PROGRESS IN BASIC RESEARCH OF WOUND REPAIR AND ITS APPLICATION TO CLINICAL MANAGEMENT OF PROBLEMATIC WOUNDS

Organizers: John M. McPherson and Richard A. Clark

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Poster Sessions

Progress in Basic Research of Wound Repair and its Application to Clinical Management of Problematic Wounds

The Extracellular Matrix in Repair and Development (Joint)

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

It has recently become apparent that the thrombospondins (TSPs) are encoded by at least four homologous genes. TSP1, the commonly recognized protein isolated from platelets, is similar to TSP2 in structure. Both proteins contain NH_2-terminal, COOH-terminal, and procollagen homology domains, and type I (TSP or properdin), type II (EGF-like), and type III (Ca^{2+}-binding) repeats. However, the two TSPs differ in amino acid sequence and in the regulation of their expression. TSP1 is rapidly induced by serum and growth factors. A serum response element (SRE) and a binding site for the transcription factor NF-Y have been shown to mediate the serum response of the human TSP1 gene. On the other hand, TSP2 is far less responsive to serum than TSP1 and lacks the promoter elements that mediate the serum responsiveness of TSP1.

TSP3 and TSP4 (previously described as COMP or cartilage oligomeric matrix protein) resemble TSP1 and TSP2 in their COOH-terminal domains and type III repeats, but contain four rather than three type II repeats and lack type I repeats and a procollagen homology. The NH_2-terminal domains of all four proteins are substantially different from one another. The TSPs demonstrate characteristic patterns of expression in the developing and adult mouse. It is therefore likely that each protein subserves a discrete function. We have begun to study the role of TSP1 during the angiogenesis that accompanies endometrial cycling and during the process of cord formation by endothelial cells *in vitro*. Our results suggest that TSP1 plays an inhibitory role in neovessel formation.

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

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

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

R 003 *Abstract Withdrawn*

Progress in Basic Research of Wound Repair and its Application to Clinical Management of Problematic Wounds

The Role of Inflammation in Wound Repair

R 004 INFLAMMATION AND WOUND REPAIR, Peter Henson, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

The inflammatory process is incomplete without the consequent repair of the tissue, optimally by restoration of normal structure and function, but all too often by fibrosis. Since inflammation is characterized by influx of migratory cells, alterations in vascular permeability and usually by local tissue damage the first step in repair has to involve removal of fluid, proteins, cells and cell debris — not to mention any microorganisms or exogenous material that might have initiated the response. It is suggested that in the later phases of an acute inflammatory reaction, incoming monocytes mature along a number of different and mutually exclusive pathways to orchestrate these various reparative processes. 1. Phagocytosis of digestible particulate matter leads to expression of new surface receptors for phosphatidylserine as well as to increased synthesis of TGF-ß, PDGF and lysosomal hydrolases by mechanisms that involve autocrine action of lipid mediators, TGF-ß, PKC and perhaps engagement of surface integrins. The PS receptors are suggested to participate in removal of cell membranous material and, in particular, of cells that have undergone programmed cell death (apoptosis) a process that is accompanied by loss of membrane asymmetry and therefore surface expression of phosphatidylserine. This is suggested to account for the removal of inflammatory cells from the lesion preparatory to the repair process. 2. Exposure of maturing macrophages to connective tissue elements such as hyaluronic acid induces on the other hand a pathway that leads to increased expression and release of the growth factor IGF-1. Acting through CD44, this stimulus initiates its own cytokine-driven pathway involving the autocrine action of TNF. 3. Stimulation with polyribonucleotides (or bacterial endotoxin) induces yet another pathway of maturation that results via mechanisms that involve calcium and tyrosine and serine/threonine phosphorylation in a cytocidal cell. Here too a requirement for autocrine TNF action has been demonstrated, and yet the pathway is quite exclusive of that induced by hyaluronidate. The difference appears to be whether or not the cells synthesize interferon. In its presence the cytocidal pathway is induced by TNF, in its absence, cells that synthesize IGF-1. These various pathways of maturation can proceed independent of external cytokines because of the extensive autocrine feedback processes. However, they are not surprisingly also extensively modulated by cytokines derived from other cells in the environment. Equally important, the cytokines and growth factors produced by the macrophages during the maturation diffuse into the inflammatory lesion and would be expected to significantly contribute to the reparative processes.

R 005 CYTOKINES AND INFLAMMATION IN THE EARLY STAGES OF WOUND REPAIR, S. M. Wahl, M. E. Brandes, N. McCartney-Francis, J. B. Allen, L. T. Furcht and J. B. McCarthy. Laboratory of Immunology, NIDR, NIH, Bethesda, MD and Laboratory Medicine and Pathology, Biomedical Engineering Center, University of Minnesota, Minneapolis, MN.

Inflammation is a complex tissue response to an injury or foreign substance in which a network of chemical signals initiates and maintains inflammation by acting on endothelial cells, evoking leukocyte recruitment, and augmenting the biochemical, endocytic and synthetic capacities of phagocytic leukocytes. Among the earliest of these signals is TGF-β, a cytokine released by activated platelets, as well as inflammatory cells, which binds to specific receptors expressed on the surface of target leukocytes to exert diverse biological effects. Not only is TGF-β an extremely potent chemoattractant, but it also modulates integrin expression to promote cell-cell and cell-matrix adhesion. Upregulation of α5 and β1 subunits promotes increased binding to fibronectin which is found in extracellular matrices, basal laminae and on cell surfaces, and appears to be crucial to targeting and retention of leukocytes to the site of inflammation. Disruption of these early events by antagonizing TGF-β or by blocking adhesion with polypeptides derived from the 33 kD carboxyl terminal heparin-binding region located on the A chain of fibronectin interrupts inflammatory sequelae. Once at the site of inflammation, exposure of recruited mononuclear phagocytes to a plethora of cytokines results in activation, modulation of their receptor repertoire, and altered sensitivity to ligand signalling. As part of a cytokine cascade, TGF-β increases both TNFα and TNFα receptor binding in undifferentiated blood monocytes, facilitating the multiple actions of this cytokine. Although the cytoplasmic domains of the monocyte TNFα receptors contain no intrinsic kinase activity, TNFα receptor-ligand interactions indirectly stimulate rapid and transient tyrosine kinase activity. Tyrosine phosphorylation of a 43 kD protein may represent an initial intracellular biochemical signalling event leading to TNF-mediated oxygen radical generation and enhanced cytokine mRNA expression. Inhibition of the tyrosine kinase interferes with the ability of TNFα to regulate these inflammatory mediators, and suggests a powerful tool for dissecting and regulating inflammatory events.

Cell Attachment Proteins in Repair and Development (Joint)

R 006 SPARC AND TYPE I COLLAGEN ARE FUNCTIONAL PROTEINS IN ANGIOGENESIS, E. Helene Sage, University of Washington, Seattle, WA 98195.

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

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

Progress in Basic Research of Wound Repair and its Application to Clinical Management of Problematic Wounds

Granulation Tissue Deposition During Soft Tissue Wound Repair-I

R 007 A ROLE FOR SIALYL LEWIS-X/A GLYCOCONJUGATES IN CAPILLARY MORPHOGENESIS, Mai Nguyen[1,2], Naomi A. Strubel[1], and Joyce Bischoff[1,3], Departments of Surgery, Children's Hospital[1], Brigham and Women's Hospital[2], and Department of Cellular and Molecular Physiology, Harvard Medical School[3], Boston, MA 02115

Growth of new capillaries (angiogenesis) is a complex and highly regulated process which occurs normally during certain physiologic events. To identify cell surface molecuales involved in this process, we have used an *in vitro* model for caillary morphogenesis in which bovine capillary endothelial (BCE) cells can be induced to form capillary-like structures on fibronectin-coated dishes. Since carbohydrates are known to mediate many cell-cell interactions, we tested the effect of various anti-carbohydrate monolclonal antibodies on capillary tube formation *in vitro*. Antibodies directed against the tetrasaccharides sialyl Lewis-X and sialyl Lewis-A inhibited capillary tube formation, while antibodies directed against Lewis-A, Lewis-B, A, B, H, and Vim-2 carbohydrate determinants had no effect. For each ant-carbohydrate antibody, the number of binding sites on BCE cells was measured using an enzyme-linked immunoassay. With the exception of Vim-2, all of the antibodies exhibited detectable binding to BCE cells. Furthermore, BCE tubes express sialyl Lewis-X/A-determinants on a small percent of their asparagine-linked oligosacchardes. Since human endothelial leukocyte adhesion molecule-1 (ELAM-1), also referred to as E-selectin, is known to bind to sialyl Lewis-X and sialyl Lewis-A structures, we used a cDNA encoding the lectin domain of human E-selectin for Northern blot analysis of RNA isolated from BCE cells. BCE cells induced to form capillary tubes were enriched in a 3kb RNA species that hybridized to the human E-selectin probe. We then isolated a 2.85 kb cDNA clone from a BCE tube cDNA library by nucleic acid hybridization using the same probe. The bovine capillary cDNA encodes a protein that is 71% identical to human E-selectin with the execption that the fourth and fifth complement regulatory repeat domains of human E-selectin are absent from the BCE cDNA. We postulate that this alternate form of E-selectin, expressed in capillary endothelial cells, interacts with sialyl Lewis-X/A-containing ligands and that this interaction is important during capillary formation.

R 008 MECHANISM OF ACTION AND IN VIVO EFFECTS OF PLATELET-DERIVED GROWTH FACTOR, C.-H. Heldin[1], S. Mori[1], A. Eriksson[1], E. Rupp[1], A.-K. Arvidsson[1], F. Vassbotn[1], M. Andersson[1], L. Rönnstrand[1], A. Östman[1], B. Westermark[2], and L. Claesson-Welsh[1], [1]Ludwig Institute for Cancer Research, Box 595, BMC, S-751 24 Uppsala, [2]Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden.

Platelets contain a number of factors of potential importance for wound healing. One is platelet-derived growth factor (PDGF), which originally was characterized as a mitogen for connective tissue cells, but which recently also have been shown to act on other cell types. PDGF stimulates mitogenesis as well as chemotaxis of its target cells, and was recently shown to be weakly angiogenic in vivo (Risau et al., Growth Factors, in press).

Structurally, PDGF is composed of two disulfide-bonded chains, denoted A and B. The homo- and heterodimeric isoforms exert their functions via binding to two structurally similar protein tyrosine kinase receptors, denoted α and β. The receptors dimerize after ligand binding; since the A-chain binds to α-receptors only but the B-chain both to α- and β-receptors, the various PDGF isoforms will induce the formation of different types of receptor dimers.

Both α- and β-receptors mediate a potent mitogenic response, whereas only the β-receptor mediates chemotaxis. Neomycin at high concentrations was found to inhibit the binding of the B-chain to the α-receptor, but not to the β-receptor. Since the chemotactic activity of PDGF-BB on fibroblasts, having both α- and β-receptors, was increased in the presence of neomycin, it is likely that the α-receptor, mediates an inhibitory signal for chemotaxis.

The structural and functional properties of the PDGF α- and β-receptors are currently being analyzed by expression of wild type and mutated cDNAs in porcine aortic endothelial cells, which lack endogenous PDGF receptors. Two novel autophosphorylation sites in the PDGF β-receptor have been identified in the C-terminal tail, Tyr-1009 and Tyr-1021. Mutation of these residues to phenylalanine residues inhibits the PDGF-induced phosphorylation of phospholipase C-γ, but this receptor mutant still transduces a mitogenic response. Data from other laboratories show that other known substrates for the receptor kinase bind to other autophosphorylated regions of the receptor. Taken together, the observations suggest that the interaction with down-stream components in the signal transduction pathway is determined by specific interactions involving autophosphorylation sites in the receptor. Moreover, ligand binding induces polyubiquitination of the β-receptor. Polyubiquitination is not seen in a kinase negative mutant of the receptor or in the receptor mutant with Tyr-1009 and Tyr-1021 altered to phenylalanine residues. The functional importance of polyubiquitination is not known; the possibility that it mediates cytoplasmic receptor degradation is currently explored.

In order to explore the mechanism behind ligand-induced receptor dimerization we have produced monomeric PDGF B-chain by identifying and mutating the cysteine residues in the B-chain that form interchain disulphide bridges. The monomeric PDGF retained the ability to bind PDGF receptors; however, the receptor interaction was not antagonistic, rather, monomeric PDGF was shown to have agonistic activity in receptor dimerization and autophosphorylation assays. This could be due to the formation of non-disulphide bonded dimers of the ligand or, alternatively, to the induction by the monomeric ligand of a conformational change in the receptor which promotes receptor dimerization. The regions in PDGF that are of importance for receptor binding are currently being localized by a mutational approach.

R 009 EXPRESSION OF HB-EGF AS A RESPONSE TO INJURY, Michael Klagsbrun[1], Penny Powell[2], Rosemary Jones[2], Moshe Marikovsky[1], Susan Dluz[1], Elof Eriksson[3], Michael Reidy[4], Shigeki Higashiyama[1], and Judy Abraham[5], [1]Children's Hospital, Boston, MA, [2]Massachusetts General Hospital, Boston, MA, [3]Brigham and Women's Hospital, Boston, MA, [4]University of Washington, Seattle, WA, and [5]Scios Nova Inc., Mountain View, CA.

Heparin-binding EGF-like growth factor (HB-EGF) is a newly-identified member of the EGF family that is a potent mitogen for fibroblasts, smooth muscle cells (SMC) and keratinocytes. It is an 86 amino acid heparin-binding polypeptide that appears to consist of at least two functional domains: a C-terminal, EGF-like domain and a heparin-binding N-terminal extension of 30-35 amino acids that has no counterpart in EGF and TGF-α. HB-EGF is synthesized by a number of cell types in culture including macrophages, SMC and keratinocytes. *In vivo*, expression of HB-EGF may be part of the response to injury as can be demonstrated in three animal model systems including wound healing, hyperoxic lung, and inter-arterial balloon injury as follows: i) Partial thickness excisional wounds were made in the back of a pig and wound fluid was collected daily. HB-EGF was not present in wound fluid one hour after injury but appeared one day after injury, was maximal after two to three days, and was no longer detectable by eight days. The wound fluid-derived HB-EGF was mitogenic for fibroblasts and keratinocytes, suggesting a role in wound repair. ii) Rats breathed 87% oxygen at normbaric pressure resulting in the remodeling of lung microvessels. New SMC developed from intermediate cells or interstitial fibroblasts. Vascular proliferation thickened the vessel wall and narrowed the vessel lumen resulting in pulmonary hypertension. Normal lung expressed a low level of HB-EGF mRNA but in the hyperoxic lung, the transcript levels increased by over 100-fold by day 7, and returned to normal levels by day 14. Analysis of hyperoxic lung by *in situ* hybridization indicated that the only cells expressing HB-EGF mRNA were eosinophils and that these cells localized around microvessels. Since HB-EGF is a potent fibroblast and SMC mitogen, it is suggested that the eosinophil-derived HB-EGF might be active in the remodeling of lung microvessels. iii) HB-EGF is not only a potent SMC mitogen and chemotactic factor but is also synthesized by SMC. HB-EGF expression *in vivo* was examined in rats in which interarterial injury was induced with a balloon catheter that disrupts medial SMC. HB-EGF mRNA was induced in rat arteries within 2 hours after balloon injury, expression levels were maximal at 6 hours and returned to normal levels by 24 hours. It is suggested that SMC-derived HB-EGF might contribute to the migration and proliferation of SMC that occurs as a result of balloon injury, a process that also occurs in restenosis following angioplasty. At present, the goals are to identify within these various processes the cells producing HB-EGF post-injury and to determine whether this mitogen is involved in the repair of injured tissue.

R 010 *GRO* GENES AND WOUND REPAIR: OVEREXPRESSION OF 9E3/CEF4 AND ITS RELATIONSHIP TO CELL GROWTH AND WOUND HEALING, Manuela Martins-Green, Life Sciences Division, Cell and Molecular Biology Department, Lawrence Berkeley Laboratory, Berkeley, CA 94720 (after 1 July 1993: Department of Biology, University of California, Riverside, CA 92521).

During the early phase of wound response, a number of wound factors, growth factors and cytokines are released from constitutents of the blood and from damaged cells. These molecules are chemoattractants for inflammatory cells, stimulate cells to divide, and are also important in granulation tissue formation during the latter phases of wound repair. Over the past four years, a number of genes, sometimes referred to as the *gro* family, have been discovered that have sequence and structural similarites to cytokines. The chicken 9E3/CEF4 gene belongs to this cytokine-like family.

My colleagues and I have shown previously that in young chicks both the 9E3 mRNA and its protein are expressed in connective tissue, tendon and bone and are not expressed in epidermis, muscle and bone marrow. After wounding, there is a marked elevation of expression which continues throughout wound healing in the granulation tissue and the tissues adjacent to it. Overexpression of 9E3 is particularly evident in areas of neovascularization and in areas around developing Rous sarcoma virus (RSV)-induced tumors.

By western blot analysis using an antibody to the 9E3 protein, the supernatant of RSV-transformed cells shows three proteins ranging from 14-6 kD in size. Studies are underway to purify each of these proteins for biological assays and to determine if these molecules are isoforms or if the different molecular weights are a result of post-translational modifications of a single protein. Pulse-chase experiments to study the kinetics of protein secretion in normal and RSV-transformed cells show that the 9E3 protein is synthesized and secreted in less than 10 minutes in both cell types. In the supernatant of normal CEFs, the protein is stable, whereas in the supernatant of RSV-transformed cells, the protein gets degraded with time. These results lead us to speculate that 9E3 is stable in normal tissues and could play a role in tissue remodeling and that, upon the proper stimulus (for example, wounding), the 9E3 protein is secreted at higher levels very rapidly, participates actively in tissue repair and then is degraded to normal levels.

A number of cytokines that are structurally and biochemically related to 9E3 have been shown to have their biological activity localized in the C-terminus of the protein. Therefore, we synthesized the 28aas of the C-terminus of the 9E3 protein to obtain a peptide that we then used *in vivo* to investigate protein function(s). The abundant expression of 9E3 in areas of neovascularization suggested a role for this protein in angiogenesis *in vivo*. To test this possibility we used the 9E3 peptide and the CAM assay. Pellets containing 200 ng of the 9E3 peptide were deposited on the CAM of 9-day-old chick embryos and incubated at 39°C for 5 days. Pellets containing the peptide showed numerous blood vessels growing toward them whereas pellets containing the same amount of BSA protein as control did not show any blood vessel growth. The angiogenic activity of this peptide coupled with our previous finding that 9E3 expression is enhanced in regions of neovascularization strongly suggest that the 9E3 protein is angiogenic *in vivo*.

R 011 MECHANISM OF ACTIVATION OF LATENT TGF-ß. Daniel B. Rifkin, Soichi Kojima, Mayumi Abe, John Harpel and Irene Nunes. Department of Cell Biology, New York University Medical Center, New York, NY 10016.

The proliferative response of the vascular system is under tight regulatory control. Endothelial cells can remain quiescent for years but then be stimulated to proliferate rapidly by various angiogenic stimuli. The proliferative response usually is eventually eliminated and the endothelium resumes a state of quiescence. A number of positive and negative regulatory molecules have been implicated in this process. One of the potential negative regulators is transforming growth factor ß (TGF-ß) that can block endothelial mitosis, migration, and invasive capacity. However, since TGF-ß appears to be initially released from cells in a latent form the generation of the active molecule is of potential importance. We have found that contact between endothelial cells and smooth muscle cells or pericytes stimulates the transformation of latent TGF-ß to TGF-ß. This process requires cell-cell contact, the surface generation of plasmin, and the participation of several proteins that serve to create a cell surface activation assemblage. These additional proteins are the cation-independent mannose-6-phosphate/IGF-II receptor, the latent TGF-ß binding protein and the type II tissue transglutaminase. Interference with anyone of these three proteins prevents TGF-ß formation in heterotypic cell cultures. TGF-ß can be formed in homotypic cultures after treatment of the cells by agents such as retinoids or basic fibroblast growth factor. The details of these processes and specific consequences will be discussed.

Granulation Tissue Deposition During Soft Tissue Wound Repair-II (Session Sponsored by Celtrix Pharmaceuticals, Inc.)

R 012 CELL ADHESION AND TGF-ß CONTROL FIBROBLAST CELL CYCLE PROGRESSION AT G1/S, Richard Assoian, Thomas Guadagno, Edward Han, and Stephen Dalton, Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032

We are interested in identifying the mechanisms and genes that are regulated by adhesion and TGF-ß1 during stimulation of cell cycle progression. We prepare parallel cultures of adherent and non-adherent, G0-synchronized NRK fibroblasts, expose them to mitogens (FCS/EGF), and follow their progress through the cell cycle. We find that attachment or TGF-ß1 is required solely for G1/S transit. Mapping experiments position this attachment/TGF-ß1-dependent transition shortly before activation of the G1/S histone H1 kinase (a marker for cdc2-like kinase/G1 cyclin complexes). We have also performed a random mutagenesis of NRK fibroblasts and have identified mutant cell lines that have lost their adhesion/TGF-ß1 requirement for G1/S transit while maintaining their normal mitogen requirements for proliferation. Overall, these data demonstrate that (i) the attachment/TGF-ß1 requirement for fibroblast proliferation can be explained in terms of a single cell cycle transition, (ii) the two major control points in the fibroblast cell cycle (G0/G1 and G1/S) are regulated by distinct extracellular signals and (iii) the mitogen and adhesion/TGF-ß requirements for cell cycle progression are genetically separable.

In related studies, we have tried to identify integrins that might mediate progression through the attachment-dependent transition at G1/S. We find that α5ß1 integrin, but not αVß3 integrin, is selectively removed from the surface of non-transformed fibroblasts upon lost of attachment and coincident with loss of cell cycle progression. Addition of TGF-ß1 to these non-adherent cells restores α5ß1 surface levels coincident with restoration of cell cycle progression. Moreover, the NRK mutant lines that have lost their attachment/TGF-ß1 requirement for G1/S transit maintain α5ß1 integrin on their surface despite loss of attachment. These three lines of evidence closely associate surface expression of the major fibronectin receptor with active cell cycle progression in fibroblasts.

R 013 INFLUENCE OF THE EXTRACELLULAR MATRIX ON MESENCHYMAL CELL BEHAVIOR, Thomas Krieg, Beate Eckes, Ewa Kozlowska, Dagmar Röckel, and Cornelia Mauch, Department of Dermatology, University of Cologne, Cologne, Germany.

The interaction of fibroblasts with the surrounding extracellular matrix is known to induce a variety of changes in their morphology, metabolism, and several specific cellular functions. So, growing fibroblasts in three-dimensional contact with collagen fibrils results in an induction of collagenase activity, and a downregulation of collagen synthesis. Run-on experiments demonstrate that induction of collagenase activity is mainly due to enhanced gene expression. In contrast, downregulation of collagen production occurs via more complex mechanisms including reduced transcriptional activity as well as decreased collagen α1(I) mRNA stability. Reprogramming of fibroblast metabolism in response to the contact with the surrounding matrix represents an important regulatory event for control of matrix deposition during wound healing and in fibrosis. We therefore attempted to find out which receptors are involved in collagen-fibroblast interactions. Using function-blocking antibodies against several integrin subunits it could be demonstrated that the α2β1 integrin is required for transmission of the mechanical forces leading to contraction of collagen lattices by fibroblasts. However, α2β1 integrin is not the receptor mediating the signal required to downregulate collagen synthesis. Further studies suggest that induction of collagenase gene expression is probably regulated via binding of collagen to the α2β1 integrin. These experiments provide evidence that fibroblasts show a complex interaction with the surrounding collagen involving different receptor proteins from the integrin family, each responsible for mediating distinct information. In addition, fibroblasts obtained from patients with progressive fibrotic processes seem to have an impaired recognition of the extracellular matrix proteins.

R 014 THE ROLE OF TGF-ß IN SOFT TISSUE WOUND REPAIR. D. Olsen, H. Higley, P. Carrillo, C. Gerhardt, and G. Ksander. Celtrix Pharmaceuticals, Santa Clara, CA. 95054-1815.

TGF-ßs promote a healing response and induce the deposition of granulation tissue in several models of dermal wounding. We have examined the effects of TGF-ß using a model of impaired healing in which full-thickness dermal wounds are covered with the semi-permeable dressing, Opsite®. In this model wounds covered with Opsite® contain significantly less granulation tissue as compared to air exposed wounds. Addition of TGF-ß to Opsite® covered wounds restores the levels of granulation tissue to those seen in air-exposed wounds. Analysis of mRNAs from impaired wounds treated with TGF-ß demonstrated elevated levels of α1(1), α1(III), and TIMP-1 transcripts. In addition to their effects on extracellular matrix production, TGF-ßs are also known to be potent inhibitors of keratinocyte growth *in vitro*. In the wounds described above, no significant differences in the degree of wound epithelialization was noted in TGF-ß treated wounds as compared to wounds receiving vehicle. We have performed additional *in vitro* studies to examine the effects of TGF-ß on keratinocyte gene expression. Our studies revealed that TGF-ß treatment of keratinocytes results in the activation of numerous genes whose products could further promote wound healing. These genes include aFGF, IL-6, TGF-α, EGF-R, ß4 integrin, and type VII collagen. Our results suggest that TGF-ßs can activate matrix gene expression in impaired wounds and that TGF-ß activated keratinocytes may be a significant source of growth factors and matrix molecules which would potentiate the cascade of events required for wound healing.

Osteoinduction in Hard Tissue Wound Repair (Joint)

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

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

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

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

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

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

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

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

Inductive and Conductive Strategies for Clinical Enhancement of Hard and Soft Tissue Wound Repair

R 018 THE RETINAL RESPONSE TO BIOCHEMICAL GROWTH FACTORS: THE FUTURE OF THE NEW APPROACH TO THE TREATMENT OF RETINAL DISORDERS. Bert M. Glaser, M.D., John T. Thompson, M.D., Ray N. Sjaarda, M.D., and Robert P. Murphy, M.D. The Retina Center at Saint Joseph Hospital, Baltimore, Maryland.

Great strides have been made in the treatment of retinal disorders over the past 20 to 30 years. Most approaches have utilized either laser or mechanical manipulation of retinal tissues. Recent advances have allowed an understanding of the role of biochemical growth factors in retinal and choroidal healing. We now will present results using growth factors in clinical studies for the treatment of retinal disorders. These data will demonstrate a new approach for the treatment of retinal disorders.

R 019 CLINICAL APPLICATIONS OF PEPTIDE GROWTH FACTORS FOR SOFT TISSUE REPAIR -- ANIMAL MODEL PREDICTIONS AND EARLY CLINICAL RESULTS, Mustoe, T.A., Wu, L., Zhao, L.L., Mockros, N.E., Ladin, D.A., Division of Plastic Surgery, Northwestern University Medical School, Chicago IL 60611

Surgical incision and dermal ulcer models have predicted potential clinical efficacy for PDGF, TGF-β, bFGF (FGF-2), GMCSF, IGF-1 with IGFBP-1, KGF, EGF and FGF-4 in our laboratory. However, we believe an ischemic open wound or surgical incision model of impaired healing is more predictive of growth factor effects clinically than other models of impaired wound healing or normal healing, because problem wounds are frequently hypoxic. In the hypoxic wound healing dermal ulcer model, bFGF has not improved healing despite an angiogenic effect in the surrounding tissue, while in the nonischemic situation, bFGF is highly effective. Clinical studies have demonstrated a preliminary positive effect in pressure sores (non ischemic) while other studies using leg ulcers and diabetic ulcers (locally ischemic) with bFGF were negative. The animal data suggests an possible interpretation for the clinical results.

Treating ischemic wounds with hyperbaric oxygen has made basic FGF effective, confirming the role of oxygen in FGF actions. A synergistic effect was seen with TGF-β or PDGF and hyperbaric oxygen, while hyperbaric oxygen by itself was minimally effective.

In both the ischemic and nonischemic dermal ulcer model, PDGF was effective in accelerating healing. Clinical studies have been reported for PDGF with preliminary positive effects in pressure ulcers. Recently, in a phase II trial of 40 patients with pressure ulcers (Mustoe et al) the positive effects of PDGF have been confirmed. However, the clinical role of growth factor therapy remains to be defined--a biological effect and improvement in healing rates has not yet translated into an absolute increase in healed wounds in these early clinical trials.

This work was funded in part by NIH grant #GM41303A.

R 020 CENTRAL ROLE OF TRANSFORMING GROWTH FACTOR-β (TGF-β) IN HEALING OF SOFT AND HARD TISSUES, Anita B. Roberts and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Transforming growth factor-β (TGF-β) is a multifunctional peptide which plays a key role in repair of both soft and hard tissues (1). Although 3 isoforms of this peptide are found in mammalian tissues, expression of TGF-β1, which is found in highest concentrations in platelets and bone, accounts for most of the activities of TGF-β in repair. This isoform of TGF-β is released from degranulating platelets at the site of wounding, and initiates a cascade of events including recruitment of cells, formation of new blood vessels, and synthesis of collagen; autoinductive processes amplify and extend the activity of TGF-β1 at the wound site. Subcutaneous injection of TGF-β induces the formation of granulation tissue in uninjured skin, and subperiosteal injection of TGF-β induces chondrogenesis and new bone formation in uninjured bone. Topically applied TGF-β promotes healing in a large variety of wound models in rats, rabbits, or pigs, and a single systemic injection of TGF-β also primes wound healing. Several lines of evidence demonstrate that endogenous TGF-β is important in tissue repair. First, use of antibodies to TGF-βs 1 and 2 improves the organization of the newly synthesized matrix in the wound bed, reducing scarring (2). Second, the concentrations and ratios of TGF-β isoforms in wound fluid of fetal wounds that heal without scarring are different from those of adult wounds which scar; concentrations of TGF-β2 are dramatically reduced in adult compared to fetal wound fluid, whereas concentrations of TGF-β1 are comparable in the two. Lastly, the concentrations of TGF-β in wound fluid are reduced in parallel to effects on healing in animals treated with dexamethasone.

The role of TGF-β in tissue repair is not limited to wounds involving connective tissue. Systemic administration of TGF-β has been shown to have protective effects in experimental models of myocardial, mesenteric, and cerebral ischemia-reperfusion injury (3). These effects are mediated, in part, by its ability to block adhesion of neutrophils to endothelium and subsequent cytokine release. TGF-β also has direct effects on cardiac myocytes, maintaining the rhythmicity of cultured myocytes and blocking the induction of reactive nitrogen intermediates in response to cytokines. Overall, the data suggest that TGF-β will be clinically effective in improving repair of a variety of acute and chronic wounds.

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Epidermal Regeneration in Soft Tissue Wound Repair

R 021 THE CLINICAL UTILITY AND WOUND HEALING POTENTIAL OF CULTURED EPITHELIAL CELLS IN THE TREATMENT OF BURN WOUNDS, Carolyn Compton[1], Nathan Trookman[1], Dawn Roy[1], William Press[1], Kathleen Nadire[1], Yiai Tong[1], John T. Fallon[1], Sigrid Regauer[1], Gregory Gallico[1], and Nicholas O'Connor[2]. [1]Massachusetts General Hospital and Shriners Burn Institute, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA.

With the development by Rheinwald and Green of an in vitro method for the propagation of human keratinocytes, it became possible to generate enough epidermis to cover an entire human body surface within 3-4 weeks from a small skin biopsy. Since autograft availability is critically limited in massive burns, cultured epithelial autografts (CEAU) constitute an effective means of providing rapid, permanent wound coverage in burns and can be lifesaving. Recognition of the wound healing promotional capabilities of cultured keratinocytes has lead to their use in the treatment of many other types of disease and injury in addition to burns.

Cultured human keratinocytes are known to produce a wide variety of cytokines including growth factors such as transforming growth factor (TGF)-α and TGF-β (all three isoforms). Such cytokines are thought to function in both the autoregulatory control of epidermal growth and differentiation and of stromal responses during normal wound healing. When CEAU are grafted to full-thickness cutaneous wounds, they form a permanent epidermis that regenerates all normal features of native epidermis except appendigeal structures. Within 5 years postgrafting, regeneration of dermis from wound bed connective tissue is observed subjacent to CEAU, whereas postgrafting age-match contrrols of meshed split-thickness graft interstices show scarred subepithelial connective tissue. When cultured epithelial grafts of allogeneic origin (CEAL) are applied to partial-thickness wounds or chronic, nonhealing wounds (e.g., venous stasis ulcers), they promote endogenous reepithelialization. Furthermore, CEAL more strongly promote outgrowth of follicular keratinocytes within isolated dermal sheets maintained in organ culture than topically applied, epithelial stimulatory growth factors. Both the induction of dermal regeneration by CEAU and the promotion of endogenous epidermization by CEAL are likely to be mediated through growth factor expression by the grafts. Biological variables that affect growth factor gene expression by cultured keratinocytes and, therefore, potentially alter the clinical impact of cultured grafts have been explored. By mRNA extraction and quantitative analysis of Northern blots, we have found that cultured keratinocytes vary in their capacity for TGF-α and TGFβ1 gene expression according to the body -site origin of the donor skin, the number of passages in culture, and the biological age of the donor. In second passage cultures, the type most commonly used for clinical purposes, TGF-α gene expression is lowest in keratinocytes of palm and thorax origin and highest in cells of scalp and axilla origin. TGF-β1 gene expression is also lowest in cells from the palm and thorax, but highest in cells from the leg and buttock. TGF-α gene expression is unaffected by serial passage (x12) in vitro whereas TGF-β gene expression goes up with passage. In contrast to existing data suggesting that IL-1 expression by cultured keratinocytes is decreased with increasing biological age of the donor, TGF-α gene expression by sex- and body site-matched cultured keratinocytes is unaffected by biological age (ages 6 wk through 82 yr), whereas TGF-β1 gene expression increases with age. Thus, both the selection of donor sites, the number of serial passages, and the age of the patient may all may influence the clinical impact of cultured grafts on wound healing processes.

R 022 **EGF AND TGF-α IN WOUND HEALING: ANALYSIS OF WOUND FLUIDS AND EFFECTS OF EXOGENOUS FACTORS ON WOUND HEALING,** Gregory Schultz, Neil Bennett, Scott Rotatori, Shawn Macaully, and Mike Moser. Institute of Wound Research, University of Florida, Gainesville, FL 32610.

Peptide growth factors and their receptors appear to play important roles in regulating healing of wounds. EGF and TGF-α are structurally related proteins that stimulate mitosis and migration of a variety of cells involved in wound healing including epithelial cells and fibroblasts. To assess if EGF of TGF-α are present in the environment of human wounds, fluids were collected from mastectomy drains or from open chronic wounds covered with occlusive dressing. Mastectomy fluids contained high levels of mitogenic activity for cultures of normal human skin fibroblasts, and contained physiologically significant levels of immunoreactive TGF-α and IGF-I, and receptor active TGF-ß but no detectable immunoreactive EGF. In contrast, fluids collected from chronic open wounds secondary to arthritis, diabetes, or peripheral vascular disease were not mitogenic and blocked the mitogenic activity of mastectomy fluid or serum. TGF-α, TGF-ß and IGF-I were detected in chronic wound fluids but the concentrations were usually substantially less than in mastectomy fluids. Human wound fluids were also analyzed for general protease activity using Azocol as substrate. Mastectomy fluid contained low levels of protease activity (average = 0.74 μg/ml wound fluid). In marked contrast, fluids from open chronic wounds contained approximately 170-fold higher levels of proteolytic activity (average = 125 μg/ml wound fluid). Gelatin zymogram polyacrylamide gels detected two major bands of proteolytic activity at 127,000 and 61,500 MW. Incubation of the wound fluids with the serine protease inhibitor PMSF did not significantly decrease protease activity. In contrast, incubation with EDTA inhibited 90% of the protease activity. Chronic wound fluids also degraded EGF extensively while mastectomy fluids did not. Addition of EDTA prevented approximately 70% of the degradation of EGF by chronic wound fluids. Treatment of spontaneously healing wounds or chronic wounds with EGF or TGF-α has been reported to enhance healing. Treatment of wounds with combinations of EGF or TGF-α and protease inhibitors may synergistically enhance healing. These results indicate that the molecular environment of closed, healing wounds is substantially different than the environment of open, chronic wounds with respect to mitogenic activity, growth factors and proteases. Supported by NIH EY05587 and US Army Contract No. DAMD17-91-C-1095.

Matrix Function in Repair and Development (Joint)

R 023 CYTOKINE INFLUENCES ON ECM METABOLISM DURING WOUND HEALING, Jeffrey M. Davidson, Kenneth N. Broadley, Stephen I. Benn[1], Departments of Pathology and [1]Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232 and DVA Medical Center, Nashville, TN.

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

R 024 **MOLECULAR COMPLEXITY OF THE CUTANEOUS BASEMENT MEMBRANE ZONE: PERSPECTIVES ON WOUND HEALING,** Jouni Uitto, Angela M. Christiano, Linda C. Chung-Honet, Daniel S. Greenspan*, Kehua Li, and Katsuto Tamai, Departments of Dermatology, Biochemistry and Molecular Biology, Jefferson Medical College, Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107; *Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI.

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

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

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

Pathological Fibrosis: Its Causes and Clinical Strategies for Prevention (Session Sponsored by Genzyme Corporation)

R 025 HYALURONIC ACID AS A SURGICAL AID IN THE PREVENTION OF ADHESIONS, James W. Burns, M. Jude Colt, and Kevin C. Skinner. Genzyme Corporation, Cambridge, MA.

Postoperative adhesions are the joining of normally separate tissue surfaces by connective tissues. The formation of postoperative adhesions is the culmination of a series of biochemical and cellular events that are intended to repair tissue surfaces which have been damaged during the course of surgery. The ultimate goal of this repair process is remesothelialization of injured serosal surfaces prior to permanent adhesion formation. Damage to serosal surfaces which can lead to adhesion formation can occur because of tissue desiccation, surgical gauze abrasion, ischemia due to suturing, and foreign body reaction. Adhesions, which form in response to these types of surgical trauma, can often cause unwanted and clinically significant complications. For example, 80% to 90% of postoperative intestinal obstruction result from adhesions. Major and sometimes catastrophic injury occurs following 5% of all reoperative procedures of previous open heart surgeries due to adhesions binding the heart to the sternum. We have developed two biopolymer products for adhesion prevention based on the naturally occurring polysaccharide hyaluronic acid (HA). These products act to limit postoperative adhesion formation by purely mechanical means. They are intended to either (1) limit the extent and severity of serosal tissue injury due to desiccation and manipulative trauma, or (2) physically separate severely injured tissue surfaces. We have shown in multiple animal models that dilute HA solutions effectively protect serosal surfaces from damage during surgery when the solutions are applied prior to standardized tissue trauma. In a rat cecal abrasion model, precoating tissues with a 0.4 wt% HA solutions reduced adhesions by 80% compared to treatment with buffered saline alone. The HA solutions also reduced the extent of serosal damage due to the abrasion procedure as determined histologically. Similar results were achieved in a canine pericardial adhesion model. Membranes based on modified HA were developed to limit adhesion formation when unavoidable tissue damage occurs. These membranes were designed to have a 3 to 7 day in vivo residence time. When used in a rat sidewall defect model the membranes effectively reduced adhesions to the sidewall defect while allowing normal remesothelialization to occur. Additionally, the membranes reduced adhesion formation when they were placed on the cecum of rats following cecal abrasion. The effect of the HA based membranes on healing and wound strength was investigated in a rabbit bowel anastomis model. The membranes did not affect the normal healing of bowel anastomosis as determined histologically nor was the bursting strength of the anastomosis affected by the presence of the membrane. We have demonstrated the utility of using hyaluronic acid based materials to limit adhesion formation without clinically affecting healing of surgical wounds. Our approaches to adhesion prevention will hopefully allow the surgeon to reduce the incidence, extent, and severity of both deliberate and unintentional tissue damage which occurs during the course of normal surgical procedures.

R 026 TGF-β REGULATION OF CELL LOCOMOTION IS MEDIATED BY THE HYALURONAN RECEPTOR RHAMM.
Arnold H. Greenberg[1,2,4], Shanti K. Samuel[1], Nasreen Khalil[1,3], Rashmin Savani[1,2], Baihua Yang and Eva A. Turley[1,2], [1]Manitoba Institute of Cell Biology, Departments of [2]Pediatrics, [3]Medicine and [4]Immunology, University of Manitoba, Winnipeg, MB, Canada

Consequent to bleomycin-induced lung injury, alveolar macrophages are induced to synthesize large amounts of active TGF-β_1, while increased TGF-β_2 and -β_3 production by epithelial cells are observed later in the response. The TGF-βs are powerful chemotactic factors which are hypothesized regulators of cell movement in the pulmonary inflammatory and reparative responses that often lead to fibrosis. TGF-β enhances the synthesis of the glycosaminoglycan hyaluronan (HA), which can directly stimulate cell locomotion through the HA receptor RHAMM, a 58 kD membane associated glycoprotein. Increased amounts of RHAMM were detected on alveolar macrophages in bleomycin injured lungs at the peak of TGF-β_1 synthesis, and then RHAMM localized to fibroblasts and extracellular matrix in fibrotic areas. TGF-β_1 enhanced the synthesis and membrane expression of RHAMM on fibroblasts, which was coincident with TGF-β_1 induction of the locomotory response in vitro. Locomotion of macrophages, smooth muscle cells, and fibroblasts was suppressed by antibodies to RHAMM fusion protein that prevented HA:RHAMM interactions. Furthermore, peptides corresponding to the HA binding motif of the RHAMM protein inhibited HA binding and cell motility. Locomotion of cells secreting TGF-β_1 was suppressed by TGF-β_1 anti-sense oligonucleotides and by TGF-β neutralizing antibodies. This suggests that TGF-β stimulates cell locomotion by activation of an HA:RHAMM autocrine motility pathway, a response that may be a key regulator of cell locomotion during tissue repair.

R 027 MECHANISMS AND CONSEQUENCES OF FIBRONECTIN MATRIX ASSEMBLY, John A. McDonald[1], Chuanyue Wu[1], Rudolph L. Juliano[2], Jeffrey S. Bauer[2], [1]Samuel C. Johnson Medical Research Center, Mayo Clinic Scottsdale, Scottsdale, AZ 85259 and [2]Department of Pharmacology, University of North Carolina, Chapel Hill, NC.

The assembly and organization of a fibronectin (FN) matrix by cells requires multiple interactions between FN and the cell surface[1]. Several sites on FN and the corresponding receptors on the cell surface are involved in this process. Binding of FN to $\alpha5\beta1$ integrin receptors which recognize the RGD sequence is pivotal in initiating matrix assembly. Chinese hamster ovary cells expressing $\alpha5\beta1$ integrin receptors assemble a pericellular FN matrix, whereas cell lines that do not express $\alpha5\beta1$ integrins cannot. Restoring $\alpha5\beta1$ integrin expression by transfecting with a full length cDNA encoding the human $\alpha5$ subunit restores matrix assembly to normal. This genetic reconstitution experiment reveals definitively that $\alpha5\beta1$ integrin receptors function in an early and essential step in FN matrix assembly. However, expression of $\alpha5\beta1$ integrins is not sufficient to confer matrix assembly activity. Many cell types expressing $\alpha5\beta1$ integrins cannot assemble a FN matrix. However, certain mesenchymally-derived cells possess a distinct binding activity on their surface interacting with FN's 29 kDa amino terminal domain. Engagement of $\alpha5\beta1$ receptors with ligand results in increased binding of the 29 kDa domain. Conversely, recombinant fragments or proteolytic fragments containing FN's 29 kDa matrix assembly domain bind to the cell surface and by doing so competitively inhibit FN matrix assembly.

A homophilic interactive site in FN has been identified and the relevant peptide sequence identified. This site, located in the first type III repeat, binds to as an yet unidentified complementary site in FN. Although cells with established FN matrices use this interactive site to bind additional FN, it appears to play a minimal role in de novo of FN assembly.

These studies in vitro with cultured fibroblastic cells are only beginning to be extended to in vivo. As will be discussed in this meeting by others, deletion of the FN gene is an early embryonic lethal. Accordingly, we are attempting alternative strategies to create dominant mutants in which FN matrix assembly or interaction of cells with FN can be inhibited in a controlled fashion during embryonic development. Some of these strategies will be discussed.

[1]Mosher, D.F., Sottile, J., Wu, C., and McDonald, J.A. Assembly of extracellular matrix. *Current Opinion in Cell Biology*, 1992,4:810-18.

R 028 **THE INFLAMMATORY CYTOKINES (TNF-α & IL-1) ACT IN PULMONARY FIBROSIS BY MODULATING ICAM-1 AND PLATELET TRAPPING,** Pierre F. Piguet, Department of Pathology, 1211 Geneva, Switzerland.

We have examined pulmonary fibrosis (PF) elicited in mice by the intra-tracheal instillation of bleomycin or silica. In these two models, collagen deposition in the lung was markedly decreased by the administration of antagonists to TNF-α (rabbit anti TNF IgG or the human recombinant soluble TNF receptor) or IL-1 (IL-1 receptor antagonist). These antagonists were effective when given during the collagen deposition (i.e 7-15 days after instillation) and also in the treatment of established fibrosis (i.e. 25-32 days after instillation)[1,2].
Since TNF-α and IL-1 are potent inducers of ICAM-1 [3], the ligand of the leukocytic integrin (CD-11/CD-18), we treated incipient or established PF with mAbs to these molecules. Anti CD-11a or b, anti CD-18 or anti ICAM-1 mAbs induced a pronounced decrease of the lung collagen, in either incipient or established PF [4]. Furthermore, these mAbs also decreased lung collagen from normal mice. The study of histologies indicated that the main effect of the anti CD-11/CD-18 mAbs was to prevent platelet sequestration in the lung capillaries, a finding in accord with the fact that inspite of their names, the CD-11/CD-18 "leukocytic integrins"are also expressed on platelets[5]. This possibility was further documented by an evaluation of the distribution of ^{111}In labelled platelets; platelet pulmonary localization was markedly increased by a systemic injection of bleomycin and this increased was abrogated by TNF or IL-1 antagonists as well as by anti CD-11/CD-18 mAbs. Platelets have been reported to fuse with endothelial cells[6]. Our investigations suggest that this fusion, resulting in a loading of the lung with fibrogenic factors, is modulated in normal or fibrotic lung by the inflammatory cytokins (TNF-α and IL-1), acting on the expression of ICAM-1, which regulates the capture of the CD-11/CD-18 bearing platelets.

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Late Abstracts

THE ROLE OF CHEMOATTRACTANTS FOR LEUKOCYTES IN EARLY WOUND REPAIR AND PHARMACOLOGIC MODULATION OF LEUKOCYTE ACCUMULATION. Bruce N. Cronstein, Department of Medicine, New York University Medical Center, 550 First Ave. New York, NY 10016.

The first step in wound repair is inflammation. To accumulate at inflamed sites leukocytes must adhere to the blood vessel wall and then find their way into the extravascular site of injury or microbial invasion. Until recently it was thought that the major stimuli for leukocyte emigration were chemoattractants generated at the site of inflammation such as activated complement components (C5a), cytokines (Il-8), lipid products (LTB_4 and PAF), bacterial chemoattractants (FMLP) and growth factors (TGFβ). Leukocytes possess receptors for these chemoattractants, many of which belong to the family of 7-transmembrane spanning, G protein-linked receptors (C5a, Il-8, FMLP, LTB_4). Occupancy of chemoattractant receptors is rapidly coupled to phospholipid remodelling, fluxes in $[Ca^{++}]_i$, phosphorylation, formation of actin filaments and, ultimately, migration of the cell. Once the leukocyte has encountered a high concentration of chemoattractant it stops moving, generates toxic oxygen radicals and releases the contents of its granules.

More recently it has been appreciated that both the endothelium and leukocytes possess adhesion molecules for each other which are critical for the first interaction between leukocytes and endothelium. The adhesive molecules of the neutrophil include L-selectin and the β2 integrins CD11a,b\CD18. L-selectin mediates rolling of the neutrophil along the vascular wall and is shed after exposure to high concentrations of chemoattractants. The integrins must be "activated" to become adhesive for the endothelium, often after exposure to a chemoattractant. The endothelium constitutively expresses ICAM-1, a ligand for CD11a,b\CD18, but after stimulation with endotoxin or cytokines (Il-1 or TNFα) expresses P-selectin and E-selectin which bind sialylated glycoproteins on neutrophils. The sequential interaction of all of these adhesive proteins is required for the accumulation of neutrophils at an inflamed site, the first step in wound healing.

The mechanism of action of many of the antiinflammatory agents now used is only poorly understood. We have observed that three different antiinflammatory agents, methotrexate, glucocorticoids and colchicine modulate the expression or function of one or another of the adhesive molecules discussed above. Thus, we have observed that methotrexate promotes adenosine release at sites of inflammation and that adenosine, acting at its receptor on leukocytes, inhibits activation of CD11b\CD18 on neutrophils and thus prevents their adhesion to endothelium in response to stimulation with chemoattractants. Steroids, presumably working at the level of transcription, diminish expression of E-selectin and ICAM-1 by stimulated endothelium. Colchicine promotes the loss of L-selectin from the surface of neutrophils and alters the function and topologic distribution of E-selectin on the surface of endothelium.

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

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

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

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

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

The Role of Inflammation in Wound Repair;
Cell Attachment Proteins in Repair and Development

R 100 SRC-RELATED PROTEIN TYROSINE KINASES ARE INVOLVED IN INTEGRIN-MEDIATED COLLAGEN RE-ORGANISATION BY FIBROBLASTS, Michael V. Agrez, Paul Tooney and Gordon F. Burns, Cancer Research Unit, Faculty of Medicine, The University of Newcastle, N.S.W., Australia.

A characteristic of fibroblasts is their ability to bind strongly to collagen and induce collapse of collagen matrices *in vitro*. This process is known as collagen lattice contraction, and has been considered analogous to wound contraction *in vivo*. We have recently shown that Arg-Gly-Asp (RGD)-containing peptides enhance fibroblast-mediated collagen lattice contraction through exposure of cryptic collagen receptors on the αv-associated integrins (Agrez et al, *Molec.Biol.Cell* 1991, **2**:1035). We now report that an inhibitor of src-mediated tyrosine phosphorylation, herbimycin A, is able to abolish the process of fibroblast-mediated collagen contraction whether as a result of constitutively expressed collagen receptors or as a result of receptors exposed by synthetic RGD-containing peptide. This effect is accompanied by disruption of actin filaments and removal of herbimycin A results in functional recovery of the fibroblasts including restoration of cytoskeletal integrity. To determine the effect of herbimycin A on fibroblast spreading mediated by integrins, cells were cultured on substrates of monoclonal antibodies (mAbs) against specific integrin subunits. Herbimycin inhibited fibroblast spreading mediated by the $\alpha 3$ integrin but not by the $\alpha 2$, $\alpha 5$ and αv subunits. The enhanced spreading observed on anti-$\beta 1$ substrate in the presence of herbimycin A was inhibited by soluble anti-αv mAb. The possibility of matrix synthesis in this cell-spreading assay is under investigation. Our findings suggest that the transmembrane link between actin fibres and the extracellular matrix which mediates collagen lattice contraction is stabilised rather than disrupted as a consequence of tyrosine phosphorylation of cytoskeletal proteins and/or integrin collagen receptors.

R 101 THE SVL30-MEDIATED ANOXIC FIBROBLAST RESPONSE: CONTROLLED EXPRESSION IN WOUND DEBRIDEMENT, UNCONTROLLED EXPRESSION IN MALIGNANCY. Garth R. Anderson, Carmine Volpe*, Daniel Stoler, Beth Firulli, and Cheryl Russo, Departments of Molecular and Cellular Biology and (*) Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263.

Fibroblasts respond to anoxia by inducing a multi-stage response which begins with induction of an endogenous complex retrotransposon, SVL30, progresses through induction of intracellular proteins centered on a metabolic switch to glycolysis and cessation of DNA synthesis, and then moves to secretion of the metalloproteinases procathepsin D and procathepsin L and a specific endonuclease. Anoxic fibroblasts are viable for three days and the response is reversible.

The fibroblast response to anoxia is consistent with the presence of fibroblasts within the anoxic interior of healing wounds during the debridement phase, where metalloproteinase secretion is appropriate for release of damaged cell fragments and the endonuclease for destruction of chromatin released by ruptured nuclei. Direct measurement of wound healing tissue in polyvinyl alcohol sponges implanted beneath the ventral panniculus carnosus in rats has confirmed expression of the anoxic response in healing wounds.

Most malignant tumors constitutively express the same anoxic response, but independent of oxygen tension. Cells transformed by those sarcoma viruses which contain recombined SVL30 elements also constitutively express the anoxic fibroblast response. Characterization of SVL30 sequences has revealed a transactivation system akin to that of lentiviruses which normally regulates and amplifies the anoxic response during wound healing. Mutation within this control system appears to make key contributions to tumor cell invasiveness and genomic instability.

R 102 RECEPTOR-MEDIATED SCAVENGING OF PLATELET-DERIVED GROWTH FACTOR/α_2-MACROGLOBULIN (PDGF/α_2M)-PROTEASE COMPLEXES BY MACROPHAGES. James C. Bonner, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Alveolar macrophages secrete a number of different growth-promoting cytokines that have been postulated as mediators of pulmonary inflammation and tissue repair. Alternatively, these same cytokines have been implicated in chronic inflammation leading to lung fibrosis. These factors include the proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) as well as transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF). Once stimulated by activating agents such as lipopolysaccharide, macrophages increase their cytokine production several-fold. A prolonged increase in cytokine expression in the pulmonary microenvironment is thought to drive mesenchymal cell proliferation and extracellular matrix production leading to fibrosis. Thus, elucidation of the mechanism(s) by which the overproduction of cytokines can be controlled is certainly of interest in developing strategies for preventing lung fibrogenesis. α_2-Macroglobulin (α_2M) could be an important cytokine modulator inasmuch as it serves as a binding protein for all of the factors mentioned above. In the present study, we describe the macrophage-mediated clearance of PDGF/α_2M complexes that is triggered by the protease plasmin. Rat alveolar macrophages obtained by lavage were allowed to attach to 24-well tissue culture dishes and then were incubated with [^{125}I]PDGF-BB (1 ng/ml) in the absence or presence of α_2M-plasmin. At various time points up to 24 hrs, the supernatants were removed and aliquots assayed by SDS-PAGE followed by autoradiography to quantitate [^{125}I]PDGF-BB. We observed a time-dependent clearance of PDGF-BB by macrophages in the presence of α_2M-plasmin (>80% PDGF-BB cleared within 1 hr), but only minimal clearance in the absence of this binding protein. Using either [^{125}I]PDGF-BB or [^{125}I]α_2M receptor assays, we demonstrated that these macrophages possess high-affinity receptors for α_2M, but not for PDGF-BB. Therefore, we postulated that macrophage clearance of PDGF by α_2M-plasmin was a specific α_2M-receptor-mediated event. Using a 39 kDa antagonist of the α_2M receptor that blocked the specific binding of [^{125}I]α_2M to rat alveolar macrophages, we observed that this 39 kDa antagonist completely inhibited clearance of [^{125}I]PDGF in the presence of α_2M-plasmin. These findings suggest that protease-activated α_2M could serve as an extravascular "scavenger" of secreted PDGF in the lung during inflammation and tissue repair.

R 103 CYTOKINE REGULATION OF PDGF PRODUCTION FROM HUMAN MACROPHAGES AND ENDOTHELIAL CELLS. S N Breit, S B Por, T Magoulas, R Penny, S C Thornton.
Centre for Immunology, St. Vincent's Hospital, and University of NSW, Sydney, Australia.

Platelet derived growth factor (PDGF) is a potent mitogen for fibroblasts and smooth muscle cells that is important in both normal wound healing and pathological fibrosis. Its presence has been detected at a number of chronic inflammatory sites including the rheumatoid synovium, the lung in pulmonary fibrosis, and in atherosclerosis. In chronic inflammation, two of the most important sites of production are the macrophage and the endothelial cell.

To try to elucidate the signals responsible for synthesis/secretion of PDGF from macrophages and endothelial cells, human culture derived macrophages, and umbilical vein endothelial cells (HUVEC) were treated with a variety of different cytokines. Cell associated and secreted PDGF were then measured using a specific bioassay, and PDGF-B mRNA by Northern blot analysis.

Cell associated PDGF-B in macrophages could only be induced only by IFN-γ. A second signal, delivered by either IL-1 (α or β), IL-2, IL-6, TGF-β, GM-CSF or endothelin, was required to induce PDGF-B secretion. Its synthesis could be inhibited by IL-10, and secretion by IL-4.

In contrast to macrophages, no cell associated PDGF could be detected in HUVEC. Density arrested cells could not synthesise any PDGF. Replication, as would presumably be induced *in vivo* by local endothelial injury, appears to be a pre-requisite, and is itself associated with very low levels of PDGF-B synthesis. Replicating endothelial cells were responded to a number of different cytokines such as IL-2, IL-1, TGF-β, IFN-α, IFN-γ and TNF-α with increased synthesis of PDGF-B. Cytokine combinations were more potent in inducing PDGF-B synthesis, the most effective being IL-2 and IFN-γ. Only one cytokine, IL-4, by acting at the trtanscriptional level inhibited PDGF-B synthesis.

Whilst in general, the same cytokines regulate the synthesis and secretion of PDGF-B in macrophages and endothelial cells, there appear to be different mechanisms invovled. However, lymphocytes, through the opposing effects of cytokines such as IFN-γ and IL-2 on the one hand and IL-4 and IL-10 on the other, may play an important role in regulation of the synthesis and / or secretion of PDGF-B from both macrophages and endothelial cells.

R 104 FETAL WOUND HEALING - THE MCV EXPERIENCE, I. Kelman Cohen, M.D., Robert Diegelmann, Ph.D. Division of Plastic and Reconstructive Surgery, Medical College of Virginia, Richmond, Virginia 23298

The differences between adult and fetal wound healing should provide solutions to many adult healing problems and facilitate human fetal surgery. A summary of this laboratory's studies in third trimester fetal rabbit fetal wounds demonstrates that primarily closed wounds are scarless on routine histology and electron microscopy. There is a negligible inflammatory response and collagen deposition is minimal. Primarily closed fetal wounds regain tensile strength at a rate significantly greater than adults. Following wounding, there is a rapid increase in both collagen and non-collagen protein synthesis, but only the essential amount of extracellular matrix is deposited in the wound site. Total RNA has been isolated from both fetal wounds and normal skin and analyzed by Northern-blot hybridization. Fetal wound m RNA from four days after wounding showed significant collagenase m RNA expression thus suggesting that early remodeling may contribute to the orderly and normal deposition of collagen in the fetal wound. A polyvinyl alcohol sponge implant has shown that there is negligible collagen deposition and a lack of inflammatory response. In contrast to the adult collagenous wound matrix, the fetus matrix is rich in hyaluronic acid. Removal of the hyaluronic acid or addition of TGFbeta, PDGF, bacteria or hyaluronic acid degradation products to the sponge produces an adult-like response within the implants characterized by inflammation and recruitment of fibroblasts and collagen deposition. Open fetal rabbit wounds do not contract but do "close" when shielded from the amniotic environment. These non-contracting wounds do not contain myophfiberblasts.

R 105 VITRONECTIN EXPRESSION IN HUMAN YOLK SAC CARCINOMA AND NORMAL MOUSE DEVELOPMENT, Sue Cooper, William Bennett, Sherry Roach, and Martin F. Pera, CRC Growth Factors, Department of Zoology, Oxford University, South Parks Road, Oxford, OX13PS, U.K.

Cultured cell lines derived from metastatic human yolk sac carcinomas both secrete and adhere to the serum protein vitronectin. Autocrine production of vitronectin (Cooper and Pera, Development 104:565, 1988; Gladson and Cheresh, J. Clin. Invest 88:1924, 1991) may account for the serum independence of these cells in vitro and their malignant behaviour in vivo, compared to cell lines derived from embryonal carcinomas. Thus in confirmation of earlier data on protein secretion, vitronectin transcripts may be detected in yolk sac carcinoma cell lines but not in embryonal carcinoma cell lines, which rely on exogenous vitronectin for attachment and spreading. In order to assess the possible role of vitronectin in normal development, we studied expression of of this adhesion factor in post-implantation mouse embryos using antisera raised against highly purified mouse vitronectin. At day 6.5, staining was restricted to a zone in the decidua surrounding the conceptus. At days 10.5 and 12.5, the visceral and parietal yolk sac and placenta were stained, but not the embryo proper. Immunoprecipitation analysis confirmed secretion of vitronectin by isolated yolk sacs radiolabelled in vitro. By day 16.5 of development, many connective tissues throughout the embryo reacted with the antiserum, as reported in adult human tissue, but no staining of the liver was observed. Extraembryonic tissues, particularly yolk sac, appear to be a major source of vitronectin in normal mouse development, as was suggested by our studies of human developmental tumours. The function of the molecule in development remains to be elucidated.

This work is supported by the Cancer Research Campaign.

R 106 **Autocrine Functions of CINC**
B. Crippes, J. Zagorski, L. Carr, A. Wittwer, G. Dolecki and J. De Larco
Monsanto Company, St. Louis, Missouri 63167.

Rat Cytokine-Induced Neutrophil Chemoattractant (CINC) is an 8kDa polypeptide originally purified from media conditioned by interleukin-1β stimulated 52E, an epitheloid clone derived from normal rat kidney (NRK) cells. Using a fibroblastic clone of the NRK cells, 49F, we found expression of the CINC gene to be induced by either serum or cytokines in growth arrested cultures within one hour of stimulation. There was no observable CINC expression in exponentially growing cells in the absence of cytokine stimulation. CINC protein had no significant effect on ^{3}H-thymidine incorporation or growth rate of NRK49F. We have observed that CINC is constitutively produced by some transformed NRK cells, clone RC20, suggesting an association with the expression of a transformed phenotype. We have examined the possible autocrine functions of CINC and its possible links to the expression of the transformed phenotype by these cells. The use of a blocking CINC polyclonal antibody demonstrated that CINC did not function as an autocrine growth factor for RC20 cells. Though CINC is a potent chemoattractant for neutrophils, it did not induce migration of either RC20 or 49F cells. CINC only moderately promoted adhesion of RC20 cells when used as a matrix protein. These data are consistent with CINC having a role in wound healing, but do not support the hypothesis that production of CINC by RC20 cells provides an obvious advantage for the transformed cells constitutively producing it.

R 107 EXPRESSION OF THROMBOPONDIN 1 IN EARLY WOUNDS. Luisa A. DiPietro and Peter J. Polverini, Shock Trauma Institute, Loyola University Medical Center, Maywood, IL 60153, and Department of Oral Medicine, Pathology, and Surgery, University of Michigan School of Dentistry, Ann Arbor, MI 48109.

Thrombospondin 1(TSP1), a member of a family of multifunctional extracellular matrix glycoproteins, has been recently shown to be a negative modulator of angiogenesis. Interestingly, TSP1 is produced by many of the cell types which are critical to wound repair, including fibroblasts, endothelial cells, and macrophages. TSP1 has been shown to be present in healing wounds, but the cellular source has not been characterized. To investigate the active production of TSP1 in wounds, wound derived RNA was subjected to Northern analysis with a TSP1 probe. When compared to normal skin, wound derived RNA exhibited a dramatic burst of TSP1 mRNA at day 1; TSP1 mRNA levels in wounds subsided to baseline by day 5. In situ hybridization with a TSP1 specific probe revealed that the cellular source of the TSP1 transcript in early wounds was a group of brightly labeled cells located directly beneath the acute inflammatory infiltrate. Labeled cells were confined to an area below the wound, and did not extend into the adjacent, unwounded skin. Histologic analysis suggested that the labeled cells were primarily of macrophage origin. The finding that the angiogenic modulator TSP1 is actively produced in wounds suggests a functional role for this molecule in wound angiogenesis. The results support the hypothesis that capillary growth within wounds is regulated through the coordinate production of both positive and negative regulators of angiogenesis.

R 108 FETAL LYMPHOCYTE ADHESION AND THE EXTRACELLULAR MATRIX, Peter Dillon, Kerry Keefer, Vincent Adolph, John Bleacher, and Thomas Krummel, Department of Surgery, Pennsylvania State University, Hershey, PA 17033

Lymphocyte adhesive interactions with the extracellular matrix play an important role in embryogenesis, tissue regeneration, and inflammation. However, since fetal wounds heal with minimal inflammation, the adhesive interactions of fetal lymphocytes with extracellular matrix (ECM) proteins are unknown. We examined the adhesive properties of thymic lymphocytes from fetal, newborn, and adult mice to fibronectin, vitronectin, collagen types I, III, IV, V, VI, and hyaluronic acid - ECM proteins involved in fetal and adult wound environments.

Thymic lymphocytes were isolated from murine fetuses (22 groups), 1 week newborns (15 groups), and 6 month adults (15 groups). A 4 hour microplate adhesion assay with crystal violet staining was performed on plates coated with the proteins listed above. Percent adhesion (±SEM) was calculated with data analysis by one-way ANOVA with p values by T-test with Bonneferoni correction.

Early fetal thymocytes (16 day gestation) demonstrated enhanced adhesion to fibronectin ($6.5^{\pm}1.1\%$), while late fetal (18 day gestation) newborn and adult results were the same ($p<.001$). Adhesion to collagen type I was higher in early fetal lymphocytes ($4.9^{\pm}1\%$) with no difference in late fetal, newborn, and adult levels ($0.9^{\pm}0.1\%$)($p<.001$). With vitronectin marginally enhanced binding was detected at day 16 ($2.1^{\pm}0.3\%$) as compared to adult binding ($0.2^{\pm}0.8\%$)($p<.05$). There was no binding in any age group to collagen types III, IV, or hyaluronic acid. Adhesion was not affected in any group by the addition of IL-1 or IFN-γ.

Fetal lymphocytes demonstrate increased adhesion to a limited number of ECM proteins. These results suggest a role for selective ECM receptor expression during lymphocyte differentiation.

R 109 MODULATION OF HUMAN MONOCYTE SECRETION BY ADHERENCE TO EXTRACELLULAR MATRIX AND BASEMENT MEMBRANE PROTEINS, Paul W. Gudewicz, Lynn A. Heinel and Mary Beth Frewin, Department of Physiology and Cell Biology, Albany Medical College, Albany, NY 12208

Peripheral blood monocytes (MO) must come in intimate contact with basement membrane and extracellular matrix proteins as they extravasate to areas of tissue injury. We examined the effects of human MO adherence to an endothelial cell derived basement membrane (BM) and extracellular matrix proteins on respiratory burst activity and the release of arachidonic acid metabolites. Elutriation-purified MO were added to 16 mm tissue culture wells precoated with bovine serum albumin (BSA), collagen type I (C-I), and collagen type IV (C-IV). To model the interactions with a provisional matrix at the site of tissue injury, MO were also adhered to denatured collagen (Gelatin), fibronectin-coated gelatin (Fn-Gel) or a BM derived from calf pulmonary artery endothelial cells. MO were adhered to the various surfaces for 1 hr at 37°C in serum-free DMEM, washed to remove nonadherent cells, and incubated for an additional 18 hr at 37°C prior to measuring phorbol myristate acetate (PMA)-stimulated superoxide production and release of prostanoids. Approximately 30-50% of the MO plated remained adherent to BSA, C-I, C-IV, gel, Gel/Fn, while less than 20% of the MO remained adherent to BM. Adherence to C-I or BM significantly increased PMA-stimulated superoxide secretion when compared to MO adherent to BSA, C-IV, Gel or Gel/Fn. In contrast, the release of two major prostanoids, PGE_2 and TXB_2, were inhibited by MO adherent to C-I containing surfaces. These results suggest that MO respiratory burst activity may, in part, be modulated by the inhibition of prostanoid release as a result of adherence to extracellular matrix proteins. (PO1-GM-40761)

R 110 REGULATION OF PROTEASE NEXIN 1 BY IL-1 AND DEXAMETHASONE IN HUMAN FORESKIN FIBROBLASTS. Denis C. Guttridge and Dennis D. Cunningham, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717, USA.

Injury and inflammation are characterized by a series of complicated events, one of which includes the release of proteases involved in coagulation and degradation of the extracellular matrix. These proteases are secreted from vascular and connective tissue, as well as macrophage/mononuclear cells. During the tissue repair process, cells undergo proliferation, a new matrix is laid down, and proteases are removed. This latter process is largely performed by protease inhibitors, also secreted from cells in the surrounding tissues. One of these protease inhibitors is protease nexin 1 (PN-1). PN-1 is a potent inhibitor of the serine proteases, thrombin, urokinase, and plasmin. Since these proteases have been implicated in inflammation, our interest has been to study the regulation of PN-1 by factors known to be present during an inflammatory response. Two such factors which effect the synthesis of PN-1 are IL-1, and the glucocorticoid, dexamesathone (DXM). In culture, IL-1 increased PN-1 secretion from human foreskin fibroblasts as early as 3 hours, and by 24 hours levels of secreted PN-1 were approximately 10 times that of untreated cells. Treatment with DXM gave an opposite result. PN-1 was decreased at 24 hours, and was 4 times lower than control cells by 48 hours. Examination of messenger RNA for PN-1 was consistent with what we had observed at the protein level; treament with IL-1 increased PN-1 RNA while DXM reduced it. Experiments employing cyclohexamide to examine the regulatory mechanism of these two factors indicated that control was at the transcriptional level. We are currently performing nuclear run on assays to verify this result. Our findings indicate that PN-1 is specifically regulated by factors involved in the inflammatory process. The expression of PN-1 may be necessary in such an event to remove proteases like thrombin,urokinase, and plasmin from the vasculature and connective tissue, in order to maintain a needed balance between proteases and protease inhibitors.

R 111 TRANSFUSION-SUPPRESSED REPAIR OF INTESTINAL ANASTOMOSES IS STIMULATED BY INTERLEUKIN-2, Thijs Hendriks, Tamer Tadros and Theo Wobbes, Department of General Surgery, University Hospital Nijmegen, Nijmegen, The Netherlands.

We have shown recently that blood transfusions severely impair anastomotic healing and significantly increase susceptibility to intra-abdominal septic complications in the rat. We now report on the effects of interleukin-2 (IL-2) in this model.

Sixty male Lewis rats (225-275 g) underwent resection (1 cm) and anastomosis of both ileum and colon. Subsequently, they received either 3 ml saline (control group, n=20) or 3 ml blood from Brown Norway donors (transfusion group, n=40). From operation onwards half the animals in the transfusion group received 5.4 x 10^5 IU rhIL-2/day subcutaneously (transfusion/IL-2 group), while the other rats received 5% dextrose. Half of the animals in each group were killed at 3 and 7 days after operation.

The average bursting pressure (± SD, n=10) of ileal anastomoses in the control group was 86±15 and 293±35 mm Hg at 3 and 7 days after operation, respectively. Values in the transfusion group were $32\pm8^{**}$ and $227\pm16^{**}$ mm Hg, and in the transfusion/IL-2 group $63\pm9^{\#\#}$ and $299\pm18^{\#\#}$ mm Hg. The hydroxyproline content of ileal anastomoses in the control group was 95±20 and 198±53 μg/5mm after 3 and 7 days, respectively. Values in the transfusion group were $68\pm7^{*}$ and $142\pm29^{*}$ μg/5mm, and in the transfusion/IL-2 group $88\pm12^{\#}$ and $208\pm81^{\#}$ μg/5mm (*, $p\leq0.05$; **, $p\leq0.001$ vs control group. #/##, id. vs transfusion group). In addition, abscesses were found around 60% of the ileal anastomoses in the transfusion group, while no abscesses were seen in the control group and the transfusion/IL-2 group. Similar differences for all parameters were found in colonic anastomoses. Thus, administration of IL-2 enhances early anastomotic strength and accumulation of collagen and prevents anastomotic abscess formation in transfused animals. We conclude that IL-2 reverses, at least partly, the negative effects of blood transfusion on the healing of intestinal anastomoses.

R 112 **CO-LOCALIZATION OF F-ACTIN AND FIBRONECTIN IN WOUNDED HUMAN CORNEAL ENDOTHELIUM,** **Robert W. Lambert, Jack L. Weiss, Perry S. Binder and Janet A. Anderson, Ophthalmology Research Laboratory, Sharp Cabrillo Hospital, San Diego, CA, 92110**

Distribution of fibronectin and F-actin in wounded, pseudophakic and normal corneal endothelium was examined by fluorescence microscopy using a double staining technique. In normal human corneal endothelium both fibronectin and F-actin fibers are distributed cortically. At 24 hours after wounding, cells migrating to cover Descemet's membrane show reorganization of F-actin fibers in the direction of migration. Fibronectin staining was intracellular and punctate over the cytoplasm with particularly intense staining over the F-actin fibers. At 48 hours after wounding, fibronectin and F-actin fibers were distributed over the entire cell cytoplasm but did not demonstrate any peripheral staining. By 144 hours after wounding, cells in the wound area showed a cortical fibronectin staining however F-actin fibers were still diffusely distributed over the cytoplasm. In pseudophakic corneas with minimal endothelial cell dropout, fibronectin and F-actin showed a cortical distribution similar to that observed in normal endothelium. However in pseudophakic corneas with moderate (<2,000 cells/sq mm) to high (< 1,000 cells/ sq mm) endothelial cell loss the distributions were altered. In these corneas the endothelial cells were usually present in groups of 20 or more cells separated by large areas of bare Descemet's membrane. Cells on the periphery of these groups appeared to have spread onto Descemet's membrane and stained densely for F-actin fibers and fibronectin at the edges adjacent to Descemet's membrane. Cells towards the center of these 'islands' show peripheral fibronectin staining together with a complex distribution of clumps of F-actin fibers over the entire cytoplasm. The study provides an insight into the role of fibronectin and the cell cytoskeleton in migrating and dysfunctional corneal endothelium. An understanding of the acute and the chronic responses of the endothelium to wounding can be used to reduce cell loss after cataract surgery and corneal transplantation.

R 113 DEVELOPMENT OF WOUND-INDUCED TUMORS IN CHICKS INFECTED WITH ROUS SARCOMA VIRUS (RSV), Manuela Martins-Green, Nancy Boudreau and Mina J. Bissell, Life Sciences Division, Cell and Molecular Biology Department, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

When newly hatched chicks are injected with RSV, a tumor will develop at the site of injection. In spite of the presence of virus in the blood, no other tumors are found distant from the site of inoculation during the life span of the animal (4-6 weeks). However, if a wound is made away from the primary tumor, a secondary tumor develops at the site of wounding (Dolberg et al., Science 230, 676-678, 1985). Work in our laboratory has shown that these tumors do not develop as a result of metastasis (Sieweke et al., Cancer Research 49, 6419-6424, 1989), therefore factors released upon wounding must contribute to the development of the secondary wound tumors. In particular, we showed (Science 248, 1056-1660, 1990) that TGF-β, a growth factor implicated in wound healing, can replace wounding in tumor development. However, we also showed that EGF and TGF-α, growth factors that also have roles in wound healing, do not induce tumors. Here we show that: (i) wound tumor development correlates with the presence of circulating virus; (ii) the virus is present in serum and in heterophyls of the peripheral blood; (iii) cell division at the site of wounding precedes the expression of viral proteins; and (iv) in addition to TGF-β, aFGF and bFGF also can replace wounding in tumor development. These three factors which promote tumors also induce inflammation whereas EGF and TGF-α do not. Taken together, these results suggest that inflammation induces leakage of the blood vessels, allowing the virus to leave the circulation and to integrate into the genome of cells dividing at the site of wounding, in this way giving rise to transformed cells that in due time form a tumor. Preliminary results using Evans blue are consistent with vascular leakage induced by those factors that induce tumors and no leakage induced by factors that do not induce tumors. We are presently testing the hypothesis further by using anti-inflammatory drugs that specifically inhibit blood vessel leakage to see if wound tumor development can be prevented.

R 114 MECHANISM OF BRADYKININ INHIBITION OF GROWTH FACTOR- INDUCED DNA SYNTHESIS Bradley S. McAllister and Merle S. Olson, Departments of Biochemistry and Periodontics, University of Texas Health Science Center, San Antonio, TX 78240

Strategies to enhance wound healing have involved primarily the addition of positive modulators of wound healing. An alternative approach to enhance wound healing would be to minimize therapeutically the negative modulators involved in the wound healing process. We have taken explants from healthy human connective tissues and established fibroblast and osteoblast cell lines. In these cells both epidermal growth factor (EGF) and platelet derived growth factor (PDGF) were found to stimulate DNA synthesis. This growth factor- induced DNA synthesis was found to be inhibited by bradykinin in a time- and concentration-dependent fashion. Peak inhibition was observed when a bradykinin concentration of 100nM or higher was added 2 hours after EGF or PDGF stimulation. The inhibitory action of bradykinin was abolished with indomethacin, which completely blocks the bradykinin-induced prostaglandin E_2 (PGE_2) release. PGE_2 also inhibits the growth factor- induced DNA synthesis in a concentration dependent manner. The time course for PGE_2 inhibition closely paralleled the time course for bradykinin-induced inhibition. These findings support a mechanism of bradykinin inhibition that involves the production of PGE_2. The cAMP analog dibutryl cAMP and forskolin, an activator of adenylate cyclase, both were able to mimic the bradykinin inhibition in a concentration dependent fashion, suggesting an inhibitory action involving a cAMP-dependent mechanism. In conclusion, we have demonstrated bradykinin acts as a negative regulator of growth factor activity and that the mechanism involves PGE_2 release and is a cAMP-dependent process. Additionally, this negative modulation has been shown to be therapeutically controlled through the use of non-steroidal anti-inflammatory drugs which block the bradykinin stimulated release of PGE_2. Supported by NIH grant DE00152.

R 115 THE REGULATION OF PEPTIDE GROWTH FACTORS INVOLVED IN WOUND HEALING PLAYS A ROLE IN ESTROGEN MEDIATED UTERINE GROWTH. K. Nelson[1], M. Bidwell[2], K. Ross[1], B. Eitzman[1], and J. McLachlan[1]. [1]Laboratory of Reproductive and Developmental Toxicology, NIEHS, RTP, NC, [2]Obstetrics and Gynecology, Duke University, Durham, NC.

The uterus is a dynamic tissue undergoing cyclic changes in growth and differentiation under the influence of steroid hormones. Many of the early responses of the uterus to estrogen such as the accumulation of white blood cells resembles the acute phase of inflammation. In fact, estrogen has been reported to modulate the expression of several growth factors and cytokines in reproductive tissues that have also been implicated in wound healing. In the present study, the effects of estrogen on the RNA expression of mouse uterine transforming growth factor alpha (TGFα), transforming growth factor $beta_{1,2,\&3}$ (TGFβ), insulin-like growth factor 1 (IGF1), and platelet derived growth factor A (PDGF A) and B (PDGF B) and their receptors alpha and beta is investigated. Expression of the mRNAs for all of these growth related molecules increase in the mouse uterus within 1 to 6 hrs following a single injection of estrogen (17β-estradiol or diethylstilbestrol, 10-20μg/kg) and decrease significantly within 12 hrs. The induction of these cytokines appears to be specific to estrogen; non-estrogenic steroids do not modulate the RNA expression. Estrogen induction of RNA for these growth factors occurs before the major peak of DNA synthesis (16hr) takes place which strongly implicates these ligands in the regulation of uterine growth in response to estrogen. Collectively our data provides evidence that there are many similarities between wound healing and estrogen regulation of reproductive tract growth in that each event involves the modulation of an array of potent regulatory molecules that act to coordinate the growth of a multicellular tissue. Estrogen may act as an inductive signal that triggers an acute inflammatory response that initiates a growth scenario involving the integrated response of the various uterine cell types and inflammatory cells similar to that found in wound healing.

R 116 THE ROLE OF RXR IN RETINOIC ACID-MEDIATED SUPPRESSION OF COLLAGENASE GENE EXPRESSION.
Luying Pan and Constance E. Brinckerhoff, Department of Medicine, Dartmouth Medical School, Hanover, NH 03755
Collagenase, a metalloproteinase which degrades extracellular matrix, is involved in the process of wound repair. Previously, we have shown that suppression of collagenase gene expression by all-*trans* retinoic acid is mediated through retinoic acid receptors (RAR)-α, β, and γ in an RAR-specific manner, and that the interaction of multiple DNA elements within the collagenase promoter with RAR and other transcriptional factors plays a central role in this process (Nucleic Acid Res. 20:3105, 1992). We now report that 9-*cis* retinoic acid (gift of Dr. P. Sorter), a ligand for retinoic acid X receptor (RXR), also inhibits the steady-state levels of collagenase mRNA in HIG82 cells (a rabbit synovial fibroblast cell line), and induces mRNAs for RAR. In addition, Northern blots probed with the RXR-α cDNA (gift of Dr. J. Grippo) show that RXR-α mRNA is present in these cells, but does not appear to be regulated by either 9-*cis* or all-*trans* retinoic acid. Using gel shift analysis, we show that nuclear extracts from HIG82 cells treated with retinoic acid and phorbol ester bind specifically to oligos which represent sequences from the rabbit collagenase promoter, and that addition of antibodies to RAR or RXR (gifts of Dr. P. Chambon) to mobility shift reactions gives rise to "super-shift bands". These results suggest that both RAR and RXR are involved in complex formation with the collagenase promoter sequences. Thus, we conclude that RAR/RXR heterodimers may involved in retinoic acid-mediated suppression of collagenase gene expression.

R 117 CELL:MATRIX INTERACTIONS INFLUENCE COLLAGENASE EXPRESSION BY KERATINOCYTES ACTIVELY INVOLVED IN WOUND HEALING, William C. Parks, Ulpu Saarialho-Kere, Steven Kovacs, Alice Pentland and Howard G. Welgus, Dermatology Division, Jewish Hospital and Washington University School of Medicine, St. Louis, MO 63110

Interstitial collagenase is a member of the matrix-degrading metalloenzyme family and is the only known mammalian protease that cleaves insoluble, fibrillar type I collagen. At physiologic temperature, cleaved collagen fibers denature and become susceptible to complete digestion by multiple other proteases. Thus, collagenase catalyzes the initial step required for collagen remodeling. Using *in situ* hybridization, we found that collagenase mRNA was prominently expressed by basal keratinocytes bordering the sites of active healing associated with re-epithelialization of a variety of ulcerative lesions. Collagenase production was most intense in migrating epidermal cells closest to the ulcer edge. Weak signal for collagenase mRNA was seen in only occasional dermal fibroblasts. No collagenase mRNA was detected in normal epidermis or in non-ulcerative specimens. TIMP, a specific inhibitor of collagenase, was expressed by perivascular and stromal cells in all samples, but away from sites of collagenase expression. This distinct localization suggests that keratinocyte-derived collagenase is allowed to act without impedance from TIMP. As demonstrated by immunostaining for type IV collagen, collagenase-positive keratinocytes were not in contact with an intact basement membrane and were probably migrating over the dermal matrix. These observations suggest that induction of collagenase expression is directed by cell:matrix contact. Indeed, cultured keratinocytes grown on basement membrane proteins (Matrigel) did not produce significant levels of collagenase whereas cells grown on a type I fibrillar collagen substratum expressed markedly increased levels. Furthermore, collagenase production was proportional to the concentration of collagen fibers on the culture dish. We hypothesize that migrating basal keratinocytes actively involved in re-epithelialization acquire a collagenolytic phenotype upon contact with the dermal matrix.

R 118 **EXPRESSION OF SPARC AND THROMBOSPONDIN 1 IN WOUND REPAIR: IMMUNOLOCALIZATION AND IN SITU HYBRIDIZATION,**
Pauli Puolakkainen*, May Reed, Timothy Lane, David Dickerson, Paul Bornstein**, and Helene Sage, Department of Biological Structure and Biochemistry**, University of Washington and Bristol-Myers Squibb Pharmaceutical Research Institute*, Seattle, WA 98121

Wound repair involves complex interactions between cells and the extracellular matrix. We describe the temporal and spatial distribution of SPARC (Secreted Protein, Acidic and Rich in Cysteine) and thrombospondin 1 (TSP1), proteins expressed in tissues that exhibit high rates of cellular proliferation and remodeling, during wound repair.

Full-thickness incision wounds were made in rats and sampled at 12 hours, 1, 2, 3, 5, 7, and 14 days. Affinity purified antibodies were used for immunolocalization (IHC) and riboprobes derived from cDNA sequences were used for in situ hybridization (ISH).

Two different antibodies against SPARC revealed a few positive cells on the wound edges at day 2. By 3 d, there was an increase in the proportion of the positive cells at the wound edge, as compared to the normal dermis. Immunoreactivity in the newly formed tissue was maximal at 7 days as determined by computerized analysis. ISH corroborated IHC. Combined IHC/ISH (ED-1 monoclonal antibody/SPARC riboprobe) showed maximal number of macrophages at 3 days; occasional macrophages expressed SPARC mRNA. Immunostain for TSP1 at the wound edges was positive at 12 hours and was maximal at 3 days. TSP1 protein was extracellular and no mRNA was seen with ISH.

The differential expression of SPARC and TSP1 suggests that they play complementary roles in wound repair. SPARC is secreted de novo by cells at the wound site to facilitate remodeling, while TSP1 is transiently deposited at the site of injury early in the repair process to augment the action of other ECM proteins.

R 119 CELLULAR INFILTRATION AND BREAKDOWN OF BOVINE COLLAGEN MATRICES *IN VITRO* AND *IN VIVO*. Abdul Sattar[1], Colleen Middleton[1], Gillian Robson[1], Robin Baillie[1], Kenneth N. Broadley[2], Mark WJ Ferguson[1], 1 Dept. of Cell & Structural Biology, University of Manchester, Manchester UK. 2 J&JBMG Scottish Metropolitan Alpha Centre, Stirling University Innovation Park, Stirling UK.

Bovine dermal collagen sponges, with and without laminates, or pastes were studied using either *in vitro* or rat dermal wound models, to investigate the possibility of actively controlling wound healing by providing an implantable primary "scaffold".

In vitro all the sponge matrices were well infiltrated by bovine aortic endothelial cells, smooth muscle cells and both adult and fetal human skin fibroblasts. Pastes showed little infiltration by any of the cell types. Directed cell infiltration was only observed in laminated sponges. Using either incisional or subcutaneous wound models, sponge matrices were almost completely resorbed by day 7 whilst pastes persisted until 56 days in the subcutaneous model. The presence of laminates in the sponges delayed their complete resorption to 14 days. Acute inflammatory cells surrounded and infiltrated the implants, particularly the sponges. In both models cellular infiltration followed the line of the laminates where these were present. The inflammatory reaction tended to demolish the implant before subtle "scaffolding effects" could be observed, except in laminated sponges. Matrices were more rapidly resorbed in the incisional model. No adverse long term inflammatory reaction was observed in any experiments.

Supported by the SERC Link grant with Johnson & Johnson Medical Biopolymer Group

R 120 REGULATION OF PROTEASE NEXIN-1 SYNTHESIS AND SECRETION IN CULTURED NEURAL CELLS BY INJURY RELATED FACTORS AND THE THROMBIN RECEPTOR. Patrick J. Vaughan and Dennis D. Cunningham, Department of Microbiology and Molecular Genetics, University of California, Irvine, California, USA.

Protease nexin-1 (PN-1) is a 43kDa protease inhibitor that can inactivate several serine proteases although its physiological target is probably thrombin. PN-1 is identical to the glial-derived neurite promoting factor or glial-derived nexin (GDN). This neurite promoting activity of PN-1 is mediated through it's ability to inhibit thrombin, a protease which can retract processes on both neurons and astrocytes. It is interesting in this respect that much of the PN-1 in human brain occurs around blood vessels where it may play a protective role against extravasted thrombin following injury within the brain. Other studies also indicate that PN-1 may play key roles after injury and may be important in certain pathological conditions. In the present studies we have utilised the neuroblastoma cell line SK-N-SH to examine the regulation of PN-1 synthesis and secretion by factors known to be produced after injury and in inflammatory processes. This cell line comprises two distinct cell types, one with the properties of glial cells and one with the properties of neuronal cells. Using cloned derivatives of each of these two types we have found that PN-1 is produced only by the glial cell type and that the secretion of PN-1 by these cells is stimulated by four factors ; interleukin-1, transforming growth factor-β, tumor necrosis factor α and platelet-derived growth factor. All of these factors have important roles in the wound repair process, thus supporting the hypothesis that following injury PN-1 plays an important protective role in the brain. In addition, activation of the thrombin receptor acted synergistically with IL-1 and TGFβ to further stimulate PN-1 secretion. Of further interest was the observation that the neuronal cell type secreted two thrombin inhibitors that were distinct from PN-1. We are currently investigating the nature and the regulation of these inhibitors.

R 122 RAPID OPTIMIZATION OF ENZYME SUBSTRATES FOR MATRIX METALLOPROTEASES USING DEFINED SUBSTRATE MIXTURES. Jeffrey Wiseman, Michael Green, Judd Berman, Rob Anderegg, Dan Kassel, David S. Millington, Daniel Norwood, Jerry McGeehan and D. Mark Bickett, Department of Molecular Biochemistry, Glaxo Inc. Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709

A strategy is described for the rapid optimization of k_{cat}/K_m for protease substrates. Selected positions of a given peptide substrate sequence are varied through synthesis with mixtures of amino acids. Incubation of the resulting peptide mixture with the enzyme of interest and analysis by high pressure liquid chromatography provides a direct measure of analogs with enhanced k_{cat}/K_m. High pressure liquid chromatography/continuous flow fast atom bombardment mass spectrometry is used to assign structure to each peak in the chromatogram. As an example of the utility and efficiency of "substrate mapping" we describe optimization of the matrix metalloprotease substrate Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2 (where Dnp is dinitrophenyl) at P_2, P_1, P_1', and P_2' positions. Sixteen different mixtures were prepared (representing 320 different synthetic substrates) for evaluation with MMP1 and MMP9. "Substrate mapping" has led to Dnp-Pro-Cha-Abu-Cys(Me)-His-Ala-D-Arg-NH_2, a substrate that possesses a 35-fold improved k_{cat}/K_m relative to Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2 with MMP1.

R 121 EXPRESSION OF ALTERNATIVELY SPLICED FIBRONECTIN mRNAS DURING FETAL WOUND HEALING. D.J.Whitby, L.F. Brown, M.T. Longaker, and L. Van De Water, Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, MA, 02215; Department of Surgery, UCSF, San Francisco, CA, 94143.

Scarless, fetal wound healing differs from adult wound healing in the rapid re-epithelialization of the wound defect and in the enhanced organization of collagen fibrils in the wound bed. The provisional matrix deposited in adult wounds provides a scaffold for cell migration essential for both processes. The structure of this matrix may thus determine the rate of re-epithelialization and the extent of subsequent collagen organization and scar formation in the wound. Fibronectins (FNs) are a family of glycoproteins present in tissue matrices and in blood plasma. FNs arise by transcription of a single gene to produce mRNAs which can be alternatively spliced in three regions, termed EIIIA , EIIIB and V. Plasma FN (pFN), that lacks the EIIIA and EIIIB domains, is a major constituent of the blood clot and the early provisional wound matrix. During adult wound healing pFN is supplemented by locally synthesized, alternatively spliced FN mRNAs which include the EIIIA and EIIIB domains. In this study the pattern of FN mRNA expression in fetal and adult mouse lip wounds was examined by in situ hybridization. Upper lip incisional wounds were created in fetal (16 d gestation) and adult mice and harvested from 1 to 72 hrs post wounding. The overall level of expression of FN mRNAs in normal, unwounded fetal skin was higher than in the adult tissues. Moreover, these FN mRNAs included forms containing the EIIIA and EIIIB domains. By 6 hrs following fetal wounding, the level of FN mRNAs increased above those found in unwounded fetal skin and persisted until approximately 48 hrs post wounding, a time after which FN mRNA expression within the fetal wound declined. The early expression of alternatively spliced FNs may underlie differences in the rate of wound closure and in the organization of collagen in healing fetal wounds.

Granulation Tissue Deposition During Soft Tissue Wound Repair (Session Sponsored by Celtrix Pharmaceuticals, Inc.)

R 200 PDGF INDUCES MITOGENESIS IN ENDOTHELIAL CELLS FORMING CORDS/TUBES IN VITRO

Edouard J. Battegay#, Joachim Rupp$, Luisa Iruela-Arispe*, Michael Pech$ and Helene Sage*, # Dept of Research and Internal Medicine, University Hospital, 4031 Basel, Switzerland. $ Hoffmann La Roche Inc, 4002 Basel, Switzerland. * Dept of Biological Structure, University of Washington, SM-20, Seattle, WA 98195, USA.

Angiogenesis depends in part on the response of endothelial cells to different growth-regulatory molecules. Although PDGF generally has no effect on macrovascular endothelial cells, some microvascular endothelial cells have been shown to respond to PDGF. To investigate whether PDGF might be important in angiogenesis, we assessed the effects of this molecule on bovine aortic endothelial cells that display spontaneous cord and/or tube formation in vitro (sprouting AOEC). PDGF-BB increased [^{3}H]-thymidine incorporation in sprouting AOEC but not in aortic endothelial cells that cannot form cords and/or tubes. The response of sprouting AOEC to PDGF-BB homodimer was assessed at different stages of capillary tube formation. The extent of [^{3}H]-thymidine incorporation in response to PDGF-BB (30 or 100ng/ml) increased as tubes formed (monolayer with sprouts: 120$\pm$9% of diluent control, early stage of cord/tube formation: 138$\pm$29%, intermediate stage of cord/tube formation: 158$\pm$19%, later stage of cord/tube formation: 171$\pm$17%). We utilized immunological detection of BrdU-labeled nuclei to localize cells that synthesized DNA in response to PDGF-BB. Compared with diluent-treated cells, an increase of 196$\pm$24% labeled nuclei was observed in response to PDGF-BB at an intermediate stage of cord/tube formation. Immunological studies with a rabbit polyclonal antibody raised against the rat PDGF receptor ß-subunit ektodomain showed that PDGF receptors were expressed in sprouting AOEC but not in cells that do not form cords/tubes. Moreover, PDGF receptor expression was restricted to sprouts and cord/tube structures. Clustering of PDGF receptor-ligand complexes occurred with PDGF-BB but not with PDGF-AA, suggesting that PDGF receptor ß-subunits are expressed. These observations support a potential role for PDGF in angiogenesis.

This research was supported by Swiss National Science Foundation grants 32-30801.91 and 32-31948.91 to EJB.

R 201 CONTROL OF CORNEA WOUND HEALING BY *IN VIVO* INHIBITION OF CORNEAL FIBROBLAST PROLIFERATION BY MUSCARINIC ANTAGONISTS, Sek Jin Chew[1,2], Roger W. Beuerman[2], The Rockefeller University[1], New York, NY; LSU Eye Center[2], New Orleans, LA

The M1 muscarinic receptor is a G_{PLC}-linked receptor which can induce *c-fos* expression and initiate cell cycle progression. In the cornea, cholinergic agonists promote epithelial regeneration. Little is known of their effects on stromal wound healing. Using human neonatal fibroblasts in culture, we found that the nonselective muscarinic inhibitor atropine and the M2-selective agonist, oxotremorine, inhibited growth and cell cycle progression, and downregulated the epidermal growth factor receptors. In contrast, carbachol upregulated EGF receptors, and promoted cell cycle progression. Its effect was blocked by the M1-selective anatgonist, pirenzepine. To study their effects in the rabbit model of corneal wound healing, we produced alkali burns, and performed superficial keratectomies and excimer laser ablations of the central cornea in 30 adult NZW rabbits. Ten animals were saline-treated controls and twenty adults received topical atropine or pirenzepine (100 μM daily). Tandem scanning confocal microscopy was used to evaluate the extent of fibrosis and corneal opacification postoperatively. Both atropine and pirenzepine significantly reduced keratocyte density and interstitial fibrosis at 10, 12, 14, and 21 days after wounding. Corneal light transmission was also 10-30% better in treated eyes; the effect was most marked in the central 2 mm. Pirenzepine was more potent than atropine. We suggest that crosstalk between muscarinic and growth factor receptors may be important in the regulation of ocular fibroblast growth. Muscarinic antanonists may offer a more physiological alternative to cytotoxic drugs and steroids in regulating ocular fibroblast proliferation.

Supported by NIH EY04074 (RWB), and Research Award from Sigma Xi (SJC).

R 202 MESENCHYMAL STEM CELLS FROM HEART, Martin L. Dalton, Richard L. Harvey, Bruce Jackson Taylor, Jr., Debra Warejcka, Henry E. Young, and Paul A. Lucas, Department of Surgery, Mercer University School of Medicine, Macon, GA 31207

We have isolated cells from adult rat skeletal muscle capable of differentiating into a number of mesenchymal phenotypes when treated with a non-specific differentiating agent such as dexamethasone. We have termed these cells mesenchymal stem cells (MSCs). We tested newborn rat hearts to see if they also contained MSCs. The hearts were removed, rinsed, minced, and the cells isolated by digestion with collagenase/dispase and cultured in gelatin-coated dishes in DMEM media with pre-selected horse serum until confluent. The cells were trypsinized and frozen in 7.5% DMSO at -80°C. The cells were then thawed and cultured in the same media supplemented with 10^{-6} to 10^{-10}M dexamethasone. After 5 weeks in culture control cultures contained cells with a stellate morphology, typical of mesenchymal stem cells or smooth muscle. However, in cells treated with dexamethasone, the following phenotypes were observed: short, multinucleated cells that spontaneously contracted in culture (skeletal myotubes), nodules of rounded cells whose extracellular matrix stained with Alcian blue, pH 1.0 (cartilage), rounded cells whose extracellular matrix stained with Von Kossa's stain for mineral (bone), round cells with large vesicles that stained with Sudan black B (adipocytes), rounded cells in a cobblestone pattern typical of endothelial cells, and granulated and fibrillar cells (connective tissue). These results point to the presence of a population of pluripotent mesenchymal stem cells in heart. Although heart "heals" by scar tissue following myocardial infarction, the MSCs are not limited to differentiation into scar fibroblasts. If they can be appropriately manipulated, actual tissue regeneration could be achieved in the heart as opposed to the formation of scar tissue.

Supported by funds from the Medcen Foundation

R 203 GROWTH FACTORS AND CYTOKINE EXPRESSION ARE DEMONSTRATED BY POLYMERASE CHAIN REACTION ANALYSIS OF CORNEAL TISSUE FROM A RAT EPITHELIAL SCRAPE WOUND MODEL. Alejandro Espaillat[1], C. Stephen Foster[1,2], Soon Jin Lee[1], Robert B. Colvin[3] Rhoads Molecular Immunology Laboratory[1], Hilles Immunology Laboratory[2], Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital[3], Harvard Medical School, Boston, Massachusetts, 02114.

We used the Polymerase Chain Reaction (PCR) technique to demonstrate the expression of messenger RNAs coding for TNFα, TGFβ and IL-1, during epithelial wound healing in a rat cornea wound model. A central 4mm area was denuded of epithelium with a blade. At 0, 15, 30, 45 minutes, 1, 2, 4, 8, 16, 24 and 48 hours after wounding; cDNA was synthesized from extracted RNA and specific primers sequences of TNFα, TGFβ and IL-1 were amplified by PCR. TNFα, TGFβ and IL-1 were detected in normal cornea. The level of mRNA expression of these cytokines showed different kinetics. TNFα and TGFβ had an initial increase in the early time points and a gradual decrease thereafter; IL-1 had an initial increase, follow by a decrease of expression and subsequent increase to a maximum in the later time points. These results suggest that corneal cells produce cytokine and growth factors which are capable of regulating, by an autocrine or paracrine effect of the different cornea cells, the wound healing process. The biological value performed by these cytokines are yet to be elucidated, but our data suggest that TNFα, TGFβ and IL-1 may play a role during corneal wound healing.

Supported by NIH Grant 37CA208822

R 204 HYPERTHERMIA INDUCES EXPRESSION OF TGF-ßs IN CARDIAC CELLS IN VITRO AND IN VIVO, Kathleen C. Flanders, Thomas S. Winokur, Michael G. Holder, and Michael B. Sporn, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892

Hyperthermia changes expression of transforming growth factor-ß (TGF-ß) mRNA and protein in cultured cardiac cells, as well as in the heart in vivo. Six to 12 h following hyperthermia, primary cultures of neonatal rat cardiomyocytes show decreased expression of TGF-ß mRNAs which returns to control levels by 48 h post-heat shock. In secondary cultures of rat cardiac fibroblasts, expression of TGF-ß mRNAs increases 6-48 h post-heat shock, while fetal bovine heart endothelial (FBHE) cells show little change in TGF-ß expression following hyperthermia. In each case, mRNAs for TGF-ßs 1, 2, and 3 are regulated similarly. By immunoprecipitation analysis, TGF-ß2 protein secreted by cells changes in concert with mRNA levels, while there is little change in TGF-ß1 protein levels. Hearts isolated from animals exposed to hyperthermia show an initial decrease in TGF-ß 1 and 3 mRNA levels which then return to control levels by 24 h and subsequently are elevated above normal levels 48-72 h post-heat shock. There is little change in TGF-ß2 mRNA expression in the heart following hyperthermia. Expression of TGF-ß 1 and 3 protein, localized intracellularly in myocytes, follows the same pattern as the mRNA expression. By 72 h, subpopulations of myocytes show hyperstaining for TGF-ß1. Staining for extracellular TGF-ß1/3 exhibits the opposite time course, being most intense 3-6 h post-heat shock and returning to control levels by 48 h. The increase in TGF-ßs following hyperthermia occurs with the same time course as the reported cardioprotective effects of heat shock (Karmazyn, M., Mailer, K., and Currie, R. W. 1990. Am. J. Physiol. 259:H424-H431), suggesting it may play a role in mediating the thermotolerance response.

R 205 AN ULTRASTRUCTURAL STUDY OF IMPAIRED CONNECTIVE TISSUE REPAIR IN DIABETES - MORPHOMETRICAL INDICATIONS OF DECREASED TISSUE CONTRACTION.

Lennart E. Franzén and Karin Roberg, Department of Pathology, University Hospital, S-581 85 Linkoping, Sweden.

Connective tissue repair as it occurs in the perforated rat mesentery model has earlier been shown to be hampered by diabetes. In the present study we evaluated the ultrastructure of such impaired healing applying morphometrical/sterological techniques.

Mesenteric perforations were collected various days after operation (1,3,5,7,10) and transmission electron micrographs taken in defined areas close to the wound margin or close to the center of already healed perforations. A test grid consisting of points and cycloid arcs constructed for vertical sections was superimposed on the micrographs and the numbers of points falling on the cells and intersections between the plasma membrane and arcs were counted. The surface volume density, (S_V) was then calculated for each wound.

The results showed a significantly higher S_V for wound healing cells of controls than of diabetic animals days 1 - 10 postoperatively ($p<0.009$).

The finding indicates a lower plasma membrane area per unit volume of cells in diabetic animals with impaired healing. This is compatible with less plasma membrane protrusions and spikes, alterations that are typical of actively migrating cells, and in agreement with the hypothesis of tissue contraction as a result of cell locomotion. The mesentery is not rigidly anchored to the surrounding tissues and contraction in healing perforations are therfore not inhibited. Contraction may thus be an important healing mechanism in connective tissue repair in the perforated rat mesentery.

R 206 FIBROBLAST CHEMOTAXIS TOWARDS FIBROBLAST GROWTH FACTORS. Saumya G-Nathan, M.S. and Martin J. MacPhee, Ph.D., Holland Laboratory, American Red Cross, Rockville, MD 20855.

Cell motility is an essential component of normal development of tissue, inflammation, tissue repair, angiogenesis, tumor invasion and wound healing. Fibroblasts play an important role in laying down collagen and forming a part of the connective tissue sheath underlying the wound. Despite extensive studies of the effects of the fibroblasts growth factors (FGFs) on fibroblast activity, their potential role as chemoattractants for fibroblasts has not been examined. The primary objective of this study was to determine whether fibroblasts would migrate in response to FGF-1, 2 & 4. To determine chemotaxis, modified Boyden's chambers were constructed using Millicell-*PCF* (12.0 μm pore size) inserts. These were placed in 24 well plates to form the upper and lower chambers of a chemotaxis chamber. Cells were then added to the upper chamber, and growth factors added to the upper &/or lower chambers to examine random migration, negative and positive chemotaxis. Cells were then allowed to migrate for 4h at 37°C, followed by fixation and staining of the filter inserts. The upper side of the filters was then scraped free of cells. The number of cells on the lower side of the filters was then counted using light microscopy. Both murine NIH.3T3 and human dermal (hdf) fibroblasts exhibited positive chemotaxis to FGF-1, 2 & 4 with typical bell shaped profiles. NIH.3T3 cells showed maximum response to FGF-1 & 4 at 10 ng/ml, and 1 ng/ml for FGF-2 ($p\leq 0.05$). Hdf showed maximum response to FGF-1 at 1 ng/ml and 10 ng/ml for FGF-2 and FGF-4 ($p\leq 0.05$). We concluded that fibroblasts migrate in response to a positive gradient of FGF-1, 2 or 4. Thus the release of FGF's during wound healing and other tissue remodelling may be partly responsible for the recruitment of fibroblasts to the site.

R 207 SEQUENTIAL CHANGES IN HISTOLOGICAL PATTERN AND EXTRACELLULAR MATRIX DURING THE HEALING OF CHRONIC VENOUS LEG ULCERS, Sarah E. Herrick[1], Grenham W. Ireland[1], Philip Sloan[2], Charles N. McCollum[3], M.W.J. Ferguson[1], Department of Cell and Structural Biology[1] and Oral Medicine and Surgery[2], University of Manchester, Manchester, M13 9PT and Department of Surgery[3], Withington Hospital, Manchester, M20 8AR. United Kingdom

Sequential biopsies were taken from the margins of venous leg ulcers during their healing. The changing patterns of tissue architecture and extracellular matrix synthesis during healing were documented histologically and immunocytochemically. Initial biopsies were similar in appearance; prominent fibrin cuffs, variable inflammation, haemosiderin and red blood cell extravasation. So called "fibrin cuffs" were highly organised structures composed of laminin, fibronectin, tenascin and collagen as well as trapped leucocytes and fibrin. Fibronectin was absent from the ulcer tissue although collagen was abundant. Major histological changes were observed after two weeks pressure bandage therapy; haemosiderin, acute inflammation and granulation tissue with the deposition of fibronectin had all increased and epithelial migration had commenced. Complete epithelialisation was frequent by the fourth week of treatment but the basement membrane was incomplete, haemosiderin and red blood cell extravasation had decreased and "fibrin cuffs" were virtually absent although chronic inflammation remained. The complex organisation of the capillary cuffs may inhibit angiogenesis, cause tissue ischemia and prevent oxygen, nutrient and cytokine diffusion but offer protection against increased venous pressure. Oxygen deficiency in ulcerated tissue results in tissue breakdown and necrosis. The effect of hypoxia on the synthesis of extracellular matrix molecules by ulcer and age-matched normal fibroblasts was compared *in vitro*. The results indicate a decrease in total protein synthesised by fibroblasts in hypoxic conditions, but no significant differences in the synthesis of individual matrix molecules or between ulcer and normal fibroblasts.

Supported by a grant from North West Regional Health Authority

R 208 **THE ULTRASTRUCTURE OF CARTILAGE AND SKIN IS PRESERVED BY HIGH PRESSURE FREEZING**

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Proteoglycan is a major component of the extracellular matrix. The overall structure of this anionic molecule is highly dependent on a locally hydrated environment, and without specific stabilization proteoglycans are extracted or collapsed during processing for electron microscopy. The purpose of these experiments is to determine a method by which the structure of proteoglycans might be stabilized for ultrastructural evaluation.

Chick sternal cartilage and human skin were cryostabilized for TEM by high pressure freezing (HPF) followed by freeze substitution in acetone/OsO_4 prior to embedding in epon 812. The resulting ultrastructure of both cartilage and skin is significantly dissimilar to that following conventional procedures. Following standard processing, much of the connective tissue space of skin and cartilage appears empty. Particularly stable structures, such as collagen fibrils, microfibrils, and membranes are easily contrasted within this space. Following HPF, much of this empty space is filled with a homogeneous, non-structured substance. The density of the matrix is so high following staining that in cartilage, individual collagen fibrils are difficult to distinguish. In skin, banded collagen fibrils are seen to be decorated in a pattern identical to that following decorin directed immunogold labeling. Given the high density of this substance in areas surrounding elastin and the DEJ, many components, such as matrix microfibrils and anchoring fibrils, are difficult to distinguish. The retained material is presumed to be composed largely of proteoglycan, which is extracted or collapsed during conventional TEM processing.

R 209 **POLYMERASE CHAIN REACTION (PCR) ANALYSIS OF CORNEAL FIBRONECTIN ISOFORM GENE EXPRESSION IN A RAT ALKALI-BURN MODEL OF WOUND-HEALING.**

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The polymerase chain reaction (PCR) was used to study the corneal gene expression of fibronectin (FN) isoforms, during wound-healing following alkali-burning of rat corneas. Following alkali-burning, healing corneas were collected at several time points, RNA was extracted, and cDNA was synthesized. PCR was performed using primers specific for different FN isoforms resulting from alternative mRNA-splicing. Total FN mRNA expression, as well as the mRNA expression of the EIIIA, EIIIB, and V FN isoforms, showed polyphasic kinetics. Maximal total corneal FN RNA expression occurred 4 days post-wounding. Up-regulated gene expression of EIIIA+, EIIIB+, and V+ FN isoforms was observed 30 min, 4 hr, and 4 days post-wounding, while up-regulated gene expression of the EIIIA- and EIIIB- FN isoforms was observed 1 hr, 4 days, 1 and 3 weeks post-wounding, compared to basal levels in uninjured corneas. Differences in the kinetics of expression of FN isoforms suggest that these isoforms may play different roles during the wound-healing subsequent to corneal alkali-burning.

Supported by NIH Grant RO1CA208822

R 210 PRODUCTION OF ANGIOGENIC ACTIVITY BY HUMAN MONOCYTES (Mφ) REQUIRES AN L-ARGININE/NITRIC OXIDE-SYNTHASE (NO-SYNTHASE)-DEPENDENT EFFECTOR MECHANISM,

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Human mφ require activation with substances such as bacterial endotoxin (LPS) to produce angiogenic activity. Activation of mφ with LPS (5μg/ml) in the absence of L-arginine (L-arg) greatly reduced production of angiogenic activity, compared to production in the presence of L-arg (0.6mM). Angiogenesis was assessed *in vivo* in rat corneas and *in vitro* by chemotaxis of human umbilical vein endothelial cells. D-arg did not substitute for L-arg in the production of angiogenic activity by mφ. The NO-synthase inhibitors N^g-monomethyl L-arginine (L-NMMA) and N^g-nitro L-arginine methyl ester (L-NAME) both inhibited production of angiogenic activity by activated mφ in the presence of L-arg. Neither of these substances directly inhibited mφ-derived angiogenic activity. LPS-induced production of the angiogenic cytokines TNFα and IL-8 was not reduced when mφ were incubated in the absence of L-arg. Also, L-NMMA and L-NAME did not reduce the LPS-induced production of these cytokines in the presence of L-arg. These results suggest that the activation-dependent generation of angiogenic activity by mφ requires an L-arg-dependent/NO-synthase effector mechanism that may be independent of the generation of TNFα and IL-8.

Supported in part by NIH grants GM29135 (SJL), AR41492 (AEK) and HL39926 (PJP).

R 211 IN VIVO RESPONSE TO INJECTED TGF-ß ISOFORMS INDIVIDUALLY AND IN COMBINATIONS.

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Transforming growth factor-ßeta (TGF-ß) is a cytokine which plays a central role in tissue repair. TGF-ß influences each stage of tissue repair including inflammation, reepithelialization, angiogenesis, and extracellular matrix synthesis and degradation. Three isoforms of the TGF-ß family are present in mammals (ß1, ß2, and ß3) and the specific roles of each of these isoforms in repair have not been well defined. Numerous studies have documented the vulnerary effect of TGF-ß1 on repair. Similarly, TGF-ß2 stimulates fibrosis and enhances healing. The in vivo effect of TGF-ß3 is unknown.

Fetal wounds heal without the inflammation and scarring seen in adult repair. We have recently shown that there is more TGF-ß in fetal compared to adult wound fluid. Furthermore, the ratios of TGF-ß1 and TGF-ß2 vary between fetal and adult wound fluid, with a predominance of TGF-ß2 in fetal wounds. These data suggest that concentrations of TGF-ß isoforms present in the wound may influence their biologic activity.

The purpose of this study was to identify possible differences between these 3 TGF-ß isoforms, and to determine the effect of various isoform combinations by in vivo injections. Two hundred newborn mice pups were subcutaneously injected daily with each isoform individually (800 ng/day injected SC daily for 2 or 4 days) or in combination. Tissues were fixed in 10% formalin in preparation for 1 micron Epon sections. Injections of TGF-ß1, ß2 and ß3 alone showed similar results with extensive subcutaneous fibrin deposition, edema, inflammation and fibroblast recruitment. The vascular endothelium was activated with dilation and congestion. The data from 4 injections showed progression of the findings present at 2 injections. Interestingly, tissue injected with TGF-ß3 showed a striking hyperplasia of nerve sheaths not seen with TGF-ß1 or TGF-ß2. There were no qualitative or quantitative differences when isoform combinations were injected. These data demonstrate that 1) TGF-ß3, like TGF-ß1 and TGF-ß2, induces vascular permeability and edema, inflammation, angiogenesis and fibrosis when injected into unwounded tissue; and 2) combinations of TGF-ß, as would be present in the wound environment, induce a similar tissue reaction as seen with individual isoforms.

R 212 MESENCHYMAL STEM CELLS FROM GRANULATION TISSUE, Paul A. Lucas, Andrew F. Calcutt, Paul Ossi, Henry E. Young, and Sheila S. Southerland, Department of Surgery, Mercer University School of Medicine, Macon, GA 31207
Previously, we have isolated cells from adult rat skeletal muscle capable of differentiating into a number of mesenchymal phenotypes when treated with a non-specific differentiating agent such as dexamethasone. We have termed these cells mesenchymal stem cells and have postulated they may be present in granulation tissue. Stainless steel wound chambers were implanted subcutaneously into 7 week old male rats. They were removed 7 or 14 days post-implantation and scraped of adhering tissue. The cells were isolated by digestion with collagenase/dispase and cultured in gelatin-coated dishes in media with pre-selected horse serum until confluent. The cells were trypsinized and frozen in 7.5% DMSO at -80°C . The cells were then thawed and cultured in the same media supplemented with 10^{-6} to 10^{-10}M dexamethasone. Cells from both time points behaved similarly in culture. Control cultures contained cells with a stellate morphology, typical of mesenchymal stem cells. However, upon treatment with dexamethasone, the following phenotypes were observed: long, multinucelated cells that spontaneously contracted in culture (skeletal myotubes), nodules of rounded cells whose extracellular matrix stained with Alcian blue, pH 1.0 (cartilage), rounded cells whose extracellular matrix stained with Von Kossa's stain for mineral (bone), round cells with large vesicles that stained with Sudan black B (adipocytes), and granulated and fibrillar cells (connective tissue). These results point to the presence of a population of pluripotent mesenchymal stem cells in granulation tissue. These cells are not limited to differentiation into fibroblasts. If they can be appropriately manipulated, actual tissue regeneration could be achieved as opposed to the formation of scar tissue.

Supported by funds from the Medcen Foundation

R 213 ION EXCHANGE PARTICLES ACCELERATE INCISIONAL WOUND HEALING IN A CHARGE AND SIZE SPECIFIC MANNER, Mockros[1], N.E., Roth[2], S.I., Mustoe[1], T.A. (1) Division of Plastic Surgery, (2) Department of Pathology, Northwestern University Medical School, Chicago, IL 60611
Previously we had demonstrated that electrically charged particles (Sephadex beads) had stimulated incisional healing, with findings of increased macrophages and TGF-β around the beads. However it could not be determined whether the effects were chemical, due to a charge effect or due to an ion exchange effect. Paired dorsal rat incisions were made with application of several ion exchange materials, fixed charge particles and both cation and anion exchangers of varying size. Tensiometry demonstrated that after 7 days, wounds with agents of strong anion exchange capacity and large size (100μ) had a 20-50% increase in breaking strength over control ($p< 0.01$). Negatively charged, uncharged, nonion exchange particles, or small particles (<5m) had no effect on healing. Histological analysis revealed increased numbers of macrophages. Anion exchange properties of the compounds rather than the charge itself appear to be contributing to the increased breaking strength. The data confirms previous experiments on the stimulatory effect of charged beads on wound healing (Plast Reconstr Surg, 89: 891, 1992), but also defines the effect as due to anion exchange rather than chemical (due to the DEAE Sephadex interaction), or purely a charge effect. DEAE Sephadex beads are most effective of the anion exchange particles tested. This work adds new insights into the mechanisms of charged particle effects on wound healing.
This work was funded in part by NIH grant #GM41303A.

R 214 CHARACTERIZATION OF COLLAGEN SYNTHESIS IN RABBIT CORPUS CAVERNOSUM IN ORGAN CULTURE, Robert B. Moreland, Philip M. Newhall, Abdulmaged Traish, and Iñigo Saenz de Tejada, Department of Urology, Boston University School of Medicine, Boston, MA.
In order to investigate the regulation of fibrosis in corpus cavernosum *in vitro* , an organ culture model was developed in which the cell-cell interactions are preserved. The viability of this system for the study of gene regulation and protein production was examined using four criteria. First, corpus cavernosum explants, studied up to 24 hours in organ culture, showed reduced cell damage as measured by lactate dehydrogenase release. Second, these explants engaged in *de novo* protein synthesis as measured by incorporation of radiolabeled leucine into acid precipitable proteins. Third, mRNAs for α1(I), α2(I) and α1(III) collagens, collagenase, TGF-ß1, and glyceraldehyde-3-phosphate dehydrogenase were detected using Northern blot analysis. Fourth, *de novo* collagen biosynthesis was measured by the incorporation of radiolabeled proline into collagenase sensitive, acid precipitable protein. When this material is analyzed on 6.5% SDS-polyacylamide gel electrophoresis with and without collagenase treatment, α1(I), α2(I) and α1(III) collagens could be identified. Thus, this organ culture system provides a means to examine the molecular processes involved in collagen biosynthesis in the corpus cavernosum under these conditions. To determine if pharmacologic agents which effect extracellular matrix deposition may act on this experimental system, we examined the changes in collagen mRNA and protein production in response to exogenous TGF-ß1, a cytokine that induces collagen mRNA expression and protein production. Maximal incorporation of radiolabeled proline into collagenase sensitive, acid nonprecipitable material was observed with 45pM TGF-ß1. This concentration of TGF-ß1 produces similar increases in corpus cavernosal fibroblasts in tissue culture. In conclusion, this system provides a means of studying the acute effects involved in the molecular regulation of collagen biosynthesis, and ultimately, fibrosis in the corpus cavernosum.

R 215 EXTRACELLULAR MATRIX (ECM) IS REQUIRED FOR MONOCROTALINE (MCT)-INDUCED ANGIOGENESIS *IN VITRO*. Jack W. Olson, Bernadette Salone, Chiung-Ying Huang, Santosh Arcot, David W. Lipke and Mark N. Gillespie. Univ. of KY, Coll. of Pharmacy, Lexington, KY.
While the mechanisms eliciting pulmonary angiogenesis in rats treated with the pneumotoxin MCT are unknown, growth factors, proteases and the ECM probably play essential roles. To test if a specifc ECM environment is required for MCT induction of angiogenesis *in vitro*, porcine pulmonary artery endothelial cells (EC) were grown on type I collagen 3-D gels or plastic and exposed to either MCT or vehicle for 24 hours. MCT stimulated ECs to invade the underlying 3-D gel matrix, and by day 13 to organize into a network of tube-like structures, while control ECs were confined to the gel surface 4 weeks later. ECs on plastic elongated and developed raised, large vacuole-like structures 6 days after MCT exposure, while controls remained as monolayers for at least 3 weeks. Prior to these morphological changes, MCT caused ECs on plastic to increase fibronectin (FN) secretion, and to modulate mRNA transcripts: collagens III & IV & thrombospondin decreased after day 1; FN & laminin increased & then decreased after day 3; basic fibroblast growth factor, transin & tissue inhibitor of metalloproteinase increased after day 3; interestingly, type I collagen did not change. These data suggest that although MCT caused ECs on plastic to differentially regulate ECM expression and to apparently initiate the multi-stage angiogenic process, the formation of capillary-like tubules required a type I collagen substrate. Supported in part by NIH HL-36404.

R 216 THE EXPRESSION OF LACTOFERRIN IN THE HUMAN ENDOMETRIUM BY INFLAMMATORY GRANULOCYTES AND GLANDULAR EPITHELIUM: A POSSIBLE ROLE IN GROWTH REGULATION C Padin[1], K Nelson[2], J McLachlan[2], D Walmer[1]. [1]Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, [2]Laboratory of Reproductive and Developmental Toxicology, NIEHS, RTP, NC. With each menstrual cycle, the human endometrium undergoes a process of injury. The post-menstrual repair that occurs under the control of estrogen provides a spectacular example of the wound healing process. This involves the influx of inflammatory cells and a regeneration of the entire surface epithelium. A number of signalling molecules thought to play a role in growth and wound repair have been shown to be under estrogen regulation in the reproductive tract. One such molecule is lactoferrin (LF), a peptide found in the secondary granules of neutrophils, in most exocrine secretions, and in the proliferating epithelium of the mouse uterus. The function of this glycoprotein is unknown. In our investigation of the human endometrium, lactoferrin was immunolocalized predominantly to neutrophils and to the epithelial cells of the basal glands. Two distinct staining patterns were observed in the glandular epithelium, one showing cytoplasmic localization and the other intense nuclear staining. This has led to our hypothesis that, in addition to its role as a secretory protein, LF may be transported into the nucleus and serve some autocrine function. Our results suggest that lactoferrin is localized to a region of the human endometrium that contains the epithelial stem cells which are responsible for the regeneration of the epithelium following menstrual injury. Studies are being done to examine in more detail this differential expression of LF. Correlations are being made with the proliferative index of the tissue using proliferating cell nuclear antigen as a marker of DNA synthesis. In addition, the localization of LF to the secondary granules of neutrophils provides a marker to analyze the influx of these cells during the menstrual cycle.

R 217 THROMBIN PROMOTES FIBROBLAST CONTRACTION BY CLEAVAGE OF ITS CELL SURFACE RECEPTOR. B.K. Pilcher and J.J. Tomasek. Department of Anatomy, University of Oklahoma Health Science Center, Oklahoma City, OK 73190.
Fibroblast contraction is proposed to play an important role in tissue contraction during wound healing and pathological diseases such as Dupuytren's contracture. Regulation of fibroblast contraction is unclear. A G-protein linked thrombin receptor is present on the surfaces of fibroblasts. Activation of this receptor is through a novel mechanism by which the enzymatic activity of thrombin generates a new NH_2 terminus which acts as a tethered ligand. The purpose of this study was to determine the role thrombin plays in promoting the contraction of fibroblasts. Fibroblast contraction was evaluated utilizing an in vitro collagen lattice contraction assay. Adult human fibroblasts were cultured within stabilized type I collagen lattices for five days. Release of the lattice from its points of attachment results in a rapid, symmetrical contraction of the collagen lattice within ten minutes after release. Contraction is cell-mediated, dependent upon an organized actin cytoskeleton, and dependent on the presence of fetal bovine serum (FBS). Human alpha-thrombin, added at various concentrations in place of FBS, resulted in contraction in a dose dependent manner, with maximal activity at 0.1 U/ml. Enzymatic activity of thrombin is necessary to promote contraction as the specific thrombin inhibitors hirudin and PPACK inhibited thrombin promoted contraction. To test whether thrombin promotes fibroblast contraction through cleavage of its receptor, a novel synthetic peptide representing the activated NH_2 terminal portion of the receptor was synthesized. The synthetic peptide promoted contraction in a dose dependent manner. Thus, the enzymatic activity of thrombin and cleavage of its specific receptor can promote fibroblast contraction. These studies demonstrate that thrombin can interact with fibroblasts and induce fibroblast-mediated collagen lattice contraction. The ability of thrombin to promote fibroblast contraction may play an important role during tissue contraction.

R 218 SYNTHESIS OF GLYCOSAMINOGLYCANS IN RESPONSE TO A WOUND CREATED BY THE INSERTION OF A POROUS POLYMER INTO THE CORNEA, V. Trinkaus-Randall, C. Brown, X.Y. Wu, and H.M. Leibowitz, Department of Ophthalmology, Boston University School of Medicine, Boston, MA, 02118

We demonstrated that fibroplasia occurred after a blown microfiber was inserted into corneal interlamellar pockets. The wound was made by separating the collagen lamellae. After 42 d, a force of 200g was required to pull the polymer from the cornea indicating that it was anchored in place. We have examined the synthesis of glycosaminoglycans (GAGs) and their core proteoglycans using this model. Immunofluorescence was conducted initially to examine the overall profile. Guanidine-HCl extracted GAGs were then purified using anion exchange chromatography. Sulfated GAGs were quantified colorimetrically using dimethylmethylene blue. Individual GAG types were determined using selective enzymatic digestion. Proteoglycans were determined using Western blot analysis. Endogenous levels of TGF-ß were followed. By 14 d, dermatan sulfate only was detected in the disc. No other GAGs were present in detectable levels. The amount of keratan sulfate in the control and surrounding level were similar while the concentrations of heparan sulfate and chondroitin - 4 sulfate were at least 2 fold greater in the surrounding tissue than the control. By 42 d, immunofluorescent micrographs showed an even distribution of keratan sulfate throughout the cornea and the disc; however, the biochemical analyses indicate that the level in the stroma is greater than in the disc µg of dry weight. The data indicate that 1) levels increase in the surrounding tissue; 2) an increase in the disc is next detected; and 3) the levels in the surrounding tissue decrease and are comparable to that of the control unwounded tissue.

R 219 POROUS COLLAGEN-GAG GRAFTS DELAY WOUND CONTRACTION BY LENGTHENING THE MIGRATION/PROLIFERATION STAGE OF WOUND HEALING, Karen S. Troxel, I. V. Yannas, Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

Porous collagen-GAG matrices which have been used to partially regenerate the dermis in human and in guinea pig also delay the onset of wound contraction in the guinea pig model. During wound contraction the wound edges move over the wound base toward the center of the wound thereby closing the wound. (Myo)fibroblasts are thought to generate the mechanical stress required for wound closure. A histological study of control and collagen-GAG grafted wounds was undertaken in order to determine the mechanism by which collagen-GAG matrices delay the onset of contraction. In control wounds (1.5 cm X 3.0 cm full-thickness skin wounds on the back of guinea pig), the fibroblasts in granulation tissue were arranged in multilayers with their long axes parallel to the wound base. At the beginning of contraction, the multilayers were only a few cells thick. During the course of contraction the granulation became increasingly thick as a result of central movement of the fibroblasts during wound closure. At the initiation of contraction, this rearrangement of fibroblasts is evident only in the granulation tissue adjacent to the wound edge and does not appear to involve the fibrobalsts in the center of the granulation tissue. This supports the "picture-frame" hypothesis of the location of stress transfer in wounds, in which only the fibroblasts which attach the wound edge to the wound base are required for wound contraction. Histology of porous collagen-GAG grafted wounds shows that fibroblasts migrate into the interior of the porous grafts. The number of fibroblasts which fill the grafted woundbed increases approximately tenfold over control (ungrafted) wounds. The onset of contraction in grafted wounds coincides with the accumulation of the maximum number of cells. This study suggests that porous collagen-GAG grafts delay the onset of contraction by extending the proliferation and/or migration phase of wound healing, and that contraction in initiated only after sufficient numbers of fibroblasts have accumulated at the wound edge - wound base interface to generate sufficient mechanical stress to initiate contraction.

R 220 TGFβ EXPRESSION IN THE CYCLING HUMAN FEMALE REPRODUCTIVE TRACT:PARALLELS WITH WOUND REPAIR

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Cyclic changes in the female reproductive tract are orchestrated by the ovaries through the production estradiol and progesterone. Scrutiny of these events in the vagina and endometrium demonstrate numerous parallels between estrogen-induced proliferation and wound repair. These events include the chemotactic recruitment and activation of inflammatory cells, remodeling of extracellular matrix, proliferation of resident cells and angiogenesis. Since a family of signaling peptides known as TGFβs are known to mediate several of these events in other tissues, we suspected that they may be important in the regulation of events in the female reproductive tract as well. In the proliferative endometrium, $TGF\beta_1$ is weakly expressed in comparison to TGFβ2&3 in both the epithelium and myometrium. $TGF\beta_{2\&3}$ are expressed in the endometrial epithelial cells at higher levels than in the stromal cells. Also, expression of $TGF\beta_2$ is quite prominant in the uterine blood vessels which provides an opportunity to visualize angiogenesis in the cycling reproductive tract. Intense immunolocalization of $TGF\beta_3$ is found associated with the myometrium in comparison to $TGF\beta_{1\&2}$. Analysis of the expression of the TGFβs in the human vagina has revealed distinct localization patterns. Immunodetection of lactoferrin, a marker for neutrophils, is also being used to correlate TGFβ expression with neutrophil traffic patterns during the menstrual cycle. Colocalization studies of TGFβs with other factors such as integrins, extracellular matrix proteins and growth factors may provide insight into the regulation of growth and differentiation of the various uterine cell types by steroid hormones. This study demonstrates that $TGF\beta_{1,2\ \&3}$ are differentially expressed in the uterus and vagina and may function as important mediators of steroid hormone action in the reproductive tract.

R 221 DIFFERENT EFFECTS OF FGF-2 AND FGF-4 IN ISCHEMIC WOUND HEALING.

Liancun Wu, Lily L. Zhao, David Rogers, Thomas A. Mustoe, Division of Plastic Surgery, Northwestern University Medical School, Chicago, IL 60611; Genetics Institute, MA 02140

FGF-2 (bFGF) had been found to induce and promote wound healing in several wound healing models including diabetic wound healing models, but properties of FGF-4 (K-FGF) in wound healing are unknown. FGF-2 and FGF-4 have similar effects in vitro on stimulating fibroblast and endothelial cell proliferation without difference in angiogenesis in vivo. In the rabbit dermal ulcer model and a new incisional model, the effects of FGF-2 and FGF-4 were examined under ischemic and nonischemic conditions (Fig. 1, 2, Table 1). FGF-2 was active under only nonischemic conditions in the dermal ulcer model (Mustoe TA, et al. J Clin Invest 87: 694;1991) in contrast to FGF-4. This suggests they bind differential FGF receptors under hypoxic conditions. This is a novel finding relating the importance of oxygen level to growth factor effects.

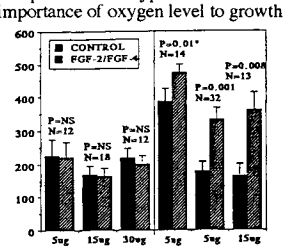

Fig. 1, New Epithelium (μm)

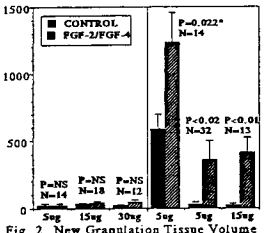

Fig. 2, New Granulation Tissue Volume (μm3x1000)

Ischemic and Nonischemic* Dermal Ulcer Model, Day 7 Postwounding. All data were expressed with Mean±SEM

Table 1, Breaking Strength (N, Day 10) of FGF Treated Incisions

	FGF-2*	FGF-2	FGF-4*	FGF-4
Control	2.63±0.2	1.31±0.183	2.47±0.17	1.23±0.147
FGF	2.84±0.18	1.37±0.133	2.85±0.196	1.56±0.199
N/P value	14/0.46	10/0.83	19/0.024	12/0.046

* Nonischemic wounds, all data were expressed with Mean±SEM

This work was support by NIH grant GM41303-A

R 222 THE ROLE OF HYALURONAN RECEPTOR RHAMM IN CELL LOCOMOTION.

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We have previously shown that hyaluronan (HA) promotes ras transformed cell locomotion. The mechanism by which it does this is unclear, but interactions with an HA receptor, RHAMM (Receptor for Hyaluronic Acid Mediated Motility), mediate its effect on locomotion. RHAMM exists at the cell membrane as determined by FACS analysis and analysis of purified membranes, in the extracellular component and in the cytosol. To begin to study the role of extracellular RHAMM in cell locomotion, we prepared RHAMM fusion protein by expressing RHAMM cDNA with pGEX-2T in E. coli HB101. We then added different concentrations of the GST-RHAMM fusion protein, shown to retain its HA binding ability by transblot assays, to ras transformed C3 and 10T1/2 fibroblast cells and analyzed their locomotion with image analysis. The RHAMM fusion protein, at 60 ng/ml, inhibited cell locomotion and prevented cells from being stimulated by HA. We considered it likely that the fusion protein was acting by binding to HA thereby competitively inhibiting locomotion. To begin to assess this possibility, we added HA binding peptides ($peptide^{aa401-417}$ and $peptide^{aa423-432}$), which mimic the HA binding domains of RHAMM, for their effect on cell locomotion. We found that cell locomotion was reduced 2-fold by addition of these peptides at a concentration of 1ug/ml. We then deleted the HA binding domains in RHAMM cDNA and expressed the modified cDNA as a fusion protein. Its inability to bind to HA was confirmed by a transblot assay. This altered RHAMM fusion protein did not affect C3 fibroblast locomotion. These results indicate that soluble RHAMM can act as a negative regulator of HA-promoted locomotion and that it apparently does so as a result of its ability to bind HA, thereby preventing this glycosaminoglycan from interacting with the cell surface.

R 223 MUCOPOLYSACCHARIDE (MPS) AND COLLAGEN IN ISCHEMIC WOUNDS TREATED WITH HYPERBARIC OXYGEN, PDGF & TGF-β.

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MPS and collagen are major components of the provisional extracellular matrix deposited within healing wounds. We have previously demonstrated (Am J Path 1992;140:1375) that PDGF enhances MPS while TGF-β decreased MPS in healing wounds at 10 days. We have developed a sustained dermal ulcer moldel in the rabbit ear in which healing is delayed due to chronic ischemia. 24 rabbits were used, with one ear serving as control. The PDGF or TGF-β was applied as a single dose at the time of wounding. The 12 animals receiving HBO were placed in a hyperbaric oxygen chamber at a standard dose of 1.5 hours at 2 atmospheres, 100% O_2 5 days/week, with a finding of total reversal of the ischemic healing deficit (Surg Forum 1992;43:711). At 7 days, the animals were sacrificed and the wounds were excised, formalin fixed and embedded in paraffin. Serial 6 μm sections through the center of the wound were stained with H&E, Alcian blue at pH 2.5 (for MPS), and Sirius red (with polarized light for collagen). The amount of MPS and collagen in the wound granulation tissue was estimated by two independent observers, and are described as percentage of the wound granulation tissue area and intensity of staining (0-4+) for MPS and percentage and amount (0-4+) for collagen.

Ischemia treated by:	MPS	Collagen
PDGF alone	47% (2.0+)	9% (0.4+)
PDGF+HBO	72% (2.4+)	15% (0.6+)
TGF-β alone	31% (1.6+)	43% (1.3+)
TGF-β+HBO	33% (1.1+)	54% (1.3+)
None	10% (0.5+)	0% (0+)

PDGF without HBO and TGF-β with or without HBO enhanced MPS in the ischemic wound, while PDGF+HBO further enhances MPS production. In contrast, PDGF produces a minimal increase in wound collagen at 7 days which is not affected by HBO. TGF-β causes an increase in wound collagen which is slightly augmented by HBO. This data suggests that the mechanisms of action of TGF-β and PDGF in promting wound healing in ischemic wounds are distinct. HBO augments the production of extracellular matrix without producing major alterations in the type of matrix induced. Supported in part by NIH grant GM41303A.

Osteoinduction in Hard Tissue Wound Repair; Inductive and Conductive Strategies for Clinical Enhancement of Hard and Soft Tissue Wound Repair

R 300 **EVALUATION OF SUBSTITUTED DEXTRAN (CMDBS) IN RABBIT SPLIT CALVARIAL DONOR SITE BONE REGENERATION.** Daniel Albo, M.D., Anne Meddahi, M.D, Charles C. Long, M.D., Mark Granick, M.D., Nirag Jhala, M.D., Barbara Atkinson, M.D, Jacqueline Josefonwicz, Ph.D., Jean-Pierre Caruelle, Ph.D., Denis Barritault, Ph.D., Mark P. Solomon, M.D.

Objective: Autogenous free outer table bone grafts are commonly used in craniofacial reconstruction. The surgically created defect heals by scarification with a contour deformity. For this reason, split calvarial bone graft can only be harvested once. Therefore, a system for promoting bone regrowth suitable for re-harvesting would be advantageous. This experiment was designed to test the efficacy of bone regrowth in split calvarial bone grafts donor sites in rabbits using a CMDBS (named AMG), which belongs to a family of functionalized biopolymers with CarboxyMethyl, Benzylamide and Sulfonate groups derived on a Dextran polymer (40.000 Dalton).
Methods: New Zealand white rabbits were utilized. Four split calvarial defects were made. Both cephalic sites were used as controls, while right and left posterior sites were treated with collagen and CMDBS respectively. The defects were studied with light microscopy at 3, 4, 8, and 12 weeks.
Results: In this study, the defects treated with CMDBS showed both qualitative and quantitative differences with both the control and collagen groups. Earlier and more complete bone regeneration was obtained in the treatment group, achieving regenerated bone showing the characteristics of mature bone, more rapidly than controls.
Conclusions: This study demonstrated that CMDBS improved bone repair when applied to a surgically created bone defect. This improvement consisted of both an overall acceleration of the healing process and the achievement of a more mature bone which was thicker and bilaminar in structure. The possible role of growth factors and clinical implications are presented.

R 301 **TRANSFORMING GROWTH FACTOR-β1 BOUND TO TRICALCIUM PHOSPHATE ACCELERATES BONE FORMATION WITHIN SKULL DEFECTS .** L. S. Beck[1], Y. Xu[1], L. DeGuzman[1], W. P. Lee[1] T. Nguyen[1] and P. Plouhar[2]. [1]Genentech Inc., South San Francisco, CA 94080, [2] DePuy, Warsaw IN 46581.

Recombinant human transforming growth factor β1 (rhTGF-β1) induces dose dependent formation of bone within calvarial defects in rabbits. Previously reported studies indicate a single application of rhTGF-β1 formulated in a viscous methylcellulose gel was sufficient to initiate the cascade of bone induction resulting in complete defect closure within 28 days (Beck, et al, JBMR 6: 1257). A clinically relevant formulation using tricalcium phosphate (TCP) granules incubated with rhTGF-β1 was evaluated in 12 mm skull defects of rabbits. Radiographs of defect sites after 28 days indicate complete closure of the defect with 14 μg rhTGF-β1 in 150 mg TCP. The new bone formed within defects administered rhTGF-β1/TCP, was characterized histologically as a mixture of immature (woven) and mature (lamellar) bone. This indicates active formation and resorption processes that are natural to bone healing occurred. This was confirmed upon histomorphometric evaluation of the defect sites. The rhTGF-β1/TCP formulation induced significant increases in selected histomorphometric parameters of bone formation including trabecular bone volume, osteoid width, osteoid volume, osteoid surface and osteoblast/osteoid. In addition, total resorption surface, an index of remodeling increased with the rhTGF-β1/TCP formulation. This study continues to support the potential utility of rhTGF-β1 in a more clinically relevant formulation.

R 302 ASCORBATE INDUCES COLLAGEN SYNTHESIS IN HUMAN DERMAL FIBROBLASTS THROUGH A PRO-OXIDANT MECHANISM, Richard A. Berg, Jeffrey C. Geesin, Laura J. Brown, and Joel S. Gordon, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854 and Johnson and Johnson Consumer Products, Inc., Skillman, NJ 08558

Ascorbic acid stimulates collagen synthesis in cultured human dermal fibroblasts by stimulating the rate of transcription of the collagen genes via a mechanism which is independent of the role of ascorbic acid as a cofactor in the hydroxylation of proline. In the present studies we have examined the effect of ascorbate on fibroblasts cultured on plastic culture dishes or on a three dimensional matrix (dermal equivalents) to explore the possible role of ascorbate in wound healing.

We have also examined the ability of ascorbate-induced lipid peroxidation to mediate this process. Ascorbic acid causes lipid peroxidation in fibroblasts at concentrations similar to those which affect collagen synthesis. Inhibitors of ascorbate-induced lipid peroxidation, such as lipophilic antioxidants, iron chelators, singlet oxygen quenchers and molecules which interfere with the role of iron in the production of lipid peroxidation, are capable of inhibiting ascorbate-stimulated collagen synthesis. The effect of ascorbate is shown for collagen protein synthesis or mRNA levels for fibroblasts grown in monolayer cultures or in contracted collagen gels. The time- and concentration-dependence for the effect of ascorbic acid on collagen synthesis are similar in both culture models. Additionally, these studies indicate that aldehyde products of lipid peroxidation, hydroperoxide mimics of such products, and poly-ADP-ribosylation do not play a role in this process. However, ascorbate-induced lipid peroxidation can alter the response of fibroblasts to growth factors, such as TGF-ß. The combination of TGF-ß and ascorbic acid is much more effective in stimulating collagen synthesis than either treatment alone, either in monolayer or contracted collagen gel cultures.

Since ascorbic acid stimulates collagen synthesis through a mechanism which involves a "prooxidant" activity, there apppears to be an oxidant sensitive process in dermal fibroblasts which can be activated, leading to alterations in gene expression including the production of collagen specifically. During the healing process after wounding, neutrophils and macrophages release oxygen radicals. At the wound boundaries, fibroblasts may respond by producing a matrix which serves to wall off the area of destruction from the healthy tissue.

R 303 RECONSTRUCTION OF A HUMAN LIVING SKIN : AN IN VITRO MODEL FOR WOUND HEALING, A SKIN SUBSTITUTE FOR GRAFTING IN HUMANS. Bernard COULOMB, Corinne LEBRETON, Laurence FRITEAU and Louis DUBERTRET. Laboratory of Dermatology, INSERM 312, Henri Mondor Hospital, 94010 Créteil, France.

A simplified skin was the first organ to be reconstructed in vitro (Bell et al., Science, 1981) and was composed, as in vivo, of a dermis and an epidermis. Our laboratory has been developing this model with a view of both grafting in humans and evaluating physiology, pathophysiology and pharmacology of the human skin. This reconstruction is a two steps procedure: the first step provides a dermal equivalent, the second, an overlaying epidermis.

In vitro model for wound healing. Dermal equivalent is the association of the two major components of the dermis in vivo, i.e. fibroblasts and collagen. When dermal fibroblasts are incorporated into a three-dimensional collagen matrix, they reorganize the collagen fibrils and contract the matrix, producing a tissue-like structure. Due to the restoration of the cell-matrix interactions, fibroblasts recover an in vivo-like differentiation (Coulomb et al., J Invest Dermatol, 1983). Particularly, fibroblasts growth and density become regulated. The dermal equivalent permits thus to study fibroblasts homeostasis in a tissue and contractility of fibroblasts (Coulomb et al., J Invest Dermatol, 1984), two important functions that occurs during wound healing.

Skin equivalent: We developed an epidermalization method consisting of placing epidermal biopsies on the dermal equivalent. Keratinocytes grow outwards from the biopsy: an epidermis gradually spread over the dermal equivalent, a situation similar to that which occurs during wound healing, from the edges of the wound. A differentiated epidermis is formed, in addition, epidermal growth can be quantified (Coulomb et al., Br J Dermatol, 1986).

Dermal-epidermal interactions: One of the advantages of this human skin model is the possibility to vary associations between the dermis and the epidermis and thus to evaluate fibroblasts-keratinocytes interactions. We could demonstrate that normal fibroblasts regulate epidermalization (Coulomb et al., J Invest Dermatol, 1989), and are able to modulate the effect of pharmacological agent on epidermal growth (Sanquer et al., J Invest Dermatol, 1990). Psoriasis can be considered as an abnormal epidermal wound healing. With the skin equivalent we could show that psoriatic fibroblasts induce hyperproliferation of normal keratinocytes (Saiag et al., Science, 1985).

Skin substitute: The preliminary clinical evaluation was made on burn patients but also on patients suffering from giant naevi. The most important observation is that the living dermal substitute permits rapid neosynthesis of elastic tissue leading to the reappearance of cutaneous micro-relief and to mechanical properties (e.g. elasticity) closer to those of normal skin than the one obtained with simple epidermal substitutes.

R 304 ENHANCEMENT OF DERMAL REGENERATION BY A NATIVE COLLAGEN SPONGE WITH ELASTIN AND FIBROBLAST CELL SEEDING, Henry J.C. de Vries, Esther Middelkoop, Charles H.R. Wildevuur and Wiete Westerhof. Department of Dermatology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.

In full thickness skin defects little to no dermal regeneration occurs. In stead, these wounds heal with severe wound contraction and scarring. A porous matrix structure can serve as a template for the regeneration of new dermal structures. In a porcine wound model we tested the effect of native collagen type I sponges on dermal replacement. The collagen sponges were linked with extracellular matrix proteins like hyaluronic acid, elastin and fibronectin or left untreated (all n=9). Dermal sponges were also tested in combination with autologous fibroblast cell seeding in a concentration of $10^5/cm^2$ (all n=9). Per pig, 16 3x3cm full thickness wounds were created. The wounds were covered with, the test materials and split skin mesh grafts. A semi permeable wound dressing (Exkin®) protected the wounds from infection and excessive fluid loss. Reference wounds were treated with split skin meshed grafts without dermal substitution or were left open. Wound healing was evaluated on cosmetic aspect, wound contraction, dermal thickness and regeneration of mature collagen fibers (visualised with sirius red staining and polarized light). With pontamin sky blue staining and confocal microscope techniques elastin fiber regeneration was visualised.

Native collagen matrices and fibroblast cell seeding both had a positive effect on dermal regeneration and wound contraction. Compared to the matrix without additions, the hyaluronic acid treated matrix stimulated granulation tissue formation but halted dermal regeneration. Fibronectin induced epidermal abberations. The elastin treated collagen matrix reduced cell proliferation. This resulted in optimal dermal regeneration (as judged by newly synthesized collagen fibers and elastin fibers).

Native collagen fibers, elastin, and fibroblast cell seeding all inhibit the formation of granulation tissue and stimulate collagen fiber formation.

R 305 PYOGENIC GRANULOMA (GP): A MODEL TO STUDY ANGIOGENSIS IN VIVO, Reinhard Gillitzer, Eckhart Kämpgen, Eva-Bettina Bröcker; Department of Dermatology, University of Würzburg, Würzburg, Germany.

GP is a benign vascular lesion which is characterized by intensive proliferation of endothelial cells (EC). This results in budding of small, incompletely canalized vascular chanels. Most notably, GP formation is a self-limiting process. We have investigated the possible growth factors and the corresponding receptors which may trigger GP growth in vivo. Using RNA-RNA in situ hybridization with 35S-labeled probes and immunohistochemistry we studied the expression of PDGF-A, PDGF-B, the alpha- and beta chain of the PDGF-receptor, aFGF, bFGF, TNF-alpha and PD-ECGF in vivo. All lesions investigated exhibited high levels of PDGF-B mRNA and protein as well as the corresponding PDGF-receptor beta-chain exclusively in areas of EC proliferation but not in adjacent tissue. The mRNA expression of all other growth factors was below the detection level. The expression pattern with most signals accumulated around EC of the tumor suggest autocrine stimulation mechanism. As compared to PDGF-B the expression of the PDGF-R beta chain RNA was significantly lower on the mRNA level Therefore, we may conclude that PDGF-B is one of the hallmark cytokines in GP-tumor development. Since the tumor formation is a self-limiting EC proliferation and the regulatory elements controlling the process may in particular influence PDGF-B and PDGF-R beta-chain expression, GP represents an adequate model to study the regulation of these factors with respect to angiogensisis in vivo. We are currently trying to localize and identify various factors (cytokines, oncogenes) which may regulate PDGF-B expression in vivo.

R 306 ELECTRON MICROSCOPIC EXAMINATION OF THE MOUSE DRESSING-INHIBITED WOUND TREATED WITH rTGF-B2 IN A LYOPHILIZED COLLAGEN MATRIX. H. Higley, C. Gerhardt and G. Ksander, Celtrix Pharmaceuticals, Santa Clara, CA. 95054.

Application of a film dressing to a full-thickness dermal wound in the mouse markedly inhibits connective tissue formation and this healing impairment is reversible by treatment of the wound with TGF-B (Ksander et al.,1990). The present study examines the ultrastructure of the cells and matrix of this inhibited wound following treatment with rTGF-B2 in a collagen matrix (CM) and compares the time course of healing to that seen in the untreated wound. Punch biopsy wounds created in the skin of mice were treated with either a single application of 1ug rTGF-B2 in CM or placebo CM, then covered with Opsite® film dressing. Untreated control wounds were covered with Opsite® alone or left air-exposed. Wounds were prepared for light and electron microscopic examination at 3, 7 and 14 days after treatment. Both air-exposed wounds and those treated with rTGF-B2/CM had the most pronounced granulation tissue response. At the electron microscopic level, activated fibroblasts, abundant collagen fibril deposition and normal microvascular endothelial cell morphology were evident. Basement membranes and hemi-desmosomes formed by keratinocytes covering wounds treated with rTGF-B2/CM were also normal. Wounds covered by Opsite® alone or treated with placebo CM showed minimal granulation tissue induction and normal re-epithelialization. These findings suggest that inhibited wounds whose healing is augmented by rTGF-B2/CM treatment retain ultrastructural features consistent with normal dermal repair.

R 307 AUGMENTED INCISIONAL WOUND HEALING INDUCED BY AN INSULIN-LIKE GROWTH FACTOR-I /IGF-BINDING PROTEIN-1 COMPLEX, Robert W. Jyung, Thomas A. Mustoe, Walker H. Busby, and David R. Clemmons, Department of Otolaryngology, Washington University, St. Louis, MO 63110; Department of Plastic Surgery, Northwestern University, Chicago, IL 60611; Department of Medicine, University of North Carolina, Chapel Hill, NC 27599

The role of insulin-like growth factor-I (IGF-I) in cutaneous wound healing is incompletely understood, although the presence of endogenous IGF-I and the vulnerary effect of exogenous IGF-I in healing cutaneous wounds have been previously demonstrated. Furthermore, the influence of IGF-binding protein-1 (IGFBP-1) on IGF-I activity *in vivo* has not been evaluated so far.

When applied to our model of surgical incisional healing in dorsal rat skin, IGF-I alone showed no effect on wound breaking strength (WBS). However, when incisions were treated with a complex of IGF-I/IGFBP-1 in an 11:1 molar ratio, WBS was increased 32% compared to paired incisions treated with IGF-I only ($P < 0.001$). This WBS increase was accompanied by a 67% increase in wound hydroxyproline content, suggesting that the IGF-I/IGFBP-1 combination may have directly influenced type I collagen synthesis. IGFBP-1 alone, an equimolar complex of IGF-I/IGFBP-1, and an 11:1 molar complex of IGF-I with phosphorylated IGFBP-1 all showed no effect on WBS. We conclude that dephosphorylated IGFBP-1 can enhance *in vivo* vulnerary activity of IGF-I when supplied in an optimal molar ratio.

R 308 PULSED ELECTROMAGNETIC FIELDS TO ACCELERATE WOUND HEALING. Stacey Kelpke, Dale Feldman Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL 35294

Introduction

Pressure sores are localized regions of tissue necrosis that occur when soft tissue is compressed between a bony prominence and an external surface for a extend period of time. These sores are found in 20-30% of spinal cord injury patients, 3-10% of nursing home residents, and in 3-11% of acute injuries. Patients with pressure sores incur at least an additional $15,000/year in health care cost with the number rising to over $30,000 for stage IV sores. Conventional treatments of whirlpool and wet to dry dressing changes are not always adequate to prevent complications and to guarantee wound healing and surgery is often required.

One technique being investigated to enhance healing is the employment of pulsed electromagnetic fields (PEMF). Though many studies have examined various types of PEMF and shown benefits, the best frequencies and intensities to use are unknown. The goal of this study was to evaluate the effects of PEMF in an animal model at various frequency and intensity levels in order to prove the validity of the following hypotheses: 1) PEMF can significantly increase healing over controls and 2) both the intensity and frequency of the PEMF affect the healing rate.

Materials and Methods

Sixteen Whites New Zealand rabbits were divided into eight groups of two rabbits each: one control group, and seven treatment groups. All of the rabbits received four 3 cm x 3 cm full thickness defects on the dorsal side. Tracings of each wound was taken and each wound received a Tegraderm dressing. The rabbits in the treatment groups were placed in a chamber and subjected to a PEMF at various combinations of frequencies and intensities for two hours per day for a duration of 2 weeks (Figure 1). The control group was also placed in the same chamber with no PEMF for the same 2 hours per day.

After two weeks the animals were sacrificed, tracings were made of the wounds, and tissue samples were taken. Half of the tissue samples were stained with both trichrome and hematoxylin and eosin. Epithelization and contracture rates (mm/wk) along with volume fractions of neutrophils, macrophages, fibroblast, collagen, and blood vessels were determined. An Instron machine with a cross head speed of 1 in/sec was used for tensile testing. A strip of the newly formed granulation tissue (3 mm x 10 mm) was removed from near the wound center. Balsa wood pieces were glued to the ends to prevent slippage.

A response surface design was used to help determine the optimal frequency and intensity for the PEMF. Each parameter (cell or tissue volume fraction, healing rate, and mechanical strength) was plotted as a contour map where the maximum or minimum points represented optimal values.

Results and Discussion

All groups treated with PEMF were further along in the healing process as evidenced by the amount of tissue formation, type of cellularity, and mechanical strength. Within the PEMF treated groups, lower intensities led to the most fibroblast and newly laid collagen, resulting in the healed wounds with the greatest tensile strength. As frequency levels increased, cellular activity, represented by neutrophils, macrophages, and fibroblast, was increased. It was concluded that PEMF accelerates overall healing as well as decreases contracture. In addition both the frequency and the intensity at which the PEMF is used influences the healing rate. In this study, optimal regenerative healing occurred at the lowest intensity.

Future studies will concentrate on the lower levels or possibly combinations of different treatments at different phases of the healing process.

This research was funced by NSF RII8996152 and NIDRR H133B80012.

R 309 EFFECT OF IGF-I AND IGF BINDING PROTEIN-1 ON WOUND HEALING IN THE RABBIT EAR DERMAL ULCER MODEL.

D. Ladin, L.L. Zhao, R.D. Galiano, J. Cox, T.A. Mustoe, Division of Plastic Surgery, Northwestern University Medical School, Chicago, IL 60611 and Synergen, Boulder, CO

Insulin-like Growth Factors (IGFs) exist in plasma and other biological fluids bound to IGF binding proteins (IGFBPs), which are thought to modulate their actions. The present study tested the effects of IGF-I and/or IGFBP-1 on wound healing in the rabbit ear dermal ulcer model. 20 young adult New Zealand rabbits (3.0-3.5 kg) were used, divided into 5 groups. IGF-I and/or IGFBP-1 (Synergen, Boulder, CO) in varying ratios or control buffer were applied at the time of wounding, and occlusive dressings applied. Wounds were harvested at 7 days for histologic analysis. New granulation tissue formation was assessed according to previously published procedures. Data were analyzed by Student's t-test. IGF-I or IGFBP-1 alone did not have a significant effect on healing compared with controls. The combination of IGF-I with IGFBP-1 showed significantly increased wound healing compared with controls. The enhancement was greatest when the molar ratio of IGF-I to IGFBP-1 was 11:1.

#	Treatment	Comparison	New Granulation
1	Control		
2	IGF	1 versus 2	NS
3	BP	1 versus 3	NS
4	IGF:BP (5:1)	1 versus 4	p<.001
5	IGF:BP (11:1)	1 versus 5	p<.0001

These results indicate that IGF binding protein modulates the effects of IGF in promoting wound healing. Further studies are planned to determine the mechanism by which this occurs.

Supported by NIH Grant GM41303A

R 310 HISTOPATHOLOGY OF CELL MATRIX RESPONSE IN CHRONIC TENDON INJURY, Wayne B. Leadbetter, M.D., Visiting Scientist, Naval Medical Research Institute, Wound Repair Enhancement Division, Bethesda, Maryland; Clinical Assistant Professor of Orthopaedic Surgery, Georgetown University, Washington, D.C.

A study of tendon biopsy specimens in a series of 26 athletes with overuse tendon injury was performed to document the observed pathology and connective tissue response. Tendon biopsy specimens were obtained in a series of 25 adult athletes requiring surgery for failed non-operative care. The surgical indication was refractory pain and loss of performance; all were treated before clinical rupture. H+E, Alcian blue stains, were analyzed for cell type, cellularity, collagen fiber patterns, vascular neogenesis, heterotopic calcification and matrix degenerative change. Electron microscopy was done on selected cases. All specimens displayed varying degrees of the following: 1) tenocyte hyperplasia; 2) blast-like change in morphology from normal tenocyte appearance; 3) prominent small vessel in-growth with accompanying mesenchymal cell populations; 4) perivascular collections of histiocytic or macrophage-like cells; 5) collagen fiber disorganization or degenerative change (fatty, hyaline, mucoid); 6) micro-tears with rounding collagen fiber separations; 7) endothelial hyperplasia. Lymphocytic cells were isolated to the synovium, peritendon structures, surrounding areas of intertendinous calcific deposit, or at sites of previous steroid injection. EM revealed cellular stress responses consistent with hypoxic injury (swollen microchrondia) associated with aging changes, lipid deposition, nuclear chromatin change, rapid deposition of collagen with variable fiber size as well as areas of increased protein synthesis with pronounced rough ER. Paradoxically reparative cells were prominent in some location implying a detective or modulated synthetic function. Lymphocytic and polymorphonuclear cell types were notable low in number. All findings will be illustrated. The pathology of sports-induced overuse tendon injury is characterized by persistence, degenerative change and failed reparative response. Inflammatory response is manifested singularly in the synovial sheath, and after tendon rupture with vascular disruption. Based upon this observed pathology pattern, current anti-inflammatory therapy and modalities may be ineffective methods to modulate cell-matrix repair response. Findings in this series are consistent with previous reports and the observations. The tendon lesions reflect an adaptive cell-matrix stress response to load or overuse. Based upon these observations, a theoretical model is proposed for the pathogenesis of sports-induced tendinosis (1.e. degeneration) as well as the therapeutic role for cell-matrix modifiers such as growth factors.

R 311 THE GLYCYL-HISTIDYL-LYSYL-COPPER COMPLEX STIMULATES COLLAGEN GENE EXPRESSION IN EXPERIMENTAL WOUNDS. François-X. Maquart, Georges Bellon, Brahim Chaqour, François Chastang, P. Birembaut*, Leonard M. Patt**, Ronald E. Trachy**, Philippe Gillery, Jacques P. Borel. *Lab. Biochemistry, CNRS ERS F0017, (*) INSERM U 314, Faculté de Médecine, 51095 Reims Cedex, France and (**) Procyte Corporation, Kirkland, WA 98034-6900.*

The tripeptide-copper complex glycyl-L-histidyl-L-lysine-Cu^{++} (GHK-Cu) was first described as a growth factor for differentiated cells. *In vitro* data showed that it possesses several properties of a potential activator of wound repair. We investigated the effects of GHK-Cu on collagen gene expression *in vivo*, using the wound chamber model described by Schilling et al (Schilling, J.A. et al, *Surgery*, 1959, *46*, 702).

Two wound chambers per animal were inserted subcutaneously on day 0 on the back of 10 rats. A first series of 5 rats (controls) received in both wound chambers the injection of 0.2 ml Dulbecco's Phosphate Buffered Saline (DPBS) on day 2, then every 3 days until day 17. The other series received on the same days 2.0 mg GHK-Cu dissolved in the same volume of DPBS. A control and a GHK-Cu-treated rat were sacrificed on day 3, 7, 10, 14, 21. Both chambers were collected and immediately frozen in liquid nitrogen. One chamber was used for histological examination and the other for RNA extraction and Northern-blot and slot-blot analysis using collagen pro α1(I) (gift of Pr E. Vuorio, Turku, Finland), rat Transforming Growth Factor-ß1 (gift of Pr M. Sporn, Bethesda, MD) and glyceraldehyde-3-phosphate-dehydrogenase (gift of Pr P. Fort, Montpellier, France) specific cDNA probes.

Histological examination of the GHK-Cu-injected chambers showed an intense inflammatory cellular infiltrate and the rapid appearance of large, dense and well organized fibrosis areas. These phenomenons were clearly delayed and less intense in control chambers. Messenger RNA analysis showed a large increase of collagen gene expression in the GHK-Cu-treated versus control chambers, from day 3 to day 14. On the other hand, TGF-ß1 mRNA was not increased.

These results demonstrate that GHK-Cu is able to increase collagen gene expression in wounds and that its effects are not mediated by an increased expression of TGF-ß1.

R 312 A PREOPERATIVE PROTEIN SPARING MODIFIED FAST (PSMF) DOES NOT DECREASE COLLAGEN ACCUMULATION POSTOPERATIVELY FOR OBESE PATIENTS,
L.F. Martin, LSU Department of Surgery, New Orleans, LA 70112
Preoperative weight loss is often suggested as a means of reducing operative risk in obese patients requiring laparotomy. Evidence to support this contention is not extensive, and recommendations regarding the extent to which weight loss can be pursued preoperatively are not available. To determine if weight loss would reduce postoperative complications without adversely affecting wound healing, we asked 100 consecutive severely obese patients requesting gastric bypass to diet by consuming a 420 kcal, 70 gm/day liquid diet for at least one month before surgery. To measure accumulation of wound collagen deposition some of the "dieters" had a 10cm piece of PTFE tubing implanted subcutaneously at surgery. The tubing was removed 7 days postoperatively to measure hydroxyproline content of the granulation tissue. Dieters were compared to similarly obese "non-dieters" and other obese patients who were significantly lighter to determine how size and weight loss affected wound healing in the obese patients.

Results	Dieters (N=17)	Non-Dieters (N=18)	Liteweights (N=12)
% Ideal Body wt.	224 ± 13	237 ± 9	185 ± 18
Preop wt. Loss (kg)	20 ± 12	None	None
Collagen mg/cm	4.9 ± 2.8	3.6 ± 1.0	5.5 ± 2.7

The 47 dieters who lost 17 ± 10 kg. (x ± S.D.) had significantly fewer postoperative pulmonary and thrombotic complications than 53 similar weight non-dieters.
Conclusions: Severely obese patients can use a PSFM for one month preoperatively without adversely affecting collagen production or wound healing. This approach decreases pulmonary and thrombotic complications by improving antithrombic III levels and by increasing lung volumes.

R 313 ACIDIC FIBROBLAST GROWTH FACTOR ACCELERATES WOUND REPAIR IN THE HEALING IMPAIRED GENETICALLY DIABETIC MOUSE,
Theodore N. Mellin, Doreen E. Cashen, John J. Ronan, Robert J. Mennie, and Kenneth A. Thomas, Department of Growth Factor Research, Merck Research Laboratories, Rahway, NJ 07065
Acidic fibroblast growth factor (aFGF) is a potent mitogen *in vitro* for many cells of ectodermal and mesodermal origin including skin-derived epidermal keratinocytes, dermal fibroblasts and vascular endothelial cells. We have previously shown that topically applied aFGF promotes healing of full-thickness wounds in normal rodents (Mellin, *et al.* Growth Factors, **7**, 1-14. 1992). In this study full-thickness mid-dorsal circular excisional wounds (2.0 cm^2) were made in 11 week old female diabetic C57BL/KsJ-db/db mice (N=20/group) and covered with transparent occlusive dressings. Wound area was measured twice weekly. Recombinant human aFGF (3.0 $\mu g/cm^2$), applied topically immediately after wounding and on days 3 and 7 post-injury, resulted in significant ($P<0.01$) wound closure (expressed as percent of initial wound area) as early as day 7 post-injury, with a 41% improvement seen at day 14 ($P<0.01$) compared to vehicle-treated controls. Treatment with aFGF decreased the time to achieve 50, 70 and 90% area closure by 6.4, 11.5 and 19.5 days, respectively ($P<0.01$). aFGF treatment also decreased the mean time to complete closure of 50, 70 and 90% of the wounds by 25.7, 29.7 and 31.5 days, respectively. Epidermal closure was confirmed histologically and no morphological differences were seen between aFGF- and placebo-treated wounds.

R 314 ENHANCED HEALING OF DIABETIC ULCERS BY TOPICAL TREATMENT WITH PC1020 [IAMIN®],
Gerit Mulder, Wound Healing Institute, Aurora CO, Lee Sanders, VAMC. Lebanon, PA, Julio Rosenstock, Diabetes and Endocrine Center, Dallas, TX, Morton Altman, VAMC, Albuquerque, NM, Leonard M. Patt, Marie Hanley, Ronald Warner, and Gordon Duncan, ProCyte Corporation, Kirkland Wa.

A randomized, vehicle-controlled, evaluator-blinded clinical study of the safety and effectiveness of PC1020 (Iamin®) in the healing of lower extremity ulcers in diabetic patients was completed. In addition to PC1020 or vehicle treatment, all patients were enrolled into an aggressive wound treatment protocol consisting of daily dressing changes, initial sharp debridement, standardized pressure relieving footwear, and patient education. Safety and wound healing evaluations were obtained at weekly intervals throughout the study duration.
Analysis of the safety data indicated no significant difference in overall adverse event rates between the Vehicle and the 2% PC1020 groups. The incidence of target ulcer adverse events and infections was significantly lower in the plantar ulcers treated with 2% PC1020. Treatment with 2% PC1020 initiated at the time a patient was enrolled in the aggressive wound care program also significantly increased the healing of plantar ulcers in these diabetic patients relative to vehicle treatment. PC1020 increased the mean percent healed at the end of the study to 73.0 ± 10.2% (P=.015, n=27) compared to 10.3 ± 21.1% (n=32) for the vehicle treated group. The enhancement of healing by 2% PC1020 treatment was greater in the larger plantar ulcers (greater than 100 mm^2 surface area at entry to the program) due to the failure of this size of ulcer to respond adequately to conventional wound treatments. Treatment with 2% PC1020 increased the mean percent healed of this group to 72.2 ± 10.4% (P=.002, n=14) compared to an average exacerbation of -13.4 ± 20.8% (n=16) of the ulcer in the vehicle control group.
We acknowledge the nurses, clinical investigators, and the members of the ProCyte wound healing development team who contributed to the success of this study.

R 315 THROMBOSPONDIN ACTIVATES LATENT TGF-ß SECRETED BY ENDOTHELIAL CELLS,
Joanne E. Murphy-Ullrich and Stacey Schultz-Cherry, Department of Molecular and Cellular Pathology, University of Alabama at Birmingham, Birmingham, AL 35294

Factors which regulate the conversion of latent to active TGF-ß are keys to controlling TGF-ß activity. The platelet and extracellular matrix protein, thrombospondin 1 (TSP), forms specific complexes with active transforming growth factor-ß1 (TGF-ß) in platelet releasates. TSP-TGF-ß complexes inhibited bovine aortic endothelial (BAE) cell growth: growth inhibition was partially due to TGF-ß. However, BAE growth inhibition by TSP stripped of TGF-ß activity (sTSP) was also sensitive to antibodies to TGF-ß. Thus, we investigated whether sTSP activates latent TGF-ß secreted by BAE cells. BAE cells were cultured with sTSP and the conditioned media (CM) were tested for the ability to support NRK-49F cell colony formation in soft agar, a measure of TGF-ß activity. sTSP-CM showed a dose and time-dependent ability to stimulate colony formation. A maximal response was observed with ~0.3 ug/ml (0.7 nM) sTSP. Stimulation of TGF-ß activity occurred as early as 2hr after addition of sTSP to BAE CM and persisted for at least 48 hr. Activation was serum-independent. Colony formation was blocked by antibody to TGF-ß and was EGF-dependent. TGF-ß activity was not observed in SPARC, tenascin, fibronectin, laminin or BSA-treated CM. sTSP-BAE cell interactions are not necessary for stimulation of TGF-ß activity, since incubation of sTSP with CM removed from cells stimulated colony formation. sTSP also stimulated TGF-ß activity in MvlLu and CHO CM. The 50 kDa chymotryptic fragment of sTSP, which contains the procollagen-like homology and the type I repeats, binds TGF-ß and contains the site on sTSP which stimulates TGF-ß activity. Although the mechanism of TGF-ß activation by sTSP is unknown, plasmin does not appear to play a role since no plasmin activity was detected in sTSP and serine-proteases did not inhibit activation. However, cysteine protease inhibitors completely blocked sTSP-stimulated TGF-ß activity. Our data suggest that sTSP may be a major physiologic regulator of TGF-ß activity. TSP peptides could potentially be used to therapeutically amplify TGF-ß activity.

R 316 FIBRIN SEALANT MATRIX, A CLINICALLY RELEVANT VEHICLE FOR DELIVERY OF THERAPEUTICS THAT ENHANCE WOUND HEALING: PROLONGED RELEASE OF EFFECTIVE CONCENTRATIONS OF ANTIBIOTICS, Hernan A. Nunez, Roger Hennigh, Anthony Campagna, Shannan Harding, William N. Drohan and Martin MacPhee, Holland Laboratories, American Red Cross, Rockville, MD 20855.

Fibrin sealant matrix (FS) is a gel similar to a natural clot formed by mixing concentrates of virally-inactivated fibrinogen and thrombin solutions. FS has been used mostly as a hemostatic agent, but it also has potential as a biodegradable delivery system for both drugs and biologics in humans. In this latter context, antibiotic-containing FS (AB-FS) has been shown to be useful for combating localized infections in several clinical settings. The relatively short AB release period, however, limits its usefulness. We found that the inclusion of certain AB in FS increases the stability of FS, a necessary prerequisite for achieving sustained drug release. Disks of FS were degraded in 1 to 2 weeks *in vitro*. FS stabilization was achieved by allowing diffusion of TET or CIP solutions into the FS. This stabilization was also accomplished by incorporation of relatively insoluble forms of these AB into the matrix. To achieve sustained release, highly insoluble forms of tetracycline (TET) or ciprofloxacine (CIP) were incorporated into FS disks. The 2.5x6mm disks were placed in 2ml of buffer which was changed either daily or continuously at a rate of 3.5ml/day. Release of TET, formulated at concentrations of 2 to 25mg/ml of FS, lasted 5 days. At concentrations of 50 and 100mg/ml this period was increased to 3 and 4 weeks, respectively. CIP, at 100mg/ml, was released for a period of 5 to 6 weeks. The concentrations of released TET and CIP reached saturation levels during the first part of the release period, then plateaued and finally decreased gradually, possibly as a function of AB solubility and diffusion through the FS. At plateau level the AB concentrations ranged from approximately 10 to 100ug/ml. The released AB are biologically active when tested for bacterial growth inhibition on agar plates. *In vivo* studies involving both drugs and biologics targeted to applications in skin wound repair, periodontal disease, prosthetic implants and bone repair, are underway.

R 317 POSSIBLE MECHANISMS FOR THE PROMOTION OF WOUND HEALING BY LOW INTENSITY LASER IRRADIATION

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Low Intensity Laser Irradiation (LILI) is a novel clinical modality for the treatment of superficial wounds. Although there has been a considerable increase in the amount of research into LILI stimulated wound healing, the basic biological mechanisms remain unclear. Cells of the haemopoeitic system are involved in the complex tissue repair process; the purpose of the present investigation was to examine the putative photobiomodulation of two haemopoeitic cell lines, HL-60 and U937, following irradiation at 660nm.

Initial experiments have looked at the effect of directly irradiating both cell suspensions. Cells were suspended at a concentration of $1x10^6$ cells/ml in serum free medium. Irradiation was with a GaAlAs laser (660nm, 12mW, 5kHz) at energy densities ranging from 1.0 to 11.5J/cm^2. Twenty four hours after irradiation the cells were assayed for their ability to incorporate tritiated thymidine during a three to four hour pulse. Some energy densities greater than 5.8 J/cm^2 produced a significant decrease in thymidine uptake, into both cell lines, when compared to control values ($p<0.05$, one way factorial ANOVA test). Further experiments are examining the effect of conditioned media from these two cell lines on non-irradiated cells of the same type, and also the possibility that the irradiated cells are differentiating to more mature stages.

It is hoped that these results will contribute important information to our knowledge of the mechanisms of action of low intensity lasers and thus lead to the safe and effective use of this modality for wound healing and other clinical applications.

R 318 Embryonic collagen is ideally suited as implantable materials. Mikio Ohno, Takao Nagasawa . University of Medicine and Dentistry of N.J., Dept.of athology Piscataway, New Jersey, 08854

Possible criteria defining an ideal soft tissue implant are; 1) chemical, physical and mechanical properties similar to the original tissue, 2) the implant should promote cell attachment, migration and growth, 3) the implant should not trigger an adverse response, 4) the implant should be replaced by normal host tissue in time, and finally, 5) the production cost should be reasonable. Bovine collagen, from calf/adult skin, have been used widely as biomaterials but there have been problems and limitations. Recently we have isolated native collagen molecules from the skin of bovine embryos. Preliminary data obtained from the investigation of this material indicate that this embryonic collagen(EC) may satisfy the major part of all the criteria mentioned above. These ideal properties may make embryonic collagen based materials very useful.The embryonic collagen fiber is 3 ~ 4 times stronger than rat tail tendon fiber under wet conditions and in the dry state, comparable to metal wire. Fibroblasts spread well onto the surface of the EC fiber. EC fibers were stable up to 4 weeks. After EC fibers resorbed there was no visible scar formation. Embryonic tissue has distinct advantage over mature tissue as a collagen source. Monomeric collagen is easily extracted due to the lack of lysin oxydase cross-linking. X-ray diffraction studies indicate a high degree of crystallinity for the EC fibers, which may explain the unusual mechanical properties. The reason for the lack of tissue rejection associated with the EC is unknown.

R 319 TOPICAL GROWTH HORMONE EVERY THIRD DAY INCREASES FORMATION OF GRANULATION TISSUE IN THE RAT, Steenfos H., Garbasch C. and Rasmussen L., Department of Plastic Surgery B, Gentofte University Hospital, DK-2900 Hellerup, and Medical Anatomic Institute A, Panum Institute DK-2200 Copenhagen, Denmark

Daily application of growth hormone (GH) increased formation of granulation tissue by 50%, and human studies have shown that topical GH increased healing in chronic wounds. This study was undertaken to study whether a less frequent application of GH was able to increase formation of granulation tissue, and to study the amount and concentration of hydroxyproline.

Methods. Sprague Dawley rats had four wound cylinders implanted subcutaneously on the dorsum. Every third day the cylinders were injected with either placebo, 0.05 units GH, or 0.2 units GH. After 16 days the granulation tissue was analyzed for wet weight (WW), dry weight (DW), hydroxyproline (HX), and concentration of hydroxyproline (HX conc.).

Results.

	Placebo	0.05 U GH	0.2 U GH
WW mg	114±9	139±8	155±14*
DW mg	13.2±1.0	16.5±1.2	19.2±1.0**
HX ug	282±91	302±113	407±115***
HX conc. ug/mg DW	21	18	21****

* = $p<0.01$ ** = $p<0.01$ *** = $p<0.012$ **** = NS

Discussion. This study shows that less frequent application of topical GH increases formation of granulation tissue. Also the total amount of hydroxyproline is increased, while the concentration is the same as placebo indicating that the granulation tissue is qualitatively similar, but quantitatively increased in the GH cylinders. GH is not an ordinary growth factor, but may prove to be useful in wound healing.

R 320 **MECHANISMS OF REPAIR OF CRITICAL SIZE DEFECTS BY TRANSPLANTATION OF OSTEOGENIC CELLS IN COLLAGEN GELS**, Thomas M. Sweeney, Lynne A. Opperman, John A. Persing, and Roy C. Ogle, Depts. of Orthopedics and Plastic Surgery, University of Virginia Medical Center. Charlottesville, VA. 22908.

Bone grafting is limited by the quantity of autograft or increased risk of rejection, donor site morbidity, and infection when allografts are used. As an alternative, gels of extracellular matrix proteins were used alone and with transplanted osteogenic cells to repair critical size defects (CSD) in rat calvariae. Gels of reconstituted basement membrane (RBM), laminin, and type I collagen were implanted into circular CSD's in the parietal bones of 6 month old Sprague-Dawley rats (5/group). Bone repair was evaluated using three dimensional reconstruction of computed tomographic scans, gross examination and histology. Control animals showed a mean of 7.0% (±4.5% SD) healing of defects 3 months following craniectomy. In groups without transplanted cells, the greatest repair at three months occurred in animals which received type I collagen gels (87.5% ± 4.7 SD). This was significantly greater than using RMB or laminin gels (55.5%± 8.8 and 46.3%± 13 respectively, $p<0.01$). Most of the repair induced by type I collagen occurred in the first 4 weeks (61.3% ±6.3) and by 5 months, 92.5%±10.6 healing of defects was observed. Transplantation of neonatal calvarial osteoblasts within type I collagen gels significantly increased the rate of repair of CSD's from 61.3%±6.3 to 81.5%±10.9 at 1 month, and 87.5%±4.7 to 94.2%±4.2 three months after gel implantation. Transplantation of neonatal rat skin fibroblasts significantly inhibited repair. Histology revealed that control CSD's healed by fibrous connective tissue formation. CSD's in animals treated with type I collagen gels showed repair by new bone growth into the defect on gross inspection and histologically. Structures consistent with sutures were frequently observed. Alcian blue staining and immunohistochemistry using antibodies directed at collagen types II and X did not demonstrate evidence of endochondral ossification, suggesting an intramembranous mechanism of repair.

R 321 RAPID ACCELERATION OF WOUND HEALING IN YOUNG AND OLD RATS INDUCED BY APPLICATION OF A COMPLEX CARBOHYDRATE, Ian R. Tizard[1], David Busbee[1], Maurice C. Kemp[2], [1]Texas A&M University, College Station, TX, 77843, [2]Carrington Laboratories, Inc., College Station, TX, 77845.

The complex carbohydrate acemannan, a polydispersed β-(1,4)-linked acetylated mannan, has been shown to be a potent activator of macrophage function. Thus it causes a significant increase in the production of IL-1, IL-6 and TNF-α by mouse peritoneal macrophages. Given the known ability of these cytokines to affect the course of normal wound healing, it was postulated that local infiltration of healing second intention wounds with acemannan could affect the rate of wound healing. 30 μg acemannan was therefore applied to healing 6mm punch biopsy wounds in 3 month-old F344xBN rats. The acemannan solution was dropped into the raw wound and subsequently injected under the eschar three times weekly. This treatment resulted in an increase in the rate of healing that was statistically significant by three days following wounding. The healing $T_{1/2}$ dropped from 8 to 5 days and time to complete closure dropped from 17 to 13 days. When the same material was applied to biopsy wounds in 24 month-old calorie deprived F344xBN rats a similar acceleration was noted with the healing $T_{1/2}$ dropping from 9.5 to 5.5 days and time to complete closure dropping from 20 to 13 days. This acceleration also occurred in aged rats fed ad libitum. The treatment thus reduced wound healing time in aged rats to that of treated young rats. The rapidity of the acceleration within the first three days suggests that wound contraction was significantly enhanced. Histological examination of treated wounds confirmed this difference in the rate of healing between treated and untreated animals. The effect was specific for this strain of rats in that acemannan had no effect on the rate of healing of biopsy wounds in Sprague-Dawley rats. This suggests that the wound healing response to acemannan was genetically determined.

R 322 COLLAGEN-BASED DELIVERY VEHICLES FOR GROWTH FACTORS: THEIR POTENTIAL USE IN WOUND HEALING. Sandra L. Watt, David Buck, and J. Peter Bentley, Dept. of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201

It has recently been recognized that a number of growth factors (FGF, EGF, PDGF, TGF-β) can influence the rate of wound healing in soft tissues such as the skin. A major drawback, however, has been the lack of a slow release delivery vehicle so that the effect of a continuous presence of the growth factor on wound healing can be studied. We present here two potential slow-release delivery vehicles for these growth factors based on pepsin-digested type I bovine collagen. The first is based on iodine- gelled collagen, which is mixed and poured directly onto a wound where it rapidly gels, filling in and adhering to the wound. This material is useful for the treatment of dermal wounds. The second is based on DOPA crosslinked collagen, which can be molded to the shape of various defects and may prove useful in the repair of cartilage and bone. We show here that these delivery vehicles are biologically inert and can be readily shaped to conform to the wound. When fibroblast growth factor (FGF) is incorporated into these collagen-based gels, about 20% of the growth factor is released over 30 minutes, and the rest acts as a very slow release reservoir for the growth factor. The rate of gelling or of release of growth factor is unaffected by the further addition of heparin to these gels. Using animal and human models we demonstrate that DOPA crosslinked and iodine collagen gels alone do not adversely affect wound healing and that when growth factors such as PDGF or FGF are incorporated into these gels, they stimulate cellular infiltration and thus potentiate wound healing. Wound healing models used include a pig skin wound model, rabbit ear cartilage replacement, a subcutaneous disc implant in rats, and human donor site wounds.

In conclusion, we show in this report that DOPA crosslinked and iodine- gelled bovine collagen type I can be used as an inert, slow-release delivery vehicle for growth factors. This system can be used to study the process of wound healing, and has obvious potential in stimulating wound healing in humans.

R 323 **MUSCLE MORPHOGENETIC PROTEIN INDUCES SKELETAL MUSCLE REGENERATION**, Henry E. Young, Paul A. Lucas, and Cato T. Laurencin, Division of Basic Medical Science and Department of Surgery, Mercer University School of Medicine, Macon, GA 31207; Department of Surgery, Medical Center of Central Georgia, Macon, GA 31206; Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139; and Department of Orthopaedic Surgery, Massachusetts General Hospital Harvard Medical School, Boston, MA 02115

Recent studies have revealed that bone, traditionally viewed as providing structural support and calcium homeostasis, is also a repository for factors that influence cellular proliferation, chemotaxis, angiogenesis, chondrogenesis and osteogenesis. Water soluble proteins (WSP) extracted from bovine bone and bovine serum albumin (BSA) were examined in vitro with pluripotent mesenchymal stem cells and implanted in vivo in adult wound repair models to ascertain the existence of additional differentiative-inducing activities. After six days, all cultures incubated with BSA demonstrated confluent mononucleated cells, fusion indices less than 1% and a myosin content of 1.5 ng Myosin/μg DNA. Treatment with WSP elicited confluent linear and branched multinucleated cells demonstrating spontaneous contractility, fusion indices of 50-90% and myosin contents from 30-60 ng Myosin/μg DNA. After 14 days in situ, implantation of BSA elicited a connective tissue scar. Implantation of WSP in situ elicited a range of responses from myotubes embedded within a connective tissue scar to a muscle bundle with an intact neurovascular triad surrounded by a thin connective tissue scar. The results reveal a novel factor capable of inducing myogenic lineage-commitment in vitro and stimulating skeletal muscle regeneration in vivo.

Supported by Rubye Ryle Smith Charitable Trust and the Medical Center of Central Georgia.

Epidermal Regeneration in Soft Tissue Wound Repair

R 400 HEALING RATES OF CORNEAL WOUNDS IN THE PRESENCE OF AN RGD-CONTAINING PEPTIDE CONJUGATE. **Janet A. Anderson[1], Nancy J. Sipes[2], Perry S. Binder[1], [1]Ophthalmology Research Laboratory, Sharp Cabrillo Hospital, San Diego, CA 92110 and [2]Telios Pharmaceuticals, Inc., San Diego, CA 92121**

Re-epithelialization following corneal epithelial abrasion in human, organ-cultured corneas was found to be more rapid when the wounded corneas were treated with an RGD-containing peptide conjugate, $p<0.02$. The stimulation of healing rates was lost when epidermal growth factor (EGF) and insulin were removed from the serum-free culture medium, although the concentrations of EGF (10 ng/ml) and insulin (5 μg/ml) present in the medium were insufficient to enhance wound healing rates in the absence of the peptide conjugate. Immunohistochemical studies after complete re-epithelialization (from 2 to 3 days of healing) showed that EGF/insulin enhanced the concentrations of the extracellular matrix (ECM) components fibronectin, type IV and type VII collagens in the wounded area. The synthesis of beta 4 integrin, a component of the hemidesmosomes of the corneal epithelium, appeared to be independent of the presence of the growth factors. Using these methods no effect of the RGD-peptide conjugate on ECM components was observed at this early time after wounding.

Corneas cultured for one week in the EGF/insulin serum-free medium before wounding showed greatly reduced wound healing rates when compared to corneas wounded immediately after extraction. The addition of the RGD-containing peptide conjugate reversed this loss of healing capability and returned the rates to those observed earlier, $p<0.001$. Removal of vitamin A from the culture medium further reduced the healing rates, $p<0.01$.

Since EGF and vitamin A are found in the normal tear film, these data suggest that a reduced tear flow, such as that found in some types of keratoconjunctivitis sicca, could have an effect on the rate of corneal epithelial wound healing. The data further indicate that RGD-containing ECM molecules work synergistically with growth factors to enhance rates of corneal re-epithelialization.

R 401 CYTOKINES AND GROWTH FACTORS REGULATE KERATIN GENE EXPRESSION IN WOUND HEALING AND OTHER PATHOLOGIC PROCESSES OF THE EPIDERMIS, Miki Blumenberg, Chun-Kui Jiang and Irwin M. Freedberg, Ronald O. Perelman Department of Dermatology, NYU Medical Center, New York, NY 10016

In healthy epidermis, basal keratinocytes are attached to the basement membrane and express keratins K#5 and K#14. As they detach from the substratum, they start differentiating and switch to expressing keratins K#1 and K#10. But in various pathological processes, such as would healing, keratinocytes become activated. They produce and respond to many growth factors and cytokines, and become migratory. Activated keratinocytes produce keratins K#6 and K#16.

Reasoning that the signaling by the extracellular factors regulates keratin gene expression, we have examined this regulation at the transcriptional level. DNA constructs in which the upstream regions of keratin K#5, K#6, K#10, K#14 and K#16 genes drive expression of the CAT reporter were transfected into keratinocytes that were then cultured in the presence or absence of various growth factors. We found that EGF and TGFα have a pronounced and specific effect: they induce transcription of K#6 and K#16, the keratins of the activated keratinocytes. Both factors have defined concentration optima. By deletion analysis and site -directed mutagenesis, we have defined the EGF responsive element in the K#16 promoter. A nuclear protein binds to this element; the analogous sequence from the K#6 gene competes for the binding, but not the consensus sequences for the AP1, AP2, AP3, Sp1, NF1, RAR or TBP transcription factors.

Whereas EGF and TGFα promote growth of keratinocytes, TGFβ inhibits them. The effect is reversible, and the differentiation markers are not induced. TGFβ induces expression of K#5 and K#14 keratin genes. Other genes tested were unaffected. Deletion analysis identified the putative TGFβ responsive elements. Apparently, the effect of TGFβ is to promote a basal cell-like phenotype. Basal keratinocytes divide much more slowly than the activated ones, which may be the mechanism of inhibition by TGFβ. Our results mean that the extracellular signaling molecules, growth factors and cytokines, in addition to regulating cell growth, directly regulate relevant genes, such as keratin genes.

R 402 IMMUNOHISTOCHEMICAL LOCALIZATION OF TGF-B1,2,3 IN OVINE WOUND REPAIR, Rafael C. Cabrera[1], Jon Canete[2], John W. Siebert[1], Michael T. Longaker[1], and Leslie I. Gold[2], [1]Institute of Reconstructive Plastic Surgery and [2]Department of Pathology, New York University Medical Center, New York, NY 10016

Transforming Growth Factor-Beta (TGF-B) influences wound repair as a chemoattractant, an angiogenic agent, and a modulator of cell surface integrin expression. TGF-B1 stimulates collagen, fibronectin, and glycosaminoglycan production. TGF-B enhanced the epithelial covering of exposed dermis and increased tensile strength in incisional wounds. In mammals, three isoforms, TGF-B1,2,3, are highly conserved across species. Characterization of promoters for the human TGF-B1,2,3 genes, provides a mechanism for differential spatial and temporal expression, suggesting unique biological activitivities. The present study investigates the localization of TGF-B isoforms throughout tissue repair of incisional and excisional wounds of adult sheep. Using isoform specific antibodies for TGF-B's, we examined excisional wounds at 1,2,5,7,10,14, and 21 days post wounding.

Immunostaining of unwounded sheep skin revealed TGF-B1 to be concentrated in the stratum corneum of the epidermis with only faint staining in the deeper layers. TGF-B2 was found to stain moderately in the strata granulosum, spinosum, and basalis but was absent in the most superficial epidermis. Similarly, TGF-B3 was not found in the stratum corneum but stained strongly in the deeper layers of epidermis. TGF-B3 also stained strongly in endothelial cells, sebaceous and merocrine sweat glands, and outer root sheath of hair follicles. This pattern persisted through day 14 in the epidermis adjacent to the excisional wounds but with more intense staining. Interestingly, the migrating epithelium showed no staining of any isoform until complete reepithelialization by day 7. The inflammatory exudate over the granulation tissue of the excisional wounds revealed a dense band of neutrophils and macrophages which stained faintly for all three isoforms, while the cells at the exudate-wound interface exhibited intense staining for all three isoforms with TGF-B2,3>TGF-B1. Inflammatory exudate separated from underlying granulation tissue by migrating epithelium was devoid of staining. The Dermis showed increased immunostaining of all isoforms but was most intense along the wound margins with TGF-B,2,3>TGF-B1. At 21 days staining for the three isoforms was similar to that of unwounded skin. The differential expression of the TGF-B isoforms mirrors the dynamic process of wound repair.

R 403 THE EFFECTS OF EXTERNAL ENVIRONMENTAL COMPONENTS ON THE GROWTH OF NORMAL HUMAN DERMAL FIBROBLASTS AND EPIDERMAL KERATINOCYTES IN CHEMICALLY DEFINED SERUM-FREE MEDIUM, Daley, J.P. and Donovan, J.M., Biomedical Research and Development, Life Technologies Inc., Grand Island New York, 14072

The establishment of pure populations of normal human primary cells such as dermal fibroblasts and epidermal keratinocytes is dependent upon the liquid environment in which the cells are cultured. In the absence of serum, it is usually necessary to supplement this external environment, or basal medium, with specific cytokines and/or attachment factors. The growth of a single tissue specific cell type, from a usually heterogeneous population of primary tissue cells, is dependent upon establishing the proper external environment for the cells desired. We have investigated the role of selected cytokines and attachment factors on the growth of normal human dermal fibroblasts and epidermal keratinocytes in defined basal tissue culture media. Using standard growth assays, we have studied the affect of these external components on cell cycle times in both primary and later passage cells. Using fluorescence activated cell sorting, we have studied the relative proportion of specific cells within our primary cultures and later passages to determine both the purity of our "final cell population" and heterogeneity of our initial primary tissue. Since both human dermal fibroblasts and epidermal keratinocytes require attachment for growth in vitro, we have studied the ability of our later passage cells to grow in an attachment independent manner. Cytogenetic analysis of both first passage cells and later passage cells was also carried out to evaluate possible changes in ploidy as a result of growth in culture.

R 404 KERATINOCYTE-FIBROBLAST CO-CULTURE HUMAN SKIN EQUIVALENT, Raul Fleischmajer, E. Douglas MacDonald, Jerome S. Perlish, Department of Dermatology, Mount Sinai School of Medicine, New York, NY 10029

The purpose of this study is to present a new human keratinocyte-fibroblast co-culture and its potential as an "in vitro" model for wound healing. Fibroblasts were grown in a 3-dimensional nylon mesh for 4 weeks and then recombined with keratinocytes in an air-liquid interface for additional 4 weeks (Advanced Tissue Sciences, La Jolla, CA). Semi-thin sections revealed an epidermis consisting of epidermal, squamous granular and horney layers, a basal lamina and a well developed dermis containing numerous fibroblasts and a rich extracellular matrix. Electron microscopy revealed a well differentiated epidermis (desmosomes, tonofilaments, keratohyaline granules and keratinosomes) anchoring filaments, a lamina densa, anchoring fibrils, elastin-associated microfibrils and collagen fibrils. Immunochemistry revealed K-10 keratin, fillagrin, trichohyaline; laminin, type IV collagen. heparan sulfate and nidogen. The anchoring zone revealed type VI and VII collagens, fibronectin and tenascin. The dermis contained type I, III, V collagens, decorin and bFGF. Wounds were performed with a 30 gauge needle. At 0 time, distinct wounds were noted perforating the epidermis and dermis. After 2 weeks in culture, there was complete repair of the epidermis and dermis. Epidermal proliferation, however, expanded deep into the dermis, enclosing numerous islands of connective tissue. The keratinocyte-fibroblast model present in this study permits the reconstruction of human skin and may be used to study epidermo-dermal interactions during development and wound healing.

R 405 EPITHELIAL REGULATION OF SAA3 EXPRESSION BY CORNEAL STROMAL CELLS (KERATOCYTES), Marie T. Girard, Katherine J. Strissel, William B. Rinehart, Jacqueline Magner and M. Elizabeth Fini, Department of Dermatology, Harvard Medical School and MGH/Harvard Cutaneous Biology Research Center, Mass. General Hospital, Charlestown, MA 02129

Although an interaction between the corneal stroma and epithelium is required to initiate stromal wound repair, the factors produced by this interaction which promote healing have never been identified. Recently, we have shown that the matrix metalloproteinases, collagenase (CL) and stromelysin (SL), are synthesized by the stroma of the rabbit cornea within 24 hours after injury. Once initiated, CL/SL synthesis is maintained for many months while the repair tissue undergoes remodeling. Stromal cells isolated from intact corneas do not synthesize CL/SL. However, synthesis occurs when stromal cells are cultured with conditioned medium harvested from epithelial cell cultures. As stromal fibroblasts become established in culture, they develop the capacity to produce CL/SL without a requirement for the factor produced by the epithelium. It appears that an autocrine factor, produced by these fibroblast cultures, can substitute for the factor that was produced by the epithelium. In an attempt to identify this factor, we examined the induction of proteins synthesized when freshly-isolated fibroblasts were either cultured with epithelial cell conditioned medium or become established in culture. A protein having a molecular weight of 14 kDa was synthesized under both culture conditions. We have identified this protein immunologically and by cDNA analysis as the SAA3 form of serum amyloid A. This protein has been reported to be an autocrine inducer of collagenase expression by rabbit synovial fibroblasts (Science 243:655, 1989). We determined that the stimulatory effects of epithelial conditioned medium on CL or SAA3 expression could be neutralized by the addition of either interleukin-1 receptor antagonist (Il-1RA) or antiserum to Il-1α. This demonstrates that Il-1 is the epithelial stimulator of synthesis for both proteins. In addition, a substantial amount of Il-1α was detected by radioimmunoassay in established, but not freshly plated fibroblast cultures. Addition of Il-1RA to established cultures decreased CL/SL expression, indicating that Il-1 is an autocrine stimulator of these proteins. Our data suggest that Il-1 might be a primary mediator of the interaction between the corneal epithelium and stroma during wound repair and may act as an autocrine factor in established fibroblast cultures; SAA3 might play a modulating role in this interaction.

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R 406 COMPARISON OF REPEATED AUTOGRAFTING VERSUS CULTURED KERATINOCYTES FOR THE TREATMENT OF VERY LARGE BURNS David G. Greenhalgh, MD, Shriners Burns Institute, 3229 Burnet Ave., Cincinnati, Ohio 45229

Before accepting the common use of cultured keratinocytes for the treatment of patients with very large burns, the new methodology should be compared with the "gold standard": repeated autografting. Currently, patients suffering from burns greater than 60% TBSA are treated by excision of the burn over 10-15 days. At the initial grafting procedure, meshed autograft is used to cover as much of the wounds as possible, with the coverage of functional and cosmetic areas (hands, face) taking preference. The remaining excised wounds are covered with allograft (human cadaver) with or without cyclosporine immunosuppression. As available donor sites heal, allograft is replaced with autograft. The results of repeated autografting were compared to several patients with large burns who were referred to the institution after treatment with cultured keratinocytes. Many of these patients received cultured keratinocytes despite having adequate donor sites. The average "take" of reharvested autograft was 85-90% compared to a reported "take" of < 50% for cultured keratinocytes. Large areas covered with cultured keratinocytes lost at least part of the keratinocytes and required repeated grafting. One patient reporting that several grafted areas requiring 5-6 regraftings. Because of the need for repeated placement of keratinocytes, the length of hospital stay was not shortened and septic episodes were not decreased. For comparison, patients with burns greater than 85% TBSA, treated with repeated autografting were completely covered as quickly as 68 days. A major difference between the two groups was seen in the long term functional and cosmetic results. Patients covered with cultured keratinocytes developed severe contractures that required extensive reconstructive procedures within a few months of their burns. Their grafts lacked durability and blistered for months to years after "taking". Many of the cultured keratinocyte-treated patients had grafts placed on fascial excisions (tissue cut to the fascia) that further worsened the functional and cosmetic results. In contrast, those patients treated with repeated autografting had functionally and cosmetically superior results, and required less reconstructive procedures. The autograft was significantly more durable. In conclusion, although the use of cultured keratinocytes or skin substitutes is a goal of all burn centers, the current techniques do not yet approach the success of the current "gold standard". Research should be continued to develop a viable alternative to repeated autografting but the current technologies are not yet ready to replace the "gold standard".

R 407 KERATINOCYTE CONDITIONED MEDIUM STIMULATES PHOSPHATIDYLCHOLINE HYDROLYSIS, ONCOGENEACTIVATION AND PROLIFERATION OF CULTURED HUMAN FIBROBLASTS. Anders Haegerstrand, Gunnar Kratz, Helena Wikström, Alexandra Dennerman, Bror Jonzon and Anna Hultgårdh-Nilsson. Departments of Anatomy, Clinical Pharmacology and Medical Cell Genetics, Karolinska Institue, S-10401, Stockholm, Sweden.

Allografts of cultured keratinocytes stimulate healing of chronic leg ulcers probably by releasing factors that activate dermal components as well as the surrounding epithelium. We have shown that keratinocyte conditioned medium (KCM) exhibit potent growth stimulatory effects on cultured human cell types related to the wound healing process, including endothelial cells, fibroblasts and keratinocytes. This effect was in part attributable to factors not yet characterized. In the KCM several known growth factors are likely to be present. In wound healing many growth factors may play a role and single growth factors sometimes fail to promote wound healing both in experimental models and in humans. Thus, the net effect s of KCM may be of clinical relevance.

In this study we investigated the effects of KCM on intracellular messenger systems, i.e. formation of cyclic adenosine monophosphate (cAMP), hydrolysis of phosphatidyl inositol (PI) or hydrolysis of phosphatidyl choline and on stimulation of the proto-oncogenes c-myc, c-fos and c-jun in cultured human fibroblasts.

KCM was inneffective in stimulating cAMP- formation whereas the control forskolin (10μM) induced a significant increase. KCM caused an insignbificant increase in PI-hydrolysis whereas thrombin (1 U/ml), used as a positive control, caused a significant increase in IP_3-formation. However, KCM caused a significant (40± 6% $p<0.001$) release of radioactivity from p^{32}-choline labelled fibroblasts indicating an increased hydrolysis of phosphatidylcholine. Phorbol myristate acetate (PMA, 100μM), used as positive control, caused a 68±9% increase in phosphatidyl-choline hydrolysis. Serumstarved fibroblasts stimulated with KCM showed a marked increase in c-fos and c-myc transcripts, whereas c-jun transcription was not increased.

The results from this study suggest that KCM stimulates hydrolysis of phosphatidylcholine, which in turn leads to the activation of the proto-oncogenes c-fos and c-myc, this possibly via formation of diacylglycerol (DAG). These effects of KCM may in part explain its wound healing properties exhibited *in vivo* and *in vitro*.

R 408 A NEW *IN VITRO* MODEL FOR HUMAN WOUND HEALING; EFFECT OF KCM, AMNIOTIC FLUID AND IGFs, Kratz G, Hægerstrand A, Dennerman A, Wikström H, Gidlund M, Palmer B. Department of Anatomy, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden.

Human wound healing is a complex process that involves interaction between a variety of cell types thougth to be regulated by a network of autocrine and paracrine acting growth factors. We have recently presented evidences for growth promoting effects on cell types involved in wound healing by growth factor preparations; keratinocyte conditioned medium (KCM) and amniotic fluid AF) as well as single growth factors; insulin like growth factor (IGF)-1 and-2. One major problem in this work has been the lack of an experimental model of the wound healing process that can be reproducibly analysed in a non-subjective, well controlled manner and that is as close to the in vivo situation as possible. In the present study we used biopsies from human skin in which partial thickness wounds were made. The wounds were cultured for 7 days and the media were changed every second day. Paraffin embedded cross sections, 10-20 µm in thickness, were stained using the hematoxylin-eosin technique and reepithelialization was determined by light microscopy. Two % FCS was not able to induce any wound healing in the wounds whereas 10 % FCS resulted in a total reepithelialization of all wounds treated. No reepithelialization was seen in the wounds kept in KCM without any serum added. However, when 2 % FCS was added to the KCM, 100% of the wounds (n=10) were reepithelialized after the incubation period. KCM 50 % with 2 % FCS was still able to induce reepithelialization. AF 100% to 25 % resulted in a complete reepithelialization even without any serum present. When wounds were incubated with EGF no reepithelialization was observed even in the presence of 2 % FCS. IGF-1 in combination with 2% FCS was shown to induce reepithelialization of the wounds whereas IGF-2 was dependant on the presence of IGF binding protein-1 (IGFBP-1). This findings indicate the presence of factors important for wound healing in KCM and AF. The need for small amount of FCS when wounds were healed with KCM could be due to additional growth factors or other components, such as matrix proteins, present in amniotic fluid and serum but not in KCM. Furthermore, the findings suggest that IGFs and IGFBPs participate in the autocrine-paracrine growth factor system controlling the healing of wounds. The in vitro model used in this study includes a viable epidermis and dermis and may be of great value for studying the local response to individual and combinations of growth factors and thus to the investigation of the complex network of autocrine and paracrine acting factors released from different cell types involved in the wound healing process.

R 409 THE ROLE OF DERMIS IN IMPROVING KERATINOCYTE GRAFT TAKE

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Keratinocytes can be cultured into sheets suitable for grafting major epidermal defects in burns and chronic non-healing wounds, such as leg ulcers. However, graft take is poor on full-thickness wounds and the resulting epidermis is fragile possibly due to the slow maturation of the basement membrane zone. Keratinocytes can be subcultured onto a variety of dermal substitutes including collagen gels and deepidermalised dermis to provide composite culture grafts. A dermis can also be provided by two stage grafting using cadaver allogenic dermis to pretreat the wound bed and applying the epidermal sheets. A comparative study of keratinocyte sheets versus composite culture grafts and two stage grafting has been performed in both full-thickness wounds in a porcine model and on a variety of clinical non-healing wounds. The take of autologous keratinocytes in pig was poor (4%) and was not improved by composite culture grafting. However, the take of keratinocytes onto wounds pretreated with autologous dermis in two stage grafting was greatly improved to 48%($p<0.001$). The resulting epidermis was clinically stable and histology showed an epidermis with rete ridges unlike that of cultured keratinocytes alone. The poor take of composite culture grafts probably reflected delay in vascularisation of the dermis with consequent loss of epidermis. The two stage grafting permitted a vascularised dermis to maintain keratinocyte perfusion and nutrition.

R 410 WOUND HEALING IN CHICK AND MOUSE EMBRYOS

Paul Martin[1], Jane McCluskey[1], Catherine Nobes[1], James Hopkinson-Woolley[1] & Julian Lewis[2]. [1]Dept. Human Anatomy, Oxford University, South Parks Rd. Oxford, OX1 3QX & [2]ICRF Developmental Biology Unit, Zoology Department, Oxford OX1 3PS

Simple excisional lesions of the limb bud in chick and mouse embryos heal rapidly and without scarring. We have shown that, just as in adult healing, an embryonic wound closes through a combination of connective-tissue contraction and epidermal forward movement (Martin & Lewis, 1992). More recent dye-marking experiments of ours have allowed us to quantify these two tissue movements accurately and have revealed that each contributes about 50% to the overall closure of the wound. However, it appears that the mode of epidermal forward movement in the healing of embryonic wounds is quite different from the lamellipodial crawling seen at an adult wound edge: embryonic wounds appear to be drawn closed by a purse string of actin operating in the basal epidermal wound front cells (Martin & Lewis, 1992).

We have begun to investigate the immediate early induction of transcription factors, for example *c-fos* (Martin & Nobes, 1992), and the slightly later expression of growth factors, particularly the TGFβs, that are induced at a wound site, since these might play crucial roles in the embryonic repair process, and in particular in the tissue movements that lead to wound closure.

Studies of cultured mouse embryos using BrdU, to label cells in S-phase, have provided preliminary data suggesting that a wave of cell division begins in the cells at the epidermal margin some hours after wounding. This may supply the additional cells needed to restore the epithelium to a normal population density.

As well as attempting to modulate the healing of embryonic wounds by the addition of exogenous growth factors to the wound healing environment we have also begun to investigate the role of macrophages in embryonic and foetal repair since these are believed to be the major sustained source of endogenous growth factors at an adult wound site.

Martin, P. & Lewis, J. (1992). Actin cables and epidermal movement in embryonic wound healing. Nature 360, 179-183.

Martin, P. & Nobes, C.D. (1992). An early molecular component of the wound healing response in rat embryos - induction of c-fos protein in cells at the epidermal wound margin. Mech. Dev. 38, 209-216.

R 411 HYALURONIC ACID PROFILES IN HUMAN SKIN AS A FUNCTION OF AGE. L.J.M. Meyer and R. Stern, University of California, San Francisco, CA. 94143.

Hyaluronic acid (HA) is a major component of the extracellular matrix of skin. The water of hydration associated with HA may be a key mechanism of skin hydration. As such, decreasing levels of HA deposition might be expected in the normal process of aging. To test this hypothesis, HA levels were determined in extracts of full thickness human skin specimens taken at various ages, from fetal stages to senescence. No significant differences were found, with levels ranging from 17.1 to 23.2 ug HA/mg protein. Sequential extraction [1)0.1% Triton X-100, 2)4M guanidine HCl, and 3)papain] was then performed, to obtain progressively more tightly tissue-associated species of HA. Steadily increasing levels of HA were obtained in extracts 2 and 3. The percent ratios ranged in extracts 1:2:3 from 68/24/7 in the fetal to 42/37/21 in the senescent sample. Finally skin specimens were analyzed histologically with an HA-specific stain utilizing a biotinylated cartilage-derived HA-binding peptide. Significant differences of compartmentalization were noted. We conclude that not the total amount, but higher levels of tightly bound and protein linked-HA, and its relative compartmentalization may be the basis for the changes in hydration that occur in human skin as a function of age. Supported by the DFG and NIH grant HD 25505.

R 412 DEMONSTRATION OF HUMAN TYPE IV COLLAGENASES IN BLISTERING DISEASES, SUCTION BLISTERS AND IN EARLY WOUND HEALING

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Type IV collagenases (72 kDa and 92 kDa forms) belong to the matrix metalloproteinase family (MMP) which degrade the basement membrane collagen IV, anchoring fibril collagen VII and some other non-fibrillar collagens as well as elastin and fibronectin.

In the present study we wanted to find out whether type IV collagenases could participate in the pathogenesis of various skin and mucous membrane blistering diseases. We also studied their induction in the experimental suction blisters during the re-epitelization by applying the blister skin either by vehicle or glucocorticoid creams. In addition we examined by *in situ* hybridization the expression of type IV collagenases in human mucosal wounds during the wound healing process from day 1,3, and 7. The blister fluid samples were analyzed by zymography.

The results indicate that there were especially high level of 92 kDa form in bullous pemphigoid patient, but also that both 92 kDa and 72 kDa forms are present in experimental suction blisters and they are clearly induced during 24 and 72 h after the blister formation. Topical glucocorticoid treatment clearly decreased the 92 kDa form expression. By *in situ* hybridization studies the 72 kDa form was localized mainly to fibroblasts and the 92 kDa form into the epihtelial and endothelial cells. Interestingly, day 7 wound healing samples showed especially high expression of 92 kDa type IV collagenase in the granulation tissue.

In the conclusion we suggest firstly, that the benefical role of glucocorticoids may in some blister diseases be caused by the mechanism where they could decrease the tissue destructions partially by decreasing the type IV collagenase activity. Secondly, that in incisional wounds the major role of the 92 kDa type IV collagenase could be in the remodelling of the wound bed matrix by the granulation tissue cells.

This study was supported by the Academy of Finland and Juselius Foundation.

R 413 KERATINOCYTE INDUCED GROWTH FACTOR GENE EXPRESSION IN COCULTURED MESENCHYMAL FEEDER CELLS, Smola H.[1,2] Thiekötter G.[1] Krieg T.[2], Fusenig N.E.[1], [1]Dept. of Tumor Cell Regulation, German Cancer Cesearch Center, 6900 Heidelberg, FRG, [2]Dept. of Dermatology, University of Cologne, 5000 Cologne, FRG

In human skin dermal cells influence the equilibrium of epidermal cell proliferation and keratinization. Mostly, these effects have been ascribed to soluble mediators synthesized and secreted by dermal cells. In vitro, keratinocyte proliferation is grossly enhanced by coculture with postmitotic fibroblasts illustrating the importance of epidermal-dermal cell-cell interactions. Whether other dermal cell types, apart from fibroblasts, are able to support keratinocyte proliferation is not known to date. Furthermore, little is known about the molecular mechanisms being involved. Here we demonstrate that in addition to dermal fibroblasts (HDF) human dermal microvascular endothelial cells (DMEC) effectively stimulate keratinocyte growth from clonal cell densities in vitro. Studies into the molecular mechanism revealed that keratinocytes induce growth factor mRNA levels (KGF, IL 6, GM-CSF) in cocultured feeder cells (HDF, DMEC) which then may stimulate keratinocyte proliferation in turn. This indicates complex cell-cell stimulation acting in a paracrine fashion. In three dimensional organotypic cocultures the effects of HDF and DMEC on epidermal cell proliferation and keratinization patterns were analyzed. Keratinocytes were cultured on top of collagen gels at the air medium interface (controls) or on top of dermal equivalents containing either HDF or DMEC. In the presence of HDF epidermal proliferation was detectable at least until day 7 resulting in a well structured epithelial architecture also obtained with DMEC. In contrast, without mesenchymal influences keratinocyte proliferation ceased at day 2 with formation of a thin almost completely keratinzed epithelium. Keratinization markers (keratin 1 and 10) were induced in all organotypic cultures with close codistribution. However, in cocultures with HDF or DMEC expression of K 1 and 10 was restricted to suprabasal layers of the *neo*epidermis giving the impression of a hyperplastic phenotype. In conclusion, keratinocytes induce growth factor mRNA levels in HDF and DMEC feeder cells with the potential to keratinize in organotypic cultures.

R 414 EFFECT OF EPIDERMAL GROWTH FACTOR ON EPITHELIAL CELL MATRIX METALLOPROTEINASE EXPRESSION, Veli-Jukka Uitto, Jim Firth, Yu-Min Pan and Edward Putnins, Department of Oral Biology, Univ. of British Columbia Vancouver, B.C. V6T 1Z3, Canada.

We have recently shown that proliferating oral epithelial cells are capable for both extracellular and intracellular degradation of collagen (Salonen et al., Matrix 11:43, 1991). In this study we examined in detail the expression of matrix metalloproteinases (MMP) by epithelial cells of human gingiva and porcine periodontal ligament (epithelial cell rests of Malassez) under different culture conditions. Since these cells have been found to express high numbers of EGF receptors (Thesleff, J Perio Res, 22:419, 1987) we examined the regulation of MMP expression by EGF. The MMP expression was analyzed by Northern blot analysis using 72 kD gelatinase and stromelysin cDNA probes, and by Western blot using specific MMP antibodies. The functional enzymes were detected by gelatin and casein enzymography. In quiescent cultures both cell types produced gelatinolytic enzymes of 95 kD (MMP-9) and 68-72 kD (MMP-2). Rapidly proliferating cells produced, in addition, caseinolytic enzymes of 140 kD, 85 kD and 43 kD (MMP-3, stromelysin). Periodontal ligament epithelial cells cultured on a polycarbonate membrane to form multilayers showed a selective directional secretion of the MMP's. While the MMP-2 was secreted preferably to the basal direction the 140 kD, 85 kD and 43 kD (MMP-3) proteinases were secreted primarily apically. EGF increased markedly production of all these MMP's. The enzyme expression of MMP-9 and MMP-3 was more pronounced than that of MMP-2. The increase in the proteinase expression by EGF was both concentration- and time-dependent. Measurement of the mRNA levels indicated that EGF caused a burst of the MMP induction for about 6 hr, followed by downregulation of the expression.

R 415 FIBRONECTIN ISOFORM EXPRESSION IN THE WOUNDED CORNEAL EPITHELIUM: POLYMERASE CHAIN REACTION ANALYSIS IN A RAT EPITHELIAL SCRAPE WOUND MODEL. A.T. Vitale, J.J. Liu, X.X. Cai, S.J. Lee, C.S Foster, and R.B. Colvin. Rhoads Molecular Immunology Laboratory, Harvard Medical School, Massachusetts Eye & Ear Infirmary, Boston, MA.

Purpose. Epithelial expression of fibronectin (FN) isoforms during corneal epithelial wound healing was studied using the polymerase chain reaction (PCR). Methods. Epithelial samples were collected at different time points following scrape wounding of rat corneal epithelium. RNA was extracted and cDNA was synthesized from these samples. PCR was performed using oligonucleotide primers specific for 1) various EIIIA, EIIIB, and V domain RNA splicing patterns and 2) a domain common to all known FN transcripts. Results. Total FN RNA was maximally expressed at one and 48 hours following corneal epithelial wounding. EIIIA+ FN, not found in normal cornea, was upregulated immediately following wounding with biphasic kinetics observed thereafter. Likewise, V+ FN was detected at one hour post wounding with subsequent polyphasic kinetics and maximal expression at 48 hours. Upregulation of EIIIA- and V- FN was observed 48 hours after wounding while EIIIB-FN was detected at 1, 4, 24, 48 hours. Conclusion. These observations indicate, for the first time, that the corneal epithelium participates in wound healing by altering the synthesis of different FN isoforms.

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R 416 ANALYSIS OF FGF FUNCTION IN THE SKIN BY TARGETED EXPRESSION OF A DOMINANT-NEGATIVE FGF RECEPTOR MUTANT.

Sabine Werner[1,4], Wendy Weinberg[3], Xiang Liao[1], Stuart Yuspa[3], Richard Weiner[2] and Lewis T. Williams[1]. [1]Cardiovascular Research Institute, and [2]Department of Gynecology and Obstetrics, University of California, San Francisco CA 94143, USA, [3]National Cancer Institute, National Institute of Health, Bethesda, MD 20892, USA. [4]Present address: Max-Planck-Institut für Biochemie, 8033 Martinsried, Germany.

Recent studies have provided evidence for an important role of Fibroblast growth factors (FGFs) during wound healing and most likely also in normal skin. In order to assess the biological function of FGF receptors in the epidermis, we used a dominant-negative FGF receptor mutant to selectively block FGF function in the epidermis of transgenic mice. The mutant receptor, known to block signal transduction in cells when co-expressed with wild-type receptors, was targeted to suprabasal keratinocytes by the use of a keratin 10 promoter. The transgene was expressed specifically in the skin and highest expression levels were found in the tail. Expression of the mutant receptor disrupted the organization of epidermal keratinocytes, induced epidermal hyperthickening and resulted in aberrant expression of keratin 6. This suggests that FGF does not play an important role in stimulating growth of suprabasal keratinocytes but is essential for their morphogenesis and for the establishment of the normal program of keratinocyte differentiation.

Matrix Function in Repair and Development; Pathological Fibrosis: Its Causes and Clinical Strategies for Prevention (Session Sponsored by Genzyme Corporation)

R 500 AN ULTRASTRUCTURAL STUDY OF THE HEALING OF HUMAN FETAL SKIN WOUNDS.

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Wound healing is a complex series of processes that lead to restoration of tissue integrity. In the adult, the end point of the process is a scar. This is loosely defined as a clinically apparent, disorganized deposition of collagen which may affect tissue function and growth. It is a morphologic entity and, as such, the only way in which to study it critically is to examine its structure. Animal studies show variation of scar severity depending on the model and gestational age of the fetus. The human fetus heals cutaneous wounds without clinically apparent scarring. A model using human, fetal skin transplanted onto and under the skin on the backs of *nu/nu* mice was used to study the ultrastructure and probe the directing forces involved in scarless wound healing.

We previously found that wounded human fetal skin transplanted subcutaneously healed without scar whereas that transplanted cutaneously healed with a scar. Scanning and oriented transmission electron microscopy of the latter revealed large masses of collagen formed by day 7 post wounding. This collagen remained in disorganized form up to 28 days post wounding. In contrast, the subcutaneously placed grafts healed with little excess or disorganized collagen. Immunoelectron-microscopy revealed direct binding of tenascin to the collagen fibers in the latter instance but not in the former. It may be that tenascin plays a role in directing the organization of collagen leading to scarless fetal cutaneous wound healing. The similarity between fetal and adult wound healing and that between scar forming and scarless healing indicate the possibility of exploiting small differences in these processes to lead to scarless adult cutaneous wound healing.

R 501 EXPRESSION OF TRANSFORMING GROWTH FACTOR-BETA IS ASSOCIATED WITH PRESENCE OF BONE MORPHOGENETIC PROTEIN IN ABNORMAL CONDITIONS OF SCAR,

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Bone Morphogenetic Proteins (BMPs) are molecules capable of inducing bone and/or cartilage formation in vivo. Transforming Growth Factor-Beta (TGF-B) has known growth regulatory properties[1] and is thought to play an important role in the regulation of wound healing. BMPs 2(BMP-2A),3,4(BMP-2B),5,6 and 7 are all members of the TGF-B superfamily of molecules and may be linked to developmental regulation.[2] Hypertrophic, keloid, and heterotopic ossified scars are products of abnormal wound repair. Early passage fibroblasts (3-5) cultured from normal adult and fetal dermis, and hypertrophic, keloid, and ossified scar tissues were evaluated for transcript levels of TGF-B and C-Myc. No significant differences in levels of TGF-B were noted among normal fibroblast lines (134 T, HLS-NF, SN-NF, 145 T), fetal fibroblasts (FF 1217), or three of four keloid fibroblast lines (BKF, FDK, SKF). Fibroblasts from one keloid (HLS-KF) and an ossified scar (ROS-1) had 2-3 times this level of TGF-B transcript. Low levels of C-myc were consistently observed among all cell lines, indicating similar degrees of cellular proliferation. Immunofluorescent staining of ROS-1, HLS-NF, and HLS-KF was performed using monoclonal antibodies to BMPs 2-7. Positive staining for BMPs 2 and 4 was observed in fibroblasts from keloid and ossified scar (ROS-1, and HLS-KF) but not in normal fibroblasts (HLS-NF). These results suggest that (1) elevated transcript levels of TGF-B are associated with the presence of BMPs 2 and 4 in fibroblasts from abnormal scar, and (2) formation of these lesions may involve abnormalities in cell growth and/or differentiation.

[1] Roberts, A.B., et al. 1985. Proc. Natl. Acad. Sci. U.S.A. 82: 119-123.
[2] Weeks, D.L., et al. 1987. Cell 51: 861-867.

R 502 IMMUNOHISTOCHEMICAL LOCALIZATION AND mRNA EXPRESSION OF TGF-ß1, TGF-ß2, AND TGF-ß3 IN THE NORMAL HUMAN LUNG AND IN IDIOPATHIC PULMONARY FIBROSIS (IPF), Leslie I. Gold,[1] Babita Saxena,[1] Thomas V. Colby,[2] and John A. McDonald,[3] Department of Pathology, New York University Medical Center, NY, NY 10016,[1] The Mayo Clinic, Rochester, NY 55905, [2] and The Samuel C. Johnson Medical Research Center, Mayo Clinic, Scottsdale, AZ 85259.[3]

The bioactivities of TGF-ß, although critical to the wound repair process are also involved in the pathogenesis of chronic inflammation and fibrotic disease. TGF-ßs are potent chemotactic agents for leukocytes and fibroblasts and regulate cytokine and growth factor production by these cells. In addition to the inflammatory component of fibrotic disease, TGF-ßs stimulate fibroblast proliferation and induce the production of extracellular matrix components, such as collagens. Three isoforms of TGF-ß exist in mammals, and although they demonstrate similar *in vitro* bioactivities, recent studies indicate that there is a differential spatial and temporal expression of TGF-ß isoforms throughout wound repair (Levine et al. Am. J. Pathol, in press). Since IPF consists of both an inflammatory and severe fibrotic component, we examined the expression of TGF-ß isoforms by *in situ* hybridization and immunohistochemistry, using isoform specific probes and antibodies to TGF-ß, respectively. IPF biopsies from patients in mid-course were compared to normal lung.

In the non-fibrotic lung, diffuse cytoplasmic immunostaining for all three isoforms of TGF-ß was observed in the epithelial cells lining the bronchi, bronchioles, and terminal bronchioles, but not respiratory bronchioles. However, there was a difference in intensity of immunostaining: TGF-ß2=TGF-ß3>TGF-ß1. A population of macrophages showed striking granular immunoreactivity for all three isoforms while others were completely negative; this was also observed for other inflammatory cells. The smooth muscle cells of both the vasculature and bronchial walls showed intense staining for TGF-ß2 and TGF-ß3, but slight for TGF-ß1; all TGF-ßs were present in endothelial cells. The IPF biopsies showed an identical cellular distribution of each TGF-ß isoform. However, the intensity of immunostaining was markedly increased, especially in areas of greater inflammation and fibrosis with the order of intensity TGF-ß3>TGF-ß2>TGF-ß1. Additionally, in IPF, different cells were also immunostained. In areas of active remodeling, type II pneumocytes were intensely positive and in the interstitial areas, fibroblasts, and mesenchymal cells were immunoreactive. The pattern of mRNA expression coincided with protein localization. The dramatic increase in TGF-ß expression in IPF compared to non-fibrotic lung suggests its involvement in the remodeling and fibrosis associated with this disease.

R 503 **DIFFERENTIAL EFFECTS OF TUMOR NECROSIS FACTOR-ALPHA ON GROWTH AND COLLAGEN SYNTHESIS IN NORMAL AND KELOID FIBROBLASTS,** **Patricia A. Hebda, Robert Rodgers, Margaret Collins, Linda Benedict and Michael D. Tharp,** Department of Dermatology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15261.

Tumor necrosis factor-alpha (TNF-α) is stored in mast cells and macrophages and may be released during the process of wound healing. Named because of its cytotoxic effects on some tumor cells, TNF-α is now known to be a cytokine for various normal cells including skin fibroblasts. It stimulates mitosis and decreases collagen synthesis as demonstrated by others and reconfirmed in our experiments. However, our studies demonstrate that TNF-α's effects on keloid fibroblasts are quite different. It failed to stimulate mitosis and in some experiments slightly inhibited growth of keloid fibroblasts. Collagen synthesis was decreased by TNF-α to a greater extent in confluent cultures of keloid fibroblasts compared with normal fibroblast lines. Keloid fibroblasts also showed a greater decrease in noncollagenous protein synthesis in the presence of TNF-α. In biopsies of keloid tissue, mast cells are found around the margins and it has been speculated that they may be involved in keloid pathogenesis. Our studies suggest that mast cells may be acting to contain or "wall off" the keloid by releasing TNF-α locally to downregulate keloid fibroblasts and favor the growth of normal fibroblast populations.

R 504 TENDON ADHESION FORMATION IS STIMULATED BY EXOGENOUS GROWTH FACTORS, Joyce ME, Manske PR. Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis MO 63110.

Flexor tendon injury often results in devastating disability. Tendon repair fails from either a rupture at the tendon anastomosis site, or by the formation of restrictive adhesions. Using an tendon laceration model we've demonstrated endogenous TGF-ß, bFGF, and PDGF at the site of tendon repair. In vitro studies show that these factors stimulate cellular proliferation and matrix synthesis in tendon sheath fibroblasts. These studies suggest that growth factors may stimulate adhesion formation following tendon injury. The present study investigates the stimulation of tendon adhesions by exogenous growth factors in a non-traumatic model. 24 Leghorn chickens were injected with either 5.0 ul of growth factor or PBS within the flexor tendon sheath. Injected limbs (n=48) were randomized to four groups: Group I (n=9) PBS controls, Group II (n=8) PDGF-BB at 100 ng, 500 ng, and 5.0 ug, Group III (n=17) TGF-ß1 at 50 ng, 200 ng, 1.0 ug, 5.0 ug, and Group IV (n=14) bFGF at 100 ng, 1.0 ug, and 5.0 ug. Animals were killed for histological analysis 2 and 4 weeks after injection. PBS injected controls were serially sectioned and demonstrated minor areas of epitenon thickening but no adhesions were seen. PDGF stimulated mild synovial hyperplasia at low dose, while 5.0 ug injections resulted in both the proliferation of tenocytes and extracellular matrix synthesis. No adhesions formed as a result of PDGF injection. Low dose TGF-ß stimulated cell proliferation within the tendon sheath and thickening of the epitenon. High dose TGF-ß stimulated tremendous cellular proliferation, throughout the tendon sheath and tendon proper, with a loss of normal cellular morphology. All specimens demonstrated areas of adhesion formation between the tendon its surrounding sheath. At the 5.0 ug dose bFGF stimulated an organized proliferation of epitenocytes and cells lining the tendon sheath while no adhesions were identified. This study show that the effect of exogenous growth factors within the tendon sheath is dependent upon both the specific growth factor and its dose. Most importantly, TGF-ß stimulated the formation of tendon adhesions. This effect was specific for TGF-ß and dose dependent suggesting that TGF-ß synthesis following tendon laceration is one mechanism for tendon adhesion formation.

R 505 DIFFERENTIAL SECRETION OF TRANSFORMING GROWTH FACTOR-β_2, AND -β_3 BY TYPE 2 PNEUMOCYTES DURING BLEOMYCIN-INDUCED LUNG INJURY N. Khalil[1,2], R. O'Connor[3], W. Shing[1], C. Whitman[1] and A. Greenberg[1], Manitoba Institute of Cell Biology[1], and the Departments of Medicine[2] and Pathology[3], University of Manitoba, Canada

The transforming growth factor-betas (TGF-β) are potent regulators of inflammation and connective tissue synthesis. Three isoforms of TGF-β are found in mammalian cells, but their expression in pulmonary injury and repair is unknown. During bleomycin-induced pulmonary inflammation and fibrosis TGF-β_1 was localized by immunohistochemistry exclusively to alveolar macrophages of only bleomycin-treated rat lungs, while TGF-$\beta_{2,3}$ were found in alveolar macrophages, epithelial cells and smooth muscle cells of bronchi and pulmonary vessels of both bleomycin-treated and normal rat lungs. Using the CCL-64 bioassay and neutralizing antibodies to TGF-β_{1-3}, we demonstrated that after bleomycin injury, explanted type 2 pneumocytes secreted primarily TGF-β_2, small quantities of TGF-β_3, and no TGF-β_1. Although the TGF-βs are usually secreted in biologically latent forms, type 2 pneumocytes secreted only biologically active TGF-β. Early in bleomycin-induced lung injury there is necrosis of type 1 pneumocytes which are replaced by actively proliferating type 2 pneumocytes. Four days after bleomycin administration, there was increased proliferation of explanted type 2 pneumocytes, while the secreton of TGF-$\beta_{2,3}$, which are potent inhibitors of epithelial cell proliferation, was decreased. However, the secretion of TGF-$\beta_{2,3}$ was increased later in the injury response and coincided with a reduction in type 2 pneumocyte proliferation. Furthermore, the addition of TGF-β in cultures of actively proliferating type 2 pneumocytes resulted in inhibition of thymidine incorporation. Our findings suggest that differential secretion of TGF-$\beta_{2,3}$ by type 2 pneumocytes during bleomycin-induced lung injury may regulate type 2 pneumocyte proliferation.

R 506 **MORPHOLOGICAL STUDIES ON THE EFFECTS OF FIBROQUEL DURING WOUND HEALING OF SURGICAL WOUNDS IN RATS.**
Krötzsch, G.F., Guerrero-Padilla, E. and Díaz de León, L., Dept. of Develop. Biol, Inst. Invest. Biomédicas., UNAM, A.P. 70228 Mexico, City, Mexico, 04510.
Wound healing is a reparative process mounted as a biological response to different types of aggressions or lesions to skin. It constitutes a series of events mainly characterized by inflammation, scar formation, synthesis and deposit of granulation tissue and presence of cellular infiltrate (fibroblasts and mononuclear cells) in the areas of lesion. In some connective tissue disorders (scleroderma, keloids, hypertrophy), scar formation constitutes a serious problem, by interfering with the normal functioning of the skin after the area of lesion has been repaired. Several compounds have been assayed to modify this process with the idea of decreasing scar tissue formation by altering collagen metabolism during wound healing. Previous observations suggest that a mixture of type I collagen and an inert polymer (FIBROQUEL™) induces scar tissue mobilization in hypertrophic scars and keloids. For this reason, in this study we have evaluated the effects of intradermal administration of fibroquel during the reparative process of surgical wounds induced in rats. Fibroquel (1 ml) was applied to surgical wounds before suturing. Morphological studies were performed at 5, 7, 14 and 21 days after surgery. The experimental groups were compared with control groups (untreated surgical wounds). The results obtained showed that fibroquel-treated wounds in comparison with control groups contained greater proportions of less structurated collagen with different tinction properties, together with increased amounts of granulation tissue (5 and 7 days). At days 14 and 21 the presence of immature collagen was evident, although skin appendages (sebaceous glands and hair follicles) were present. The results obtained suggest that fibroquel modifies the metabolism and turnover of collagen during wound healing.

R 507 TRANSFORMING GROWTH FACTORS ß1 AND ß2 SYNERGISTICALLY INCREASE COLLAGEN GENE EXPRESSION IN FETAL FIBROBLASTS BUT NOT IN ADULT FIBROBLASTS, H. Peter Lorenz, James Chang, Michael J. Banda, Departments of Surgery and Radiobiology & Environmental Health, University of California, San Francisco, CA 94143

Multiple isoforms of the transforming growth factor-beta (TGF-ß) cytokine family exist in mammals. TGF-ß1 and TGF-ß2 stimulate scar and fibrosis in postnatal wounds. Unlike the adult, the midgestation fetus heals skin wounds without scar. Fetal wounds fluid contain more TGF-ß than adult wounds and the relative amount of TGF-ß2 is increased.

The purpose of the present study is to determine the influence of TGF-ß1 and TGF-ß2 on collagen gene expression on both fetal and adult fibroblasts. Human fetal fibroblasts (23 weeks gestation, ATCC-CRL 1475) and human adult fibroblasts (35 yrs old, NIA-AG 2602) were cultured to confluence. In serum-free conditions, cells were exposed for 24 hours to total TGF-ß concentration 5.0 ng/ml in varying proportions: ß1 or ß2 alone, 9:1, 3:1, or 1:1 ratios of ß1:ß2. Total cellular RNA was extracted and subjected to Northern and slot blot hybridization analysis with radiolabeled alpha$_1$(I) collagen cDNA.

Fetal fibroblast expression of the alpha$_1$(I) collagen gene was increased by application of either TGF-ß1 or TGF-ß2. However, the greatest increase (6.5 fold) occurred when TGF-ß was applied in a ratio of 9:1 (ß1:ß2). All of the ratios tested were more effective at stimulating alpha$_1$(I) collagen gene expression than either isoform applied alone.

Adult fibroblast alpha$_1$(I) collagen gene expression was increased 3-fold by either TGF-ß1 or TGF-ß2. However, this increase did not significantly vary by isoform or ratio. The relative alpha$_1$(I) collagen mRNA increases above untreated cells are shown below:

TGF-ß Ratios (ß1:ß2)	Fetal Fibroblasts	Adult Fibroblasts
100% ß1	2.4	3.3
90%/10%	6.5	3.3
75%/25%	5.9	3.0
50%/50%	4.9	3.2
100% ß2	3.3	3.4

These data demonstrate 1) both fetal and adult fibroblasts respond to TGF-ß1 or TGF-ß2 with an increase in collagen alpha$_1$(I) gene expression; 2) this expression is synergistically increased only in fetal, but not adult, fibroblasts by combinations of both TGF-ß1 and TGF-ß2; 3) TGF-ß2 application enhances collagen alpha$_1$(I) gene expression to a greater extent than TGF-ß1 in fetal fibroblasts.

R 508 DIFFERENTIAL REGULATION OF CYTOKINE-INDUCED IL-8 AND COLLAGENASE GENE EXPRESSION BY TGF-β.
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A complex cytokine network controls connective tissue deposition in normal or disease states, such as in tissue repair, tumor metastasis and inflammation. In this study, we have investigated the molecular mechanisms by which TGF-β interacts with pro-inflammatory cytokines, IL-1, TNF-α, and leukoregulin (LR), in modulating fibroblast gene expression *in vitro*. We focused on the expression of the genes coding for interstitial collagenase and IL-8, two essential factors of the inflammatory reaction. Using Northern blot hybridization, we showed that TGF-β blocks the induction of collagenase gene expression induced by all three cytokines studied. By contrast, it does not interfer with the induction of IL-8 gene expression, as determined at the mRNA steady-state level. These effects are transcriptional since transient transfection experiments with collagenase or IL-8 promoter/CAT constructs showed similar results. Using gel retardation assays, we showed that TGF-β does not counteract the binding of cytokine-induced nuclear proteins to the region -101/-63 of the IL-8 promoter, responsible for gene activation by cytokines. Furthermore, TGF-β does not counteract cytokine-induced NF-kB-binding activity, nor does it affect the activation of an NF-κB/SV$_2$CAT construct in transient cell transfections. These data suggest that TGF-β does not counteract the activation of genes under the control of NF-κB, such as the IL-8 gene.

In contrast to the NF-κB driven promoters, TGF-β blocks cytokine activation of an AP-1-tk/CAT construct in transient transfection experiments. IL-1, TNF-α, and LR induce high levels of *c-Jun* mRNA, independently of the presence of TGF-β, and increase *Jun-B* mRNA levels to a lesser extent. TGF-β has no effect on the levels of *c-Jun* mRNA but is a potent inducer of *Jun-B* mRNA, in the absence or in the presence of the other cytokines This induction of *Jun-B* by TGF-β explains the down-regulation of cytokine-induced collagenase gene expression since *Jun-B* is a well known inhibitor of *c-Jun* trans-acting activities, responsible, at least in part, for the induction of collagenase by cytokines.

R 509 NITRIC OXIDE AND HYDROXYL RADICAL IN THROMBOXANE B_2 AND TRANSFORMING GROWTH FACTOR-$ß_1$ PRODUCTION IN RAT ALVEOLAR MACROPHAGE EXPOSED TO SILICA DUST IN VITRO, Urszula Orlinska, Douglas C. Kuhn, Lesley J. Gaydos and Lawrence M. Demers, Department of Pathology, Penn State University, Hershey Medical Center, Hershey, PA 17033.

Repeated inhalation of silica dust can lead to inflammation and lung fibrosis in human and in experimental animal models. The alveolar macrophage is believed to play a pivotal role in this process. Macrophage-derived arachidonic acid metabolites and transforming growth factor-$ß_1$ (TGF-$ß_1$) have been shown to contribute to inflammation and fibrosis. In this study it was investigated a role for nitric oxide (NO) and hydroxyl radical (OH·) in thromboxane B_2 (TXB_2) and TGF-$ß_1$ production in silica-activated alveolar macrophages. It was observed that TXB_2 was produced in time-dependent manner and achieved a maximum after 6 hours of silica activation. The synthesis and the release of TGF-$ß_1$ was at a maximum after 15 minutes, declined in time and approached the lowest level at 24 hour. There was 2 fold drop in level of intracellular NO after 3 minutes of silica activation followed by a restoration to control value in 24 hours; extracellular NO was maximum after 30 minutes then declined to control value within 6 hours. Intracellular OH· peaked after 10 minutes of silica activation then gradually declined to nearly 0 in 24 hours; extracellular OH· was detectable after 2 hours and gradually increased in 24 hours. N^G-Monomethyl-L-Arginine (NMMA) or allopurinol inhibited NO or OH·, respectively, and TXB_2 production, in a dose-dependent manner with the maximum inhibition of 65% and 31%, respectively. UK 38,485, an inhibitor of thromboxane synthase, at 100 μM abolished intracellular NO but elevated 6 fold intracellular OH·. TGF-$ß_1$ synthesis and release did not change in the presence of either of these inhibitors. These data suggest that in silica-exposed rat alveolar macrophage both NO and OH· are involved in the regulation of TXB_2 production but not in TGF-$ß_1$ synthesis or release.

R 510 POLYMERIC CARRIERS OF CIS-HYDROXY-L-PROLINE: POTENTIAL AGENTS FOR INHIBITING COLLAGEN ACCUMULATION. G.J. Poiani, D.J. Riley, K.F. Gean, J. Fox, H.C. Strong, and J. Kohn, UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ 08854.

Cis-hydroxy-L-proline (cHyp) is an L-amino acid that inhibits collagen accumulation in vivo but is toxic if administered over long periods of time. To circumvent this problem, we developed two polymeric carriers of cHyp and tested their bioactivity in vitro. We prepared poly(ethylene glycol) conjugates in which cHyp was attached to a polymeric backbone, poly(PEG-Lys), by the carboxylic groups of the lysyl residues. cHyp was bound to the PEG based carrier through a hydrolyzable ester linkage or a more stable amide bond. Biological activity was investigated by the ability of the polymeric cHyp derivatives to inhibit growth of cultured fibroblasts, an antifibrotic effect since cells grown on plastic require secretion of collagen to attach and grow. The polymeric cHyp derivatives were added to cultures at 75 μg/ml, a non-inhibiting concentration for free cHyp; growth at 6 days was inhibited by 39% (ester-linked conjugate) and 62% (amide-linked conjugate) compared to nonbioactive trans-Hyp in equimolar amounts. There was no inhibitory effect of poly(PEG-Lys) plus nonconjugated cHyp. Cells grown on a collagen substrate in the presence of these compounds were not inhibited, showing that growth inhibition was due to an antifibrotic effect. Cell viability was not affected. We conclude: (1) copolymers of cHyp are nontoxic to cells and have prolonged antifibrotic effects, (2) the antifibrotic effect requires conjugation of cHyp to poly(PEG-Lys), and (3) an equimolar concentration of the amide polymer has a greater inhibitory effect on fibroblast growth than the ester polymer. The more prolonged bioactivity of the amide polymer is probably due to the more stable amide bond producing gradual release of the agent from the polymeric backbone. This delivery system which releases cHyp slowly may improve the antifibrotic effect of cHyp in vivo by allowing targeting of the agent to sites of fibrosis and may reduce toxicity.

R 511 EXPRESSION OF HYALURONAN (HA) AND THE HA RECEPTOR RHAMM FOLLOWING BLEOMYCIN-INDUCED LUNG INJURY Rashmin C. Savani, Chao Wang, Robert Stern, Nasreen Khalil, Arnold Greenberg and Eva Turley. Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada.

In bleomycin-induced pulmonary fibrosis, the migration of macrophages and fibroblasts into areas of lung injury precedes the onset of fibrosis. The synthesis of hyaluronan (HA) is acutely increased at the time of macrophage infiltration after bleomycin. Hyaluronan has been implicated in cell migration, and RHAMM, an HA receptor, mediates HA-stimulated cell motility in macrophages and fibroblasts. The synthesis of HA and the expression of RHAMM are both regulated by transforming growth factor-ß (TGF-ß) in vitro and increased expression of TGF-ß in alveolar macrophages has been described following bleomycin injury. We have investigated the expression of HA and RHAMM following intratracheal instillation of bleomycin in rats. Rat lungs were harvested at various times following injury. Immunocytochemical localization of RHAMM and HA was acheived using an antibody specific for RHAMM and biotinylated aggrecan respectively. RHAMM was expressed constitutively in bronchiolar epithilium and smooth muscle and in resident alveolar macrophages, while HA was found in the extracellular matrix surrounding the bronchiolar smooth muscle. Two hours after injury, reduced staining for RHAMM in the alveolar macrophages and for HA around the smooth muscle was noted. RHAMM and HA staining was again detected by 24 hours, and at 4 days after bleomycin, both HA and RHAMM expression was significantly increased in macrophages accumulating in areas of injury. From 7 to 14 days after injury, RHAMM and HA were found in fibroblasts and in extracellular matrix in fibrotic areas. The increased expression of RHAMM in macrophages following injury was confirmed by immunoblot analysis of macrophages isolated by bronchoalveolar lavage. Using timelapse cinemicrography, the motility of isolated macrophages after bleomycin injury was measured, and an antibody monospecific for RHAMM was able to significantly inhibit this locomotion. We conclude that both HA and RHAMM expression is regulated following bleomycin lung injury and that the changes observed closely follow those described for TGF-ß. These results may be important in the mechanism of cell migration to areas of lung injury.

R 512 EXTRACELLULAR DOMAIN OF TRANSFORMING GROWTH FACTOR-ß (TGF-ß) RECEPTOR TYPE II ENCODES A SOLUBLE TGF-ß BINDING PROTEIN. Patricia Segarini, David Olsen, Desmond Mascarenhas, Pedro Carrillo, David K. Schmidt, Martha Scheer Moritz and James Dasch, Celtrix Pharmaceuticals, 3055 Patrick Henry Drive, Santa Clara, CA 95054-1815.

Transforming growth factor-ß (TGF-ß) is a potent regulator of extracellular matrix deposition and there are a number of fibroproliferative diseases that express high levels of TGF-ß including idiopathic pulmonary fibrosis, glomerulonephritis, liver cirrhosis and proliferative vitreoretinopathy. These findings have suggested that a strategy for the treatment of fibrotic diseases may be the development of high affinity antagonists that would bind excess TGF-ß. In at least one animal model, fibrotic disease has been suppressed by the addition of an antibody that binds TGF-ß1 (Border et al., Nature **346**: 371 - 374, 1990). The recent cloning and availability of the high affinity type II and type III TGF-ß receptor proteins (Lin et al., Cell **68**: 775 - 785, 1992; López-Cassillas et al., Cell **67**: 785 - 795, 1991; Wang et al., Cell **67**: 797 - 805, 1991) has made them ideal therapeutic candidates. In order to develop a receptor antagonist, we have initially sought to express the proteins in a soluble form. We have expressed the extracellular domain of the type II receptor in both bacterial and transient mammalian systems. This truncated receptor (sßRII) is a soluble protein and in a solution binding assay, sßRII is bound and crosslinked with ^{125}I-TGF-ß1. We find that COS produced sßRII binds TGF-ß1 but not TGF-ß2. Two antisera prepared against peptides of the carboxy-terminal region of sßRII have been characterized and identify the protein in COS cell supernatants and bacterial cell extracts. A ligand blotting assay that utilizes biotinylated TGF-ß1 or TGF-ß2 has also been developed for the detection of sßRII. Recently, the soluble forms of several cytokine receptors have been applied in disease models to reduce the level of circulating cytokine. The sßRII may be an effective antagonistic therapeutic for the treatment of disorders that are characterized by high levels of TGF-ß.

R 513 HYALURONIDASE IS A HYALURONAN-BINDING PROTEIN, AND IS PRESENT IN PREPARATIONS OF HYALURONAN. Gregory W. Tapper, Michael Stern, Albert Chang, Daniel Schwartz, and Robert Stern. Departments of Ophthalmology and Pathology, School of Medicine, and the Department of Oral and Maxillofacial Surgery, School of Dentistry, University of California at San Francisco.

Hyaluronidase is a hyaluronan-binding protein. Evidence for the presence of hyaluronidase was found in several commercial preparations of hyaluronan (HA). This enzyme was active at a neutral pH. Incubation of HA at neutral pH led to increasing degradation of the polymer in a time dependent manner, as documented by molecular sieve Sepharose CL-4B chromatography. Incubation at acid pH 3.8 did not lead to such degradation, suggesting that the substrate-associated enzyme was a neutral hydrolase. Heparin is a potent inhibitor of hyaluronidase. The presence of 100 u/ml of heparin during incubation prevented degradation of HA. Enzymatic activity was also eliminated by heat treatment. Preincubation of the HA preparation at 70° also prevented HA degradation. The binding of hyaluronidase to HA was confirmed by dot blot analysis, using rabbit polyclonal antisera obtained against purified porcine liver hyaluronidase. Hyaluronan is prominent in both adult and fetal wound healing. Naturally occuring HA-bound hyaluronidase, may be implicated in the modulation of HA in the course of wound healing. Supported by the Chartrand Foundation and by NIH, DHHS grant CA-iis44768.

R 514 ROLE OF INTERFERON ALPHA 2B IN HYPERTROPHIC SCARRING IN VITRO AND IN VIVO, E Tredget, H Shankowsky, Y Shen, B Medelec, C Dodd, P Scott, A Ghahary. Department of Surgery, University of Alberta, Edmonton, AB, Canada T6G 2B7.

Hypertrophic scarring (HTS) commonly occurring after thermal injury is a fibroproliferative disorder of the dermal matrix wherein components of the inflammatory process in wounds including the fibrotic growth factor, TGF-ß appear to activate dormant fibroblasts leading to cellular proliferation and excessive matrix synthesis.

To investigate the potential role and mechanism of interferon alpha 2b (IFN-alpha2B) in controlling excessive collagen production in HTS fibroblasts, dose response, timing of onset of action and duration of action was investigated in HTS fibroblasts *in vitro* and compared to site-matched normal fibroblast pairs obtained from 4 patients following burn injury. IFN-alpha2b reduced collagen protein synthesis and type I mRNA levels after treatment but the onset of action required approximately 72 hours before apparent in both HTS and normal fibroblasts. Significant reductions in collagen synthesis occurred in 4 other pairs of HTS and normal fibroblasts ($p<0.05$), as well as reductions in type I procollagen mRNA ($p<0.05$) and type III procollagen mRNA but to a lesser extent. HTS fibroblasts recovered completely from the effects of IFN-alpha2b on procollagen type I mRNA within 48h of cessation of treatment in contrast to normal fibroblasts where type I mRNA levels remained suppressed to 72h post-treatment.

Treatment of one burn patient with severe HTS using IFN subcutaneously over 6 mos resulted in clinical improvement in scar volume and scar assessment. Immunohistochemistry using anti-LC and CC TGF-ß antibodies demonstrated marked reductions in TGF-ß in the dermis of HTS coincident with alterations in the expression of type I and III collagen and the proteoglycans DS-PGI and DS-PGII. These data suggest that IFN-alpha2b may normalize collagen and connective tissue protein synthesis by HTS fibroblasts *in vitro* and *in vivo* possibly by a direct effect on HTS fibroblasts and by a reduction in TGF-ß expression in the dermal matrix of HTS.

R 515 ANTI-TGF-β1 ANTIBODIES SUPPRESS PATHOLOGICAL FIBROSIS IN EXPERIMENTAL ANIMALS

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TGF-β, a multifunctional cytokine, plays an important role in the regulation of many physiological and pathological processes. We have previuosly shown in TGF-β1 transfected cells (TIA) xenograft in a nude mouse model that levels of plasma TGF-β1 is substantially elevated 2 wk after TIA-inoculation in contrast to the absence in plasma of control mice. The present studies were carried out to investigate whether administration of anti-TGF-β1 antibodies attenuates a variety of pathological changes in different organs in nude mice inoculated with TIA cells. Histologically, TIA-inoculation caused fibrotic changes, being more pronounced in the liver and the lungs, but no significant fibrotic changes occurred in the kidneys, the heart and other organs. In addition, the red pulp of the spleen almost disappeared in company with extensive granulopoiesis in the region. Furthermore, injection of anti-TGF-β1 antibodies markedly prevented the development of pathological fibrosis in different organs in nude mice. To evaluate more directly the collagen deposition in different organs, the total hydroxyproline content were determined. After TIA-inoculation to animals, the hydroxyproline content of the lung, the liver, and the spleen was increased by 40% after 2 wk as compared with the control mice. This increase was nearly completely prevented by injection of anti-TGF-β1 antibody. Our results are consistent with a key role for TGF-β1 in at least some types of several organ fibrosis.

R 516 COLLAGEN EXPRESSION IN PLEURAL AND ALVEOLAR EPITHELIAL CELLS FROM RAT LUNGS

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Fibrous processes of the lung are characterized by an enhanced deposition of collagen type I and type III either in interstitial or intra-alveolar spaces. While lung fibroblasts, pericytes and smooth muscle cells are known to be the main sources for connective tissue proteins in interstitial lung fibrogenesis, lung epithelial cells may participate in collagen synthesis of post inflammatory alveolar fibrosis and in the development of mesotheliomas.

Material and Methods: For further investigations alveolar type II epithelial and mesothelial cells from rat lungs were isolated either after elastase digestion of the alveolar structure or by treatment with a collagenase-dispase solution of the pleura surface. Isolated cells were cultured in DMEM, 10% FCS, in tissue culture flasks at 37°C in a H_2O saturated atmosphere containing 5% CO_2. The cells were characterized by phase contrast microscopy and typified using antibodies against α-SMC actin, desmin, vimentin, cytokeratins 1-8, 18, and 19, macrophages, and factor VIII associated antigen. Synthesis of surfactant proteins B and C could be demonstrated by in situ hybridization using cDNAs for SP-B and SP-C (gift of Dr. J.C. Clark, Cincinnati, Ohio). The presence of collagen types I and III was visualized by indirect immunofluorescence while the expression of collagen-mRNA was observed using cDNAs for the α-1(I) chain and the α-1(III) chain (gift of by Dr.Th. Krieg, Köln, Germany) for non-isotopic in situ hybridization.

Results and Discussion: Type II and mesothelial cells showed typical epithelial growth patterns when confluent. After immunomarkation collagen types I and III were present in both cell types. In situ hybridization revealed a higher proportion of type I collagen regarding the number of granules/cell. But in comparison with mesothelial cells, the expression of collagen type I was very weak in type II cells indicating that pulmonary epithelial cells are different from each other although one of their main matrix proteins obviously is collagen type I.

Late Abstract

LOCALIZED PRODUCTION OF PAI-1 mRNA IN MOUSE SKIN AFTER STIMULATION WITH THE TUMOR PROMOTER PHORBOL MYRISTATE ACETATE.

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In vitro, a variety of hormones, tumor promoters and cytokines have been shown to regulate the expression of type-1 plasminogen activator inhibitor (PAI-1) in a number of different cell lines. Since cultured cells are not necessarily representative of the cells in the intact organism from which they are derived, respecting the production of the different components of the plasminogen activation system, we have treated intact mouse skin with the tumor promoter Phorbol myristate acetate (PMA). After 5 hrs of PMA exposure analysis by in situ hybridizations have shown that cells in the dermis synthesize uPA and PAI-1 mRNA. After 48 hrs of PMA treatment PAI-1 is also expressed by the basal layer of the migrating keratinocytes as in wound healing. Biochemical control experiments by PCR analysis and Northern blotting showed the presence of both uPA and PAI-1 mRNA in RNA samples isolated from the mouse skin. These results suggest that PAI-1 mRNA synthesis is a fast response to PMA treatment also in vivo, and that the production is restricted to as yet unknown cell types in the dermis. The biological importance of the presence of PAI-1 in the dermis in unkown.