordon W. Laurie, and Hynda K. Kleinman, Laboratory of Developmental Biology and Anomalies, National Institute for Dental Research, National Institutes of Health, Bethesda, MD 20205

Basement membranes contain heparan sulfate proteoglycan as their major proteoglycan. We have studied the synthesis, structure and interaction of the basement membrane specific proteoglycan by using EHS tumor tissue as well as tumor cells in culture. Components previously isolated from this tumor are identical to those found in authentic basement membranes.

Different forms of the proteoglycan can be isolated by sequential extactions with first saline and then 7 M urea. Saline extracted a high density proteoglycan whereas the urea extracted a low density proteoglycan. Based on gel filtration, the low density proteoglycan is larger than the high density proteoglycan but both contained heparan sulfate side chains of 65,000 MW. Preliminary electron microscope examination of the high and low density proteoglycan confirmed their size differences. Amino acid and hexosamine analyses showed the low density proteoglycan contained 40% protein whereas the high density proteoglycan contained only 10% protein. The necessity of denaturing solvents to extract the low density proteoglycan suggests that this proteoglycan interacts strongly with other basement membrane components present in the matrix.

Antibodies against each of these proteoglycans stained basement membrane structures in normal tissues. The antibodies were shown by ELISA to cross react with both proteoglycans and to be directed against the protein core. The low density proteoglycan, however, contains antigenic sites not present on the high density proteoglycan. These observations suggest that the proteoglycans are related and pulse chase studies with $^{35}SO_4$ show the conversion of the low density to the high density form. Antibodies to both proteoglycans immunoprecipiated a single protein (M_r=400,000) from EHS cells pulsed with ^{35}S-methionine. Pulse chase studies show this 400,000 MW protein to be converted to a proteoglycan. This core protein was found to be synthesized by other basement membrane producing cells, such as muscle and epithelial cells, but not by skin fibroblasts.

These studies indicate that the heparan sulfate basement membrane proteoglycan is initially synthesized by the addition of heparan sulfate glycosaminoglycans to a 400,000 MW protein to form a high molecular weight, low density proteoglycan. A portion of the protein core is removed from the low density proteoglycan to yield the smaller high density proteoglycan. The various size heparan sulfate proteoglycans reported for different basement membrane producing cells probably arise, in part, from differences in post-translational modifications of the 400,000 MW core protein.

1550 PROTEOGLYCANS SYNTHESIZED AND SECRETED BY CULTURED VASCULAR CELLS. Thomas N. Wight, Michael G. Kinsella and Susan Perigo. Department of Pathology, University of Washington School of Medicine, Seattle WA 98195.

Proteoglycans accumulate in blood vessels and have been implicated in many cellular events fundamental to arterial development and disease.[1,2] Cultured aortic endothelial and smooth muscle cells have been used to determine whether proteoglycan metabolism is modulated when these cells are stimulated to proliferate and/or migrate. Proteoglycan analysis has been performed on isotopically labelled (^{3}H-glucosamine, ^{35}S-sulfate) 4 M guanidine HCl extracts of culture medium and cell layer. Endothelial cells synthesize and deposit large amounts of proteoheparan sulfate and hyaluronic acid and little proteochondroitin sulfate.[3] This pattern differs from that of smooth muscle which synthesize and secrete large amounts of proteochondroitin sulfate and proteodermatan sulfate but little proteoheparan sulfate and hyaluronic acid.[4,5] Each family of proteoglycan synthesized by each cell type differs with regard to charge density, hydrodynamic size, glycosaminoglycan type and molecular size, oligosaccharide content, and ability to form high molecular weight aggregates.[5] These molecules are present throughout the extracellular matrix and their ultrastructure[6] resembles that described for intact blood vessels.[7] Smooth muscle cells made quiescent by culture media containing low serum (0.1%) increase sulfated proteoglycan synthesis approximately 3-4 fold when stimulated to proliferate by the addition of 5% serum. This increase occurs during the G_1 part of the cell cycle and is accompanied by a shift toward the synthesis of proteochondroitin sulfate. Endothelial cells forced to migrate by disruption of intact cell monolayers by "multiscratch" wounding exhibit increased proteoglycan synthesis up to 48 hrs after injury. This increase is shown to involve a specific increase in the synthesis of proteochondroitin sulfate. This culture system is offered as a useful model to study parameters involved in the regulation of proteoglycan synthesis by vascular cells.

1. Toole, B.P., in Cell Biology of Extracellular Matrix, ed. E. Hay, Plenum Press, NY, 259-294, 1981.
2. Wight, T.N., in Progress in Hemostasis and Thrombosis, ed. T. Spaet, Grune and Stratton, NY, 5:1-39, 1980.
3. Oohira, A., Wight, T.N. and Bornstein, P., J. Biol. Chem. 258:2014-2021, 1983.
4. Wight, T.N. and Hascall, V.C., J. Cell Biol. 96:167-176, 1983.
5. Chang, Y., Yanagishita, M., Hascall, V.C. and Wight, T.N., J. Biol. Chem. 258:5679-5688, 1983.
6. Wight, T.N. and Ross, R., J. Cell Biol. 67:660-674, 1975.
7. Chen, K. and Wight, T.N., J. Histochem. Cytochem., in press, 1983.

Fibronectin and Cell Attachment Proteins

1551 FIBRONECTIN AND OTHER CELL ATTACHMENT PROTEINS: AN OVERVIEW, Kenneth M. Yamada, Takayuki Hasegawa, Etsuko Hasegawa, Wen-Tien Chen, Martin J. Humphries, Bruno Bernard, Kenneth Olden, and Steven K. Akiyama, Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, N.I.H., Bethesda, MD 20205, and Howard University Cancer Center, Washington, DC 20060

Adhesive glycoproteins such as fibronectin, laminin, and serum spreading factor (vitronectin) are providing rapidly-expanding insights into the molecular basis of cell interactions with extracellular materials. Such multifunctional proteins can mediate or promote cell adhesion, migration, growth, and other complex events. The structure and functions of several better-characterized cell attachment proteins will be reviewed, with emphasis on fibronectin as a prototype. Fibronectin and laminin consist of a series of discrete, protease-resistant domains; e.g., those of fibronectin are specialized for binding to collagen, fibrin, heparin, other molecules, and the cell surface. Mapping and protein sequencing studies reveal the modular organization of fibronectin, which can be dissected by proteases and partially reconstituted by forming hybrid recombinant protein molecules from isolated polypeptide regions.

A single gene appears to account for the two major forms of fibronectin, and it has been isolated in recombinant DNA clones. Structural analyses of this genomic material and protein sequencing reveal a repetitive gene and protein structure, suggesting that fibronectin evolved by massive gene duplication of small exon units. Fibronectin antigenic determinants are found throughout the animal kingdom, although invertebrate "fibronectin" remains to be characterized further. There is a high degree of evolutionary conservation of functional domains and protein sequences, but inter-domain regions may have greater variability. For example, the cell-binding domain is highly conserved; its activity can be mimicked or competitively inhibited by synthetic peptides containing a conserved sequence.

The receptors for fibronectin and laminin on the cell surface can be characterized by standard ligand-binding methods. The molecular identity of such receptors and the mechanisms by which they may interact with the cytoskeleton are under intensive investigation. Candidates for the fibronectin receptor include 47K and 140K glycoproteins and gangliosides. A 70K receptor for laminin has been isolated and characterized in vitro. Fibronectin and other attachment proteins should continue to provide valuable model systems for understanding cell adhesion and other important cell interactions with the extracellular milieu.

Selected Reviews:

1. Hay, E.D., Cell Biology of Extracellular Matrix, 417 pp. (1981).
2. Yamada, K.M., Ann. Rev. Biochem. 52: 761-799 (1983).

1552 FIBRONECTIN AND WOUND HEALING, Frederick Grinnell, Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The healing of excisional cutaneous wounds involves three interrelated processes: recruitment of connective tissue cells, formation and remodeling of granulation tissue, and re-epithelialization. Fibronectin (FN) has been implicated in all of these processes. In normal skin, fibronectin has been localized at the dermal-epidermal junction, in association with fibroblasts in the dermis, and in the basement membrane region of blood vessels. The collagenous component of the dermis, however, generally lacks fibronectin. Following wounding, FN can be found coating the fibrin of the blood clot. As a result, an interface is formed between the fibronectin-rich fibrin matrix and the fibronectin-poor dermal collagen matrix. The presence of fibronectin at this interface may provide in part the chemotactic stimulus for migration of connective tissue cells into this region. Moreover, adhesion and migration of fibroblasts on the fibrin matrix probably requires the presence of the fibronectin coating on the fibrin. The initial source of fibronectin in the wound bed is from the plasma pool. As connective tissue cells migrate into the wound bed, granulation tissue formation begins. During this period, fibroblasts deposit a neo-matrix composed largely of Type III collagen. Fibronectin is co-deposited with the Type III collagen and may provide the scaffolding on which the collagen is organized. This fibronectin appears to be derived from the local connective tissue cells, especially fibroblasts and endothelial cells. When the granulation tissue matures, it is replaced by neo-dermis that is rich in Type I collagen and most of the fibronectin disappears. In the event, however, that the granulation tissue reaction is sustained (e.g., in hypertrophic scarring) then the levels of fibronectin and Type III collagen remain high. Similarly, in other sclerotic reactions high levels of Type III collagen and fibronectin are found in the tissue. Concurrent with granulation tissue formation, re-epithelialization of the wound surface begins by migration of epidermal cells from the wound edges (or hair follicles). The migrating epidermal cells are in direct contact with the fibronectin-rich granulation tissue, and the presence of fibronectin may be required for adhesion and migration of the epidermal cells on this substratum. Epidermal cells distal to the migrating edge form a neo-basement membrane containing laminin and Type IV collagen, and fibronectin may be necessary for the basement membrane components to be organized. Finally, throughout the period of wound healing, extensive removal of tissue debris and tissue remodeling occur. Generally, cells of the monocyte/macrophage lineage have been implicated in these activities, and fibronectin has been proposed as an opsonin that can promote uptake by these cells of fibrin and collagen degradation products. Fibroblasts also extensively phagocytose fibronectin-coated particles and may be more important than monocyte/macrophages when fibronectin is the opsonin. (The author's research has been supported by grants from N.I.H., CA14609 and GM31321.)

1553 RECONSTITUTION OF EXTRACELLULAR MATRIX COMPONENTS INTO DEFINED SUPRAMOLECULAR COMPLEXES Hynda K. Kleinman, Mary L. McGarvey, Gordon W. Laurie, John R. Hassell, Srinivasan Chandrasekhar, Vicki L. Star, and George R. Martin, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205

Extracellular matrices contain tissue-specific components including distinct collagens, proteoglycans, and glycoproteins. Recent studies show that the precise interactions between these components give rise to defined supramolecular complexes which fuse to form the extracellular matrix. Using the assembly of the basement membrane components as a model system for studying the formation of extracellular matrices, we report here on the specific interactions of basement membrane components which result in the deposition of material comparable in appearance to the observed in basement membranes. Laminin, type IV collagen and heparan sulfate proteoglycan react in rather well defined proportions under physiological conditions and form an ordered precipitate. Additional proteins from basement membranes induce the precipitate to assemble into a gel-like form. The formation of the gel structure is increased by type IV collagen and by heparan sulfate proteoglycan but not by heparin or type I collagen. Ultrastructural analysis of the gels revealed ordered structures which resembled those observed in intact basement (Reichert's) membrane. These studies demonstrate that specific components of the basement membrane can interact to form a gel *in vitro* which is similar to the structures observed *in vivo*. By allowing the protein to reconstitute in vitro, it is possible to study the specific interactions between components involved in matrix assembly. This approach has also successfully identified a collagen binding protein (M_r=54,000) from cartilage which may modulate collagen type II fibrilogenesis. Such highly specialized matrices interact with cells via the attachment glycoproteins to form unique tissues.

Role in Development I

1554 THE ROLE OF BASAL LAMINA IN REGENERATION OF THE NEUROMUSCULAR JUNCTION. U.J. McMahan, Department of Neurobiology, Stanford University School of Medicine, Stanford, California, 94305 U.S.A.

If skeletal muscles are damaged in ways that spare the basal lamina sheaths of the muscle fibers, new myofibers develop within the sheaths and neuromuscular junctions form at the original synaptic sites on them. At the regenerated neuromuscular junctions, as at the original ones, the muscle fiber plasma membrane is characterized by infoldings and a high concentration of acetylcholine receptors(AChRs). I will present evidence that the myofiber basal lamina sheaths contain molecules that direct the formation of the subsynaptic apparatus on regenerating myofibers. I will also describe experiments aimed at identifying the molecules in synaptic basal lamina that cause aggregation of AChRs. My colleagues and I have been examining the molecular components of an extracellular matrix (ECM)-enriched fraction from the electric organ of Torpedo californica; the electric organ has a much higher concentration of cholinergic synapses than muscle. The ECM fraction is insoluble in isotonic saline and detergent, as is basal lamina, and causes AChRs on cultured myotubes to aggregate. The active molecules are extracted by treatment with high salt or low pH buffers which solubilize other extracellular matrix molecules. Antiserum against the high salt extract binds both to the AChR aggregating molecules and to basal lamina at frog neuromuscular junctions. We have purified the active molecules more than 1000-fold, have shown that they are proteinaceous and active at protein concentrations as low as 10^{-10}M, and have determined that they have a molecular weight of 50,000-100,000 daltons. Relatively small amounts of similar molecules can be extracted from Torpedo muscle. We are currently making a specific antiserum against the active molecules from electric organ to determine if similar ones are localized to myofiber basal lamina at neuromuscular junctions.

1555 THE FIBRIL/CELL PROBLEM: WHERE FIBRILS START AND END, Robert L. Trelstad and David E. Birk, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

Unanswered questions about the morphogenesis of type I collagen fibrils persist despite extensive information about the chemical structure of the molecule and its precursor forms, the necessity for enzymatic processing of carboxy and amino terminal extensions and the probable quasi-hexagonal packing of the final product. Where do fibrils begin and/or end? If there are no apparent discontinuities in fibrils seen by electron microscopy are we to assume that a fibril is deposited in a manner which is an obligate continuum from the time of initial deposition to the cessation of morphogenesis? To address this problem we have undertaken an examination of: 1) the embryonic chick tendon, a relatively simple structure in which collagen molecules and fibrils are relatively uniaxial; and 2) the chick corneal stroma which is a cholesteric liquid crystal-like structure in which the immediate fibrillar arrangement about a single cell is orthogonal. To extend our understanding of these two tissues beyond that which we've been able to garner during the past decade, tissues have been examined with a high voltage electron microscope (1000 kv) and photographed as stereo pairs. From these studies we have an entirely new view of the structural and functional topography of the 'fibroblast.' These cells are not simple oblate spheroids; their surface folds are extensive and complex. We also now realize that the extracellular space surrounding the cells in both tissues is compartmentalized and ordered. It is no longer useful to consider the extracellular space as a single compartment; around the tendon and corneal cells there are probably a dozen different 'spaces' each with a unique function. We currently consider the extracellular space to be as partitioned as the intracellular environment although our language to describe these compartments is just developing. The surface of the fibroblast is a highly convoluted one in which there are a graded series of recesses, each leading into another, but each with its own presumed function. In the smallest of these pericellular recesses we have found newly formed collagen fibrils; in large compartments enveloped by the cell we find bundles of collagen which merge into fascicles or lamellae. In the cornea a single cell contributes to fibrils, bundles and lamellae which are orthogonal; the cell processes and compartments which delimit these extracellular spaces are likewise orthogonal. Much to our surprise, in the cornea we have found that individual fibrils and bundles of fibrils execute ninety degree changes in orientation. The density of cells along a forming tendon or in the cornea suggest that the products of one cell interweave with those of another to form fibrils and bundles whose length exceed the length of individual cells. The manner by which locally deposited material becomes structurally integrated to ultimately provide an apparently continuous structure continues to elude us.

1556 MATRIX-CYTOSKELETAL INTERACTIONS IN THE DEVELOPING CORNEA, Elizabeth D. Hay, Department of Anatomy, Harvard Medical School, Boston, MA 02115

The interaction of the cytoskeleton with extracellular matrix (ECM) and the effect of this interaction on ECM synthesis can be studied to good advantage in the developing avian cornea. Synthesis of ECM is the hallmark of differentiation of both the early corneal epithelium and the mesenchyme that later invades the primary corneal stroma created by the epithelium. The cytoskeleton and polarity of these two tissue types, however, are strikingly different and there is reason to believe that epithelial and mesenchymal cells differ in distribution of matrix binding sites and reaction to ECM of the cytoskeleton and cytoskeleton-associated collagen synthetic machinary. When isolated corneal epithelia are grown with ECM molecules such as collagen or laminin, the basal cortical actin cytoskeleton forms an organized mat[1] with which the rough endoplasmic reticulum (RER) is associated. In the absence of exogenous ECM, the basal cell surface and cytoskeleton become highly disorganized and the epithelia synthesize collagen at a low rate. Since the level of collagen production and organization of the cytoskeleton are closely correlated, we investigated the effect of cytoskeletal-disrupting drugs on production of collagen by corneal epithelia isolated with exogenous ECM (basal lamina) intact[2]. Cytochalasin D disrupts the basal cortical actin mat with a concomitant effect on RER organization and drop in the level of collagen synthesis by the treated epithelial cells.[2] Thus, we suggest that the stimulatory effect of exogenous ECM on collagen production by the epithelium is transmitted across the basal plasmalemma to the RER via the cytoskeleton. The cell surface binding sites, as judged by binding of labeled exogenous ECM, are localized only on the basal epithelial plasmalemma, and separate plasmalemma proteins (65-70 KD) have been isolated that bind either laminin or collagen.[3] Normally, epithelium is not confronted with ECM on its apical surface. The mesenchymal cells that invade the primary corneal stroma, however, are surrounded on all sides by ECM and thus do not exhibit polarized basal binding sites for exogenous ECM. Isolated corneal fibroblasts have an organized actin cortical net under the entire cell surface that becomes associated with filopodia when the cells become bipolar in collagen gels in vitro.[4] The cells become flat and develop stress fibers on planar substrata and cannot be rescued from this abnormal cytoskeletal configuration by soluble exogenous ECM, whereas epithelial cells interact with either soluble or polymerized ECM[1]. These differences in cell surface characteristics will be discussed in the context of possible mechanisms of epithelial-mesenchymal transformation in the embryo.

1. Sugrue, S.P. and E.D. Hay. 1981. J. Cell Biol. 91:45-54.
2. Svoboda, K. and E.D. Hay. 1984. Anat. Rec., in press.
3. Sugrue, S.P. and E.D. Hay. 1983. J. Cell Biol. 97:319a.
4. Tomasek, J.J., E.D. Hay and K. Fujiwara. 1982. Dev. Biol. 92:107-122.

Role in Development II

1557 EPITHELIAL-MESENCHYMAL INTERACTIONS, Harold C. Slavkin, Malcolm L. Snead, Margarita Zeichner-David, Tina Jaskoll, and Barry T. Smith*, Graduate Program in Craniofacial Biology, University of Southern California, Los Angeles, CA 90089-0191, and *Department of Pediatrics, Harvard Medical School, Boston, MA 02115

One key issue in developmental biology is to understand how tissue interactions result in the induction, expression and stabilization of differential gene expression. Epithelial-mesenchymal interactions are defined as tissue interactions which result in changes to one or both tissues; changes which could not occur without the interaction (1). "Instructive" interactions describe a tissue developing unique morphological patterns and expression of differential gene products as the direct consequence of mesenchyme-derived signals. In the complete absence of exogenous humoral factors, ectomesenchyme-derived signals induce inner enamel epithelial cells to express enamel gene products (2-4). The developmental signals are derived from ectomesenchymal cell surfaces or forming extracellular matrix interposed between mesenchyme and responding epithelium. In contrast, glucocortocosteroids mediate mesenchyme-derived signals required for fetal lung epithelial differentiation into Type II cells with production of pulmonary surfactant. In response to glucocorticoids, fetal lung fibroblasts produce a low molecular weight, fibroblast pneumonocyte factor (FPF), which in turn stimulates pulmonary surfactant synthesis by Type II epithelial cells (5). During tooth and lung organogenesis, mesenchyme-derived signals influence morphogenesis as well as the expression of differential gene products. In each example, a stimulus initiates basal lamina degradation in regions of the developing organ associated with changing morphology and subsequent differential gene expression. Events which initiate the differentiation program during tooth and lung development raise a number of important issues which will be discussed. Supported in part by NIH grants DE-02845 and HL-28325.

(1) Grobstein, C. (1967) Mechanisms of organogenetic tissue interaction. Natl. Cancer Inst. Monogr. 26:279-294.
(2) Brownell,A.G., Bessem,C. and Slavkin,H.C. (1981) Possible functions of mesenchyme cell-derived fibronectin during formation of basal lamina. Proc.Natl.Acad.Sci.,USA 78:3711-3715
(3) Slavkin,H.C., Zeichner-David,M., MacDougall,M., Bringas,P., Bessem,C. and Honig,L.S.(1982) Antibodies to murine amelogenins: Localization of enamel proteins during tooth organ development in vitro. Differentiation 23:73-82.
(4) Snead,M.L.,Zeichner-David,M.,Chandra,T.,Robson,K.J.,Woo,S.L.C. and Slavkin,H.C.(in press) Construction and identification of mouse amelogenin cDNA clones. Proc.Natl.Acad.Sci,USA.
(5) Smith,B.T. and Sabry,K. (1983) Glucocorticoid-thyroid synergism in lung maturation: a mechanism involving epithelial-mesenchymal interaction. Proc.Natl.Acad.Sci.,USA 80:1951-1954.

1558 HYALURONATE-CELL INTERACTIONS IN MORPHOGENESIS AND TUMORIGENESIS, Bryan P. Toole, Gloria Chi-Rosso, Ronald L. Goldberg, Cheryl B. Knudson and Warren Knudson, Department of Anatomy and Cellular Biology, Tufts University, Boston, MA 02111.

Hyaluronate is a major component, in vivo, of the extracellular matrix which surrounds proliferating and migrating cells at early stages of morphogenesis of embryonic tissues and organs, as well as in regenerating, remodelling and healing tissues. Using various cell lines in vitro we have demonstrated two distinct modes of interaction of hyaluronate with the cell surface. The first is a stable interaction with the plasma membrane which is typical of cells with large pericellular coats of hyaluronate but is of unknown mechanism. The second involves specific, non-covalent, high affinity interaction of the polysaccharide chain with multiple binding sites in the plasma membrane. This latter mechanism is predominant in virally transformed cells. Transformation of 3T3 cells with simian virus increases the number of high affinity hyaluronate-binding sites, decreases cell surface-associated hyaluronate, increases intracellular hyaluronidase levels and increases the rate of internalization and degradation of hyaluronate.

The hyaluronate-binding protein of simian virus-40 transformed 3T3 cells has been extracted from membrane preparations in detergent solution wherein the affinity and specificity of binding becomes decreased. On reconstitution into liposome membranes, the binding affinity returns almost to the level found in the original cell membranes or at the cell surface. However the specificity is not regained since binding of hyaluronate to the reconstituted liposomes is inhibited by addition of chondroitin sulfate, whereas with the original membranes it is not.

In vivo, tumors frequently contain high concentrations of hyaluronate, yet tumor cells derived from these neoplasms synthesize low amounts of glycosaminoglycans. However, addition of tumor cells or tumor extracts to cultures of fibroblasts stimulates hyaluronate synthesis by the latter. Thus interaction of tumor and host cells in vivo appears to be important in the regulation of hyaluronate production.

Cell differentiation, e.g. myogenesis and chondrogenesis, also involves radical changes in hyaluronate-cell interactions. During chondrogenesis in the chick embryo limb, mesodermal cells enter the limb, accumulate by proliferation, condense and then form chondrocytes. The mesodermal cells initially exhibit prominent hyaluronate-containing pericellular coats, but these are lost by the time of precartilage condensation. During cartilage formation, pericellular coats are regained. These coats are now enriched in cartilage proteoglycan which is held to the chondrocyte surface via hyaluronate. Supported by NIH Grant DE 05838.

1559 REMODELING OF THE BASEMENT MEMBRANE IN EPITHELIAL MORPHOGENESIS, Merton Bernfield, Shib Das Banerjee, Joy E. Koda, Alan C. Rapraeger, Department of Pediatrics, Stanford University Medical Center, Stanford, CA 94305

The branching morphogenesis of several epithelial organs (e.g. lung, salivary glands) occurs by budding, involving cell proliferation; by folding, involving cytoskeletal-mediated changes in cell shape; and by maintaining these changes in morphology. This morphogenesis is under the influence of the associated mesenchyme. In the mouse embryonic submandibular gland, maintenance of branched lobules, the organization of actin microfilaments and localized proliferation depend on the integrity of the basal lamina which separates the epithelium from its mesenchyme. Thus, epithelial morphology is controlled by the basal lamina which it produces, suggesting that the mesenchyme acts by modifying the basal lamina.

The composition and metabolism of basement membrane materials change during submandibular epithelial morphogenesis[1]. These changes are due, in part, to the mesenchyme[2] which (i) contains a neutral hyaluronidase and which (ii) deposits fibronectin and type I collagen on the basal lamina. The hyaluronidase is soluble, non-lysosomal, able to degrade laminar glycosaminoglycan components and is developmentally regulated during morphogenesis. The effect of type I collagen was studied in a mammary epithelial cell model system. The collagen binds extracellular heparan sulfate proteoglycans produced by these cells[3], stabilizing them to degradation and enhancing their accumulation in a basal lamina[4]. The collagen also binds a cell surface heparan sulfate proteoglycan that is intercalated in the plasma membrane[5]. This membrane proteoglycan, which is more stable to degradation when cell shape is maintained, may be linked to the cytoskeleton. Thus, degradation and stabilization of basement membrane components accompany submandibular epithelial morphogenesis. Remodeling of the basement membrane may be a general mechanism for controlling epithelial behavior.

1. Bernfield, M. and Banerjee, S.D., "The Turnover of Basal Lamina Glycosaminoglycan Correlates with Epithelial Morphogenesis," Develop. Biol. 90:291-305, 1982.
2. Smith, R.L. and Bernfield, M., "Mesenchyme Cells Degrade Epithelial Basal Lamina Glycosaminoglycan," Develop. Biol. 94:378-390, 1982.
3. Koda, J.E. and Bernfield, M., "Heparan Sulfate Proteoglycan Binding to Collagen Involves the Native Fibril," J. Cell Biol. 95:126a, 1982.
4. David, G. and Bernfield, M., "Type I Collagen Reduces the Degradation of Basal Lamina Proteoglycan by Mammary Epithelial Cells," 91:281-286, 1981.
5. Rapraeger, A.C. and Bernfield, M., "Heparan Sulfate Proteoglycans from Mouse Mammary Epithelial Cells: A Putative Membrane Proteoglycan Associates Quantitatively with Lipid Vesicles," J. Biol. Chem. 258:3632-3636, 1983.

1560 STRUCTURE AND FUNCTION OF MATRIX RECEPTORS IN CARTILAGE AND MUSCLE DEVELOPMENT. Klaus von der Mark, Jürgen Mollenhauer, Uwe Kühl, Herve Lesot. Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG.

Several macromolecules of the extracellular matrix such as collagens, proteoglycans, fibronectin and laminin have been shown to regulate cellular events in embryonic development. For example, differentiation of somitic and limb bud mesenchyme to cartilage in vitro can be stimulated by exogeneous collagen substrates. Similar, the cartilage phenotype of differentiated chondrocytes is stabilized by the pericellular type II collagen matrix. There is now ample evidence that direct cell-collagen interactions exist independent of the presence of adhesion molecules. We have recently isolated from chick chondrocyte membranes a collagen-binding glycoprotein with the molecular weight 31,000 called anchorin CII. When inserted into the lipid bilayer of liposomes, the hydrophobic protein binds selectively to native type II collagen. Fab'-fragments from an antibody against anchorin CII reduce attachment of dissociated chondrocytes to type II collagen-coated dishes. The collagen-binding protein is located in clusters on the surface of freshly isolated chondrocytes, which can be induced to capping by antibody staining at elevated temperatures. Membrane proteins of similar properties have also been detected in the membranes of fibroblasts and myoblasts, yet their affinity to different collagen types remains yet to be established.
Differentiation of skeletal myoblasts to multinucleated myotubes in vitro is enhanced by collagen or gelatin substrates (Konigsberg and Hauschka, 1965). In following up this experiment, we found that myoblasts show highest affinity for type IV collagen as compared to other collagen types, and that the attachment to type IV collagen is enhanced by laminin. In attempts to elucidate the mechanism of myoblast-laminin type IV collagen interactions, we were able to isolate a laminin-protein of MW 68,000 from membranes of skeletal muscle cells. This protein is also partially hydrophobic and binds specifically to laminin when inserted into liposomes. By immunofluorescence it was also located on other epithelia such as parietal yolk sac or amnion, which indicates that this protein may be utilized by many cell types for anchoring in the basal lamina.

Role in Diseases

1561 MUTATIONS OF TYPE I PROCOLLAGEN IN OSTEOGENESIS IMPERFECTA. Darwin J. Prockop, Charlene Williams,* Wouter J. de Wet, Mon-Li Chu, Maria Sippola,* and Taina Pihlajaniemi*, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, New Jersey.

Mutations which change the primary structure of the proα1 or proα2 chains of type I procollagen have been shown to cause several forms of osteogenesis imperfecta (OI), a heterogenous group of disorders characterized by brittleness of bone. In one lethal variant, half the proα1 chains synthesized were shortened by about 100 amino acids (1,2). The shortened chains associated with normal-length proα chains to form disulfide-linked heterotrimers, but the trimers did not form stable triple-helical structures at 37^{0}C and were rapidly degraded (2). Examination of genomic DNA demonstrated that the mutation was a deletion of about 500 bp in one allele for proα1(I) chains (3). In a second lethal variant, all the proα2 chains synthesized were shortened by about 20 amino acids (4). Examination of the parents' fibroblasts indicated that the patient inherited a non-functioning allele for proα2 from her father. The allele for proα2 inherited from the mother apparently underwent a sporadic mutation. In a mild variant of OI, half the proα2 chains synthesized were shortened because of a deletion of about 30 amino acids in α2-CB4, a fragment containing amino acids 7 to 327 of the α2 chain (5). One consequence of the mutation was that triple-helical trimers containing the shortened proα2 chains unfolded with a Tm about 5^{0}C lower than normal type I procollagen. The second consequence was that trimers containing the shortened proα2 chains were resistant to cleavage by procollagen N-proteinase. The results indicate that mutations which produce shortening of proα chains may be relatively common in OI. Procollagen genes may be particularly prone to such mutations either because of their highly repetitive coding sequences, or their large number of intervening sequences. Mutations which produce large changes in length are lethal. Smaller changes are of less immediate consequence unless associated with a second mutation. Since however collagen fibers have a long metabolic life, small changes may predispose an individual to chronic disorders of connective tissue.

1. Barsh, G.S. and Byers, P.H. (1981) PNAS 78, 5142. 2. Williams, C.J. and Prockop, D.J. (1983) J. Biol. Chem. 258, 5915. 3. Chu, M.-L., Williams, C.J., Pepe, G., Hirsch, J.L., Prockop, D.J. and Ramirez, F. (1983) Nature 304, 78. 4. de Wet, W.J., Pihlajaniemi, T., Myers, J., Kelley, T.E. and Prockop, D.J. (1983) J. Biol. Chem. 258, 7721. 5. Sippola, M. and Prockop, D.J. (1983) J. Cell Biol., abs. in press.

1562 COLLAGEN AND INFLAMMATORY JOINT DISEASE: ITS DEGRADATION, SYNTHESIS AND POTENTIAL ROLE IN PATHOGENESIS, Stephen M. Krane, Edward P. Amento, Jean-Michel Dayer, James T. Kurnick, Steven R. Goldring, Mary B. Goldring, Ashok K. Bhalla and Peter A. McCroskery, Departments of Medicine and Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114

In rheumatoid arthritis, connective tissue loss occurs in areas contiguous to the inflammatory cells mass, the latter comprised of a heterogeneous cell population including lymphocytes, monocytes, and synovial cells related to fibroblasts. We have utilized cell culture models in which the potential contribution of at least partially purified populations of different cell types can be assessed. The adherent synovial fibroblast-like cells, which in primary culture have a prominent stellate appearance, synthesize and release, among other substances, collagenase and prostaglandins mostly PGE_2. The monocyte/macrophages when exposed to T cell products or other stimulants such as the Fc portion of immunoglobulins or types II or III collagens produce a soluble polypeptide called mononuclear cell factor (MCF) which has homologies with interleukin 1. MCF added to synovial cells then induces the synthesis and release of procollagenase (as shown by enzyme activity following activation as well as incorporation of labeled, amino acid into immunoprecipitable protein) and PGE_2 by the synovial fibroblast-like target cells. The synovial target cells also release enzymes such as plasminogen activator, which acts on plasminogen to yield plasmin which can activate the latent procollagenase complex. Control of activity of these proteases is also exerted via production of inhibitors of collagenase as well as other inhibitors (e.g protease nexin) of serine proteases (e.g. plasminogen activator). Interactions between T lymphocytes and monocytes have been examined further utilizing the human clonal monocyte line, U937. The U937 cells grow in suspension culture, adhere poorly to surfaces and produce no detectable basal MCF activity. When these U937 cells are cocultured with T lymphocytes in the presence of lectin MCF activity is released. The cell-free medium from lectin-stimulated T cells contains a factor which matures the U937 cells and induces MCF production. Cells present in the pannus are also exposed to endocrine factors such as $1,25(OH)_2D_3$. The U937 cells (which we found have specific receptors for $1,25(OH)_2D_3$) express the more mature macrophage phenotype when exposed to $1,25(OH)_2D_3$ anologous to that produced by the lectin-treated T lymphocyte conditioned medium but in contrast to the latter, do not produce MCF activity. When both the T lymphocyte medium (lymphokine) and the $1,25(OH)_2D_3$ are added together, however, the release of MCF by the U937 cells is enhanced. Thus $1,25(OH)_2D_3$ can provide one but not both of the signals required for MCF production by these immature monocytes.

1563 IDENTIFICATION OF BONE SPECIFIC PROTEINS AND THEIR ROLE IN OSTEOGENESIS IMPERFECTA. J.D. Termine, P. Gehron Robey, L.W. Fisher, M.A. Drum, H. Shimokawa, K.G. Thompson*, L. Denholm#, and K.M. Conn. NIDR, NIH, Bethesda, MD; *Texas A&M U., College Station, TX; #Cornell U., Ithaca, NY, USA.

Because of the development of new techniques for the non-degradative extraction of bone[1], several tissue-specific proteins have been described. Osteonectin[2], a phosphorylated glycoprotein with a molecular weight of 32,000 daltons, has been found to bind calcium, hydroxyapatite and type I collagen[3]. This protein promotes mineralization of collagen *in vitro*. The bone-specific proteoglycan[4], is composed of a core protein of 35,000 daltons to which one or two chondroitin sulfate glycosaminoglycan chains of 40,000 daltons are attached. This protein is one of the first proteins expressed in osteogenesis. The bone-specific sialoprotein[5] contains 50% protein and 50% carbohydrate with a molecular weight of 75,000, and remains in bone, albeit in a degraded form, until bone resorption occurs. These three proteins appear to be tissue-specific and may be implicated in bone disease. Recently two bovine forms of osteogenesis imperfecta (BOI-1 and BOI-2) have been described with affected calves presenting symptoms comparable to those in the human disease; i.e., multiple fractures, dentinogenesis imperfecta, joint laxity and in BOI-2, blue sclerae. Type I collagen in both disease models exhibited no major structural defects, but non-collagenous bone proteins were affected. Bone from BOI-1 calves had normal amounts of osteonectin and bone sialoprotein, but the bone-specific proteoglycan was reduced greatly. Bone from the more severely affected BOI-2 calves contained one half of the normal amount of bone sialoprotein, but virtually no osteonectin or bone specific proteoglycan. In addition, dentin from BOI-2 calves was found to be deficient in phosphophoryn, a major non-collagenous bone protein of normal dentin[6]. These results indicate that bovine osteogenesis imperfecta, like the human disease, is genetically heterogeneous. Biochemical determinations of non-collagenous bone proteins are now being studied in bone specimens from humans with variable forms of this disease. Studies aimed at determining whether the defects in bovine and human osteogenesis imperfecta bone are synthetic or degradative in origin are also under investigation in bone cell cultures. Cells obtained from bovine and human sources actively synthesize and deposit an extracellular matrix which mineralizes *in vitro*. Use of non-collagenous, bone-specific proteins as markers in bone cell culture systems appears to provide a powerful tool for study of bone function in health and disease.

[1]Termine, J.D. *et al.*, JBC 255:9760, 1980. [2]Termine, J.D. *et al.*, JBC 256:256, 1980. [3]Termine, J.D. *et al.*, Cell 26:99, 1981. [4]Fisher, L.W. *et al.*, JBC 258:6588, 1983. [5]Fisher, L.W. *et al.*, JBC 258:12723, 1983. [6]Termine, J.D. *et al.*, PNAS USA, in press.

Collagens and Elastin

1564 FURTHER BIOCHEMICAL CHARACTERIZATION OF TYPE VII COLLAGEN FROM HUMAN AMNION, Nicholas Morris, Lou Ann Marvey, Hanne Bentz, David W. Hollister and Robert E. Burgeson, Shriners Hospital for Crippled Children, Portland, OR 97201

Collagens are a multigene family of fibrous proteins. Currently, greater than 15 different polypeptide (α) chains have been identified which comprise at least 8 different molecular forms (types). However, only one distinct type of fiber structure has been described, the banded fibrile of Types I, II and III collagen. Models are emerging for the structure of Type IV collagen fibers and there is preliminary evidence for the macromolecular organization of Type VI and VII. Type VII collagen was isolated from human chorioamniotic membrane. It is a disulfide linked trimer of a single polypeptide chain, approximately 150,000 MW. The triple helical domain includes more than 90% of the molecule and is separated into two fragments, P_1 and P_2, at a pepsin sensitive site. As isolated from the tissue, Type VII is found in antiparallel molecular dimers (6 polypeptide chains) connected by intermolecular disulfide bonds within an overlap region of the P_1 ends of the molecule. P_2 contains only intramolecular disulfide bonds. Type VII contains a very large cyanogen bromide peptide spanning the pepsin site which encompasses most of P_2 and part of P_1 and is not disulfide bonded. Hence, the disulfide bonds in P_2 must be located very near the end of P_2 opposite the pepsin site. Type VII also contains a collagenase resistant disulfide bonded peptide which becomes collagenase sensitive after reduction. This peptide is found in P_1 and may be the overlap portion of that molecule. We hypothesize that Type VII collagen is the anchoring fibril protein since SLS aggregates of Type VII closely resemble the banded anchoring fibril in toad skin.

1565 TISSUE-SPECIFIC EXPRESSION OF TYPE I COLLAGEN GENES, Sherrill L. Adams, Eileen S. Allebach and Richard J. Focht, University of Pennsylvania, Philadelphia, PA 19104

The collagens are a large family of closely related extracellular matrix proteins whose synthesis is regulated in a tissue-specific way. For example, Type I collagen is synthesized at very high levels in bone and tendon, intermediate levels in skin, low levels in smooth muscle, and not at all in cartilage. We have examined the basis for this tissue-specific variation, using highly purified populations of primary cell cultures from tissues of chick embryos and have found that there are several parameters which determine this wide range of synthetic rates. (1) RNA levels differ considerably among the various cell types, indicating that the Type I collagen genes are probably transcribed at different rates. However, there is not a strict correlation between the amount of mRNA and the rate of protein synthesis. (2) The Type I collagen mRNAs differ qualitatively from one cell type to another, due at least in part to selection of alternative polyadenylation signals, giving rise to RNAs which differ by hundreds of nucleotides in the length of the 3' untranslated region. There is a very strong correlation between the type of transcript present and the translatability of the mRNAs, both *in vivo* and *in vitro*, implying that the 3' untranslated region may determine translational efficiency. (3) In at least one type of cell we have examined, the Type I collagen mRNAs are present and can be translated *in vitro*; however, no synthesis of Type I collagen is observed in the intact cells, indicating that there may also be cell-specific factors required for recognition of the mRNAs.

1566 $[\alpha 1(V)]_2\alpha 2(V)$ AND $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ COLLAGEN MOLECULES IN THE HUMAN PLACENTA, Michel van der Rest and Christopher Niyibizi, Shriners Hosp. and McGill U., Montreal, Canada.

Type V collagen was extracted from human placentas after pepsin digestion and separated from the other collagen types by differential salt precipitation. This crude type V collagen preparation contained the α1(V), α2(V) and α3(V) chains in variable proportions. Several independent methods were found to resolve these crude preparations into two fractions. Based on densitometric integration of electrophoretic bands, one fraction contained α1(V) and α2(V) in a 2:1 ratio while the other fraction contained α1(V), α2(V) and α3(V) in a 1:1:1 ratio. The methods used for this separation were: a) phosphocellulose chromatography; b) ammonium sulphate precipitation in 0.5M acetic acid; c) ion exchange h.p.l.c. (IEX-540 DEAE column). All these separations were done under non denaturing conditions. The separated molecules were shown to be triple helical by circular dichroism [for methods a) and b)] and by their resistance to a trypsin-chymotrypsin digestion [for method b)]. The fractions isolated by methods a), b) and c), as well as a crude type V preparation were analyzed by native gel electrophoresis in a first dimension and under denaturing conditions in a second dimension. Two bands were observed in the crude preparation, a fast moving band containing only α1(V) and α2(V) and a slow moving band containing the three chains in apparent equimolar ratio. All the other fractions showed single bands, on native gels, with migrations corresponding to the fast moving band of the crude preparations when they contained α1(V) and α2(V) in a 2:1 ratio and to the slow moving band when they contained α1(V), α2(V) and α3(V) in a 1:1:1 ratio. Thermal denaturation of the fractions containing α1(V), α2(V) and α3(V) showed symmetrical transitions, about 2°C lower than the corresponding $[\alpha 1(V)]_2\alpha 2(V)$ fractions. These data are best explained by postulating the existence of two heterotrimers in placental type V collagen, with $[\alpha 1(V)]_2\alpha 2(V)$ and $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ stoichiometries.

1567 BASEMENT MEMBRANE METABOLISM PURSUED BY RADIOIMMUNOLOGICAL DETERMINATION OF TYPE IV COLLAGEN FRAGMENTS IN SERUM Dietrich G. Brocks and Horst P. Neubauer HOECHST AG Frankfurt/Main Fed.Rep. of Germany

Using a specific and sensitive radioimmunoassay for 7s collagen (Risteli et al. Eur.J.Biochem 108,239 (1980)), isolated from EHS-sarkom, the metabolism of basement membrane collagen was studied in streptozotocin diabetic rats. Intravenous administration of radioactively labelled and of non-labelled 7s collagen showed that the elevated 7s collagen serumlevels in streptozotocin diabetic rats (Risteli et al. Diabetologia 23,266 (1982)) were not due to different biological half life times in normal and streptozotocin diabetic rats. Gelfiltration of serum showed the size distribution of antigenic material, one peak corresponded to a molecular weight comparable to 7s collagen, the second peak showed a considerably higher molecular weight (presumably intact type IV collagen). The percentage of antigenic material present in the peak corresponding to higher molecular weight increased significantly during the development of streptozotocin diabetes. We interprete the data in that way, that during streptozotocin diabetes there is an increased biosynthesis of type IV collagen reflected by increased serumlevels of type IV collagen as measured by 7s collagen RIA. This interpretation is supported by findings of Hasslacher et al. (Diabetologia in press) who found a good correlation of 7s collagen serumlevels and the glomerular synthesis of type IV collagen.

1568 IDENTIFICATION AND EXPRESSION OF A HUMAN COLLAGEN GENE. Kathryn S.E. Cheah, Jane R. Griffin*, Rita Tilley*, Frank G. Grosveld**, Ellen Solomon*. Department of Biochemistry, Hong Kong University, Hong Kong; *Imperial Cancer Research Fund,London U.K. and **National Institute for Medical Research, London U.K.

The 38kb human DNA cosmid clone cosHcol.1 previously isolated and characterised by us codes for a collagen gene with approximately 65% homology with other known collagen genes (Weiss et al 1981). CosHcol.1 contains, in common with other interstitial collagen genes 54bp and 108bp exons in the coding region for repeating gly-X-Y triplets found in the triple helical domain of collagen. The 3' end of this gene contains regions of conservation similar to those found in collagen α1(I), α2(I), α1(II), and α1(III) genes. (K. Cheah, N. Stoker & J. Griffin, unpublished results). The hybridisation abilities of subfragments of cosHcol.1 and overlapping cosmid clones to mRNA indicate that the entire gene is encoded within 35kB of cosHcol.1. To determine whether this clone corresponds to an expressed collagen gene its homologous mRNA transcript was sought by hybridisation to mRNA from different sources on Northern blots. A total of 26 mRNAs from a variety of tissues and cell lines synthesizing collagens types I-V were tested. The hybridisation characteristics of cosHcol.1 to these different mRNAs and to tissue sections of developing mouse embryos by in situ hybridisation will be described. CosHcol.1 may correspond to a hitherto uncharacterised collagen.

Weiss, E.H., Cheah, K.S.E., Grosveld, F.G., Dahl, H.H.M., Solomon, E. and Flavell, R.A. (1982) Nucl. Acids Res. 10, 1981-1994.

1569 STRUCTURE OF PROCOLLAGEN IV AND CLEAVAGE OF ITS TETRAMERS BY THE SPECIFIC MAMMALIAN COLLAGENASE, Liselotte I. Fessler, Keith G. Duncan and Karl Tryggvason, University of California at Los Angeles, CA 90024

Tetramers of procollagen IV molecules joined at their amino ends were prepared from mouse PF-HR9 cells (Duncan et al JBC 258, 5869, 1983), then cut with a type IV specific mammalian collagenase (Salo et al., JBC 258, 3058, 1983), and the products were separated by velocity sedimentation. Electron microscopy showed the amino end junctional complex, 7s collagen, 32 nm long, with four arms. The initial thread length of arms was 353 nm with an additional knob end, and the collagenase cut this into 89 nm arms and free 267 nm threads, to which the knob was attached. Further digestion with bacterial collagenase gave the knob, and on SDS-PAGE this yielded the ca 30K peptides, identified as the carboxyl end of proα IV chains (Fessler JBC 257, 9804, 1982). Therefore this collagenase cuts procollagen IV into two parts: an approximately 0.3 amino fragment and 0.7 carboxyl piece, and the results confirm our previous assignment of amino and carboxyl ends. Individual procollagen IV molecules were also cut with this collagenase and both amino and carboxyl fragments were found to be inter-chain disulfide linked. Disulfides must link chains within each fragment of the thread, as they are absent from the carboxyl knob. The constituent C1 and C2 carboxyl propeptides differ in electrophoretic mobility, and their interactions were investigated.

1570 ACTIVATION OF TYPE I COLLAGEN GENES IN SCLERODERMA FIBROBLASTS
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Pro 1(I)collagen mRNA levels in fibroblasts cultured from affected and non-affected skin areas of scleroderma patients were measured by nucleic acid hybridization. Total RNA was isolated from the cells, electrophoresed and transfered to nitrocellulose filters. The RNA blots were hybridized with nick-translated plasmid pCAL1, containing a 675 bp insert of cDNA for chicken pro 1(I)collagen mRNA. The rate of collagen synthesis in the same cells was estimated simultaneously with an inhibition ELISA for type I collagen and with densitometric scans of fluorograms of ^{3}H-proline labeled culture medium polypeptides. Fibroblasts grown from affected sclerodermatous areas exhibited 2 to 7 -fold higher levels of pro 1(I)collagen mRNAs than non-affected controls to account for the proportionately increased synthesis of type I collagen by the same cells. These findings suggest that a marked activation of type I collagen genes has occurred at the transcriptional level in scleroderma. Upon subculturing the affected cells gradually reduce their rate of collagen synthesis; by the 10th passage the synthesis of collagen in affected and non-affected cells proceeded at the same rate. Preliminary data, using a cDNA clone for chicken pro 2(I)collagen mRNA (pCAL2), suggests that the levels of pro 2(I)collagen mRNA closely follow those of pro 1(I)collagen mRNA.

1571 Unusual structures in the pro α2(I) collagen gene. Sirpa Aho, Mitchell Finer, Valerie Tate and Helga Boedtker, Harvard University, Cambridge, MA 02138

To precisely elaborate the structure of one collagen gene, we have extended the DNA sequence analysis of the chicken pro α2(I) collagen gene to include the 2.6 kb between exons 1 and 3 (numbered 5' to 3'), and thus located exon 2, and to include 15 exons (encoding residues 412 to 765) and about half of the introns in a 11 kb central portion of the gene. Exon 2, an 11 bp exon that contains two overlapping donor splice junctions (Tate, et al, 1983, Nucleic Acids Res. 11, 91), is located between a Pst I site and two partly overlapping consensus donor splice junctions. Thus the correct splicing of this exon requires recognition of only one of four adjacent donor splice sites.

Moreover a S1 nuclease hypersensitive site has been identified at the nucleotide level 180 bp 5' to the cap site by S1 nuclease digestion of supercoiled plasmids containing at least 200 bp of 5' flanking gene region of the α2(I) collagen gene. The S1 site is located within a stretch of forty-two pyrimidines, specifically within TCCCTCCCTTCCTCCCTCCT. (Supported by grants from the N.I.H., by the Academy of Science of Finland. S.Aho is a Fogarty International Research Fellow).

1572 EFFECTS OF CELLULAR DIFFERENTIATION ON TYPE I PROCOLLAGEN mRNA LEVELS IN PRIMARY RAT HEPATOCYTES CULTURES. MA Zern, MA Giambrone, DA Shafritz, DM Jefferson and LM Reid. Albert Einstein College of Medicine, Bronx, NY 10461.

We have recently shown that hepatocytes respond to the fibrogenic stimulation of schistosome ova by increasing Type I procollagen mRNA content (Saber et al., J. Cell Biol. 1983). In this study, we employed molecular techniques to evaluate the effects of dexamethasone (DEX) on primary cultures of adult rat hepatocytes grown on tissue culture plastic in either serum supplemented medium (SSM) or a serum-free hormonally defined medium (HDM). Cells were plated and allowed to attach for 24 hr. in a mixture of SSM + HDM. Cultures were then fed every 24 hr. for 4 days under 4 conditions with either SSM, SSM + DEX, HDM, or HDM + DEX. On the 5th day, RNA was extracted. DEX did not affect the amount of RNA isolated, nor did it influence the quantitative translation of the mRNA in the lysate system. However, SDS-PAGE revealed significant changes in specific proteins under these various conditions. DEX increased albumin synthesis in hepatocytes grown in SSM and decreased type I collagen synthesis in cells grown in either SSM or HDM. Cells grown in HDM synthesized more albumin than those grown in SSM. We then determined albumin, collagen and β actin sequence content by molecular hybridization with cDNA clones. Confirming the protein synthesis results, we found the DEX increased albumin mRNA levels in RNA extracts from cells grown in SSM and decreased collagen mRNA levels in RNA from cells grown in SSM or HDM. β actin mRNA levels remained unaffected by any condition. These results suggest that: 1) HDM seems to encourage the expression of the differentiated state, and 2) DEX reduces Type I collagen mRNA content and increases albumin mRNA content in hepatocytes, possibly by inhibiting the dedifferentiation process.

1573 CHARACTERISATION OF A HUMAN COLLAGEN GENE, Neil G. Stoker, Kathryn S.E. Cheah, Jane R. Griffin, Frank G. Grosveld* and Ellen Solomon, Imperial Cancer Research Fund, London WC2 and *National Institute for Medical Research, London, NW7.

We have isolated a human collagen gene from a cosmid library, using a chicken procollagen α1(I) cDNA clone as a probe (1). Although it was originally referred to as an α1(I)-like gene, DNA sequencing has shown it and its putative product to be no more similar to α1(I) collagen than to any other collagen gene or protein which has been sequenced. Furthermore, the gene has been mapped to chromosome 12 (2), whereas the genes encoding α1(I) and α2(I) collagen chains have been mapped to chromosomes 17 and 7 respectively.

The clone hybridises strongly to a single mRNA-species from a rat chondrosarcoma, and to a similarly sized mRNA from human tissue. Hybridisation of overlapping cosmid clones indicates that the entire gene may be just contained within the 37kb of the original clone.

An important role for the product of this gene in connective tissue synthesis is suggested by the presence of deletions in or near the gene in several patients with Ehlers-Danlos Syndrome Type II and lethal osteogenesis imperfecta, but not in control individuals (M. Pope, Northwick Park, personal communication).

Details of the sequence and organisation of this gene will be presented.

1. Weiss, E.H., Cheah, K.S.E., Grosveld, F.G., Dahl, H.H.M., Solomon, E. and Flavell, R.A. (1982) Nucl. Acids Res. 10, 1981-1994.
2. Solomon,E., Hiorns,L., Cheah,K.S.E., Parkar,M., Weiss,E. and Flavell,R.A. International Human Gene Mapping Workshop 7, Los Angeles, 1983. Cytogenet. Cell Genet. in press.

1574 A 38 BP INSERTION IN THE PROα2(I) COLLAGEN GENE IN THE MARFAN SYNDROME, Elizabeth Henke, W. Mark Leader, Sheldon Pinnell and Russel E. Kaufman, Duke University, Durham, NC 27710

Abnormalities in Type I collagen have been found to be responsible for a number of connective tissue disorders. We have attempted to characterize an alteration in the proα2(I) collagen gene of an individual with the Marfan Syndrome who was reported by Byers et al, 1981, to have a 20-25AA insertion in one of the proα2(I) collagen chains 5' to the collagenase cleavage site. We first studied by restriction enzyme digestion and Southern blotting, 20 kb of the proα2(I) gene of this individual but were unable to demonstrate any alteration in restriction endonuclease fragments. We subsequently prepared a bacteriophage gene library from this patient's DNA. The library was screened with fragments of the normal proα2(I) gene which encode the region near the collagenase cleavage site. Comparison of the Marfan library recombinants with normal collagen gene recombinants by restriction endonuclease mapping demonstrated a 400 bp Xba-EcoRI fragment which was approximately 40 bp longer than the normal control. This Xba-EcoRI fragment hybridized to a normal 1.2 kb EcoRI fragment in the proα2(I) collagen gene. The DNA sequence of this fragment and of the corresponding fragment in the control gene were identical with the exception of a 38 bp insert in the gene derived from the individual with Marfan Syndrome. This alteration may represent a useful marker for diagnosis of the Marfan Syndrome.

1575 TYPE IV COLLAGEN SYNTHESIS AND ASSEMBLY INTO THE BASAL LAMINA BY CULTURED HUMAN MICROVASCULAR ENDOTHELIAL CELLS. R.H. Kramer, P.M. Davison, AND M.A. Karasek, Departments of Anatomy, and Dental Medicine, University of California, San Francisco, CA 94143, and Department of Dermatology, Stanford University, Stanford, CA 94143.

Cultured microvascular endothelial cells (MEC) isolated from the human dermis elaborate a continuous basal lamina-like structure. Immunofluoresence studies with specific antibodies indicate that the extracellular matrix produced by the MEC contains type IV collagen, laminin, thrombospondin, and fibronectin. Analysis of radiolabeled subendothelial matrix, after electrophoresis in SDS-gels under reduced conditions, revealed the presence of two major proteins that have apparent mol. wt. of 180,000 and 400,000. Both proteins are sensitive to purified collagenase but exhibit resistance to digestion by pepsin. Although the 180,000 dalton protein is also secreted into the culture medium, the 400,000 dalton protein is mainly associated with the subendothelial matrix. The 180,000 dalton protein is partially extracted from the matrix with 4M urea or 2M guanidine HCl. In contrast, the 400,000 dalton protein is solubilized only after extraction with SDS plus reducing agent. Immunoprecipitation of culture media-associated proteins with specific antibody identifies the 180,000 dalton component as type IV procollagen monomer. The identity of the high mol. wt. collagen is not known, but it appears to represent an aggregate of type IV collagen that is cross-linked by covalent bonds that are resistant to cleavage with reducing agents under denaturing conditions and are formed in the presence of beta-aminopropionitrile fumarate. The results indicate that the MEC deposit a basal lamina-like structure that is ultrastructurally and biochemically similar to that formed in vitro, and suggests that the MEC may provide a useful system for studies on the biosynthesis of basement membrane components by mirovascular endothelial cells.

1576 TYPE VII MAY BE THE ANCHORING FIBRIL PROTEIN, Louann Murray, Nicholas Morris, Granger Cobb and Robert Burgeson, Division of Medical Genetics, Harbor-UCLA Medical Center, Torrance, Ca. 90502.

Type VII collagen, isolated from human amnion by limited pepsin digestion has a unique CnBr and V8 protease map and segment-long-spacing (SLS) pattern (H. Bentz et al., 1983, PNAS 80: 3168-3172). It has an M_r of 170,000 per alpha chain, and is at least 90% triple helical with a helical length that is 1.45 times longer than Type I collagen. Type VII SLS can occur as 424 nm monomers but usually occur as centrosymmetrically banded, 785 nm dimers. There is one pepsin sensitive site about midway along the length of the molecule, dividing it into two fragments, P1 and P2. Here, we report an analysis of SLS of the P1 and P2 fragments, showing that the P1 fragment is from the end of the molecule that is involved in dimeric overlaps and that P2 is from the other end. Rotary shadowed preparations of Type VII molecules show that dimers are common and that the molecules are aligned in an antiparallel rather than parallel manner. A comparison of Type VII SLS to anchoring fibrils reveals that they are both centrosymmetrically banded, with a remarkably similar banding pattern, and have the same length. Using conditions that will reconstitute 67 nm period fibers from Type I collagen, we have produced SLS-like aggregates from Type VII. We suggest that the fiber form of Type VII collagen may be side-by-aggregates of dimeric molecules, sometimes seen as anchoring fibrils.

1577 COMPARISON OF THE COLLAGENS FROM PEPSIN SOLUBLIZED AND PAPAIN SOLUBLIZED SHARK FIN ELASOIDIN, Ronald J. Chandross and Gerald L. Mechanic, Dental Research Center, University of North Carolina, Chapel Hill, NC 27514

Shark fin elastoidin can be solublized by prolonged treatment with pepsin in dilute acetic acid at 18°C. An equally lengthy treatment with papain at 4°C (pH6.8) splits the native ceratotrichia into small hair-like fibrils which are readily soluble in 1% acetic acid. Collagen can be precipitated from the above acid solutions by the addition of NaCl to a concentration of 1M. Both forms have α, β, and γ components, all of which migrate on SDS-4% polyacrylamide gels slightly faster than the corresponding type I mammalian components. The pepsin derived collagen (form A) has the β and γ components in relatively greater amounts than those from the papain derived collagen (form B), and the α band of form A is usually a doublet, which differs from the single α band displayed by the B form. The A form is insensitive to the presence of mercaptoethanol, while the B form is readily dissociated into subunits. On the other hand, at pH 4.8, form A is sensitive to both heat (40°C) and urea (6M), both of which degrade it into a number of small fragments. No equivalent phenomenon has been noted for form B. This latter observation suggests that the apparent α1,α2 doublet of form A is a degradation artifact rather than a true analysis of the chain composition of elastoidin collagen.

1578 COLLAGEN SYNTHESIS DURING THE DEVELOPMENT OF CHICK CORNEAS, Winston W.-Y. Kao, Jonathan Ebert, and Candace W.-C. Kao, University of Cincinnati College of Med. Cinti.

It has been demonstrated that the five different tissue layers in cornea contain different collagen types. The synthesis of these collagen types changes during the development of cornea. In present studies, a chronological map of such changes in collagen synthesis was established. Corneal epithelium and stroma were dissected from chick embryos between day 9 and 21 of the development. The rate of DNA and collagen synthesis were determined by pulse-labeling the tissues with [^{3}H]thymidine and [^{14}C]proline. The results indicated that the epithelium reach a peak in collagen synthesis after 13 days of development and then decline rapidly. At the completion of epithelial stratification at day 19, the collagen synthesis by epithelium reaches a new peak. In the stroma, the cells maintained a low level of collagen synthesis before day 13. The collagen synthesis increases rapidly as the rate of DNA synthesis decreases in stroma after 13 days of development. The rate of collagen synthesis by stroma maintained a plateau between 15 and 19 days of development. The increase in collagen synthesis is accompanied by a sharp increase of prolyl hydroxylase activities. In other experiments, the collagen types synthesized by various tissues were examined. It was found that the stroma fibroblast synthesized type I and V collagen at all developmental stages examined. In epithelium, there is a change from the synthesis of collagen(I) to collagen(IV). The epithelium primary synthesized type I collagen before day 11, when the epithelium begins its stratification at day 13, synthesis of collagen(IV) can be detected. (Supported by NIH Grant EY 04641, an RPB Research Development Award and Ohio Lion's Eye Research Foundation).

1579 CHONDROCYTE BIOSYNTHESIS OF TYPE II COLLAGEN AND PROTEOGLYCAN, B.M. Vertel, J.J. Morrell, L.L. Barkman and L. Weber, Syracuse University, Syracuse, NY, 13210.
Although chondroitin sulfate proteoglycan (CSPG) and type II collagen are produced by the same chondrocytes in culture, differences in certain details of the synthesis, secretion and extracellular deposition of these matrix molecules can be observed by double immunolocalization analysis. Polyclonal antibodies directed against type II collagen and the CSPG aggregate components (link protein and CSPG monomer) and monoclonal antibodies directed against CSPG monomer were utilized in immunohistochemical reactions. Chondrocytes grown without ascorbate supplements synthesize type II collagen and CSPG, yet deposit only CSPG in extracellular matrix. Intracellular CSPG molecules are localized primarily in perinuclear regions. Monoclonal antibodies which recognize antigenic determinants associated with sulfated oligosaccharide chains of CSPG monomer exclusively stain these perinuclear regions. In contrast, intracellular type II collagen is distributed in vesicles throughout the cytoplasm. After the addition of ascorbate, we observed a redistribution of intracellular type II collagen and CSPG within 1 hr and the extracellular deposition of fibrous type II collagen and associated CSPG within several hr. Under all conditions, we noted cytoplasmic vesicles which stained intensely with antibodies to link protein and CSPG monomer precursors and not with type II collagen antibodies. Interestingly, these vesicles did not localize monoclonal antibodies which recognize antigenic determinants associated with sulfated oligosaccharide chains. Our observations suggest that these vesicles are pre-Golgi compartments, perhaps involved in some aspect of the biosynthetic processing or intracellular transport of proteoglycans and not other secreted matrix molecules. (Supported by NIH grant AM-28433).

1580 ISOLATION OF MINOR COLLAGENS FROM SWARM CHONDROSARCOMA, Lisa Workman, S. Chandrasekhar and Gary Balian, Univ. of Virginia, Charlottesville, VA 22908 and NIDR, Bethesda, MD

The Swarm chondrosarcoma is a cartilage-like tumor found in rats which is known to contain type II collagen, cartilage specific proteoglycan and fibronectin. When the pepsin solubilized extract was salt fractionated at acid pH it was found that, in addition to type II collagen, the tumor contains ($1\alpha,2\alpha,3\alpha$) and disulfide bonded HMW collagen. Further purification of HMW collagen was achieved using the differential solubility properties of this collagen in 0.02M Na_2HPO_4 pH 9.0. The availability of a tumor that produces significant quantities of the minor cartilage collagen components provides a convenient means for their further study. SDS-PAGE of ($1\alpha,2\alpha,3\alpha$) collagen extracted from lathyritic tumor without pepsin digestion migrated significantly more slowly than the pepsinized counterparts reflecting a 10-12 kilodalton difference between pepsinized and unpepsinized chains. Type II α-chains were of the same apparent molecular weight with or without pepsin extraction. Cells were isolated from the tumor by digestion with trypsin and bacterial collagenase and grown in monolayer cultures. Metabolic labeling with [^{3}H]proline showed that types I, II and III collagen and fibronectin were the major components synthesized and released into the medium. Types II and ($1\alpha,2\alpha,3\alpha$) collagen were identified in the cell layer. The presence of the minor collagens of cartilage, ($1\alpha,2\alpha,3\alpha$) and HMW, in the rat Swarm chondrosarcoma further demonstrates the usefulness of the tumor for the study of macromolecules normally present in hyaline cartilage. The tumor may serve as a convenient model for studying the minor collagenous components of cartilage.

1581 FURTHER BIOCHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF MINOR DISULFIDE-BONDED CARTILAGE COLLAGEN, Daniel Herbage, Syvie Ricard-Blum, Jérôme Tiollier and Daniel Hartmann, Claude Bernard University, Lyon, and Pasteur Institute, Lyon, France.

Several new minor collagens have been isolated from cartilaginous chains. We extracted from fetal calf cartilage disulfide-bonded collagen, certainly related to collagenous fractions (type M, HMW-LMW, CPS...) described by different authors, but showing some major differences. This pepsin-soluble fraction is composed of at least three different helical segments revealing without reduction, 7 bands on SDS-PAGE (called X_1 - X_7). These different segments were isolated and their denaturation components analysed by mono and bidimensionnal polyacrylamide gel electrophoresis after reduction, CNBr or V_8- protease treatments.
Relationships between the different bands were observed. Parallelely these collagenous fractions were studied using physico-chemical methods such as rotary shadowing, electrical birefringence, differential scanning calorimetry and in E.M. under the form of different SLS fragments. The major molecules were present as relatively flexible rope - like filaments of 90 - 100 nm in length.

Using light and electron immunoperoxidase microscopy this collagen was located to the pericellular region of the chondrocytes with an increasing intensity from the superficial to the inner and growth plate zone of the epiphysis.

1582 LOCATION OF 64 K COLLAGEN PRODUCER CHONDROCYTES IN DEVELOPING CHICK EMBRYO TIBIAE.
Ranieri Cancedda, Olga Capasso, Fiorella Descalzi Cancedda†, Natalina Quarto and Gianfranco Tajana. 2nd Medical School and I.I.G.B. CNR†, Naples, Italy.
The synthesis of a new low molecular weight collagen by cultured chick embryo chondrocytes has been recently demonstrated (Capasso et al., 1982 Exp. Cell Res. 142: 197; Gibson et al., 1982 J. Cell Biol. 93: 767; Schmid and Conrad 1982 J. Biol. Chem. 257: 12444). By pulse-chase labeling of cells and by enzymatic digestion of extracellular proteins we have observed that in chondrocytes cultures, this collagen, by us called 64 K, is deposited extracellularly and rapidly matured into a 30 K molecular weight fragment. In the developing chick embryo, chondrocytes synthesizing 64 K collagen concentrate at the diaphysis of 9 day old and at the epiphysis of 17 day old tibiae; these regions are characterized by a remodeling of the cartilage matrix leading to the replacement of the cartilage with the bony tissue. We suggest that the 64 K collagen is a marker of a specific developmental stage of the chondrocytes. To further investigate the origin of cells competent for the synthesis of the 64 K collagen and its role during the tibia organogenesis we have isolated this collagen from tibiae of 17 day old embryos and we are preparing specific antibodies to be used in immunofluorescence studies.

1583 MATRIX INFLUENCE ON RELEASE OF THE TUMOR CELL SURFACE STIMULATOR OF FIBROBLAST COLLAGENASE PRODUCTION, Chitra Biswas, Dept. of Anatomy and Cellular Biology. Tufts University School of Medicine, Boston, MA 02111.

Increased levels of collagenase against type I collagen in cocultures of fibroblasts and tumor cells have been demonstrated previously (Biswas, BBRC, 109: 1026, 1982). This stimulatory effect was found to be mediated by a factor present in the conditioned media of tumor cells. Further experimentation has demonstrated that the level of the stimulatory factor in the conditioned media from tumor cells depends on the culture conditions even though high levels of stimulation are consistently obtained in cocultures. It has now been found that the conditioned media from B-16 cells plated on matrix produced by fibroblasts ("SAM") is consistently stimulatory as opposed to medium from cells plated on tissue culture plastic or on collagen. As a result of the above studies the possible involvement of plasma membrane-bound stimulator was explored. Addition of membrane fractions prepared from B-16 cells to rabbit fibroblasts stimulated collagenase production, whereas addition of rabbit fibroblast membrane to B-16 cells had no effect. The extent of stimulation obtained with the tumor cell membrane fraction was equivalent to the maximum obtained in cocultures of fibroblasts and B-16. These studies indicate that the stimulatory factor(s) is present at the cell surface of tumor cells and that under the influence of matrix deposited by fibroblasts this factor is released from the cell surface into the medium.

1584 AN ENDOGENOUS NON-ENZYMATIC ACTIVATOR OF PROCOLLAGENASE: ITS ROLE IN INFLAMMATION,
Jacqueline B. Weiss, Simon Elstow and Barry McLaughlin, University of Manchester Medical School, England, M13 9PT

A factor has been isolated from a variety of sources such as synovial fluid, normal and diabetic retina, human vitreous from patients with diabetic or other proliferative retinopathies and from tissue culture fluids of cultured tumour cells. The factor is capable of stimulating capillary endothelial cell mitosis and is capable of initiating angiogenesis in the chick chorioallantoic membrane (CAM) test and in the rat corneal assay. The factor has been called endothelial cell stimulating angiogenesis factor (ESAF) and has been shown by gel filtration and diafiltration to have a molecular weight in the order of 400-600. The factor activates procollagenase (prepared from normal human skin fibroblasts) and this effect can be achieved with hormonal concentrations (pg). The activation is greater than that achieved with organic mercurials but of the same order as can be obtained with trypsin.

The angiogenic effect of ESAF in the CAM test is potentiated by heparin and the effectiveness of its action on procollagenase can also be potentiated with heparin. The relevance of this information to the inflamed connective tissue will be discussed.

1585 CLEAVAGE OF PROCOLLAGEN BY CATHEPSIN D- EVIDENCE FOR A PHYSIOLOGICAL ROLE, Donald L. Helseth, Jr., Northwestern University Dental School, Chicago, IL 60611.

The enzymatic removal of the COOH-propeptide from procollagen molecules has been studied in vitro and in organ culture. A crude catheptic activity isolated from chick embryo homogenates specifically removes the COOH-propeptide from procollagen without attacking the NH_2-propeptide; this activity was similar to that identified by Davidson, et al., and was inhibited by pepstatin. Affinity-purified (1200-fold) Cathepsin D specifically removed the COOH-propeptide from procollagen. At pH values below 5.5, cleavage occurs in the C-telopeptide at position 6^C-7^C. Between pH 5.5-6.0 the cleavage site shifts to a position indistinguishable from the authentic "C-proteinase" cleavage site. The pH 6.0 cleavage could not be inhibited by inhibitors of other classes of proteinases, ruling out the possibility of a second proteinase being activated by Cathepsin D. To determine whether Cathepsin D might play a role in the in vitro processing of procollagen, chick embryo sternae were labeled with ^{14}C-Pro, and further protein synthesis was inhibited by the addition of cycloheximide. Subsequent processing of procollagen was followed in the presence or absence of various lysosomotropic amines, including chloroquine; all inhibited COOH-propeptide cleavage. Since these agents block the processing of lysosomal enzymes, the procollagen biosynthetic pathway may parallel or merge with the lysosomal biosynthetic pathway. The in vivo processing of procollagen may thus involve an intracellular pathway with Cathepsin D removing the COOH-propeptide in a slightly acid (pH 5.5-6.0) compartment, possibly the secretory granule or the immediate pericellular space. (Supported by NIH Grants AM06476 and AM13921).

1586 REMOVAL OF AMINOTERMINAL PROPEPTIDE FROM TYPE III PROCOLLAGEN: PARTIAL PURIFICATION AND CHARACTERIZATION OF ENZYME ACTIVITY, Leena Peltonen, Ritva Halila and Lasse Ryhänen, University of Oulu, Oulu, Finland

Procollagen type III aminoterminal protease was partially purified from two sources: From the cultures of smooth muscle cells of fetal calf aorta about 400 fold and from human placental tissue about 4000 fold. The purification procedure consisted of the successive affinity chromatographies on Con-A-Ultrogel, Heparin - Sepharose and pN type III collagen - Sepharose. This enzyme activity with a molecular weight about 70 000 did not cleave type I or type IV procollagen and also denatured PN type III collagen remained uncleaved. Neutral pH and Ca^{2+} were required for maximal activity and the K_m of the enzyme was 0.76 µM for type III pN collagen. The metal chelators EDTA and EGTA inhibited the activity but there was no inhibition by serum or several proteinase inhibitors.

This enzyme activity was assayed together with the quantitation of type III N-propeptide in culture medium of scleroderma cell lines with increased collagen synthesis and recessive dystrophic epidermolysis bullosa cell lines with highly increased collagenase activity. The purpose of these studies was to detect the possible differences in the conversion process of type III collagen during excessive synthesis or degradation of interstitial collagens.

The conversion processes of both type I and III collagens were further analyzed on cell cultures using known effectors of type I and type III N-proteases (1,2). Ca-free medium as well as EDTA completely inhibited removal of N-propeptides of both collagen types and Zn^{2+}-ions slightly inhibited this conversion. Addition of lysine into culture medium inhibited removal of C-propeptide of both collagen types but did not effect the removal of N-propeptide.

References: 1: Tuderman & Prockop, Eur J Biochem 125,545,-82, 2: Halila & Peltonen, Biochemistry -84, in press)

1587 *THE ROLE OF ELASTASE-LIKE PROTEASES OF SMOOTH MUSCLE CELLS AND FIBROBLASTS IN THE MODULATION OF ELASTIN BIOSYNTHESIS AND DEGRADATION. Ladislas Robert, Georgia Meimon, and William Hornebeck. Labo. Bioch.Tissu Conjonctif,GR CNRS 40,Fac.Méd.Créteil,France.*

Elastase-type proteases were isolated and characterized from aorta smooth muscle cells (SMC) and skin fibroblasts. The first is a membrane-bound serine protease, the second a metalloprotease. Neither is sensitive to alpha$_1$-antiprotease. The SMC-enzyme activity increases linearly with passage number and also with in vitro aging of confluent cultures or by adding LDL (but not HDL) to the culture medium. These same parameters influenced human and rabbit aorta elastase activity.Its reaction during aging and atherosclerosis appears to be conditioned by a direct contact between mooving cells and the elastic lamellae. Cell movement with increased secretory and increased degrading activity may be the result of phenotypic modulations during atherogenesis as suggested by the Campbells. The fibroblast enzyme is secreted (~30 % of the total activity) in the medium. It appears to be involved in degradation of skin elastic fibers progressing with age and accelerated in pathologies as atherosclerosis, LSA and possibly also in the attack of basal laminae in bullous epidermolysis. Colchicine (10^{-5}-10^{-7} M) decreased its synthesis and secretion. Colchicine administration (1 mg) to patients (micro- and macroangiopathy) during 3-4 months, resulted in a significant increase of the cutaneous elastic network estimated by histochemistry and computerized image analysis (G. GODEAU, AM.ROBERT and G. LAGRUE, in prep.). As both enzymes studied can attack tropoelastin and also microfibrils, the control of elastogenesis may well be dependent on the regulation of the above enzymes.

Proteoclycans

1588 GLYCOSAMINOGLYCAN SYNTHESIS BY DEVELOPING AND ADULT SUBMANDIBULAR GLAND SECRETORY UNITS, L. Cutler* and J. Rendell, Department of Oral Diagnosis, University of Connecticut Health Center, Farmington, CT

This study examines the nature of glycosaminoglycan (GAG) synthesis by developing and adult submandibular gland (SMG) secretory units. SMG secretory units were isolated from adult and 1 day male Sprague-Dawley rats by digestion of a glandular mince with hyaluronidase and collagenase followed by gentle sieving of the digest through a graded series Teflon screens. The acinar units were incubated for 2-3 hrs. in medium containing 50μCi/ml of [^{35}S]-sodium sulfate and [^{3}H]-glucosamine. Following the incubation the acinar units were washed and homogenized. The homogenate was digested in pronase, boiled, dialyzed against distilled water and then lyophilized. The lyophilized material was resuspended in ammonium acetate-ethanol buffer, chromatographed over a Sephadex G-50 column (0.9 x 120cm) and the total GAG peak collected. The GAG peak was subjected to sequential, specific digestion procedures. Following each digestion step the sensitive and resistant material was determined. Resistant material was lyophilized and then subjected to the next digestion step. The results indicated that 25-30% of the GAG synthesized by the adult acinar units was hyaluronic acid and 70-75% was heparan sulfate, while no chondroitinase ABC sensitive material was synthesized. The GAG synthesized by neonatal secretory units contained about 20% chrondroitinase ABC sensitive material in addition to hyaluronic acid and heparan sulfate. These data suggest that there are stage specific patterns of GAG synthesis during the maturation of SMG acini. Studies on GAG synthesis by prenatal SMG secretory units are currently in progress. This work was supported by NIH Grant DE 05632.

1589 GLYCOSAMINOGLYCANS IN POSTNATAL DEVELOPMENT OF THE RAT CEREBELLUM. William Werz, Günther Fischer and Melitta Schachner, Department of Neurobiology, University of Heidelberg, Fed. Rep. Germany

Biochemical analysis of glycosaminoglycan (GAG) content during postnatal development of rat cerebellum demonstrates three classes of GAG's: chondroitin sulfate (CS), heparan sulfate (HS) and hyaluronic acid (HA). CS and HS show relatively high levels at birth (3 and 1 μg/mg protein, respectively), which decrease until postnatal day 9 (P9) and rise again to reach adult levels of 2.5 and 0.7 μg/mg protein, respectively. HA content is 3 μg/mg protein at birth and decreases steadily to adult levels of 0.2 μg/mg protein. Relative contents of GAGs for neonatal and adult cerebellum are 43 and 73% for CS, respectively, 15 and 22% for HS and 42 and 5% for HA. Biosynthetic labeling of cerebellar explant cultures with ^{3}H-glucosamine indicates maximal synthesis of HS at P6 and HA synthesis with two peaks at P3 and P6. CS shows a steadily declining incorporation until adulthood. A correlation of content and synthesis of these GAG's with morphogenetic events in cerebellar development will be attempted.

1590 EFFECT OF NH_4Cl ON THE BIOSYNTHESIS AND INTRACELLULAR TRANSPORT OF CHONDROITIN SULFATE PROTEOGLYCANS BY HUMAN MELANOMA CELLS. J.R. Harper, R.A. Reisfeld, and V. Quaranta. Scripps Clinic and Research Foundation, La Jolla, CA 92037

Human melanoma cells synthesize a unique chondroitin sulfate proteoglycan whose core protein has an M_r = 250,000 and contains N- and O-linked oligosaccharides. Formation of the proteoglycan is completely inhibited by the monovalent ionophore, monensin, thus apparently supporting the notion that the addition of glycosaminoglycans occurs in the Golgi apparatus. However, since terminal glycosylation and maturation or the core protein is also inhibited by monensin, the question remains as to whether proteoglycan formation is contingent upon core protein maturation, as if it is regulated by an independent, post-Golgi processing step. We have now examined the effect of NH_4Cl on core protein maturation and proteoglycan biosynthesis. Pulse-chase studies performed in the presence of 15 mM NH_4Cl resulted in a marked inhibition of proteoglycan synthesis, while the kinetics of core protein biosynthesis and terminal processing were unaffected. After long-term labeling of melanoma cells with ^{3}H-leucine in the presence of NH_4Cl, we found a significant accumulation of the endo-H resistant mature 250K core protein concomitant with inhibition of proteoglycan biosynthesis. These data strongly suggest that the NH_4Cl inhibition of proteoglycan synthesis occurs at a biosynthetic step subsequent to that which is inhibited by monensin. Furthermore, since NH_4Cl induces the accumulation of a core protein that has apparently cleared the Golgi apparatus (endo-H resistant), it appears that glycosaminoglycan addition may involve an acidic post-Golgi compartment. This low pH requirement of proteoglycan biosynthesis may involve either recycling of an intracellular receptor or low pH-dependent fusion of intracellular membranes.

1591 T LYMPHOCYTES RESPOND TO MYELIN BASIC PROTEIN AND PRODUCE A MATRIX DEGRADING HEPARAN SULFATE ENDOGLYCOSIDASE, Israel Vlodavsky, Yaakov Naparstek, Zvi Fuks and Irun R. Cohen, Hadassah University Hospital, P.O. Box 12000, Jerusalem, and the Weizmann Institute, P.O. Box 26, Rehovot, ISRAEL.

During inflammatory processes of the central nervous system (CNS), lymphocytes penetrate the blood brain barrier and accumulate in the nervous tissue. Extravasation from the vascular compartment requires that the cells invade the vascular endothelium and degrade its underlying basal lamina. To learn more about processes of antigen recognition and penetration of blood vessels we studied the interaction in vitro of anti-BP effector T lymphocytes with the basement membrane-like extracellular matrix (ECM) produced by vascular endothelial cells. Both the accumulation in the brain of intravenous inoculated lymphocytes and their subsequent induction of experimental autoimmune encephalomyelitis were unique characteristics of the activated anti BP line cells. In the present study we have compared the ability of activated and non activated anti BP T cells to degrade sulfated glycosaminoglycans in the subendothelial ECM. We report that both non specific and specific antigenic activation of T cells resulted in the induction of an endoglycosidase capable of degrading heparan sulfate side chains of the proteoglycan scaffold of the ECM. Moreover, the anti-BP lymphocytes responded to BP presented by ECM by markedly enhanced elaboration of the endoglycosidase. These findings indicate that tissue-specific antigens at the vascular-tissue interface could direct lymphocyte homing by activating enzymes that facilitate penetration of the subendothelial basal lamina.

1592 BIOSYNTHESIS OF CHONDROITIN SULFATE AND OLIGOSACCHARIDES ON CHONDROSARCOMA PROTEOGLYCAN, L.Stefan Lohmander, James H.Kimura, Masaki Yanagishita and Vincent C.Hascall, Dept. Orthopedic Surgery, University of Lund, Lund, Sweden; Depts. Orthopedic Surgery and Biochemistry, Rush Medical College, Chicago, Ill.60612; Mineralized Tissue Research Branch, NIDR, NIH, Bethesda, Md.20205

Cultured chondrocytes from Swarm rat chondrosarcoma synthesize a proteoglycan containing chondroitin sulfate chains and N- and O-linked oligosaccharides. Data indicate an intracellular half life of about 100 min for the proteoglycan precursor and that at least 70% of this time is spent in the rough endoplasmic reticulum (1,2). In experiments using 1-^{3}H-glucose as precursor the kinetics of entry of label into oligosaccharides and chondroitin sulfate was investigated. The results show that initiation of O-linked oligosaccharides as well as completion, including sialylation, occurs essentially at the same time as chondroitin sulfate chain elongation takes place, during the last minutes of the intracellular dwell time of the proteoglycan. This confirms data obtained with 6-^{3}H-glucosamine as precursor (3). Ratios between label incorporated into oligosaccharide-galactose and chondroitin sulfate-galactose indicated little if any temporal separation between these two separate biosynthetic events, suggesting they both take place in the same cellular compartment, the Golgi complex. Comparison of incorporation of label from ^{3}H-glucose into galactose and xylose of the chondroitin sulfate linkage region indicated a somewhat longer lag time before linear incorporation into xylose than for galactose. Interpretation of this difference will, however, have to await the determination of the kinetics of the UDP-sugar pools. 1. J.H.Kimura et al.(1981) J. Biol. Chem. 256, 4368; 2. S.A.Fellini et al., ibid., submitted; 3. E.J-M.Thonar et al.(1983) ibid. 258, 11564

1593 IMMUNOFLUORESCENCE ANALYSIS OF CARTILAGE PROTEOGLYCAN DEPOSITION DURING CHICK CHONDROGENESIS. Maurizio Pacifici, University of Pennsylvania. Philadelphia, PA 19104

Intracellular routing and extracellular deposition of proteoglycans were studied by immunofluorescence procedures in vertebral chondroblasts in culture and in definitive chondroblasts emerging during somitic and limn chondrogenesis in vivo. A rabbit antiserum was raised against the major keratan sulfate/chondroitin sulfate-rich cartilage proteoglycan (KS:CS-PG) isolated from adult chicken sterna. The specificity of this antiserum has been described elsewhere (Pacifici et al., J. Cell Biol., December 1983 issue). This antiserum stained the abundant extracellular matrix of monolayer vertebral chondroblasts and localized primarely in a large intracellular, juxtanuclear structure. Radioautographic and electronmicroscopic evidence proves this structure is the Golgi complex. In a complementary way, antibodies to Type II collagen (provided by Dr. K. von der Mark) stained primarely numerous cytoplasmic vacuoles that were notably reduced in the Golgi complex area. In in vivo studies, both the proteoglycan antiserum and Type II collagen antibodies localized only in the peri-notochordal sheaths of stage 14 through 22 chick embryos. At these stages no overt sclerotome chondroblasts could be identified. Both antisera, however, stained definitive chondroblasts emerging in stage 23 sclerotome at the wing level and in stage 25 wing buds. These results will be discussed in terms of possible differences in intracellular routing for KS:CS-PG and Type II procollagen, and in terms of the supposed roles of extracellular matrix in the determination of the chondrogenic differentiation program.

1594 PURIFICATION OF CHONDROITIN SULFATE E PROTEOGLYCAN FROM THE MOUSE BONE MARROW-DERIVED MAST CELL AND ANALYSIS OF ITS PROTEIN CORE, Richard L. Stevens and K. Frank Austen, Harvard Medical School, Boston, MA 02115

Previously we demonstrated that when mouse bone marrow cells are cultured in the presence of interleukin 3 they preferentially differentiate into a subclass of mast cells (MC) distinguishable from serosal MC by the presence of chondroitin sulfate E proteoglycan (ChS-E) rather than heparin proteoglycan (HP) in its secretory granules. Our demonstration that the rat serosal HP-containing MC could be induced to make a phenotypic switch from synthesis of HP to synthesis of ChS-E upon removal of the protein core requirement by xyloside treatment suggested that the peptide cores of the two MC proteoglycans are distinct. ChS-E was, therefore, extracted from 6 x 10^9 differentiated MC by detergent-guanidine treatment in the presence of protease inhibitors. The proteoglycan was purified 100-fold by sequential density-gradient centrifugation, and ion exchange and gel filtration chromatographies. Incubation of the purified proteoglycan with a number of different proteases revealed that it was resistant to degradation like HP. An aliquot was incubated with chondroitinase ABC and rechromatographed on the gel filtration column. Amino acid analysis before and after chondroitinase treatment revealed that, again like HP core, ChS-E was very rich in Ser and Gly with the sum of these two amino acids accounting for 70% of the total amino acids. However in contrast to HP which is essentially only a Ser and Gly copolymer, ChS-E core was found to be rich in Glu with the ratio of Ser to Glu being 1.6:1.0. Thus, studies on purified MC ChS-E revealed that its protein core was homologous but distinct from MC HP peptide core.

1595 A DISULFIDE-LINKED, SULFATED PROTEOGLYCAN-LIKE COMPONENT OF DROSOPHILA BASEMENT MEMBRANES, Andrew G. Campbell, Liselotte I. Fessler, and John H. Fessler, Department of Biology and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Several components of basement membranes have been obtained in this laboratory from the medium of cultured Drosophila KC cells. After incubation with [^{35}S]-sulfate and precipitation with $(NH_4)_2SO_4$, the following material was purified by a combination of velocity and buoyant density centrifugation and gel chromatography. The monomeric unit, after reduction, migrates as a [^{35}S]-labeled, periodic acid Schiff's positive band of approx. apparent molecular wt. 800K on SDS acrylamide and agarose gel electrophoresis. A series of higher multimers exist before reduction. The buoyant density in CsCl/4M GuCl is approx. 1.4g/ml and the material is resistant to chondroitinase ABC. It is partly degraded by alkali/$NaBH_4$. It is specifically precipitated by an antiserum made against it, and immunofluorescence microscopy locates this antigen in the basement membranes of Drosophila embryos and larvae. (Supported by AG02128 and G.O.F.P.)

1596 REGULATION OF PROTEOGLYCAN SYNTHESIS BY AVIAN TENDON CELLS IN CULTURE, Julie A. Robinson and Mina J. Bissell, Lab. Cell Biology, Division of Biol. & Med., LBL, University of California, Berkeley CA 94720.

Chick embryos (16-day-old) were radiolabelled with ^{35}S-sulfate for 24h. The tendons were then dissected from the embryos and the proteoglycans were extracted and purified by DEAE-Sephacel chromatography. We found that the tendons synthesize mainly chondroitin sulfate proteoglycan (CSPG) and a smaller amount of dermatan sulfate proteoglycan (DSPG). Whole tendons freshly dissected from 16-day-old embryos and radiolabelled *in vitro* for 2-3h also synthesize mainly CSPG and devote 30% of their total protein synthesis to collagen. If the tendon cells are isolated from the tissue by collagenase treatment and cultured in F_{12} medium (0.2% FCS), however, collagen synthesis rapidly decreases. In addition, proteoglycan synthesis switches from CSPG's to almost exclusively DS and HS proteoglycans. If the PAT cells are maintained in culture in the presence of ascorbic acid (50 g/ml), the level of collagen synthesis increases from a low of 5% to 25% of the total protein. After 2 weeks in culture, the PAT cells synthesize predominantly CSPG again, and this PG is secreted into the extracellular matrix (ECM). In the absence of ascorbic acid, the level of collagen synthesis remains low and the PAT cells continue to synthesize DS and HS proteoglycans. We postulate that PG synthesis by PAT cells in culture is regulated by the composition of the surrounding extracellular matrix. We have tested this hypothesis by studying the PG's synthesized by PAT cells plated onto tissue culture dishes coated with rat tail collagen, fibronectin or ECM prepared from cultures of PAT cells. Supported by US DOE, Contract No. DE-AC03-76SF00098.

1597 CHANGES IN HEPARAN SULFATE PROTEOGLYCANS WITH SKELETAL MUSCLE DEVELOPMENT, Douglas M. Noonan and Ronald J. Przybylski, Case Western Reserve Univ., Cleveland, Ohio, 44106.

Primary chick breast muscle cultures were set in L-15 medium with $^{35}SO_4^{2-}$. At 24, 42 and 72 hours in culture (corresponding to proliferation, alignment and fusion stages) cells were scraped in PBS with protease inhibitors. Cells were freeze-thawed and made to 0.2% SDS. Samples were dialyzed and subsequently run on Sepharose Cl-2B and Cl-6B columns (in SDS-tris buffer) after treatment with either acetic acid and $NaNO_2$ (to degrade heparan sulfate, HS) or sham digested. Sepharose Cl-2B columns demonstrated a small, broad peak of nitrous acid insensitive material just included from the void volume. This material is probably identical to the large chondroitin-6-sulfate proteoglycan found by Guanidine HCl extraction of chick muscle cultures (Carrino and Caplan, J. Biol. Chem. 257:14145 1982). Unlike Guanidine HCl extracts, SDS extracted samples also demonstrated a large, further included peak approximately 50% of which was nitrous acid sensitive (HS). This peak increased on day 2 and appeared similar on day 3. Sepharose Cl-6B columns of day 1 muscle cells demonstrated a sharp void volume peak, only some of which was nitrous acid sensitive, and a broad peak just included from the void volume which was largely nitrous acid sensitive and probably the same as that seen in Cl-2B columns. Day 2 Cl-6B profiles were similar, but with a larger nitrous acid sensitive peak and a new, further included nitrous acid sensitive peak. Day 3 Cl-6B profiles were similar to day 2 profiles. These results indicate that a new, smaller HS-proteoglycan is synthesized with the development of chick muscle cultures, particularly associated with the alignment phase. The HS-proteoglycans of muscle appear to be tightly membrane bound.

1598 EXTRACELLULAR MATRIX PROTEOHEPARAN SULFATE IN EPITHELIAL CELL GROWTH.

Madeleine MOCZAR*, Yves COURTOIS**, Jacqueline TASSIN**, Elemer MOCZAR*.
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Glycoconjugates in the extracellular matrix synthetized by corneal endothelial cell cultures were biosynthetically labeled with ^3H glucosamine and $^{35}SO_4$. The matrix was extracted with 4M urea, 50 mM Tris, 0.05 M DTT, 0.005 M benzamidine HCl at pH 7.5, and the residue hydrolyzed with bacterial collagenase. Two types of proteoheparansulfate with different molecular size, charge density and heparan sulfate composition (PHS_1) were isolated from the urea extract, and from the collagenase digest (PHS_2).
Lens epithelial cells were grown exponentially on the radioactive labelled matrix for 3 days. Unlike metastatic tumor cells, epithelial cells released about 3% of the radioactive matrix glycoconjugate label into the medium, concomittantly to the association of PHS_1 to the cells. Extracellular proteoheparansulfate containing nonsulfated and sulfated domaines seems to be involved in the interactions of the extracellular matrix with the growing cells.

1599 THE ANCHORAGE OF ACETYLCHOLINESTERASE TO THE SYNAPTIC BASAL LAMINA IS MEDIATED BY HEPARIN-LIKE MACROMOLECULES, Mafalda Maldonado, Juan-Carlos Torres, Jorge Garrido, Enrique Brandan and Nibaldo C. Inestrosa, Department of Cell Biology, Catholic University of Chile, Santiago, P.O. Box 114-D, CHILE.

Heparin, a sulfated glycosaminoglycan (GAG) extracted collagen-tailed acetylcholinesterase (AChE) from the extracellular matrix (ECM) of the electric organ of Discopyge tschudii. The effect of heparin is abolished by protamine; other GAGs like chondroitin sulfate or hyaluronic acid were not able to extract the esterase. The solubilization of the asymmetric AChE apparently occurs through the formation of a soluble AChE-heparin complex of 30S. Affinity chromatography on a heparin-agarose column, showed a specific binding of the collagen-tailed enzyme, whereas the globular forms did not bind to the column. The interaction of the asymmetric AChE required an intact tail, because the binding is abolished by pretreatment with collagenase. Ruthenium red was used to demonstrate anionic sites on the ventral electrocyte surface. Chemical analysis showed that GAGs account for 0.8% of the total ECM and the main types were heparan sulfate and chondroitin sulfate. These experiments provide strong evidence that AChE and GAGs are associated in the ECM; they also support the concept that an heparan sulfate proteoglycan is involved in the anchorage of the collagen-tailed AChE to the synaptic basal lamina. (Supported by DIUC, grant 59/82).

1600 SYNAPTIC VESICLES AND THE SYNAPTIC EXTRACELLULAR MATRIX CONTAIN AN IDENTICAL PROTEOGLYCAN-LIKE ANTIGEN. Steven Carlson, Pico Caroni, Kathleen Buckley, Erik Schweitzer and Regis B. Kelly, University of California, San Francisco, Ca 94143

Synaptic vesicles purified from the elasmobranch electric organ contain a proteoglycan-like material attached to the inside surface of the vesicle membrane (Carlson & Kelly (1983). JBC 258, 11082). We have recently found that a large quantity of this material is also associated with the synaptic extracellular matrix. The proteoglycan-like material is present on the external surface of the nerve terminal and confined to the synaptic region. This has been determined by immunocytochemistry at the electron microscopic level on intact electric organ nerve terminals. Several monoclonal antibodies against the proteoglycan-like antigen have been used. On the nerve terminal surface the proteoglycan-like antigen is associated with extracellular matrix. We have demonstrated this by cell fractionation studies where 90-95% of this antigen is found in extracellular matrix enriched fractions. The absence of another synaptic vesicle antigen in these fractions rules out the artifact of synaptic vesicle contamination. The proteoglycan-like antigen is neuronal pathway specific. By immunocytochemistry it is only found associated with the neurons innervating the electric organ and absent in the elasmobranch brain and spinal cord. An attractive interpretation of these results is that synaptic vesicles are inserting a proteoglycan-like antigen into the synaptic extracellular matrix which is specific for a neuronal pathway. The alternative hypothesis that the vesicles are removing such a matrix component has not been ruled out.

1601 ANELASTIC PROPERTIES OF PROTEOGLYCANS. Marie-Françoise Harmand, Alain G. Lamure[+] and Colette Lacabanne[+], Université de Bordeaux II 33076 Bordeaux Cedex, [+]Université Paul Sabatier 31062 Toulouse Cedex

Proteoglycans (PG) extracted from calf cartilage of 120 days, have been deposited onto a torsional glass braid for investigation by thermostimulated creep. Two energy loss peaks have been observed at -60 and +60°C, at an equivalent frequency of 10^{-3} Hz.

The higher temperature peak is only observed in the "hydrated" PG. Three states of bound water have been distinguished: removable water stiffens chondroïtin sulfate chains by bridging them; slightly and tightly bound water are responsible of "compensation" phenomena at respectively 30 and 150°C.

The behavior of the lower temperature peak versus hydration is very different from the preceding one. In the hydrated state, is is not observed for molecular weight of proteoglycans higher than 5.10^4 probably because of the bridging of glycosaminoglycans by water molecules. The study of the fine structure of dehydrated PG has shown the existence of retardation times τ following a compensation law. Such a distribution of τ which is also observed in chondroïtin sulfates 4 and 6, is characteristic of the cooperative movements liberated at the glass transition temperature.

So this retardation mode has been associated with the microbrownian motions of chondroïtin sulfate segments. These movements might be responsible for the reversible dissociation of aggregate at 60°C.

Fibronectin, Laminin and Cell Attachment Proteins

1602 CHARACTERIZATION OF THE INTERACTION BETWEEN SOLUBLE PLASMA FIBRONECTIN AND FIBROBLASTIC CELLS IN SUSPENSION. Steven K. Akiyama and Kenneth M. Yamada, Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

The fibronectins are major extracellular matrix and blood proteins. Plasma fibronectin binds to a wide variety of macromolecules and mediates several cellular activities associated with cell-cell and cell-substratum adhesion. The biological activities of fibronectin are generally assumed to involve binding to specific receptors on the cell surface. We have characterized the cell-fibronectin interaction by examining the binding of (^{3}H)-plasma fibronectin to BHK cells in suspension in serum-free medium. (^{3}H)-fibronectin binds to these cells in a time-dependent manner. Half-maximal binding occurs in approximately 15 minutes. In competition experiments, this binding is 60% inhibited at 22°C and 37°C, and 70% inhibited at 4°C with 7.5 mg/ml unlabeled plasma fibronectin. The specific component of binding is saturable and reversible with a K_d of 2-8x10^{-7} M and 200,000-600,000 sites per cell at 22°C assuming a molecular weight of 440,000. Immunofluorescence experiments suggest that the fibronectin which is bound at 22°C is on the surface of the cells and is not bound to pre-existing cell-surface fibronectin. These experiments represent the first demonstration of the direct binding of soluble fibronectin to fibroblastic cells in the absence of an extracellular matrix under physiological conditions.

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1603 THE PROPERTIES AND MOLECULAR INTERACTIONS OF A TRANSFORMATION-SENSITIVE PROTEIN OF THE EXTRACELLULAR MATRIX. Regina M. Malczewski and Susan P. Hawkes, Michigan Molecular Institute, Midland, Michigan 48640.

Upon transfer to the temperature permissive for transformation, the extracellular matrix of chicken embryo fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus undergoes several changes. One of these is the transient increase in synthesis and/or deposition of a 21,000-dalton protein ("21K") which remains attached to the culture dishes after gentle removal of cells with 5mM EGTA. Two-dimensional electrophoresis has shown that 21K is a basic protein (pI ∿ 9.0) which is unexpectedly insensitive to trypsin both *in situ* and upon isolation. Cleveland gel analysis, immunoprecipitation, and immunoblotting results indicate that 21K and fibronectin are not related. The 21K protein is able to bind hyaluronic acid, a negatively-charged glycosaminoglycan also found in the extracellular matrix. Incubation of media, conditioned by transforming cells, with the substratum-associated material remaining after cell removal with EGTA results in the deposition of specific proteins. One of these may be related to matrix 21K; it has a similar molecular weight, a basic isoelectric point, and appears to be trypsin-resistant upon isolation. A comparison of 21K to other proteins with similar properties is currently underway. Anti-H1 histone antibody does not react with native or denatured 21K; furthermore, preliminary experiments suggest that 21K and the *ras* gene product p21 are probably not related. (Supported by NIH grant CA-27283 and The Elsa Pardee Foundation.)

1604 THE M_r = 95 000 GELATIN - BINDING PROTEIN IN HUMAN MACROPHAGES AND GRANULOCYTES T. Vartio, K. Hedman, T. Hovi, and S.-E. Jansson, University of Helsinki, Helsinki, Finland

Cultured adherent human macrophages were previously shown to produce an M_r = 95 000 gelatin-binding protein (Vartio, T. et al. J. Biol. Chem. 257, 8862-8866, 1982). The protein has no immunological cross - reactivity with another gelatin - binding protein, fibronectin, and is not produced by a variety of cultured mesenchymal or epithelial cell types (Vartio,T. and Vaheri,A. J. Biol. Chem. 256, 13085 - 13090, 1981). In the present study the M_r = 95 000 protein was found in Triton X-100 extracts of granulocytes from human blood buffy coat fraction. The protein, as isolated by gelatin - agarose, was immunologically cross - reactive with the corresponding macrophage protein in immunoblotting assay. The distribution of the protein in the blood cells was studied by immunofluorescence. The M_r = 95 000 protein was detected in the cytoplasm of the granulocytes and monocytes, but not in other blood cells. In monocyte/macrophage cultures, the cytoplasmic immunofluorescence staining of the protein increased along with the morphologic differentiation of the cells into macrophages. The results suggest that the M_r = 95 000 protein is specific for human monocyte/macrophages and granulocytes, and may function in their interactions with tissue extracellular matrices or in phagocytosis.

1605 PLATELET ADHERENCE TO LAMININ, VITRONECTIN, AND TWO DIFFERENT EXTRACELLULAR MATRICES Charles R. Ill, Eva Engvall and Erkki Ruoslahti. La Jolla Cancer Research Foundation, La Jolla, CA 92037

The binding of platelets to components in the subendothelial matrix is an initial event in hemostasis and thrombosis. The protein characterized as important in this interaction is collagen which binds, activates and induces aggregation of platelets. In our studies we demonstrate time- and concentration-dependent binding of platelets to laminin, fibronectin, vitronectin, collagen types I and IV, and two basement membrane-like extracellular matrices: that secreted by PFHR9 mouse endodermal cells (HR9) and that by bovine corneal endothelial cells (BCE). The binding to laminin, fibronectin and vitronectin is not associated with their activation as measured by ^{14}C-serotonin secretion, while the collagens do activate the platelets. The binding of platelets to fibronectin and vitronectin is morphologically similar since the platelets completely flatten on these substrates whereas on laminin they remain rounded. BCE and HR9 matrices bind but *do not* induce activation or aggregation of platelets over a three hour period. The presence of collagen type IV, fibronectin and laminin in these matrices was demonstrated by SDS-PAGE before and after collagenase treatment. When collagen-coated dishes were exposed to increasing concentrations of fibronectin, activation of platelets decreased in parallel and this was associated with increased binding of non-activated platelets, suggesting that other matrix components may influence the thrombogenic activities of collagen. These studies decribe two new glycoproteins which bind but do not activate platelets and suggest that basement membranes, while capable of binding platelets, are nonthrombogenic entities.

1606 LAMININ AND FIBRONECTIN ADSORPTION TO A NEW SURFACE FOR CELL CULTURE: EFFECT ON CELL ATTACHMENT AND SPREADING, T. A. Guhl and D. M. Umstatter, Becton-Dickinson Labware, Oxnard, CA 93030

A new polystyrene surface for cell culture was evaluated for its ability to support cell attachment and spreading after pretreatment with laminin and fibronectin. This new cell substratum called PRIMARIA, recently introduced by Falcon Labware, is highly nitrogenated, possessing a surface chemistry very similar to collagen. Cellular response to this surface has been markedly different than to standard tissue culture (TC) plastic, so the binding of these cellular attachment factors to PRIMARIA was investigated to determine possible mechanisms of this altered cellular interaction with the new surface. Baby hamster kidney (BHK) and PAM-212 mouse epidermal cells were evaluated for their ability to attach and spread on PRIMARIA pretreated with either fibronectin, laminin, collagens I and IV, or a combination of these. 20% to 50% more fibronectin was bound to PRIMARIA versus TC plastic, and complete BHK cell spreading on PRIMARIA required only one-half the amount of bound fibronectin as did TC plastic. BHK cells were not responsive to collagen IV, but when the surfaces were coated with a collagen IV-laminin complex, nearly complete cell spreading on PRIMARIA and very little spreading on TC plastic was observed. PAM-212 cells spread equally well on laminin-coated, fibronectin-coated or uncoated PRIMARIA, whereas spreading on TC plastic was mediated only by laminin or fibronectin. This data suggests that PRIMARIA's unique surface chemistry is either acting as a "cofactor", or mediator for the binding of endogenous cellular-secreted laminin to facilitate spreading, or the conformation of exogenous fibronectin, laminin and collagen when bound to PRIMARIA is such that active cell binding regions of the molecules are exposed.

1607 FIBRONECTIN IN OSTEOARTHRITIC CANINE ARTICULAR CARTILAGE, Nancy B. Wurster and George Lust, Cornell University, Ithaca, NY 14853

Extracts of cartilage from disease-free canine joints contained 0.07 ± 0.02 µg fibronectin per mg wet cartilage, but extracts of fibrillated cartilage from osteoarthritic joints contained 3.2 ± 3.8 µg per mg as determined by a monoclonal antibody in an Elisa assay (Biochem. Biophys. Res. Comm., 109: 1094-1101, 1982). The cartilage surrounding the osteoarthritic, focal area often contained more fibronectin than disease-free cartilage, but much less than the fibrillated cartilage of the same joint. Synovial fluids of disease-free canine joints had 99 ± 20 µg/ml of fibronectin whereas osteoarthritic joints contained 206 ± 142. Canine plasma contained 955 ± 211. Incubation of cartilage explants *in vitro* with [^{3}H] phenylalanine and subsequent purification of [^{3}H] fibronectin with a gelatin affinity column and characterization by Na Dod SO_4 PAGE and by immunoprecipitation indicated that disease-free and osteoarthritic cartilage explants synthesized fibronectin. Fifty per cent of the [^{3}H] fibronectin was recovered in the incubation medium. Fibrillated (osteoarthritic) cartilage produced up to fivevold more [^{3}H] fibronectin/mg than disease-free cartilage. Thus cartilage fibronectin *in vivo* could originate from the synovial fluid or from the chondrocytes themselves. Current work is directed toward resolving this question, and toward understanding the relationship of the accumulation of fibronectin to collagen and proteoglycan metabolism in fibrillated cartilage.

1608 DIRECT CONNECTION BETWEEN EXTRACELLULAR BASAL LAMINA AND CYTOPLASMIC FILAMENTS Nibaldo C. Inestrosa, Mafalda Maldonado and Jorge Garrido, Department of Cell Biology, Catholic University of Chile, Santiago, P.O. Box 114-D, CHILE.

The idea that the cytoskeleton and the extracellular matrix are part of a continuos supramolecular assemblage has important implications for considerations of the effects of extracellular matrices on cellular behavior and differentiation. Several indirect lines of evidence suggest that intracellular filaments may be functionally and spatially related to extracellular matrices. (Yamada, K.M. (1983) Ann.Rev.Biochem. 52: 761-799).

In the course of our experiments on the isolation of the basal lamina from the electric organ of Torpedinae fishes, we observed, by transmission electron microscopy, that after osmotic shock and detergent treatment the basal lamina sheets retains an almost intact histoarchitecture, moreover, the majority of the cytoplasmic filaments normally observed in contact with the post synaptic membrane appears (after remotion of the cell membrane) in direct physical connection with the extracellular basal lamina. Work in progress concerns the characterization of the molecules involved in such interaction.

(Supported by DIUC, grant 59/82).

1609 UNDIFFERENTIATED HUMAN KERATINOCYTES SYNTHESIZE BOTH LAMININ AND FIBRONECTIN BUT ONLY DEPOSIT FIBRONECTIN IN THE PERICELLULAR MATRIX, Richard A.F. Clark, Samuel E. Howell, Miyoko Kubo, D.A. Norris, National Jewish Hosp., Univ. Colorado Medical School, Denver, CO 80206

Fibronectin and laminin production by human keratinocytes cultured in serum-free, low-calcium medium without a fibroblast feeder layer were examined using several techniques. Immunohistochemical examination confirmed that the cultures were not contaminated with fibroblasts or Langerhans cells. By indirect immunofluorescence fibronectin but not laminin appeared as short radial fibrils between the cells and the substratum, and in the pericellular matrix. Synthesis of fibronectin and laminin by 7 day keratinocyte cultures were determined by 18 hr ^{35}S-methionine metabolic labelling followed by sodium dodecyl sulfate polyacrylimide gel electrophoresis and autoradiography. Fibronectin accounted for 1.1% of total synthesized protein, and half of the product was secreted. In contrast, although similar quantities of laminin were synthesized only one tenth of the product was secreted. Our results indicate that human keratinocytes under conditions that prevent terminal differentiation in vitro can synthesize, secrete and deposit fibronectin in the extracellular matrix. Although these cells synthesize laminin, they secrete very little and deposit no detectable laminin in the matrix under these culture conditions. These data suggest that fibronectin may play an important role in the interaction of epidermal cells with connective tissue matrix under nondifferentiating conditions in vivo such as wound healing or morphogenesis.

1610 THE ROLE OF FIBRONECTIN IN THE GROWTH OF PRIMARY GLOMERULAR EXPLANTS IN SERUM-FREE MEDIA, Terry D. Oberley, Pathology Service, VA Hospital, Madison, WI 53705

The role of the dimeric (MW 440,000) and multimeric forms of FN (MW>440,000) in the outgrowth of kidney glomerular cells was examined in an in vitro culture system. FN was required for adhesion of whole glomeruli. While glomerular cell outgrowth was greater in multimeric than in dimeric FN, studies of whole glomerular adhesion vs. glomerular cell mitosis indicated that the increase in glomerular cell number in multimeric FN relative to dimeric FN was due to an increase in whole glomerular adhesion rather than an increase in glomerular cell mitosis. In contrast to most reported cell lines, glomerular cell outgrowth with mitosis occurs in a basal medium with FN but without hormones or growth factors. While FN was required for whole glomerular adhesion, once outgrowth had begun, removal of FN from the medium increased the glomerular cell mitotic rate 3-fold. Insulin and/or diethyldithiocarbamate, a potential inhibitor of the enzyme superoxide dismutase, increased the whole glomerular adhesion rate, while superoxide dismutase added to the medium greatly inhibited whole glomerular adhesion. Transferrin and/or 3-amino-1,2,4-triazole, a potential inhibitor of the enzyme catalase, increased glomerular cell mitosis. Catalase added to media greatly inhibited glomerular cell mitosis, but only in the presence of transferrin. Fibroblast growth factor increased the number of glomerular cells but had only a minimal effect on whole glomerular adhesion or glomerular cell mitosis. Epidermal growth factor increased whole glomerular adhesion in the presence of dimeric FN, but increased mitosis in the presence of multimeric FN. I have used these results to develop a model of glomerular cell outgrowth in defined media, which suggests a role for the cell substrate and reactive oxygen metabolites in glomerular cell outgrowth.

1611 BIOFUNCTION OF EXTRACELLULAR FIBRONECTIN ON HUMAN FUNCTIONAL MAMMARY EPITHELIAL CELLS. Anwar A. Hakim and Charles E. Joseph. Loyola Univ. of Chicago Med. Cntr. Maywood, Ill. and Univ. Southern Calif. Los Angeles, Calif.

Several studies have been reported on the identity of the target antigen on the surface of human neoplastic cells that binds effector cells. Earlier investigation showed a correlation between immunosuppression to tumorigenicity and to sialylated cell membrane glycoproteins (Hakim, A.A. Europ. J. Cancer 14: 1249-1260, 1978; Clin. Immunol. Immunopathol. 20: 261-277, 1981), and the influence of fibronectin of the extracellular matrix on proliferation and growth promoting activities of human fibroblasts and malignant melanoma cells (Hakim, A.A. Fed. Proc. 40: 1816, 1981). The present studies were aimed at the identification of a biofunction for extracellular fibronectin. When cultivated in media supplemented with 10^{-7}M estradiol human mammary carcinoma (HMCC-GM) showed 10 fold increase, whereas human normal mammary epithelial (HNMEC-2) cells showed 2 fold increase in ^{3}H-thymidine uptake, with increased oncogenicity of HMCC-GM cells. Continuous culture of HNMEC-2 cells in presence of 10^{-7}M estradiol, for approximately 10 passages, the cells became oncogenic in athymic Nu/Nu BALB/c mice. If grown in absence, or in presence of estradiol HMCC-GM cells were oncogenic, but if grown in absencen of presence of estradiol on fibronectin-coated substrata, HMCC-GM cells became non-oncogenic and acquired normal epithelial cell growth profile and cellular characteristics. Therefore these observations strongly indicate that fibronectin as an extracellular matrix will interfere with the biofunction of estradiol. Changes in cellular mRNA coding activities suggest that fibronectin acts at the nucleic acid phase of oncogenesis

1612 DISULFIDE BONDING IN THE ASSEMBLY OF GP140 AND FIBRONECTIN IN THE EXTRACELLULAR MATRIX
William G. Carter and Robin Heller-Harrison, Hutchinson Cancer Ctr., Seattle, WA 98104
Human fibroblasts (WI-38 cells) in culture synthesized at least three molecular forms of the major, collagen-like, cell-adhesive, extracellular matrix(ECM) glycoprotein, GP140: (1) Cytoplasmic GP140 (30ng GP140/25µg cell protein) was detergent-soluble, underglycosylated, and possessed detectable levels of intermolecular disulfide bonding. (2) Matrix GP140 (90ng GP140/25µg cell protein) was detergent-insoluble, more highly glycosylated and polymerized and co-distributed in the ECM with fibronectin. (3) Released GP140 (50ng GP140/25µg cell protein) was recovered in the conditioned culture media and lacked intermolecular disulfide bonding. Cytoplasmic GP140 was the immediate biosynthetic precursor of the matrix form of GP140. In addition, various human adult and fetal tissues contained a form of GP140 that resembled the fibroblast matrix GP140 in degree of intermolecular disulfide bonding, relative molecular mass and immunological cross reactivity. Comparisons of matrix and placental GP140 to pepsin-solubilized, collagen types I-VI detected immunological and biochemical similarities between GP140 and one subunit of type VI collagen. Analysis of the sequence of events in assembly of GP140 and fibronectin in the ECM detected the following: (1) Fibronectin was first to appear in the ECM. (2) GP140 accumulated in the cytoplasm, then deposited in the ECM and co-aligned with the established fibronectin. (3) Maturation proceeded by continued intermolecular disulfide bonding. Further studies on the process of matrix deposition utilized a unique assay system and indicated: (1) Disulfide bonding of GP140 into an exogenous matrix required cell contact with the matrix. (2) The deposited GP140 derived from the cells and not the conditioned culture media.

1613 *STUDIES ON PLASMA AND TISSUE FIBRONECTIN (FN) IN DIABETES.*
Jacqueline Labat-Robert, Luu Phan Thanh, Jean-Claude Derouette. Lab.Bioch.Tissu Conj.GR CNRS 40, Fac.Méd.Créteil France.

Genetic or adult onset diabetes can be considered as a connective tissue disease characterized by thickening of the basement membranes and a general derangement of the regulation of extracellular matrix biosynthesis (L.ROBERT et al., in: "Biology and Chemistry of the Basement Membranes" ed. Kefalides (Acad.Press, NY) 1978, pp.503). FN distribution pattern in human diabetic skin biopsies was compared to a normal population. An increase of the fibronectin immunofluorescence in the thickened dermo-epidermal basal lamina, vascular basement membranes and disorganized papillary dermis was observed. The exponential increase of the plasma FN with age in the normal population was absent in the diabetic population. Lower control values were found in diabetics, the difference between males and females was also absent. These results are not influenced by the poor or good control of the disease, neither by the existence of retinopathy. Using the skin of genetically diabetic KK mice by comparison with control mice (Swiss and C57Bl) in organ culture conditions in the presence of ^{35}S-methionine followed by extraction and immunoprecipitation of FN from the tissues, we could show a significantly increased biosynthesis of FN in the diabetic KK mouse skin. Nevertheless this increased biosynthesis of skin FN does not explain the decrease of plasma FN. There may well be in addition to the local increased synthesis of FN a trapping of plasma FN by the extracellular matrix proteins. This hypothesis is based on an increased type III collagen biosynthesis in diabetic skin (P. KERN et al., Biochem. J., 1979, 182, 337). Furthermore FN reacts with glucose in a non-enzymatic glycosylation reaction which was studied and may change the metabolism of plasma FN.

1614 FIBRONECTIN BINDING TO TREPONEMA PALLIDUM, Thomas Fitzgerald and Lillian Repesh, University of Minnesota, Duluth, MN 55812.

Syphilis is both a localized and a generalized infection. In vitro, T. pallidum attaches to cultured mammalian cells and isolated capillaries. It has been suggested that in vivo attachment is an important factor in pathogenesis. We have recently reported that T. pallidum attaches to fibronectin (FN), laminin, collagen IV, collagen I, and hyaluronic acid. The purpose of this research is to further characterize this treponemal-FN interaction. Organisms were pre-incubated with anti-FN, then incubated with FN-coated coverslips, cultured cells, the extracellular matrix of cultured cells, and isolated capillaries. Anti-FN reduced treponemal attachment to each of these substractes. T. pallidum was isolated from early (7 day) versus late (14 day) infections. Radioimmunoassays indicated more FN associated with the surface of the older organisms. Direct binding of I^{125} FN confirmed this finding. Organisms were passed through glass wool columns coated with FN. Approximately 40% of the treponemes attached to the FN column. The remaining 60% of the organisms, along with the PBS controls, were incubated with cultured cells. Better attachment was observed using those treponemes that did not bind to the FN column. These findings suggest at least 2 populations of T. pallidum. One may bind to the FN present within the extracellular matrix; these organisms could then produce the localized infection of the primary stage. A second population may not bind to FN. These organisms could then make their way through the extracellular matrix, attach to laminin or collagen IV of the basement membrane of vessels, penetrate, and disseminate producing secondary stage lesions in other tissues.

1615 LOCALIZATION OF A LAMININ-LIKE PROTEIN(S) IN THE MEMBRANE ASSOCIATED CYTOSKELETON IN DIFFERENT CELLS, R. Rajaraman and G. Faulkner, Dept. of Medicine and Microbiology, Dalhousie University, Halifax, N.S., Canada.

Laminin (Lm) is a complex extracellular matrix molecule consisting of 3 α-subunits (M_r220KD) and one β-subunit (M_r400KD). Even though Lm is found in the basement membrane probably synthesized by the epithelial cells, it is now apparent that different cell types are synthesizing similar molecules. However, the role of laminin-like molecules in different systems is not well understood. We have characterized Lm and raised Lm specific antiserum (anti-Lm) in rabbits. The distribution of Lm was studied by indirect immunofluorescence in different cells such as PFHR-9, WI-38, MRC-5, CHO, 3T3, W138VA13ZRA, and K13T3. All normal and transformed cells display a high amount of intracellular submembranous network-like component cross-reacting with anti-Lm serum and not with antifibronectin serum as seen by immunofluorescence in permeabilized cells. Preabsorption of anti-Lm with increasing amounts of Lm progressively decreased the staining of the submembranous network. Anti-Lm sera from three other laboratories also showed similar staining pattern. The structural and non-secretory nature of this submembranous staining was confirmed by (a) inhibiting protein synthesis in 0.5% serum and 4 μg/ml puromycin and (b) by immunoelectron microscopy of permeabilized cells. Immunoprecipitation of ^{3}H-leucine labelled cellular proteins with anti-Lm sera showed proteins of M_r 220-210 KD in SDS-PAGE fluorography. These studies suggest that an antigen(s) crossreacting with anti-Lm sera is localized in the membrane associated cytoskeletal region where spectrin/fodrin family of proteins have been localized.

1616 FIBRONECTIN FORMS A CONSPICUOUS PERICELLULAR MATRIX DURING THE EARLY STAGES OF PULMONARY FIBROSIS. Irwin I. Singer, Douglas W. Kawka, Diana M. Kazazis, and George J. Elermann. Merck Sharp & Dohme Research Laboratories, Merck & Co., Inc., Rahway, N.J. 07065

Fibronectin (FN) gives rise to a scaffold for the attachment of fibroblasts within the early granulation tissue of healing skin wounds, and is synthesized at increased levels during interstitial lung disease. FN also forms an intimate transmembranous association with cortical actin microfilaments termed the fibronexus (Singer, I.I., Cell 16, 675, 1979). This complex is localized at the adhesive surfaces of fibroblasts in vitro, and at the plasma membranes of granulation tissue myofibroblasts, where it governs tissue-matrix cohesion (Singer, I.I. et al, J, Cell Biol, in press, 1984). We are presently studying pulmonary fibrosis induced in Fischer rats by the intratracheal administration of bleomycin sulfate, to determine if fibronectin provides a pericellular matrix for the adhesion of the increased numbers of macrophages and fibroblasts characteristic of this condition. Using high resolution immunofluorescence microscopy on 1 um thin frozen sections of fibrous lung lesions, we have observed that a fibronectin-rich extracellular matrix closely surrounds the numerous interstitial macrophages and fibroblasts found 4-7 days after drug dose. The pericellular fibronectin appears to be closely apposed to cortical actin fibers seen in the macrophages, suggesting that fibronexus-like complexes might modulate macrophage adhesion and metabolism in pulmonary fibrosis. This FN matrix also appears to form prior to the major onset of collagen synthesis at 7d, implying that FN also provides a provisional matrix for fibroblast orientation and the organization of collagen deposition in the fibrotic lung.

1617 FIBRONECTIN BIOSYNTHESIS IN A HUMAN HIBROSARCOMA CELL LINE AND ADHESION VARIANTS, Noelynn Oliver, Ron Newby, Leo Furcht and Suzanne Bourgeois

HT1080 human fibrosarcoma cells show changes in morphology and the extracellular matrix when treated with the synthetic glucocorticoid dexamethasone (dex). Dex treatment results in a 10-fold increase in the rate of fibronectin (FN) biosynthesis in HT1080 cells. Untransformed, normal human fibroblasts also show increased FN biosynthesis in the presence of dex. The glucocorticoid antagonist, RU-486, blocks the dex-induced changes, but does not alter the basal rate of FN production. Therefore, FN biosynthesis is controlled by two distinct mechanisms--one, regulating basal rates of FN production, which is transformation sensitive and glucocorticoid independent; and, another which is mediated by glucocorticoid receptors, resulting in elevated rates of FN biosynthesis upon dex treatment in both normal fibroblasts and in HT1080 cells. To study these mechanisms regulating FN expression, "strong" (SAV) and "weak" (WAV) adhesion variants of HT1080 were selected in the absence of dex. These were examined for alterations in FN biosynthesis as compared to parent cell line HT1080. 2 classes of WAVs have been identified: 1) those which are apparently unchanged for FN biosynthesis; and, 2) those which show increased FN biosynthesis both in the absence and presence of dex. 3 classes of SAVs have been identified: 1) those which are unchanged for FN biosynthesis; 2) those which make no detectable FN either in the absence or presence of dex; and 3) those which show no detectable FN in the absence of dex but near normal FN biosynthesis when treated with dex.

1618 RETROVIRUS TRANSFORMATION ASSOCIATED EXTRACELLULAR PHOSPHOPROTEINS OF MOUSE, MINK AND CAT CELLS, Kalyan Ganguly and Max Essex, Department of Cancer Biology, Harvard School of Public Health, Boston, Mass. 02115.

Using antisera to major excreted protein (MEP)of Kirsten sarcoma virus transformed NIH 3T3 (K-NIH) cells, we have identified phosphoproteins from the media of feline sarcoma virus (FeSV) transformed mink and cat cells. These secretory phosphoproteins from the FeSV-transformed mink and cat cells are of 35,000 dalton and 60,000 dalton molecular weight respsctively and they do not have autophosphorylation activity. A comparative analysis of MEP-related molecules from the media of transformed mouse, mink and cat cells was performed on the basis of proteolytic cleavage products and acid hydrolysed products of the secreted phosphoproteins. While a marked difference was observed in the peptide map, a common ^{32}P-linked molecule was observed following acid hydrolysis of all these species of phosphoproteins. Pulse-chase analysis confirmed that extracellular phosphoproteins were not released by cell lysis. The secretion of these phosphoproteins was observed to be dependent on transformation by retrovirus (regardless of the strain of virus) but the molecular weight of those polypeptides appeared to be host species specific.

1619 THE FUNCTION OF INTERACTIONS BETWEEN HEPARAN SULFATE PROTEOGLYCAN AND FIBRONECTIN, Blair T. Atherton and Mary E. Hutton, Northwestern University Medical School, Chicago, IL 60611

Heparan sulfate proteoglycan is present in basement membranes and on the surface of many different cell types. Fibronectin, another major cell surface molecule, appears to share a common distribution with heparan sulfate on cell surfaces.

We have isolated monoclonal antibodies which specifically block heparin binding sites in fibronectin and small fragments of heparin for use as inhibitors to study the biological function of interactions of heparin and heparan sulfate with fibronectin. Preliminary studies with these reagents indicate the possible involvement of heparin binding sites in fibronectin in the formation of a fibrillar matrix on the cell surface. We are in the process of determining whether the reagents affect synthesis, binding to the cell surface or assembly of fibrillar complexes between fibronectin and heparan sulfate proteoglycan.

In addition, we have begun studies of the biological activity of heparan sulfate proteoglycan isolated from normal rat kidney glomerular basement membrane and the effects of fibronectin-proteoglycan complexes on transformed cells deficient in both of these extracellular matrix materials.

1620 ABSTRACT WITHDRAWN

1621 IN VITRO GROWTH OF HUMAN AMNIOTIC ENDOTHELIAL AND EPITHELIAL CELLS ON EXTRACELLULAR MATRIX, Jai Pal Singh and Paul D. Bonin, Biomedical Product Div.. Collaborative Res., Inc., Lexington, MA. 02173

We have studied effects of extracellular matrix (ECM) on plating efficiency, morphology and growth of endothelial and epithelial cells derived from amniotic fluid. Cultures of PYS-2 cells producing ECM that contains basement membrane specific protein laminin and collagen IV were used as source of ECM. Tissue culture plates coated with ECM were prepared by first growing a monolayer of PYS-2 cells and then asceptically removing cells leaving a uniform layer of ECM attached to the plates. Amniotic fluid derived cells previously maintained on plastic dishes show a maximum of 40-50% efficiency of attachment upon replating. Plated cells remain round and do not begin to spread until 6-8 hrs. When plated on ECM coated dishes, 80-90% cells became attached to the plate surface and exhibited spreading within 2-3 hrs. By eight hours most cells were seen with flattened morphology and extended pseudopodia. First cell count performed 24 hrs. after plating on plastic dishes normally did not show increase in cell number. During same period, cells plated on ECM were increased 1.5 - 2 fold (150%-200% higher than on plastic dish). ECM supported cell attachment and growth at all densities of cell seed tested (50-5000 cells/cm^2). Largest effect was observed at lower seeding densities. These results support that higher cell growth is achieved in the presence of ECM. The major effect of ECM are probably through influences on early cellular activities required for establishment of cells in culture. The ECM culture system described here could be useful for growing cells for neonatal diagnosis.

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1622 THE FORMATION OF MESENCHYME-LIKE CELLS FROM THYROID FOLLICULAR EPITHELIUM CULTURED IN COLLAGEN GELS, G. Greenburg and E.D. Hay, Harvard Medical School, Boston, MA, 02115. A number of different epithelia of embryonic and adult origin transform to mesenchyme-like cells when cultured within collagen gels (Greenburg and Hay, 1982, JCB 95:332-339). The transformation is accompanied by a loss of epithelial phenotype and polarity, and acquisition of ultrastructural and biosynthetic properties typical of mesenchyme. Glandular epithelia, however, have been reported to maintain epithelial morphology when embedded in matrix. Here, we reexamine the possibility that a glandular epithelium, the thyroid follicle, can form mesenchyme-like cells when cultured in collagen gels. Bovine and rat thyroid follicles were isolated enzymatically with collagenase-dispase followed by cell sieving. The purity of the follicle preparation was verified by electron microscopy. Freshly isolated follicles were suspended in gelling solutions of type I collagen. Mesenchyme-like cells elongate from the basal surface of most follicles within 24 hrs., detach, and migrate radially away from the explant as individual, bipolar cells. These cells are indistinguishable morphologically from mesenchymal cells. Epithelial specializations are lost and the elongating cells acquire long parallel arrays of rough endoplasmic reticulum, and a highly filamentous cytoplasm. Follicles cultured on the surface of either plastic or collagen give rise to epithelial outgrowths consisting of compact, tightly adherent, cuboidal cells. These cells on plastic respond to TSH (10uU/ml) by forming hemicysts. Thus, the ability of a well differentiated glandular epithelium to express epithelial characteristics is dramatically dependent on the extracellular environment. Epithelia, even in the adult, appear to retain a capacity for epithelial-mesenchymal transformation heretofore attributed only to embryonic cells.

1623 THE ROLE OF BASEMENT MEMBRANE AND SCHWANN CELLS DURING AXONAL REGENERATION THROUGH LONG NERVE GRAFTS. A.K. Gulati and A.A. Zalewski, Laboratory of Neurochemistry, NINCDS, National Institutes of Health, Bethesda, Maryland 20205.
The basement membrane, which encircles Schwann cells is an important component of the extracellular matrix, and after injury to the nerve is believed to act as a scaffold within which subsequent axonal regeneration occurs. The present study was designed to determine whether the basement membrane, in the absence of Schwann cells would support axonal regeneration through long nerve grafts. Four-cm long nerve isografts (non-antigenic) of normal and a 4-wk predegenerated nerves (with no myelin and axons) were performed in rats. In order to eliminate Schwann cells, nerve grafts (normal and predegenerated) were repeatedly frozen and thawed, thereby leaving behind their basement membranes as determined by indirect immunofluorescence using specific antibodies against laminin and type IV collagen. Successful regeneration through the entire length of nerve grafts was seen only in non-frozen grafts, which possessed viable Schwann cells. In these successful grafts, the original basement membrane persisted for sometime, but as the axons grew into the graft, a new basement membrane appeared around the regenerated axons. The nerve grafts which lacked viable Schwann cells, inspite of the presence of basement membrane scaffold (frozen-thawed grafts) were unable to support axonal regeneration for distances greater than 2 cm. These results demonstrate that viable Schwann cells together with their basement membrane are essential for axonal regeneration, while the basement membrane by themselves are unable to support nerve fiber regeneration over long distances.

1624 DEVELOPMENT AND DISTRIBUTION OF FIBROUS MATRIX IN CHICK LIMB MESENCHYME, Martha Y. Janners, Michigan Technological University, Houghton, Michigan 49931

Ectoderm was stripped from limb mesoblasts after mild trypsinization. Scanning electron micrographs of the denuded mesoblasts reveal changes in both the amount and the orientation of fibrous matrix during stages 18 to 21 of limb development.

At stage 18, forelimb mesenchyme cells are irregular in shape and possess numerous vesicles which adhere to cell surfaces or lie grouped within intercellular spaces. Stage 18 hindlimb mesenchyme cells have finger-like projections which abut neighboring cells. There is more intercellular fibrous matrix in stage 18 hindlimbs than in forelimbs.

At stage 19, differences are seen within each limb. Proximal limb areas show little fibrous matrix, but the cells have numerous clumped projections on their surfaces. Mid-apical mesenchyme has significant fibrillar network between the cells.

The fibrous matrix is more well developed in all limb areas at stage 20. Furthermore, the fibrous matrix of the dorsal proximal regions of the limb appears to be preferentially arranged in an orientation parallel to the proximo-distal limb axis and perpendicular to the anterior-posterior axis of the embryo.

Sinuses of the developing vascular system first become apparent at stage 21. The mesoblast at this stage has a much more fibrous matrix than at earlier stages.

1625 HYALURONATE INTERACTION WITH THE CELL SURFACE DURING CHONDROGENESIS, Cheryl B. Knudson and Bryan P. Toole, Tufts Univ., Boston, MA 02111.

In the developing limb bud, mesodermal cells appear morphologically homogeneous until stg 21, but during stg 22-24 the chondrogenic core becomes condensed and by stg 25, cartilage is detectable. In culture, cells with large amounts of cell surface-associated hyaluronate exhibit pericellular coats which may be discerned morphologically by the exclusion of particles. Most early mesodermal cells have a hyaluronidase-sensitive coat: stg 17 lateral plate, 75% have coats; stg 19 limb bud, 49%. Cells from older limb buds lack coats: stg 21 (pre-condensation), 18%; stg 22-24 (condensed chondrogenic core), 6%; and stg 26 (detectable cartilage matrix), 3%. However, coats are reformed during subsequent cytodifferentiation: 100% of stg 38 tibial chondrocytes have coats. Hyaluronate has been shown to interact with specific binding sites on the plasma membrane of some cell lines. To detect binding of cell surface hyaluronate to such sites during chondrogenesis we attempted to displace labeled cell surface hyaluronate with exogenous hyaluronate. For both the stg 24 mesodermal cells and mature chondrocytes, greater than 50% of the cell surface hyaluronate could be displaced. Only 15% of the cell surface chondroitin sulfate was displaced by the exogenous hyaluronate in the case of the stg 24 mesodermal cells whereas greater than 50% was displaced from the chondrocytes. These results suggest that both cell types have occupied, cell surface, hyaluronate-binding sites, and that in chondrocytes, chondroitin sulfate-proteoglycan is retained by the cell surface hyaluronate. Supported by NIH Postdoctoral Fellowship GM09235, and NIH Grant DE05838, and MDA Postdoctoral Fellowship.

1626 PROTEOGLYCANS PREVENT INVASION OF EMBRYONIC PERICHORDAL SPACE BY NEURAL CREST CELLS, D.F.Newgreen, M.Scheel, V.Kastner, Max-Planck-Institut, Tübingen D7400, W.Germany

In early avian embryos a wide cell-free space surrounds the notochord and this contains a complex extracellular matrix (ECM) much of which is synthesized by the notochord. This ECM is rich in fibronectin, collagen, hyaluronic acid and chondroitin-sulphate proteoglycan , as shown by immunolabelling, alcian blue histology combined with specific enzyme digestion, and electron microscopy after ruthenium red staining. This space remains devoid of cells while nearby ECM-filled spaces are invaded by migratory neural crest (NC) cells. To test the role of ECM components in excluding cells from this space , 2-2.5 day avian embryo notochords were grown in vitro on substrates of i) collagen gels or ii) ECM of detergent-extracted fibroblasts. These substrates, designed to mimic the basic structure of the perichordal ECM, served as surfaces for migration of co-cultured NC cells. The NC cells in culture were excluded from a zone adjacent to the notochord, on both substrate-types. This exclusion did not require direct contact between NC cells and the surface of the notochord. The cell exclusion zone occurred despite Streptomyces hyaluronidase in the culture medium, but failed to appear when testicular hyaluronidase or chondroitinase ABC were used. The results indicate that notochordal proteoglycans exert an important morphogenetic control by preventing cellular invasion of the perichordal space during early embryogenesis.

1627 STRUCTURE AND FUNCTION OF A UNIQUE EXTRACELLULAR MATRIX, THE MAMMALIAN ZONA PELLUCIDA (ZP). Bonnie S. Dunbar, Dept. Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

The ZP is formed during the growth of the mammalian oocyte and its extracellular assembly coincides with follicular cell differentiation. The three major glycoprotein families of porcine and rabbit ZP each exhibit extreme charge and molecular weight heterogeneity when analyzed with 2D-PAGE. Enzymatic and chemical deglycosylation as well as immunochemical analysis with polyclonal and monoclonal antibodies demonstrates that there are distinct species variations in these structures. Intact porcine ZP were digested with a variety of proteolytic enzymes (trypsin, V8 protease, chymotrypsin and pronase). Although pronase alone results in the microscopic dissolution of the ZP structure, all enzymes cause partial proteolysis of specific proteins and result in unique 2D-PAGE and color-based silver staining protein patterns. These studies have made it possible to analyze the specific molecules essential for the structural integrity of the ZP which provides protection of the oocyte and embryo and yet allows passage of sperm during the fertilization process.

1628 THE IMMUNOHISTOCHEMISTRY OF EXTRACELLULAR MATRIX SURROUNDING OVARIAN FOLLICLES AND THE EFFECT OF HCG INDUCTION ON THE METABOLISM OF THE MATRIX, Aarno Palotie, Leena Peltonen and Hannu Rajaniemi, University of Oulu, Oulu, Finland

Cryosections of ovaries from rats treated with pregnant mare serum (PMSG) were immunostained with antibodies against collagen types I, III, IV and V, laminin and heparan sulphate proteoglycan using the peroxidase-antiperoxidase technique. A uniform belt like staining was observed between the granulosa and theca interna layers with antibodies against type IV collagen, laminin and heparan sulphate proteoglycan. Interstitial collagens type I and III stained the connective tissue in the theca externa layer but gave only faint staining in the area between the granulosa and theca interna zones. If the PMSG-treated rats were injected with human chorionic gonadotropin (hCG) the number of follicles that showed a disrupted staining reaction in the area between the granulosa and theca interna layers was clearly increased. Surprisingly in such cases also a punctate staining reaction was seen in the basal granulosa cells.

These results suggest that there is a basement membrane-like structure in Graafian follicles, between the granulosa cells and theca interna layer and that this basement membrane is disrupted after hCG stimulation.

Free collagenase activity was found to be clearly increased when assayed from tissue culture medium of dissected ovarian follicles after hCG induction in vitro. This suggesting an increased activation or release of this enzymes from ovarian cells after hCG stimulation.

1629 BLOOD-BORNE IMPLANTATION AND INVASION OF VASCULAR ENDOTHELIUM AND ITS MATRIX BY METASTATIC MELANOMA CELLS, Garth L. Nicolson, T. Irimura and M. Nakajima, Department of Tumor Biology, University of Texas-M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

A model for implantation and invasion of vascular endothelium has been developed using endothelial cell monolayers which synthesize a basal lamina-like matrix (BLM) (G.L. Nicolson, J. Histochem. Cytochem. 30:214-220, 1982). Metastatic and normal invasive cells bind to endothelial cells and stimulate retraction and exposure of BLM. Invasive cells migrate to the BLM, possibly because of an adhesive gradient established by BLM components fibronectin, laminin, collagen IV and proteoglycans. Antibodies to BLM components inhibit adhesion of human or murine melanoma cells to BLM only partially, indicating multiple components in tumor-BLM binding (G.L. Nicolson et al., Exp. Cell Res. 135:461-465, 1981). Inhibiting biosynthesis of cell surface glycoprotein (GP) saccharides results in loss of endothelial/BLM binding and experimental metastasis. Melanoma cells bound to BLM solubilize BLM components in relation to their metastatic potentials (Nakajima et al., Science 220:611-613, 1983). GP from BLM are release as nearly intact molecules, but proteoglycans and collagens are degraded. Heparin sulfate proteoglycan (HSP) is an important substrate for melanoma endo- and exoglycosidases. Metastatic cells degrade HSP with an endoglycosidase, releasing a unique HS fragment. HS from lung, EHS sarcoma and BLM are degraded into characteristic M_r components, in contrast to other GAGs which are essentially undegraded. Heparin but not other GAGs inhibit HSP degradation. Analysis of HS cleavage points via reducing terminal saccharides yields almost all L-gulonic acid indicating the enzyme responsible is an endoglucuronidase (heparanase) (Nakajima et al., J. Biol. Chem., in press). Supported by USPHS grant RO1-CA28844 and RO1-CA28867 to G.L.N.

1630 B16 MELANOMA CELL ADHESION TO DEFINED SUBSTRATA. Eve Ida Barak Briles, Dept. of Tumor Biology, U. Texas System Cancer Center, M.D. Anderson Hosp., Houston, TX 77030.

In the course of studies intended to characterize B16 melanoma cell adhesion to defined components of extracellular matrices, it was observed that some B16 sublines adhere to albumin-coated polystyrene. This was surprising, since albumin is routinely used as a negative control in adhesion assays involving a variety of cell lines, both in this laboratory and many others (Briles and Haskew, 1982, Exp. Cell Res. 138, 436). The time course of attachment was unusual, in that adhesion was preceded by a 30 min lag phase which did not occur when B16 cells attached to other substrata, e.g. collagen, under identical assay conditions. One hypothesis to explain this phenomenon is that the cells are not adhering to albumin, but rather that they are modifying the substratum so as to create a new substratum to which they can adhere. Consistent with this is the observation that adhesion to albumin is inhibited by blocking cellular protein synthesis, either by pretreatment with cycloheximide or amino acid starvation prior to assay. Among three B16 sublines tested, adhesion in vitro to albumin paralleled metastatic aggressiveness in vivo (BL6 > F10 > F1), suggesting that a tumor cell's ability to modify the host environment in order to promote its own adherence in situ may influence tumor metastasis. The studies also point out a need for caution in interpreting adhesion assays designed to test substratum-specific cell-matrix interactions.

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1631 INCREASED HYALURONATE SYNTHESIS IN COCULTURES OF FIBROBLASTS WITH TUMOR CELLS, Warren Knudson, Chitra Biswas and Bryan P. Toole, Dept. of Anatomy and Cellular Biology, Tufts University, Boston, MA 02111.
Hyaluronate (HA), an important extracellular component, is often found at high concentrations in matrices conducive to cell migration and may play a role in tumorigenesis. We have shown that some murine tumors in vivo contain elevated levels of HA as compared to normal tissues but produce low amounts of HA when grown as isolated cells in culture. These somewhat conflicting results may be explained, in part, by the involvement of tumor-host cell interactions which affect HA synthesis in vivo but are not duplicated in the studies in vitro. To explore these possibilities, cultures of human lung carcinoma (LX) were grown separately and in coculture with normal human fibroblasts (HFb). Hyaluronate synthesis, determined by ^{3}H-acetate incorporation into streptomyces hyaluronidase-sensitive, CPC precipitable material, was measured. Under serum free conditions, cocultures of LX and HFb synthesized 3x more HA than the sum of that produced by LX and HFb cultures grown separately. This stimulation continued linearly for 24, 48 and 72 hours. In the presence of serum, cocultures synthesized 2x more HA than the sum of the individual cultures implying that the induced stimulation is separate and additive to that induced by serum. HA represented approx. 80% of the total glycosaminoglycans produced by the HFb cultures as well as the cultures of HFb + LX. This stimulation could not be effected by addition of LX conditioned medium to HFb cultures or by culturing LX and tumor cells under conditions where they share the same medium but were physically separated. Cell contact between LX and HFb thus appears to be necessary for stimulation of HA synthesis. Supported by NIH-F32CA07278 and RO1DE05838.

1632 DEGRADATION OF ISOLATED BASEMENT MEMBRANE MATRIX BY METASTATIC TUMOR CELLS, Jean R. Starkey and David R. Stanford, Washington State University, Pullman, WA 99164
Invasive and metastatic potentials of tumor cell populations have been shown to correlate with the possession of enzymes able to degrade components of connective tissue and basement membrane (BM) matrices. Bovine lens capsule BM matrix is used as a substrate to elucidate the mechanisms of BM matrix dissolution and invasion by metastatic cells. The lung colonizing ability of a series of metastatic variants is shown to correlate with the ability of the cells to invade across the full thickness of the lens capsule BM and with the amount of cell-associated solubilization of bound ^{125}I from surface labelled capsules. Such matrix dissolution showed a better overall correlation with lung colonizing ability than did the abilities of the tumor cells to degrade isolated laminin, type I collagen or type IV collagen. Lens capsule BM was solubilized without the use of any preliminary enzymatic digest and the resultant bands on SDS PAGE gels visualized using silver staining. The major high molecular weight bands (150k, 180k and 400k) were found to be relatively heavily labelled from the surface radio-iodinated preparations and these were judged, from enzymatic susceptibilities, immunoblot analysis and protease cleavage patterns, to derive from laminin and collagen type IV. Patterns of cleavage products from lens capsule BM digested with various purified proteases under controlled conditions were compared with those generated during incubation of the matrix with several different metastatic tumor cell lines. Finally, morphological evaluation of the cell/matrix interaction in vitro indicates that both extracellular and phagolysosomal digestion can occur, with invasion showing localized distribution patterns. CA 32071

1633 EXPRESSION OF AN ACTIVE PROTEINASE INHIBITOR, α-1-ANTICHYMOTRYPSIN, BY BREAST EPITHELIAL CELLS. S.J. Gendler and Z.A. Tokes, Dept. of Biochemistry and Comprehensive Cancer Center, School of Medicine, U. of So. Calif., Los Angeles, Ca. 90033.

Gradual remodeling of breast tissue during ontogeny and the breakdown of tissue integrity during neoplasia may be mediated by uncontrolled proteinases. The enzymes involved in the modification of the extracellular matrix may originate from the breast cells themselves or from invading leukocytes and mast cells. The biosynthesis of a glycoprotein which was immunologically identified as α-1-antichymotrypsin, Achy, has been demonstrated using human organ cultures and cell lines[1]. The central question remained as to whether or not it was active as an inhibitor of serine proteinases. Achy which is synthesized by breast cells was purified to homogeneity using immunoaffinity chromatography. Fifty percent inhibition of 50 ng of chymotrypsin was given by 115 ng of the purified protein using a fluorometric assay which establishes the functional activity of this inhibitor. Medium containing fetal bovine serum without cells did not yield an inhibitor. Thus, an active inhibitor is synthesized and released by breast epithelial cells. The expression of Achy was further investigated using immunohistochemisty and immunofluorescence. Achy was present in sections of normal breast tissue and of primary and metastatic tumors. Considerable heterogeneity was seen and the distribution of Achy appeared to be dependent on growth patterns of the cells. Studies using chymotrypsin as the enzyme for cell detachment from tc flasks showed differing rates of release which may depend on the amount of inhibitor present. The concentration of this molecule in the immediate vicinity of the cell could significantly affect the integrity of the supporting matrix and the survival of the cell from invading leukocytes. In addition, it may have a developmental role in controlling basement membrane hydrolysis which occurs during the involution of breast tissue. NIH CA-24645. [1]Tokes, Z.A., Gendler, S.J. and Dermer, G.B. (1981) J. Supramol. Str. and Cell Bioc. 17: 69-77.

1634 SELECTION AND CHARACTERIZATION OF F9 TERATOCARCINOMA STEM CELL MUTANTS WITH ALTERED RESPONSES TO RETINOIC ACID, Lorraine J. Gudas and Sho-Ya Wang, Dana Farber Cancer Institute, Boston, MA 02115

Retinoic Acid (RA) induces the differentiation of many murine teratocarcinoma stem cell lines To elucidate the molecular mechanism of action of RA, we have selected a series of mutants which exhibit altered responses to RA. Differentiation of the mutant F9 teratocarcinoma stem cell lines in the presence of $5x10^{-7}$M RA, or RA plus dibutyryl cyclic AMP, was then assessed morphologically, and biochemically by the synthesis and secretion of plasminogen activator, laminin, and collagen IV. First, all of the mutants display abnormal morphology following addition of $5x10^{-7}$M RA. In addition, none of the mutants are resistant to the cytotoxic effects of higher concentrations of RA (greater than 75uM). After the addition of RA, one mutant, RA-3-10, does not differentiate by any of the biochemical criteria we have used; this mutant also possesses less than 5% of the wild type level of cellular retinoic acid binding protein (cRABP). Other mutants, such as RA-3-3, RA-3-4, and RA-5-1, contain the same amount of cRABP as wild type F9 cells. However, the mutant RA-3-4 exhibits only 10-20% of the wild type synthesis and secretion of laminin and collagen IV following treatment with RA.
In the mutant RA-5-1, RA treatment induces synthesis of laminin and collagen IV, but these proteins are synthesized and secreted at reduced rates relative to the rates in wild type cells. Moreover, the mutant RA-5-1 secretes collagen IV with a molecular weight that is less than that of wild type F9 cells, suggesting that RA-5-1 cells have a mutation in the collagen IV gene(s) or in the enzymes responsible for post-translational modification of collagen IV. Further studies of these mutants will be described.

1635 The Role Of Extracellular Glycoproteins In Prosotate Growth, John A. Arcadi, Whittier College, Whittier, California 90608

For thirty years I have been studying the connective tissue of the prostate in man, dog, and rat, evaluating the state of Periodic acid-Schiff (PAS), and lecten-peroxidase sencitive substances. I have noted that the PAS material in caneer of the prostate is more soluble before estrogen administration than during or after. Also the basal lamina thickens after estrogen therapy or orchiectomy. In comparing 2 month old rat prostates, I find no positively stained periacinar connective tissue with lectens conjugated with peroxidase in the 2 month old rat. The 10 month old prostate has a lot of lecten-positive "sugar residues".

1636 HUMAN MENINGIOMA CELLS PRODUCE A SPECIFIC CHEMOATTRACTANT FOR VASCULAR ENDOTHELIAL CELLS. Heikki E.J. Seppä and Silja T. Seppä, Dpt of Anatomy, University of Oulu, SF-90220 Oulu 22, FINLAND.

Meningiomas are highly vascular tumors of the central nervous system, which have been shown to be potent inducers of neovascularization in experimental assays for angiogenesis. As chemotaxis is involved in the directional migration of the neovascular sprout, we have studied the production of chemoattractants for vascular endothelial cells by cultures of human meningioma cells. Using the Boyden chamber method we found that serum-free conditioned medium from confluent cultures of human meningioma cells contains endothelial cell attractant activity. This is destroyed by heating or trypsin treatment and is not extractabke in ethanol. Production of this attractant activity by the meningioma cells is inhibited by cycloheximide. The conditioned medium is angiogenic when tested on chick chorionallantoic membrane. The 120 K molecular size of the meningioma cell derived endothelial cell attractant, assessed by gel filtration on Sephadex G-200 differs from the sizes of the major fibroblast and polymorphonuclear leukocyte attractants in the conditioned medium, which elute at the void volume and at the total volume, respectively. Thus, human meningioma cells in culture produce a specific chemoattractant for vascular endothelial cells. This may be involved in meningioma cell - endothelial cell interactions controlling the vascularity of the tumor.

1637 THE ASSEMBLY OF EXTRACELLULAR MATRIX COMPONENTS BY ENDODERMAL CELLS,
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Department Tumor Biology, M.D. Anderson Hospital, Texas Medical Center, Houston, TX 77030.

The differentiation of embryonal carcinoma cells as aggregates closely mimics the initial differentiation of the inner cell mass of the early mammalian embryo. Namely, the outer layer of pluripotent cells differentiates to form a surface layer of endodermal cells. Between the stem and endodermal cells an extracellular matrix is laid down. This matrix is rich in laminin, type IV collagen and proteoglycan. We have examined the assembly of these components using established endodermal cells which are teratocarcinoma-derived, and which assemble laminin, type IV collagen and proteoglycans. We have paid particular attention to the assembly of laminin. This is a large, complex glycoprotein having one large and three smaller subunits. Pulse chase experiments demonstrate that the formation of a trimer by the small subunits is a discrete event which occurs in the absence of the large subunit. The formation of the completed molecule occurs before the maturation of the carbohydrates is completed. Sulfation of this molecule occurs but the timing of this substitution has not yet been determined. While large quantities of laminin are secreted by these cells into the culture medium, the formation of the extracellular matrix by these asymmetric cells appears to occur by directed deposition of laminin. Exogenous laminin is very poorly bound by the apical surface of these cells and does not appear to be incorporated into the extracellular matrix. This observation is supported by dilution experiments in which the cells are grown in the presence of a large excess of potentially competing unlabeled laminin. The assembly of laminin does not appear to be disrupted by compounds which inhibit collagen expression. We are currently exploring the role that cellular interactions may play in regulating the expression of this matrix.

1638 ALTERED STRUCTURE OF TYPE I COLLAGEN FROM INFANTS WITH THE LETHAL PERINATAL FORM OF OSTEOGENESIS IMPERFECTA, Jeffrey Bonadio and Peter H. Byers, Univ of Washington, Seattle,WA 98195.

Structural mutations in type I collagen from 13 infants with the lethal perinatal form of osteogenesis imperfecta (type II OI) have been localized by cyanogen bromide peptide mapping of proαchains. In 12 cases the mutations were localized to the proα1(I) chain and in 1 case to the proα2(I) chain. Two large deletions, which significantly increased the mobility of the affected proαchain on SDS-PAGE, were identified: one encompassed the COOH-terminal Met of α1(I)CB8 while the other occurred about one-third the distance from the COOH-terminus of proα2(I). In the remaining 11 cases, the mutation produced no apparent change in the molecular weight of the affected chain, and therefore could represent either a single amino acid substitution, a short deletion, or a short insertion. Two of these mutations were localized to α1(I)CB6, 8 were localized to α1(I)CB7, and 1 was localized to α1(I)CB8. Type I procollagen molecules with abnormal chains were excessively modified during their intracellular stay, had reduced thermal stability, underwent excessive intracellular degradation, were secreted at a reduced rate, and formed thin fibrils in the extracellular matrix in vitro.

1639 ASPARTYLGLYCOSAMINURIA - A NEW HERITABLE DISORDER OF CONNECTIVE TISSUE
Risto P.K. Penttinen, Kirsti Näntö-Salonen and Lauri J. Pelliniemi, University of Turku, Turku, Finland

Aspartylglycosaminuria (AGU) is a recessively inherited storage disorder of glycoprotein and proteoglycan turnover which is detected almost exclusively in Finland. It is characterized by progressive mental deficiency and various connective tissue signs (short stature, hernias, sagging skin, compression fractures of the spine and skeletal deformations). We have shown by several methods that cultured fibroblasts from AGU patients produce only 40-60 % collagen compared with values of age-matched controls whereas total protein synthesis is not much altered. Patients also excreted decreased amounts of hydroxyproline in urine during the maximal growth period. Electron micrographs of collagen fibrils from AGU patient skin show variable fibril diameters whereas control skin samples show fibril sizes similar to published controls. We suggest that these findings can explain the connective tissue findings in AGU.

Reference: K. Näntö-Salonen and R Penttinen, J.Inher. Metab. Dis.5:197-203, 1982.

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1640 THE EFFECT OF CDGF ON CULTURED FIBROBLASTS, MICROVASCULAR ENDOTHELIAL CELLS AND DEVELOPING RAT-GRANULATION TISSUE, J. M. Davidson, M. Klagsbrun, K. G. Hill, A. Buckley, P. S. Brewer, S. C. Woodward, VA Medical Center, Salt Lake City, UT 84148

Bovine articular cartilage, as well as human and bovine scapular and costal cartilage, contain a cartilage-derived growth factor (CDGF, M_I = 1700), which is released by collagenase digestion. Previous studies *in vitro* have shown that CDGF stimulates the proliferation of mouse fibroblasts, chondrocytes and endothelial cells. We have shown that CDGF stimulates the proliferation of rat embryo fibroblasts and bovine capillary endothelial cells in a dose-dependent manner. Collagen synthesis was stimulated four-fold in the embryo fibroblasts. Similar studies are being performed with adult and neonatal rat skin fibroblasts. To investigate CDGF as a potential *in vivo* wound healing accelerator, polyvinyl alcohol sponges were implanted subcutaneously in rats. Six days after implantation, sponges were injected with 300 µg of CDGF, a dose taking into account the relative cell numbers in the sponges as compared with cell cultures. CDGF rapidly disappeared from the sponges, with only about 10% of the initial dose remaining after four hours. Despite its transient presence, CDGF caused a relative increase in sponge DNA content of 2.6-fold at 48 hours and 2.4-fold at 72 hours. Morphologically, CDGF did not evoke an inflammatory response, and its effect on proliferating endothelial cells and fibroblasts was, therefore, probably direct. Presently, the failure of CDGF to accelerate collagen accumulation *in vivo*, but not *in vitro* is being analyzed. Studies are directed at examining the possible influence of collagenase production by invading capillaries and fibroblasts, as well as extending the duration of the wound repair experiment to include the latter stages of collagen accumulation.