WOUND REPAIR

Organizers: Richard Clark, Michael Pierschbacher and Michael Sporn

April 1-7, 1991

Plenary Sessions Page

Poster Sessions

Keynote Addresses (joint with FGF meeting)

Q 001 TRANSFORMING GROWTH FACTOR-ß (TGF-ß) AND REPAIR OF TISSUE INJURY. Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892.

The three principal isoforms of TGF-ß are essentially ubiquitous molecules that play vital roles in embryogenesis and morphogenesis. Since repair of tissue injury involves processes that recapitulate embryonic development, it is not surprising that TGF- ß also is of major significance for response to injury as well. We will briefly summarize the relevant molecular and cellular biology that relates to both wound repair and formation of bone and cartilage. In addition, we will consider new applications of TGF-ß, such as the repair of cardiac injury. Finally, we will summarize new data obtained in both experimental animals and in man, indicating that TGF-ß will be an important therapeutic agent in the clinical arena.

References:
M.B. Sporn and A.B. Roberts. Transforming Growth Factor-ß: Multiple Actions and Potential Clinical Applications. JAMA 262: 938-941 (1989)

A.B. Roberts and M.B. Sporn. The Transforming Growth Factors-ß. In: Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors, ed. M.B. Sporn and A.B. Roberts, Heidelberg, Springer-Verlag, vol. 95/1, pp. 419-472 (1990)

FGF Structure and Function (joint with FGF meeting)

Q 002 RECOMBINANT EXPRESSION AND WOUND HEALING ACTIVITY OF bFGF. Judith A. Abraham, Stewart A. Thompson, Corine K. Klingbeil, and John C. Fiddes, California Biotechnology Inc., Mountain View, CA 94043.

We have recombinantly expressed the 155-residue form of basic fibroblast growth factor (bFGF) in E. coli. Basic FGF contains four cysteine residues, which lie at positions 34, 78, 96, and 101 in the 155-residue form of the protein. We and others have found that, in the bacterial recombinant bFGF, the cysteines at positions 78 and 96 can become involved in bFGF multimer formation, which apparently occurs through the generation of intermolecular disulfide bonds. Pituitary-derived bFGF, however, has not been observed to multimerize under similar conditions. To determine the source of this difference in behavior, we have conducted structural analyses of the natural and recombinant proteins. Our results indicate that in pituitary-derived bFGF, the cysteines at positions 78 and 96 are not available for multimer formation because each is disulfide-bonded to a glutathione molecule. The glutathione modification did not appear to be present when the pituitary bFGF was purified in the presence of N-ethylmaleimide (to prevent thiol oxidations or exchanges), indicating that the glutathione addition is occurring during the process of purification.

In order to assess bFGF as a wound-healing agent, we have examined the effects of topically-applied recombinant bFGF in a variety of animal models. While the bFGF appeared to increase the rate of wound healing in all models tested, the most significant increases were seen in healing impaired situations, such as in the healing of full-thickness wounds in genetically obese (ob/ob) and diabetic (db/db) mice. These results have suggested that the therapeutic usefulness of bFGF for soft-tissue healing may lie in the treatment of healing-impaired wounds such as pressure sores and diabetic ulcers.

Q 003 STRUCTURE AND FUNCTION OF ACIDIC FIBROBLAST GROWTH FACTOR,

Kenneth A. Thomas, Sagrario Ortega, Denis Soderman, Marie-Therese Schaeffer, Jerry DiSalvo, Anthony Capetandes, Theodore Mellin, Robert Mennie, John Ronan, Doreen Cashen, Thorir D. Bjornsson, Maciej Dryjski, and John Tluczek, Dept. of Biochemistry, Merck Research Laboratories, Rahway, NJ 07065

Acidic fibroblast growth factor (aFGF) contains 3 cysteine residues, two of which are conserved not only in all sequenced aFGFs but also among all known FGF family members. Conservation of Cys residues is typically the result of their requirement for either enzyme catalysis or disulfide bond stabilization of an active protein conformation. In mitogenically active aFGF, however, intramolecular disulfide bonds are neither required, present nor tolerated. Mutants in which any two or all three Cys residues are converted to serines and, therefore, can not form an intramolecular disulfide bond, are substantially more stable and less heparin dependent that the wild-type protein and mutants retaining any two Cys residues. The rapid inactivation of aFGF in culture appears to be the direct result of spontaneous oxidation, a process inhibited by complexation with heparin. Oxidation could also contribute to the inactivation of aFGF released from cells *in vivo* thereby mediating a paracrine constraint on activity by limiting its half-life and diffusional range.

Acidic FGF has therapeutically relevant activities in animal models. Topical application of the mitogen enhances repair of full-thickness dermal wounds by promoting angiogenesis followed by an increase in the volume of granulation tissue. Intravenously delivered aFGF promotes vascular endothelial repair with concomitant inhibition of myointimal thickening. Rapid inactivation of aFGF in the absence of heparin could enhance the window between efficacious responses and undesirable side effects resulting from widespread bioavailability.

PDGF

Q 004 REGULATION AND DISTRIBUTION OF PDGF AND PDGF RECEPTORS IN NORMAL HUMAN WOUND HEALING,

Hart C. E., *LaVallie C., *DeWan P., Downey B., *Bensch P., *Kindsvogel W., #Usui M., #Olerud J., *ZymoGenetics, Seattle, WA 98105, #University of Washington, Division of Dermatology.

Platelet-derived growth factor (PDGF) has been implicated as a critical factor in the normal wound healing process. This is suggested by the ability of PDGF to stimulate replication of fibroblasts and smooth muscle cells in culture, and act as a chemoattractant for fibroblasts, smooth muscle cells, neutrophils and monocytes. Additionally, in healing impaired animals, PDGF added topically to wounds has been shown to stimulate wound healing when evaluated both for wound closure rates and stimulation of granulation tissue formation. The complexity of the biological activities stimulated by the three forms of PDGF (AA, AB, BB) has been increased by the recent identification of two PDGF receptor molecules, which appear to dimerize to form three receptor phenotypes, and which have variable ligand-binding specifities. The responsiveness of any cell type to the three forms of PDGF appears to be determined by the ratio and absolute numbers of the two PDGF receptor subunits. Thus, when evaluating the biological activities of PDGF *in vivo*, it is critical to understand the expression of both the ligand and the receptor molecules.

To understand the role of PDGF in normal wound healing, we have begun investigating the distribution of the A and B-chains of PDGF and both of the PDGF receptor molecules in standardized human wounds at various times in the course of healing. The results of these studies show differential expression of the ligands and receptors, both with respect to spatial distribution within the wound and the extent of wound healing that has occurred.

As a potential antagonist to investigate the *in vivo* activities of PDGF, we have developed an expression system for the production of soluble forms of the PDGF receptor. Based upon the model that receptor dimers/multimers are required to generate high affinity ligand-binding sites, we have produced PDGF receptor/immunoglobulin fusion proteins. The proteins contain the ectodomain of either the PDGF alpha or beta receptor, and the constant domain of either human Ig heavy-chain or Ig light-chain. When expressed in a mouse myeloma cell line, these molecules associate via the Ig domains to form multimers analogous to native IgG. These soluble PDGF receptors have binding affinity and specificity equivalent to that of native membrane bound receptor.

Q 005 THE BIOLOGY OF PLATELET-DERIVED GROWTH FACTOR AND ITS RECEPTORS, Bengt Westermark and Carl-Henrik Heldin, Department of Pathology, University Hospital, S-751 85 Uppsala, and Ludwig Institute for Cancer Research, Biomedical Center, S-751 24 Uppsala, Sweden

PDGF is a 30 kDa protein made up as dimers of A and B chains. The three possible isoforms of PDGF (AA, AB and BB) have been isolated from natural sources, viz. platelets or conditioned media of human tumor cell lines. The PDGF isoforms bind with different affinities and specificities two cell surface receptors such that the alfa-receptor binds all three isoforms with high affinity and the beta-receptor binds PDGF-BB with high affinity, PDGF-AB with a tenfold lower affinity and does not bind PDGF-AA. A role for autocrine and paracrine responses to PDGF in development is suggested by the finding that PDGF receptors are present on placental cytotrophoblasts (that also synthesize PDGF) and O-2A glial progenitor cells (that respond to PDGF-AA produced by type 1 astrocytes). More recently, analyses by immunohistrochemistry using PDGF beta-receptor-specific antibodies, have revealed PDGF receptors on neuronal cells of newborn rat brains. Ligand-binding experiments on cultured rat neurons showed high affinity binding of ^{125}I-PDGF-BB only. PDGF-BB, but not PDGF-AA, added to such cultures led to a rapid increase in the synthesis of c-*fos* protein. These findings suggest a novel role for PDGF in the neuronal compartment of the central nervous system.

The paracrine growth response to PDGF was studied by co-culture experiments in which single CHO cells expressing recombinant PDGF were grown among mass cultures of human foreskin fibroblasts or Swiss 3T3 cells. PDGF-BB elicited a marked paracrine growth response, visible as focal growth of fibroblasts around the CHO-cells. PDGF-AA secreting cells had no such effect. The paracrine growth response was probably attributable to secreted PDGF that was deposited around the cells. Experiments with ^{125}I-labeled ligands showed that PDGF-BB, but not PDGF-AA, becomes rapidly and efficiently adsorbed to solid substrata, including extracellular matrix. These results imply a difference in the paracrine function of the different isoforms of PDGF. PDGF-AA may be designed to be freely diffusible in the extracellular space, whereas PDGF-BB may be deposited locally to have a short range activity.

TGF-Beta (joint with FGF meeting)

Q 006 MATRIX AND TRANSFORMING GROWTH FACTOR-ß1 INTERACTION IN DISEASE. Wayne A. Border,[1] Seiya Okuda,[1] Takamichi Nakamura,[1] Tatsuo Yamamoto,[1] Nancy A. Noble[1] and Erkki Ruoslahti,[2] Division of Nephrology, University of Utah School of Medicine, Salt Lake City, UT 84132[1] and La Jolla Cancer Research Foundation, 10901 N. Torrey Pines Rd., La Jolla, CA 92037.[2]

Glomerulonephritis is an inflammation of the kidney that is characterized by the accumulation of extracellular matrix within the damaged glomeruli. We have shown that transforming growth factor-ß1 (TGF-ß1) is unique in regulating the production of proteoglycans and matrix glycoproteins by glomerular cells in vitro (1,2). In an experimental model of glomerulonephritis in rats we found increased proteoglycan and fibronectin synthesis by cultured nephritic glomeruli, which was greatly reduced by addition of antiserum to TGF-ß1 (3). Conditioned media from glomerular cultures, when added to normal cultured mesangial cells, induced elevated proteoglycan synthesis. The stimulatory activity of the conditioned media was blocked by addition of TGF-ß1 antiserum. Glomerular histology showed mesangial matrix expansion in a time course that roughly paralleled the elevated proteoglycan synthesis by the nephritic glomeruli. At the same time there was an increased expression of TGF-ß1 mRNA and TGF-ß1 protein in the glomeruli. Administration of anti-TGF-ß1 at the time of induction of glomerulonephritis suppressed the elevated extracellular matrix production and dramatically attenuated histologic manifestations of the disease .

Subsequent studies in the glomerulonephritis model have focused on TGF-ß and matrix interaction. One of the proteoglycans regulated by TGF-ß, decorin, has been shown to be incorporated into the matrix and bind TGF-ß through its core protein. Addition of the decorin core protein or the intact proteoglycan to TGF-ß shows a strong ability to neutralize the biological action of TGF-ß. This suggests a normal control mechanism for TGF-ß activity. Additional experiments have analyzed the glomerulus to see if matrix molecules are preferentially expressed in "wound" type formation of extracellular matrix. Early results show increased deposition of tenascin and fibronectin alternatively spliced variants in the pathologic matrix, consistent with a wound healing response.

1. *Nakamura* T, Okuda S, Miller D, Ruoslahti E, Border W: Kidney Int 37:221, 1990.
2. Okuda S, Languino LR, Ruoslahti E, Border WA: J Clin Invest 86:453-462, 1990.
3. Border WA, Okuda S, Languino L, Sporn MB, Ruoslahti E: Nature 346:371-374, 1990.

Q 007 DIFFERENTIAL CONTROL OF EXPRESSION OF TGF-β'S 1, 2, and 3, Anita B. Roberts, Seong-Jin Kim, Adam Glick, Robert Lafyatis, Andrew Geiser, Michael O'Reilly, David Danielpour, and Michael B. Sporn, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892
Three distinct isoforms of TGF-β are expressed in mammalian tissues. Although the biological effects of the mature forms of each of these isoforms are usually indistinguishable *in vitro*, their patterns of expression are often distinct as seen in embryonic development and in response to treatment with members of the steroid/ retinoid family of compounds. As an example, retinoic acid is a potent inducer of TGF-β2 expression *in vitro* in cultured mouse keratinocytes and of TGF-β2 and 3 expression in a variety of epithelia in retinoid-deficient rats *in vivo*; in contrast, the steroid tamoxifen selectively increases expression of TGF-β1 in primary human fibroblasts. The 5' flanking sequences of each of these 3 genes have now been cloned and characterized with respect to known transcription factor binding sites. Major differences are that genes for TGF-β's 2 and 3, but not TGF-β1, contain cyclic AMP-response elements (CRE) with initiation of transcription just downstream of TATAA boxes. In contrast, the TGF-β1 gene lacks TATAA boxes and contains binding sites for the AP-1 Jun/Fos complex which mediate its autoregulation. The activity of the TGF-β1 promoter is also upregulated by several other oncogenes including *ras*, *src*, and *abl*, as well as by the product of the retinoblastoma gene, Rb, and by the *trans*activator of the HTLV-1 virus, Tax. Reciprocal induction by TGF-β of the c-*jun* and *tax* genes suggests that complex interrelated regulatory loops function to prolong the induction of TGF-β1 expression. Further evidence for distinct regulation of the promoters of the TGF-β isoforms has come from recent studies of the expression of the TGF-β's accompanying fusion of myoblasts into myotubes; expression of TGF-β3 mRNA is selectively upregulated as the cells differentiate. This effect has been mapped to a distinct upstream region in the TGF-β3 promoter. Further study of such defined, differentiating systems should enhance our understanding of both the regulation of expression as well as the function of the TGF-β isoforms.

For a review see:

A.B. Roberts *et al.*, Multiple forms of TGF-β: distinct promoters and differential expression, in Ciba Foundation Symposium 157, Clinical Applications of TGF-β (ed. G.R. Bock and J. Marsh), John Wiley & Sons Ltd., West Sussex, England, 1991.

Q 008 THE FAMILY OF TGF-ß RECEPTORS, Patricia Segarini, Jill Ziman and James Dasch, Celtrix Laboratories, 2500 Faber Place, Palo Alto, CA 94303
TGF-ß binds specifically and with high affinity to all cell types with few exceptions. Dissociation constants range from 1 - 100 pM and the number of receptors varies with the cell type from 200 to 100,000 receptors per cell. Several cell surface proteins that specifically bind TGF-ß1 and TGF-ß2 have been characterized as putative receptors; these proteins have been classified on the basis of their size as type I, II or III receptors. The most abundant of these proteins, the type III receptor, has been renamed betaglycan (1). Betaglycan is a membrane-bound proteoglycan (2) that is a dimer of subunits with M_r of approximately 250,000. This protein is plentiful on primary cells of mesenchymal origin but absent on primary epithelial and endothelial cells. It does not appear to be associated with TGF-ß-mediated cell responses and its function remains unknown. Type I and type II receptors are 50,000 M_r and 80,000 M_r, respectively, and contain N-linked carbohydrates. TGF-ß-mediated responses appear to be transmitted through one or both of these proteins. Recent binding data on confluent monolayers of rat skeletal myoblasts of the L6 cell line indicates that the type I and type II receptors may interact during ligand binding. We were able to visualize apparent cooperativity because TGF-ß2 binds with low affinity to the type II receptor. Saturation of the the type I receptor with native TGF-ß2 induces a 6 fold increase in binding of trace quantities of radiolabeled TGF-ß1 at the type II protein. The same results were observed when TGF-ß3 was used to saturate the type I receptor. No induction of type II receptor binding was observed on subconfluent cells indicating a density-dependent phenomenon. These data suggest that ligand binding to the type I receptor induces either a change in the type II receptor number or promotes a conformational change that increases its ability to bind TGF-ß1. Such changes in the binding state of the type II receptor may be indicative of a regulatory role that is activated by the phase of cell growth or differentiation.

1. Andres, J. L. et al. (1989) J. Cell Biol. **109**: 3137 - 3145.
2. Segarini, P. R., and Seyedin, S. M. (1988) J. Biol. Chem. **263**: 8366 - 8370.

TGF-Alpha/EGF

Q 009 REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR, Gordon N. Gill, Department Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, La Jolla, California 92093-0650

Because appropriate signaling depends on the concentration of growth factor receptors, several mechanisms operate to control cell surface receptors available for ligand activation. The EGF receptor is highly regulated by both post-transcriptional and transcriptional mechanisms. At a post-transcriptional level, attenuation of ligand-induced signaling from the cell surface occurs by occupancy-induced internalization and down-regulation of receptors. Occupied kinase-active EGF receptors are internalized through a high affinity, saturable endocytic system at rates up to 10-fold faster than empty receptors or than ligand-occupied kinase-inactive receptors. Ligand binding, tyrosine kinase activity, and sequences in the C' terminus increase the affinity of the receptor for coated pits to result in down-regulation. Signaling from the cell surface is also attenuated by a feedback loop in which activated protein kinase C phosphorylates the EGF receptor at Thr^{654}.

At a transcriptional level, activity of the promoter of the EGF receptor gene is increased in response to treatment of cells with EGF, cAMP, and TPA. Although inactive alone, TGF-β, and bradykinin each enhance TPA-induced promoter activity through distinct mechanisms. Ligand-activated thyroid hormone (T_3) and retinoic acid (RA) receptors inhibit EGF receptor promoter activity. Within the EGF receptor 5' region a 36 bp core G+C-rich promoter'enhancer has been identified. Multiple regulatory pathways converge at this core. T_3 and RA receptors and c-*jun* bind here. T_3 receptor binding is strikingly enhanced by a nuclear protein but is inhibited by c-*jun*. Transcriptional regulation of EGF receptor expression thus depends on interactions among proteins that act via the core element of a G+C-rich promoter.

Re-Epithelialization

Q 010 KERATINOCYTE ACTIVATION AND CHANGES IN CELL ADHESION AND INTEGRIN RECEPTOR EXPRESSION, Frederick Grinnell, Department of Cell Biology and Neuroscience, UT Southwestern Medical School, Dallas, Texas 75235

Keratinocytes in normal skin are specialized for differentiation. In wounded skin, they are specialized for migration. The adhesion functions of keratinocytes are activated during wounding and in cell culture. Human keratinocytes freshly harvested from skin were found to attach to collagen type I or basement membrane substrata but not to fibronectin (FN). Cells that attached to collagen type I or basement membrane could not spread, form focal contacts, or migrate. On the other hand, after cell culture for 4-7 days, keratinocytes attached to FN as well as to other substrata. Moreover, these cells could spread, form focal contacts, and migrate. Based on immunostaining for BP-antigen, the activated cells were basal keratinocytes. To learn if activation depended on loss of contact of basal keratinocytes with basement membrane, we compared the extent of activation of cells cultured on dermis with cells cultured on plastic. On dermis, differentiation was favored compared to activation. We analyzed the possible role of integrin adhesion receptors in keratinocyte activation. In adult human epidermis, integrin $\alpha 5$ subunits were absent, but these receptor subunits were prominent in keratinocytes migrating out of skin explants in culture. Also, immunoprecipitation experiments with polyclonal anti-FN receptor antibodies showed that FN receptors were expressed in cultured but not freshly isolated keratinocytes. In adult human epidermis, integrin $\beta 1$ subunits were found mostly between lateral cell borders of basal keratinocytes rather than at the epidermal-dermal interface. In keratinocytes that migrated out of skin explants, on the other hand, $\beta 1$ integrin subunits were found around and beneath the migrating cells. Also, $\beta 1$ integrin subunits were organized in focal adhesions by cultured keratinocytes. In immunoprecipitation experiments, we found that freshly isolated keratinocytes synthesized mostly immature $\beta 1$ subunits, whereas cultured keratinocytes synthesized mostly mature $\beta 1$ subunits. Based on these results, we suggest that activation of keratinocyte adhesion to fibronectin can be explained by increased expression of $\alpha 5\beta 1$ integrin receptors and that up-regulation of spreading, focal adhesion formation, and migration depends on changes in the processing of pre-$\beta 1$ subunits to mature $\beta 1$ subunits.

Q 011 HUMAN KERATINOCYTE LOMOTION, David T. Woodley, Yves Sarret, Department of Dermatology, Stanford University School of Medicine, Stanford, CA 94305

Re-epithelialization is a critical event in wound closure and involves the migration of keratinocytes. In order to directly assess human keratinocyte locomotion, we have used phagokinetic and immunofluorescent assays that employ computer-assisted image analysis and quantitate locomotion as the percentage of observed field area consumed by cellular tracks, a so-called Migration Index (MI). Human keratinocytes apposed to plastic, albumin, type V collagen or heparan sulfate proteoglycan migrated very little (MI = < 5%). In contrast, keratinocytes apposed to fibronectin or collagens I and IV exhibited high levels of motility (MI = 18 - 35%). Laminin promoted cellular growth but inhibited keratinocyte locomotion on plastic or collagen in a dose-dependent manner. This effect resided within a specific peptide sequence of the laminin A chain CSIKVAVS-NH_2. Keratinocyte migration on collagen was unaltered in the presence of TGF-Beta or basic FGF (which respectively decreased and increased thymidine incorporation into the cells) suggesting that the mechanisms of cellular locomotion and proliferation are independent. When apposed to suboptimal amounts of collagen, recombinant human EGF and bovine pituitary extract, both mitogens, had a modest stimulatory effect on keratinocyte migration. Although extracellular matrix has profound influences upon direct keratinocyte migration, to date, we have found minimal effects with most growth factors and cytokines.

Fibroplasia/Matrix Remodeling

Q 012 MODULATION OF FIBROBLASTIC CELL PHENOTYPIC FEATURES DURING NORMAL AND PATHOLOGICAL WOUND HEALING, Giulio Gabbiani, Department of Pathology, University of Geneva, Centre médical universitaire, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland

During the healing of an open wound, a proportion of granulation tissue fibroblasts acquire contractile features including the expression of α-smooth muscle actin, the actin isoform typical of smooth muscle. These features become more permanent in hypertrophic scars, fibromatosis and different types of parenchymal fibrotic changes, as well as in the stroma reaction to epithelial tumors. We have studied the conditions influencing the expression of α-smooth muscle actin in fibroblastic cells, both in vivo and in vitro. In vivo, the subcutaneous application of Granulocyte Macrophage Colony Stimulating Factor by means of an osmotic pump induces the accumulation of fibroblastic cells expressing α-smooth muscle actin. Accumulation of fibroblastic cells without expression of α-smooth muscle actin is produced by Platelet-Derived Growth Factor, Tumor Growth Factor-β and Interleukin-1. In vitro, the different fibroblastic populations studied (including cloned populations) show allways a proportion of α-smooth muscle actin expressing cells in standard cultured conditions. The number of these cells and the quantity of α-smooth muscle actin are characteristically reduced by γ-interferon and increased by heparin. We conclude that α-smooth muscle actin is a marker of contractile fibroblasts (myofibroblasts) and that cellular factors, as well as extracellular components, may modulate the expression of this protein in fibroblastic cells and thus participate in the development and evolution of normal and pathological fibrotic reactions. (Supported by the Swiss National Science Foundation, Grant nr 3.108-0.88).

Q 013 REGULATION OF COLLAGEN METABOLISM DURING WOUND HEALING
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Collagen metabolism is closely controlled during repair processes. This is achieved by the interaction of fibroblasts with the surrounding extracellular matrix. In addition, various cytokines are released, which influence collagen gene expression and control the activity of collagenases. In order to obtain direct information about collagen gene expression in vivo we used in situ hybridization to detect collagen I, III and VI mRNA containing cells. In addition fibroblasts were studied in three-dimensional collagen lattices in vitro and the influence of TNF-α, TGF-β and IFN-g were investigated. These studies demonstrate that collagen I and III is pretranslationally induced following tissue injury. After a few days, however, synthesis of collagen is down regulated. This is probably due to a close contact of fibroblasts with the newly synthesized extracellular matrix as demonstrated in vitro using collagen lattices. In addition a combined activity of TNF-α and IFN-g dramatically decreased collagen gene expression. These cytokines also affect gene expression of the α_3(VI) chain whereas α_1 and α_2(VI) chain mRNA are not altered. Since collagen VI, however, requires all three chains for building up a stable molecule the α_3(VI)- chain is rate limiting. The cytokines IFN-g and TNF-α reveal also other activities and not only reduce collagen production but can interfere with the deposition of collagen VI and other connective tissue proteins in the extracellular space. These studies indicate a very complex control of connective tissue metabolism during early and late phases of tissue repair.

Regulation of Endothelial Cell Growth (joint with FGF meeting)

Q 014 ENDOTHELIAL CELL-MURAL CELL INTERACTIONS IN VASCULAR GROWTH CONTROL, Patricia A. D'Amore and Kim B. Saunders, Childrens Hospital and Harvard Medical School, Boston, MA 02115

Endothelial cells make frequent contact with mural cells (smooth muscle cells or pericytes) throughout the vasculature. Ultrastructural studies reveal the existence of fenestrations in the internal elastic lamina and discontinuities in the basement membrane at the capillary level through which endothelial cells and mural cells contact one another. Previous studies from our laboratory have shown that coculture of endothelial cells with mural cells leads to growth inhibition of the endothelium in a contact-dependent process which involves the generation of activated TGF-B, a potent inhibitor of endothelial proliferation. In investigating the nature of this intercellular interaction we have documented the presence of functional gap junctions using either electron probe microanalysis or dye transfer in conjunction with fluorescent activated cell sorting. We speculate that the presence of the pericyte or smooth muscle cell is important in maintaining the differentiated phenotype of the endothelial cell and that the observed growth inhibition is simply a reflection of this differentiated state. To test this hypothesis we have developed a new coculture chamber that permits contact between cocultured endothelial cells and mural cells while maintaining pure cell populations. In this coculture chamber, cells are grown on opposite sides of a porous polycarbonate membrane which is supported by concentric plastic rings. Ultrastructural analysis reveals that the cells can extend processes into the pores allowing intercellular contact. The fact that the two cell populations are kept separate allows examination of the effects of intercellular contact on gene and protein expression in each cell type. Preliminary data suggest that contact with mural cells can induce changes in the profile of endothelial extracellular matrix proteins. In addition, studies using this coculture chamber as well as an under agarose assay of migration indicate a reciprocal effect of endothelial cells on smooth muscle cells and suggest that endothelial cells elaborate a soluble factor which acts as a mural cell chemoactractant. Thus, we speculate that growing endothelial cells release a factor(s) which acts to recruit mural cells. Once the mural cell has arrived at the growing blood vessel, specific contacts are established, gap junctions are formed between the two cells types and activated TGF-B is generated. These intercellular interactions and alterations in the vessel microenvironment may then lead to the expression of a differentiated endothelial phenotype including dramatically reduced turnover time and the expression of tissue-specific characteristics. (Supported by EY05318 and CA45548)

Q 015 WOUND HEALING ANGIOGENESIS: THE ROLE OF PLATELET GROWTH FACTORS IN WOUND HEALING ANGIOGENESIS. D.R.Knighton, V.D. Fiegel, and G.D.Phillips. Dept. of Surgery, Univ. of Minnesota, Mpls, MN,55455.

Studies on the chronology and morphology of wound healing angiogenesis (WHA) were performed using collagen sponges implanted subcutaneously in rat hind limbs. Vascular casts were made and viewed with scanning EM. WHA was found to occur only from venules with the first sprouts appearing at 48 hours. Sponges implanted with the products of platelet alpha granule Procuren (PDWHF) showed a markedly enhanced capillary density over control sponges. To study this in vitro, cultures of wound capillary endothelial cell (WCEC) were made from implanted sponges. These cells were positive for acetylated LDL uptake, factor VIII, and angiogenesis converting enzyme. Proliferation, chemotaxis, plasminogen activator production, and tube formation in response to endotoxin free recombinant PDGF-AB, AA, BB, and TGF-β were carried out. The ability of these platelet growth factors to stimulate angiogenesis was assayed in vivo using the rabbit corneal angiogenesis assay.

Our results demonstrated that TGF-β induces intense monocyte migration into the cornea in a dose dependent fashion which was followed by a maximal angiogenesis response. This indirect angiogenesis was totally blocked by local injection of methyl prednisolone at the time of TGF-β implantation. Injection 48 hours after implantation failed to retard the monocyte response and subsequent angiogenesis. TGF-β inhibited WHEC proliferation, had no effect on migration, and induced tube formation. PDGF-BB is a patent chemoattractant for WCEC, but does not induce direct angiogenesis at low doses ranging from 50-100 ng/implant and stimulates indirect angiogenesis at higher doses (250-500 ng). WCEC have 30-35,000 high affinity receptors/cell for PDGF-BB and competition studies show that most of the chemoattractant activity in PDWHF is due to PDGF-BB

Q 016 EXTRACELLULAR ACTIVITIES OF BFGF AND TGF-β, Daniel B. Rifkin, Department of Cell Biology, New York University Medical Center, New York, New York 10016. bFGF and TGF-β are both produced by endothelial cells and have profound effects on the endothelial cell phenotype. In most situations bFGF is a positive regulator, while TGF-β is a negative regulator. However, assumptions concerning the extracellular activity of these two growth factors must account for the fact that bFGF is a cytoplasmic protein and is not secreted and that TGF-β is secreted in a latent form (LTGF-β). In the case of bFGF, while neutralizing antibodies are able to effect the cell phenotype, it has never been clear that extracellular bFGF was not derived from dead cells. To answer this question we have performed assays under conditions in which only a single cell is present to exclude contributions from other cells. We have found that the migration of a single cell is affected by antibodies to bFGF. Since only one cell is present, this result indicates true extracellular autocrine activity of bFGF. The activation of LTGF-β appears to be a surface-mediated, proteolytic reaction involving cell surface urokinase, plasminogen, and one or more LTGF-β binding proteins. One of these is the mannose-6-phosphate/IGF-II receptor. This receptor binds LTGF-β but not TGF-β and may serve to increase the surface concentration of LTGF-β. These reactions as well as other steps in the activation of LTGF-β will be discussed.

Bone and Cartilage Repair

Q 017 COLLAGEN-CALCIUM PHOSPHATE CERAMIC-AUTOGENOUS BONE MARROW GRAFT FOR ORTHOPEDIC REPAIR: A SUMMARY, Karl A. Piez, Collagen Corporation, 1850 Embarcadero Road, Palo Alto, CA 94303. Purified reconstituted fibrillar collagen (FC), prepared from bovine dermis, and calcium phosphate ceramic (CPC), consisting of hydroxyapatite and tricalcium phosphate (65/35), are known to be biocompatible. A mixture of approximately equal parts by weight of 6.5% FC in phosphate-buffered saline, pH 7.4, and CPC in porous particulate form, 0.5-1.0 mm, was used as a bone graft substitute. It was mixed with 20% by volume of autogenous bone marrow just prior to use. The marrow was taken from a convenient site, usually the iliac crest, with a needle and syringe. The FC-CPC-marrow bone graft substitute was tested in two dog models. In one (Grundel, R.E. and Chapman, M.W., in preparation), a 2.5 cm segment was surgically removed from the mid-diaphysis of one ulna. Alignment of the ends was maintained with an intramedullary pin. Bone graft substitute was placed in the defect. The other forelimb was not operated for later use as a control. Animals were x-rayed periodically and sacrificed at 24 weeks. The experimental and control ulnae were removed and tested mechanically by rotational stress to failure and then sectioned for microradiography and histology. In the second dog model (Black, R.J. and Zardiackas, L.D., in preparation), a wedge-shaped segment of bone was surgically removed from one femur and the defect was plated to allow weight-bearing. The plates were removed at 6 months and the animals were sacrificed at 1 year. Bone grafting and testing procedures were similar in both models. In both studies, all animals receiving the bone graft substitute showed callus at 4-8 weeks and went on to union by 8-24 weeks by x-ray criteria. Mechanical testing showed that the healed bones were at least as strong as unoperated control bones. Histology and microradiography showed that the new bone was largely mature but still undergoing remodelling. The FC was entirely gone. The CPC particles were firmly embedded in new bone without evidence of a soft tissue interface or decrease in bone quality. In a controlled clinical trial conducted at 19 centers, 336 traumatic fractures (289 patients) were treated with either autogenous bone graft (positive control) or the bone graft substitute. At 1 year, the groups did not differ significantly in efficacy or safety except that the patients receiving bone graft substitute had a lower incidence of infection and were in surgery for a shorter time. These differences can be explained by the fact that a second surgical site to obtain autogenous bone graft was not necessary. Thus, FC-CPC-marrow is expected to have major medical value in orthopedic surgery.

Q 018 CELLULAR APPROACHES TO THERAPY FOR THE PREVENTION AND TREATMENT OF OSTEOPOROSIS. G.A. Rodan, Bone Biol./Osteoporosis, Merck Sharp & Dohme Res. Labs., West Point, PA 19486 Osteoporosis is a reduction in bone mass, which increases the risk of fractures. It results from an imbalance between bone resorption (destruction) and formation, in a tissue which turns over continuously to fulfill its dual function of mechanical support and ion (calcium) homeostasis. Bone resorption is carried out by osteoclasts, bone formation by osteoblasts, and strategies for preventing and/or treating osteoporosis are aimed at controlling the activity of these specialized cells. Osteoclasts are large, multinucleated cells of hematogenous origin, related to the macrophage lineage. Their generation from bone marrow precursors requires M-CSF, is enhanced by interleukin-1, TNFα, PGE_2, $1,25(OH)_2D_3$, PTH, and is inhibited by TGFβ (above 1 nM) and INF ɣ. Key steps in the action of mature osteoclasts include: attachment to bone matrix, apparently via αv/β3 integrins; acidification of an extracellular lysosomal compartment; and proteolytic digestion of the matrix. A rise in intracellular calcium reduces osteoclast activity. Empirically it was found that certain bisphosphonates, analogs of pyrophosphate which concentrate on the bone surface, block osteoclastic activity. In view of their localization bisphosphonates are highly tissue specific and are being developed for osteoporosis therapy.

Bone formation is carried out by osteoblasts, which originate from resident mesenchymal precursors, committed to the chondrogenic/osteogenic phenotype. Osteoblastic phenotypic features include response to osteotropic hormones and cytokines, including parathyroid hormone, $1,25(OH)_2D_3$, IL-1, TGFβ, possibly IL-6 and leukemia inhibitory factor as well as production of these and other factors, which participate in autocrine/paracrine regulation of bone and bone marrow activity, including bFGF and colony-stimulating factors. FGF, both a and b, EGF and PDGF are mitogenic to bone cells in culture but their role in vivo is not well-established. TGFβ stimulates or inhibits proliferation and differentiation in culture, dependent on conditions and cell type. Bone formation in vivo in animals was stimulated by TGFβ injected next to periosteum or endosteum, by bone morphogenetic proteins (related structurally to TGFβ) injected into skin or muscle and by prostaglandin E_2 injected systemically. The use of these agents for osteoporosis therapy faces difficulties regarding mode of administration and tissue specificity. In vitro osteoblast differentiation from precursor cells is enhanced by retinoic acid and glucocorticoids. Osteoblasts also possess estrogen receptors. An appealing strategy for the stimulation of bone formation would be to identify tissue "selective" agonists, which may stimulate the autocrine/paracrine anabolic responses which occur during normal bone remodelling. The tissue diversity in hormone responses via receptors of the erb A family, raises possibilities for such an approach.

Q 019 EXTRACELLULAR MATRIX PROTEINS AND HUMAN BONE FORMATION, John D. Termine, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

In addition to type I collagen, the extracellular matrix of developing and repairing human bone contains a discrete set of endogenous gene products whose composite expression reflects the differentiation and progression of the osteoblastic phenotype. Since many of these macromolecules are also found in dentin and cementum, their properties seem important to all collagen-based mineralizing tissues. Two of these gene products, osteopontin and bone sialoprotein, are heavily glycosylated proteins that can regulate skeletal cell attachment via internal RGD sequences, interacting with the vitronectin class of integrin receptors. These are important then for cell-cell and cell-matrix interactions, critical for cell differentiation and function in most connective tissues. Other gene products, like osteonectin, biglycan and decorin, seem to be involved in hard tissue matrix formation. Decorin [PGII, proteodermatan sulfate (PDS), PG40] is a small interstitial proteoglycan that binds to collagen fibrils, while osteonectin [SPARC, BM40] is a phosphorylated glycoprotein important to forming basement membrane, certain epithelia, calcified cartilage and mineralized bone structure. Osteonectin and biglycan [PGI], another small proteoglycan, are often cell-associated and are postulated to have developmentally critical functions in a variety of tissue systems. For example, biglycan is found in cellular or pericellular loci in developing cartilage, epidermis and angiogenic connective tissues. Osteonectin expression is almost always increased in rapidly proliferating and growing tissue. The combined action of all these genes, as regulated in osteogenic cells, appears to determine many features essential to bone structure and function.

Q 020 BONE MORPHOGENETIC PROTEINS AND BONE REPAIR, Elizabeth A. Wang, Vicki Rosen, John M. Wozney, Tobin N. Gerhart*, Dean M. Toriumi,** Alan Yasko,***, Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 02140-2387. *Harvard University School of Medicine, Boston, MA 02115. **Department of Otolaryngology-Head and Neck Surgery, University of Illinois at Chicago College of Medicine, 1855 West Taylor Street, Chicago, IL 60612, ***Hospital for Special Surgery, 535 E. 70th Street, New York, NY 10021. Human recombinant BMP-2 is known to induce cartilage and bone in a rat subcutaneous site. Here we demonstrate that BMP-2 can induce therapeutic bony union in segmental bone defects in three different animal models. In each model, inactive bone matrix from the same species as the test animal was prepared by acid demineralization and extraction in guanidinium chloride. Human recombinant BMP-2 was reconstituted with the bone matrix and inserted in the defect site. Inactive matrix served as the negative control. Healing was monitored weekly by x-ray. At the final time points, specimens were evaluated for union by biomechanical testing and for bone formation by histology. In the first model, a 0.5 cm gap was made in the femur of mature rats and stabilized with a polyethylene plate fixed with Kirshner wires. The final time point of analysis was 9 weeks. In the second model, a 3 cm mandibular defect in dogs was made and stabilized with a stainless steel reconstruction plate. Animals were terminated at 3 and 6 months. In the third model, an osteoperiosteal defect was created by excising a 2.5 cm midshaft segment from the femur of a skeletally mature sheep. The gap was stabilized by an anterolateral fixation plate. Animals were sacrificed at 12 weeks post-operation. In all three studies, BMP-2 mixed with inactive carrier induced bony union judged by radiography, biomechanical testing, and histology; by the same criteria, the defect implanted with the carrier matrix alone did not unite.

Soft Tissue Repair

Q 021 ABSORPTION AND DISPOSITION OF RECOMBINANT BASIC FGF APPLIED TOPICALLY TO SKIN WOUNDS, D. Bloedow, Erwin Freund, and D. Carmichael, Synergen Inc., 1885 33rd Street, Boulder, CO 80301.

Recombinant basic fibroblast growth factor (bFGF) is presently being evaluated as a wound healing therapy for diabetic and venous stasis skin ulcers in a multicenter, placebo control trial. The bFGF is being applied topically to these wounds and is expected to act locally to accelerate wound healing processes. Systemic absorption from the wound is not desirable since this dilutes the active FGF at the site and presents it to the circulation where it is potentially toxic. The absorption of FGF from wounds is too slight to be measured in the human clinical trials. Isotopically labelled bFGF of sufficient specific radioactivity is also not feasible in human trials. Using ^{35}S labeled bFGF however the absorption can be measured in animal experiments.

We have examined the absorption and disposition of biologically active bFGF in two species. ^{35}S-bFGF (60 μg/kg) was injected i.v. and s.c. to monkeys (n=6 i.v. and n=5 s.c.) and rats (n=12 i.v. or s.c.) or topically to full thickness wounds in rats (n=8). Serial blood and urine samples were collected for 48 hr in monkeys and from 6 to 24 hr in rats. Rat plasma data were pooled to provide four bFGF concentration vs. time curves for each route of administration. Biologically-active ^{35}S-bFGF and active derivatives thereof were measured by heparin affinity chromatography. Following intravenous administration, bioactive plasma bFGF declined biexponentially in rats ($t_{½}$ of 3.0 and 95 min) and triexponentially in monkeys $t_{½}$ of 0.82 min, 1.7 min, and 14 hr). Compared to rats, the initial distribution volume was smaller in monkeys (0.067 ± 0.035 vs. 0.18 ± 0.6 L/kg) and the steady-state distribution volume was larger (14 ± 10 vs. 1.6 ±0.9 L/kg). Plasma clearances were similar (monkeys, 17 ± vs. rats, 21 ±4 mL/min/kg). Bioactivity was not associated with urine radioactivity. Following subcutaneous injection, 3.0% and 4.7% of the dose reached the systemic circulation in monkeys and rats, respectively. Only 0.43% of the bFGF reached the plasma when the dose was applied topically to full thickness dermal wounds in rats. The results illustrate interspecies differences in bioactive bFGF plasma kinetics and indicate a therapeutically important localization of bFGF at the site of administration.

Q 022 PRINCIPLES OF WOUND REPAIR MODELS. Jeffrey M.Davidson, Kenneth N. Broadley, Stephen C.Woodward, Mariagabriella Giro, Lillian B.Nanney, Daniela Quaglino, Jr. Departments of Pathology, Plastic Surgery, and Cell Biology, Vanderbilt University School of Medicine and VA Medical Center, Nashville, TN 37232-2561.

The coordinated events of wound repair show remarkable interspecies variation, yet animal models are essential to understanding the biological process. Interaction between epithelium, mesenchyme, vascular and inflammatory cells makes *in vitro* modeling difficult. Animal models of tissue repair may be used to define mechanisms of repair and to evaluate the potential role of influences such as cytokines, metabolic state, and humoral factors. Many models can provide quantitative information on the rate of wound repair, as measured by numbers and types of cells present, appearance of new blood vessels, amount and type of collagen or other extracellular matrix components, biomechanical properties of the injury site, and the local presence and expression of cytokines. Key models include full and partial-thickness cutaneous excisional injuries, incisional wounds, radiation and thermal burns, tissue expanders, dead-space devices (sponges, wound chambers), and fractures. Species studied include the mouse, rat, hamster, rabbit, dog and pig. Defective wound repair and hypertrophic scarring are major clinical issues, yet it is difficult to create parallels in animal models in which healthy, young, and relatively hardy strains are used. Drugs can be used to compromise wound healing. Diabetogenic and chemotherapeutic agents as well as glucocorticoids retard wound healing significantly, having obvious clinical parallels. It is convenient to use young animals for many studies, yet young animals heal at very high rates. Aging and malnourished animals, including rats and mice, are less effective at healing wounds. Ischemic models can be created in extremities such as the rabbit ear. In contrast to healing, the study of fibrosis has largely been limited to toxic organ injury models, such as CCl_4 in the liver and bleomycin in the lung. Despite the limitations of animal modeling of human wound repair, cellular mechanisms are accessible to analysis. The local repair process can be visualized at the level of mRNA or protein expression, and defined reagents that either augment or repress individual aspects of the system can be administered in a controlled setting. Specific and phenotypically-distinct cell populations can be isolated from wounds to study their interactions. In evaluating wound models. The guiding principle for treatment of wounds has been an acceleration of the process to an end-point. Few models have been used to assess overall quality of repair, and the definition of a "healed" wound can vary enormously. Normal healing is a well-controlled process, and better models and parameters of abnormal repair are still required.

Q 023 PDGF AND PD-ECGF: PRECLINICAL AND CLINICAL STUDIES. Glenn F. Pierce, Donna Yanagihara, John Tarpley, Thomas Boone, Tsutomu Arakawa, Karen Gray, Vickie Costigan, Arlen Thomason, Amgen Inc., Thousand Oaks, CA 91320.

The precise physiologic roles of polypeptide growth factors such as platelet-derived growth factor (PDGF) and platelet-derived endothelial cell growth factor (PD-ECGF) in mediating tissue repair processes are not known. However, growth factors are considered important physiologic mediators of repair because (1) cells required for wound healing, such as macrophages, fibroblasts, and endothelial cells, secrete and respond to growth factors via autocrine and paracrine mechanisms; and (2) supraphysiologic doses of recombinant growth factors applied to wounds enhance normal repair and reverse deficient repair.

PD-ECGF is a 46 kilodalton single chain polypeptide found in platelets and placenta, and appears to be relatively specific for endothelial cells. The role of PD-ECGF in mediating angiogenic processes has been investigated. PD-ECGF triggers endothelial cell proliferation on prosthetic grafts and induces differentiation into functional neovessels, suggesting a potential therapeutic role in vessel repair. PD-ECGF has been detected in endothelial structures forming in the rat embryo, and thus also may play a role in normal development of the vascular system.

Studies of experimental wounds have demonstrated that PDGF-BB augments wound macrophage influx, provisional matrix deposition, and subsequent collagen synthesis, accelerating the entire repair process. Immunolocalization studies have shown the presence of PDGF isoforms within healing dermal wounds of both animals and man, suggesting a physiologic role for PDGF in repair. Taken together, these results indicate that PDGF-BB may stimulate dermal repair in man. Initial clinical studies suggest PDGF-BB is a promising therapeutic agent for the treatment of chronic dermal wounds.

Q 024 ARG-GLY-ASP RECEPTOR SPECIFICITY, Michael D. Pierschbacher, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, CA 92037

Most extracellular matrix proteins carry at least one Arg-Gly-Asp (RGD) sequence and, in many cases, this sequence has been shown to mediate the interaction of the protein with the cell surface. The receptors at the cell surface that recognize and bind the extracellular matrix proteins form a large supergene family and have been given the name integrins. Many of the integrins recognize an RGD sequence in their respective extracellular matrix (ECM) ligand yet they can distinguish one ECM protein from another. Data from our laboratory indicate that these integrins can distinguish the three-dimensional presentation of the RGD sequences in different proteins. As more and more members of this gene family are discovered, it is becoming clear that the interaction between the cell and its surrounding matrix is quite complex and subtle. These integrins appear to mediate the movement of cells through tissues in the body and, as such, probably play a critical role in wound healing. Our laboratory has been investigating a subtle mechanism of regulation of the function of integrins. The current extent of our knowledge in this area will be presented.

Q 025 EGF IN WOUND THERAPY, G S Schultz*, N T Bennett, D S Rotatori, Departments of Ob/Gyn*, and Surgery, University of Florida, Gainesville, FL 32610

Wound healing is a localized event that involves inflammation, migration and mitosis of wound cells, neovascularization, and synthesis and remodeling of extracellular matrix. Peptide growth factors produced at the site of injury may regulate wound healing by influencing wound cells through autocrine and paracrine pathways. An important corollary to this concept is that wounds may fail to heal properly if there are insufficient actions of peptide growth factors at wound sites. To evaluate these concepts in human wounds, fluids were collected from chest drains of mastectomy wounds to represent the environment of spontaneously healing closed wounds. In addition, fluids which accumulated under occlusive dressings applied over skin ulcers were collected to represent the environment of chronic wounds. Mastectomy fluids stimulated high levels of DNA synthesis by pure cultures of human foreskin fibroblasts or vascular endothelial cells, and mastectomy fluids contained physiologically significant levels of TGF-α, IGF-I, PDGF, and TGF-β. EGF or bFGF were not detected in mastectomy fluids. Addition of TGF-α neutralizing antibodies to mastectomy fluid decreased DNA synthesis by fibroblasts approximately 20%. In marked contrast, fluids collected from chronic wounds inhibited DNA synthesis of both fibroblasts and endothelial cells when tested alone or when combined with serum or mastectomy fluid, and levels of IGF-I in chronic wound fluids were an average of 15-fold lower than IGF-I levels in mastectomy wound fluids at four days after surgery. Animal models of wound healing also suggest important roles for TGF-α and EGF in normal wound healing. Concentrations of TGF-α in the anterior chamber of cats increase approximately 14-fold two hours after injury and then progressively decreased to pre-wound levels by 24 hours after injury. Treatment of a variety of spontaneously healing wounds in animals and humans with TGF-α or EGF has been reported to enhance healing including partial thickness burns and dermatome skin injuries (1), corneal epithelial injuries, gastric ulcers, and skin incisions. Recently EGF was reported to stimulate healing in 8 of 9 chronic wounds in patients which had failed with conventional therapies (2). Future directions of research on clinical applications of EGF and TGF-α in wound healing need to address questions of optimal vehicles and doses of the growth factors as well as combinations with other growth factors, protease inhibitors and extracellular matrix components.

1. Brown GL, Nanney LB, Griffen J, Cramer AB, Yancey JM, Curtsinger LJ, Holtzin L, Schultz GS, Jurkiewicz MJ, and Lynch JB. Enhancement of Wound Healing by Topical Treatment With Epidermal Growth Factor. New England J Med. 321:76-79, 1989.
2. Brown GL, Curtsinger L, Jurkiewicz MJ, and Schultz GS. Stimulation of Healing of Chronic Wounds by Epidermal Growth Factors. Plastic Reconstruct. Surg. In Press.

Late Abstract

EPIDERMAL CELL GRAFTS IN WOUND REPAIR. **Carolyn Compton, M.D., Ph.D., Departments of Pathology, Massachusetts General Hospital and Shriners Burns Institute, Boston, MA 02114.**

Cultured Autografts as Permanent Wound Coverage: The technique for in vitro cultivation of human keratinocytes developed by Rheinwald and Green [1] has made it possible to produce enough epithelium to cover the entire body surface within 3 weeks from a small donor skin biopsy. Use of cultured epithelial autografts (CEA) for permanent wound coverage can be life-saving in massive burn injuries in which donor sites for skin grafts are severely limited [2]. Wound healing and skin regeneration in 60 patients treated with CEA have been studied from 5 days to 7.5 years postgrafting and compared to controls of meshed skin graft interstices of comparable postgrafting age and to normal age and site-matched skin [3]. When transplanted to surgically-prepared full-thickness cutaneous wounds, CEA differentiate rapidly to form a normal epidermis within 5 days. De novo regeneration of a dermo-epidermal junction and permanent attachment to wound bed occurs within 2-3 weeks. The cultured keratinocytes are the primary source of the type VII collagen of the newly formed anchoring fibrils. Complete morphologic and morphometric maturation of regenerated anchoring fibrils requires 6-12 months. Dendritic cell repopulation of CEA-derived epidermis varies with the specific cell type. Melanocytes are present in CEA, but require weeks to months to re-establish functional epidermal-melanin units. CEA are devoid of Langerhans cells which do not survive the culture conditions, but the regenerated epidermis is rapidly repopulated by Langerhans cells via transmigration from vessels in the subjacent wound bed. Merkel cells differentiate from epithelial precursors in CEA of sole-skin origin as early as 3 weeks postgrafting [4]. The wound bed underlying CEA evolves from granulation tissue to normally healed scar within 3-8 weeks. Stromal remodelling leads to bilayered distribution of collagen and vascular architectural reorganization by 6-12 months postgrafting and coincides with rete ridge regeneration in the epidermis. By 4-5 years postgrafting elastin appears within the remodelled stroma at which point it is morphologically indistinguishable from normal dermis.

Cultured Allografts as Biologic Wound Dressings: Cultured human keratinocytes have been shown to produce a large number of growth factors and adhesion molecules many of which are known to be directly involved in and/or to promote wound healing. Topical application of cultured human allografts to chronic wounds such as venous stasis ulcers leads to complete healing within 6 weeks in the vast majority of cases [5]. The allografts themselves don not survive in the wound bed beyond 1 week but promote endogenous re-epithelialization in the recipient. The use of cultured allografts as biological wound dressings is likely to be the most important medical application of cultured human keratinocytes.

Growth Factors and Cytokines in Wound Repair

Q 100 GROWTH FACTOR EFFECTS ON THE DIRECTIONAL CAPILLARY FORMATION IN COLLAGEN GELS, Tuomo Alanko and Olli Saksela, Department of Virology, University of Helsinki, Helsinki, FINLAND. We present a new in vitro angiogenesis model that can be used to investigate the effects of growth factors (GFs) on capillary formation and migration of endothelial cells. Bovine capillary endothelial cells are seeded on a sharply demarcated area between two layers of collagen (type I) and are then overlayed by growth medium solidified with agarose. A gradient develops when the GF is pipetted into a well made outside the cell area. Basic fibroblast GF (bFGF) induces dose-dependent migration of a subpopulation of the cells forming capillary-like structures. Mitoses have been located by labeling with bromodeoxyuridine. Their density is greatest near the stimulus and diminishes sharply with growing distance from the edge of the cell spot, possibly because of effective binding of bFGF to the extracellular matrix structures. We have used this model to characterize factors affecting the diffusion of bFGF and to demonstrate the effects of GFs and proteinase inhibitors on the proliferation and morphology of the migrating cells.

Q 101 IDENTIFICATION OF AMINO ACIDS IN PDGF THAT ARE INVOLVED IN RECEPTOR INTERACTIONS AND DISULFIDE-BONDING, Maria Andersson, Arne Östman, Gudrun Bäckström, Christer Wernstedt, Ulf Hellman and Carl-Henrik Heldin, Ludwig Institute for Cancer Research, Box 595, S-751 24 Uppsala, Sweden.

Platelet-derived growth factor (PDGF) is a mitogen for connective tissue cells. It is composed of two disulfide-bonded homologos polypeptides, A and B. Three isoforms of PDGF (-AA, -AB, -BB) have been purified from natural sources. Each of the polypeptides in the PDGF dimer binds to one PDGF receptor-molecule and cause dimerization of the receptors, which leads to activation of the receptor tyrosine kinase. The PDGF α-receptor binds all isoforms of PDGF while the β-receptor only binds PDGF-BB with high affinity.

By analysing the binding specificities of 17 A- and B-chain chimeras expressed in COS cells three amino acids in the B-chain, asn-115, arg-154 and ile-158, was identified as important for the ability of PDGF-BB to bind to the PDGF-β-receptor.

Furthermore, structural analysis of partially reduced PDGF-AA suggested two of the eight cysteine residues as major candidates for forming the disulfide-bonds between the two chains in the dimer. These two cysteine residues were mutated to serine residues. The mutant was expressed in COS cells and could be immunoprecipitated, from media conditioned by metabolically labeled cells, with an antiserum raised against native PDGF-AA. The product appeared on SDS-PAGE as a monomer and was also shown to contain internal disulfide-bonds.

The results of these structural studies are now being applied in projects aimed at designing a PDGF-receptor antagonist.

Q 102 SUBSTITUTED DEXTRANS AND HEPARIN BINDING GROWTH FACTORS IN DEEP SKIN WOUND HEALING, Barritault D. (a), Bizbiz L. (b), Moulin V. (a), Gil Diez de Medina S. (a),Leandry J.(b), Loisance D.(b), Jozefonwicz J. (c), Courty J. (a), Caruelle J.P. (a). (a)LBCE, et (b) Centre d'Expérimentation Animale et Chirurgicale, Université Paris XII,CRETEIL;(c)LRM CNRS URA 502, Villetaneuse, FRANCE.

Several Heparin binding growth factors (HBGFs) are thought to play a key role in the natural processes of tissue regeneration or repair after being released by neighbouring, inflammatory or circulating cells as well as from extracellular matrix associated heparan sulfate proteoglycoaminoglycans. In order to better understand how the bioavailability of these Heparin binding growth factors can take part in the regulation of the wound healing processes, we have studied the healing effect of various chemically substituted Dextrans (SD) selected for their affinity for HBGFs, alone and in association with HBGFs. Rats were punched and skin regeneration was studied by morphometric and histological analysis. The quality of healing was evaluated by measuring the granulation tissue thickness, collagen matrix density, angiogenesis, inflammatory response, reepithelialization speed, epidermis maturation and apparent deformation of the wound. The wounds (6 mm diameter) were exactly filled with collagen plaster alone or soaked with SD, HBGF or both. Substituted Dextrans were sulfonate, benzylamine and methylcarboxylic derivatives as already published (Tardieu et al, J. Biomater. Sci. Polymer Edn., 1989,1, p 113). Three HBGFs were studied : bFGF, aFGF and HARP (Heparin Affin Regulatory Peptide). HARP is a new brain extracted angiogenic growth factor purified and sequenced in our laboratory and also described as a neurotrophic factor (Rauvala, Embo J., 1989, 8, p 2933).Some highly substituted derivatives alone in the collagen plaster were able to induce a remarkable effect both on the kinetics and on the quality of the restored skin. These results suggest that endogenous growth factors naturally released during the regeneration process could be trapped, protected and released by SD. Combination of SD with exogenous HBGF had also a remarkable effect on angiogenesis associated to the formation of the granulation tissue as well as to the quality of the restored skin.

Q 103 **EFFECTS OF GROWTH FACTORS AND CYTOKINES ON REGULATION OF EXPRESSION OF DIFFERENTIATION MARKERS IN HUMAN EPIDERMAL KERATINOCYTES,** **Miroslav Blumenberg, Chuan-Kui Jiang, Deirdre Connolly and Irwin M. Freedberg, Departments of Dermatology and Biochemistry, New York University Medical Center, 550 First Avenue New York, 10016**

Keratinization, the orderly process in which the keratinocyte moves from the basal layer through the spinous and granular layers losing its nucleus and organelles before turning into a corneocyte, is characterized by the loss of the basal layer-specific keratin pair K#5 and K#14 and appearance of suprabasal keratins K#1 and K#10. Under hyperproliferation conditions, such as in wound healing, keratins K#6 and K#16 are produced. Keratinocytes express several growth factor receptors and, when activated, can secrete a large number of cytokines. We have measured the effects of cytokines and growth factors on the transcription of keratin promoters by transfecting keratin DNA-CAT constructs into primary cultures of human epidermal keratinocytes in the presence and absence of various cytokines and growth factors in the medium. Our construct included promoter DNAs from K#5, K#6, K#10, K#14 and K#16 genes. All of these constructs are epithelial cell-specific and regulated by retinoic acid and thyroid hormones. Whereas tumor necrosis factor and insulin-like growth factor apparently do not affect the function of keratin gene promoters, epidermal growth factor, interleukin-6 and interferon-gamma have differential effects on expression of keratin genes. We discuss our results within the current concepts of the roles growth factors play in wound healing of the epidermis.

Q 104 MODULATION OF LEUKOCYTE TYPE I TRANSFORMING GROWTH FACTOR BETA (TGFβ) RECEPTORS BY INFLAMMATORY STIMULI, Mary E. Brandes, Lalage M. Wakefield and Sharon M. Wahl, Laboratory of Immunology, NIDR, NIH, Bethesda, MD, 20892.

The regulatory mechanisms which control the wide array of cellular responses to TGFβ are not understood. This study investigates whether leukocyte TGFβ receptors can be down-regulated, thus controlling the effects of TGFβ. Treatment of monocytes with interferon gamma (IFNγ) or lipopolysaccharide (LPS) for 18 h decreased [^{125}I]TGFβ binding in a dose-dependent fashion by 89% and 78%, respectively. Incubation with other cytokines did not alter the amount of TGFβ bound. In contrast, treatment of neutrophils with cytokines and chemotaxis factors did not alter [^{125}I]TGFβ binding. The decreased [^{125}I]TGFβ binding displayed by IFNγ or LPS-treated monocytes could not be attributed to competition for receptor sites by secreted TGFβ, nor did the agents directly compete with TGFβ for binding. In addition, both had no effect when the cells were treated at 4°C. Instead, the decline in binding was found to be due to a decrease in the number of type I TGFβ receptors (380/cell), the subtype primarily expressed by monocytes, with no decrease in receptor affinity (7 pM). The rate of LPS-induced monocyte receptor loss was rapid (1-4 h), in contrast to the prolonged (12 h) decline induced by IFNγ. Loss of receptors was accompanied by a diminished ability of TGFβ to induce TNFα mRNA in monocytes. Thus, this monocyte system is the first example of a heterologous agent causing the down-regulation of TGFβ receptors with a concomitant decline in a TGFβ-stimulated function. Moreover, the absence of change in neutrophil receptor number (340/cell) or affinity (50 pM) suggests differential regulation of the response to TGFβ by these two myeloid cell types.

Q 105 PURIFICATION, CHARACTERIZATION AND ACTIVITY OF RECOMBINANT HUMAN BMP-5, Josephine S. D'Alessandro, Karen A. Cox, David I. Israel, Peter LaPan, Ioannis K. Moutsatsos, John Nove, Vicki Rosen, Mary C. Ryan, John M. Wozney and Elizabeth A. Wang, Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 02140-2387.

The genes encoding seven novel regulatory proteins derived from bovine bone-inductive extracts have been cloned. Six of these human genes, BMP-2 through BMP-7, belong to a subclass of the TGF-β supergene family and are most closely related to Drosophila *dpp*, Xenopus *Vg-1*, and mouse *Vgr-1*. Recombinant human (rh) BMP-2 and rhBMP-4 each individually have been shown to induce cartilage and bone formation. We have now isolated and characterized active rhBMP-5 from CHO cells. A three-step process utilizing hydrophobic interaction, heparin affinity and reverse phase C4 HPLC chromatographies results in the purification of the BMP-5 35-40 kD dimer of greater than 80% purity. Processing, dimerization, and cleavage of the precursor protein to yield the mature form has been confirmed by N-terminal sequence analysis and is as predicted by homology to BMP-2 and TGF-β. *In vivo* cartilage and bone-forming activities of these forms in the rat ectopic bone formation model will be shown by a dose-response and time course study. With the isolation and characterization of these recombinant proteins, we can now begin to examine their individual and/or cooperative activities.

Q 106 ROLE OF THE RETINOBLASTOMA GENE PRODUCT IN REGULATING GROWTH INHIBITION BY TRANSFORMING GROWTH FACTOR BETA, Maria Frexes-Steed, J.A. Pietenpol, C.M. Murphy, C. Arteaga, and H.L. Moses. Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Transforming growth factor beta (TGF-$ß_1$) is a 25 kD polypeptide which is the most potent growth inhibitor known for epithelial cells. It has been shown, however, that many carcinoma cells have lost their normal growth inhibitory response to TGF-$ß_1$. Previous studies from this laboratory have suggested that in human keratinocytes TGF-$ß_1$ inhibits cellular proliferation through down-regulation of c-myc. Furthermore, these studies showed that binding of DNA tumor viruses to the product of the retinoblastoma gene (pRB) blocked TGF-$ß_1$ suppression of c-myc transcription. These studies thus implicate the possible role of pRB in the TGF-$ß_1$ regulatory pathway. The RB gene is known to be mutated in a large percentage of breast carcinoma cell lines. The hypothesis of this study is that an intact pRB is necessary for TGF-$ß_1$ induced growth inhibition and that loss of pRB results in insensitivity of the cells to the growth-inhibitory effects of TGF-$ß_1$. Cells from nine breast carcinoma cell lines were subjected to several growth assays (^{3}H-thymidine incorporation, colony formation in soft agar, and cell growth curves) and the growth-inhibitory effects of TGF-$ß_1$ were determined. TGF-$ß_1$ inhibited cell growth similarly in the three assays and at 10 ng/ml the following level of inhibition was achieved: MDA-MB-231 (50%), MDA-MB-361 (50%), MDA-MB-468 CA (65%), MDA-MB-435S (62%), and HS0578T (87%). In a second set of cell lines, TGF-$ß_1$ had no significant effect on cellular proliferation (MDA-MB-468 ATCC, MDA-MB-436, BT549, DU4475). Western analysis of pRB in these cell lines was performed and demonstrated that an intact pRB was present in the first group of cell lines which were sensitive to TGF-$ß_1$ while pRB was absent or altered in the second set which was not inhibited by TGF-$ß_1$. These studies provide support for the role of pRB in the TGF-$ß_1$ growth-inhibitory pathway.

Q 107 TGF-ß and IL-1 MODULATE METALLOPROTEINASE EXPRESSION BY CORNEAL STROMAL FIBROBLASTS, Marie T. Girard, Masao Matsubara and M. Elizabeth Fini, Eye Research Institute and Departments of Ophthalmology, and Anatomy and Cell Biology, Harvard Medical School, Boston, MA 02114.

Cultured corneal fibroblasts can synthesize and secrete several classes of matrix metalloproteinases (MMPs) including collagenases, stromelysins, and gelatinases. We have recently shown that primary cultured fibroblasts secrete only 72 kD gelatinase while passaged fibroblasts secrete 72 kD gelatinase as well as collagenase and stromelysin. In this study, we have determined that the cytokines, TGF-ß and IL-1, which are found in the cornea, can alter the synthesis and secretion of these enzymes in vitro. Treatment of primary corneal stromal fibroblasts with recombinant TGF-ß significantly increases the synthesis and secretion of only 72 kD gelatinase over a 3-day time course. In passaged fibroblasts, TGF-ß reciprocally regulates expression of 72 kD gelatinase and collagenase/stromelysin. While TGF-ß significantly increases the synthesis and secretion of 72 kD gelatinase, it downregulates expression of both collagenase and stromelysin. The effects of recombinant IL-1 on MMP expression by primary corneal fibroblasts are quite different from the effects of TGF-ß. IL-1 coordinately upregulates expression of 72 kD gelatinase, 92 kD gelatinase, collagenase, and stromelysin. When primary stromal fibroblasts are treated with both TGF-ß and IL-1, TGF-ß treatment is able to reduce the IL-1 induction of collagenase and stromelysin synthesis and secretion after 1 day of culture. After 3 days, the combined treatment stimulates a significantly higher level of 72 kD gelatinase secretion than either TGF-ß or IL-1 treatments alone. Our results suggest that TGF-ß and IL-1 may be important agents controlling MMP expression in corneal health and disease. The capacity of TGF-ß to repress collagenase and stromelysin expression by corneal fibroblasts suggests that this cytokine may be useful therapeutic agent in degradative disorders such as corneal ulceration. This work was supported by R01-EY08408 to MEF. MM was a fellow of Bausch and Lomb.

Q 108 CHARACTERIZATION OF THE EXTRACELLULAR DOMAIN OF EPIDERMAL GROWTH FACTOR RECEPTOR, Mary T. Harte and Larry E. Gentry, Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43699

Binding of Epidermal Growth Factor (EGF) to its cognate receptor - Epidermal Growth Factor Receptor (EGFR) - causes oligomerization of the receptors. It is proposed that it is this oligomerization that activates the tyrosine kinase function of the receptor and is therefore responsible for signal transduction across the membrane. In order to determine what residues are important both in EGF binding and in oligomer formation we decided to do a mutational analysis of the extracellular domain. To study the extracellular domain in isolation from the rest of the receptor a stop codon was introduced by site-directed mutagenesis at position 643, the first residue of the transmembrane domain. This 1.9 kb cDNA fragment (EGFR stop643) was subcloned into the mammalian expression vector CDM8 and transiently expressed in COS-1 cells. Using a crosslinking method with radioiodinated EGF, the secreted receptor was shown to specifically bind EGF. Having established that a functional extracellular domain could be produced, the EGFR stop643 was subcloned into the pUC18 vector and twenty two site-specific insertion mutants were prepared resulting in the inframe placement of four amino acids. These mutants were subsequently subcloned into the expression vector CDM8. The properties and characteristics of the mutant extracellular domains will be presented.

Q 109 PURIFICATION OF THE BACULOVIRUS EXPRESSED N51 CYTOKINE, Julia N. Heinrich, Edward C. O'Rourke and Rodrigo Bravo, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

Serum stimulation of quiescent mouse NIH3T3 fibroblasts promotes the transcriptional activation of a gene named N51 which is homologous to the human melanoma growth stimulating activity (MGSA), and human and hamster *gro*. Both MSGA and *gro* were identified first by their association with oncogenic states and the former is reported to be mitogenic, to stimulate the activation of its own gene, and to be a chemotactic for neutrophils. These properties suggest that N51 may provide a model system for studying cell cycle regulation and for delineating the pathway of signal transduction from the ligand to gene level.

High level production of the N51 protein (1-3 mg/l) was obtained by using the baculovirus expression technology. Rabbit polyclonal antibody produced against the complete N51 protein recognized both the baculovirus expressed protein as well as the human homologues from cultured cell lines. The baculovirus N51 protein was purified to near homogeneity by a cation-exchange column followed by gel filtration. The recovered protein appeared to be correctly processed since on SDS-PAGE its molecular weight (~6 kDa) was the same as that of *in vitro* translated N51 protein in the presence of microsomes. We are now in the process of designing experiments to document the bioactivity of the N51 protein.

Q 110 TGFβ REGULATES THE SECRETION OF IL-6 FROM OSTEOBLASTS, Mark Horowitz, John Phillips and Michael Centrella. Yale University School of Medicine, New Haven, CT 06510; St. Francis Hospital, Hartford, CT 06105 and the University of Connecticut School of Medicine, Farmington, CT 06030.

IL-6 is a product of activated osteoblasts but little is known about the control of its synthesis or secretion. IL-6 has been implicated in osteoclastogenesis and bone resorption. TGFβ is a potent regulator of various bone cell activities, including bone resorption, osteoblast replication and bone formation during growth, wound and fracture repair. In the present study we asked if TGFβ could regulate IL-6 secretion by osteoblasts. Primary fetal rat osteoblasts were prepared by sequential collagenase digestion of parietal bones, seeded at low density ($10^4/cm^2$), and grown to confluence. The monolayers were washed and cultured with either PTH (0.02-20 nM), LPS (1-10 μg/ml), or IL-1 (1-10 ng/ml) with or without TGFβ-1 (400 pM) for 48 hrs. The conditioned medium was recovered and tested for the presence of IL-6. In some experiments cells were exposed to TGFβ or LPS for 2 hrs., washed, and treated with PTH, LPS or IL-1. Results from these studies indicate: 1) TGFβ alone can directly induce IL-6 secretion; 2) the increase in cytokine release cannot be accounted for by the mitogenic effect of TGFβ; 3) LPS, PTH and IL-1 synergizes with TGFβ for the production of IL-6; and 4) pretreatment of the osteoblasts with either TGFβ or LPS primes them to respond to the reciprocal reagent. We can conclude from these studies that primary osteoblasts can be primed with either TGFβ or LPS to respond to a second signal, which results in a synergistic release of IL-6. Our results suggest that TGFβ is a potent regulator of osteoblast IL-6 secretion via a previously unrecognized interaction with known osteotropic molecules.

Q 111 EXPRESSION OF RECOMBINANT BMP2 IN CHINESE HAMSTER OVARY CELLS, David I. Israel, Kelvin M. Kerns, John Nove, and Randal J. Kaufman, Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 02140

Bone is a dynamic tissue that responds to many nutritional and hormonal factors including vitamin D, parathyroid hormone, calcitonin, and bone morphogenetic proteins (BMP's). Purified BMP2, in conjuntion with a suitable matrix, is sufficient to stimulate the synthesis of new bone. We have expressed recombinant human BMP2 in Chinese hamster ovary (CHO) cells using methotrexate-mediated gene amplification. Several forms of BMP2 are efficiently secreted from CHO cells; 1) a propeptide of 40-45 KDa, 2) a mature active 30KDa homodimer consisting of 18-22 KDa subunits, and 3) a small amount of unclipped 65 KDa precursor protein. The propeptide lacks cysteine residues, and therefore cannot form covalent complexes with itself or with mature BMP2. The mature, active protein is predominantly a 30 KDa homodimer consisting of three subspecies of 18 KDa, 20 KDa, and 22 KDa. A heterodimer consisting of the unclipped and mature species of BMP2 is also secreted from CHO cells. Both the mature and pro forms of BMP2 are glycosylated. The molecular and cellular events involved in the processing, secretion and extracellular fate of BMP2 will be addressed.

Q 112 CORTICOSTEROIDS DECREASE TOTAL LUNG CONTENT OF TRANSFORMING GROWTH FACTOR-BETA (TGF-β) IN BLEOMYCIN INDUCED PULMONARY INFLAMMATION BUT DO NOT AFFECT ALVEOLAR MACROPHAGE SECRETION OF TGF-β. N. Khalil, O. Bereznay, A. Greenberg, Manitoba Institute of Cell Biology, Wpg., MB, R3E 0V9.

We have previously shown in a rat model of bleomycin-induced pulmonary inflammation (BPI) and fibrosis (BPF) that TGF-β is maximally elevated 7 days after intratracheal (IT) bleomycin (BLM) administration and precedes maximal collagen synthesis. At the time of maximal TGF-β production immunohistochemical staining localized TGF-β_1 almost exclusively in clusters of alveolar macrophages (AM). Corticosteroids can inhibit macrophage influx into injured tissue. 7 days after immunosuppressive doses of methylprednisolone (MTP) given concomitantly with BLM 20% of the bronchoalveolar lavage (BAL) leukocytes were AM compared to 80% AM in the BAL after BLM alone. AM were the major source of TGF-β 7 days after BLM and since MTP inhibited macrophage influx into the lungs we demonstrated that MTP reduced the total lung TGF-β 7 days after IT BLM to 9-10% of rats treated with BLM alone. Although MTP inhibited AM influx into BLM injured lungs to demonstrate that corticosteroids have no effect on the secretion of TGF-β by AM, we obtained AM by BAL from BLM and BLM plus MTP treated rats. Conditioned media (CM) from overnight cultures of AM obtained from rats treated with BLM contained 6.0 ± 2.0 pg of TGF-β per μg DNA while CM of AM from rats treated with BLM and MTP contained 4.0 ± 2.0 pg of TGF-β per μg of DNA. These findings suggest that the decrease in total lung TGF-β in BPI after methylprednisolone administration is a result of corticosteroid induced inhibition of macrophage influx into the lungs rather than inhibition of secretion of TGF-β by alveolar macrophages.

Q 113 EFFECT OF TGF-β ON MITOGENIC RESPONSE TO BASIC FGF IN HUMAN SKIN FIBROBLASTS, Kanako Kikuchi, E. Carwile LeRoy and Maria Trojanowska. Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, Charleston SC 29425.

Multiple growth factors (e.g PDGF, FGF and TGF-β) have been identified in scleroderma and other fibrotic lesions. It is likely that interactions between different factors play an important role in mediating regulatory effects on target cells. For example, we have recently demonstrated that TGF-β enhances mitogenic potency of all three PDGF isoforms (Ishikawa, et al., J Cell Physiol, 1990). In this study we examined the effect of TGF-β on FGF induced proliferation of healthy adult human skin fibroblasts. In serum free conditions basic FGF (1 ng/ml) alone was strongly mitogenic (300% increase over control), while acidic FGF (1 ng/ml) was a very weak mitogen. Pretreatment of cells with TGF-β before FGF addition consistently resulted in synergistic enhancement of FGF mitogenic effect (600% increase over control). When both growth factors were added simultaneously, enhancement of FGF mitogenic potency was observed with delayed kinetics (at 42 hours after growth factors addition), as compared to peak of response to FGF alone (at 24 hours). The mechanism of TGF-β effect on FGF is currently under investigation.

Q 114 CYTOKINE PRODUCTION AND T CELL ACTIVATION IN BURN WOUND GRANULATION TISSUE. Gary R. Klimpel, Ed G. Brooks, David N. Herndon, and P.G. Hayward. Shriners Burns Institute and Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550.

Small fragments of granulation tissue or normal skin samples from pediatric burn patients were investigated for: 1) the presence of activated T cells, 2) the ability of the tissues to produce cytokines following *in vitro* culture,and 3) the presence of increased levels of mRNA for IL 2 and IL 6. In studying 8 different patients, we found that supernatants from overnight cultures of granulation tissue contained high levels of IL 2 and IL 6 activity. New, uncultured, granulation tissue contained high levels of mRNA for IL 2 and IL 6 when compared to normal skin using coupled reverse transcriptase/PCR analysis. During *in vitro* experiments, we observed that large populations of lymphocytes were growing out of granulation tissue fragments incubated in just culture medium. Cells obtained from these cultures were further expanded with IL 2 and T cell lines were established from 6 pediatric thermal injury patients. These T cell lines contained both $CD4^+$ and $CD8^+$ T cells with the majority of these cells bearing TcR-2. A number of cytokines (TNF, IFNγ,IL 2, IL 6) were produced by different T cell lines following stimulation with anti-CD 3 or mitogen. Further, most of these T cell lines demonstrated non-MHC restricted killing against various targets. These results were unique to granulation tissue since normal skin biopsies failed to exhibit any evidence of T cell proliferation. These results document the presence of IL 2, IL 6 and activated T cells in human granulation tissue and suggest that T cells may play an important role in wound healing.

Q 115 EVIDENCE FOR GROWTH STIMULATORY PROPERTIES OF CONDITIONED MEDIUM FROM HUMAN KERATINOCYTES; Gunnar Kratz, Anders Hægerstrand, Carl-Johan Dalsgaard. Department of Anatomy, Karolinska institutet, Box 60400 Stockholm, Sweden.
Evidence for growth stimulatory properties of keratinocyte conditioned medium (KCM) on human fibroblasts, endothelial cells, keratinocytes and smooth muscle cells as well as on 3T3 cells is presented. On fibroblasts, KCM caused a more than 400% increase in DNA synthesis as revealed by ^{3}H-thymidine incorporation and autoradiography. The proliferative effect was comparable to that of platelet derived growth factor (PDGF), but was not inhibited by PDGF antibodies, and exceeded that of transforming growth factor-α (TGF-α), epidermal growth factor (EGF) and fibroblast growth factor (bFGF). Furthermore, KCM was found to stimulate smooth muscle cells, keratinocytes and endothelial cells more potently than PDGF, EGF/TGF-a and bFGF, respectively. KCM was also potent in stimulating thymidine incorporation in 3T3 cells whereas EGF showed a 20-fold weaker stimulatory effect. Since keratinocytes have been shown to secrete TGF-a, which binds to the EGF receptor, binding of KCM to the EGF receptor was assayed. Only a minor displacement of radiolabelled EGF by KCM was found, implying that the growth stimulatory effect by KCM was not caused by binding and activation of EGF receptors. Taken together, these results suggest the presence of hitherto unidentified growth stimulatory factor/s, expressed and secreted by cultured human keratinocytes.

Q 116 WOUNDING OF KERATINOCYTES *IN VITRO* CAUSES RESISTANCE TO TGFβ1 INHIBITION, Jeffrey H. Levine, Lillian Nanney and Harold L. Moses, Departments of Cell Biology and Surgery, Vanderbilt University Medical Center, Nashville, Tn 37232
TGFβ1 is a potent promoter of wound healing. In the dermis, it promotes the formation of granulation tissue and may increase the tensile strength of collagen. This is consistent with its observed *in vitro* effects: neutrophil, monocyte and fibroblast chemotaxis, and increased collagen production. TGFβ1 effects on the epidermis are less clear. While it is known that TGFβ enhances epithelialization of wounds, it is a potent inhibitor of keratinocyte proliferation *in vitro*. In order to better understand this paradox we are studying the effects of TGFβ1 on wounded keratinocytes *in vitro* using BALB/MK cells, a continuous cell line. When rapidly growing cells are wounded and treated with TGFβ1 (10 ng/ml), all cells are inhibited except for a zone of TGFβ resistant cells at the wound margin, where cells proliferate as rapidly as untreated cells. The appearance of this zone of TGFβ resistant keratinocytes requires EGF in the media and is temporally related to the time of wounding. TGFβ1 resistant cells can also be found when CCL64 cells are wounded. Therefore, while TGFβ1 is a potent inhibitor of keratinocyte proliferation *in vitro*, enhanced epithelialization may occur *in vivo* because wounding keratinocytes induces resistance to TGFβ1 inhibition.

Q 117 TGF-β2-PRO-REGION PEPTIDE COMPLEXES CONSTITUTE LATENCY. Lioubin M.N., Purchio A.F., ONCOGEN, Seattle, WA 98121
Transforming growth factors β are secreted by cells as latent molecules and require a denaturation step (such as acidification) in order to detect optimal biological activity. Recombinant TGF-β2 expressed in high levels by CHO cells is also secreted as a latent form and acidification is needed for activation. Under neutral conditions the mature protein chromatographs as a high molecular weight complex containing pro-region proteins at an apparent molecular weight of 130 kDa; the two proteins do not resolve by hydrophobic interaction chromatography. Crosslinking experiments on neutral purified samples show non-covalent association of the mature peptide with pro-region sequences. Acidification conditions that activate TGF-β2 dissociate the mature protein from the pro-region containing complex. Acid purified pro-region molecules, when incubated under neutral conditions with purified mature TGF-β2, reconstitute latency. Crosslinking experiments of these samples also suggest non-covalent complex formation of mature and pro region proteins. TGF-β2 pro-region molecules are also capable of reconstituting latency by forming complexes with mature TGF-β1.

Q 118 EFFECT OF INSULIN AND IGF-I ON CHONDROGENESIS AND MYOGENESIS IN MESENCHYMAL CELL CULTURE, Paul A. Lucas, Henry E. Young, Jerry Padrta, and Lorna Putnam. Department of Surgery, Medical Center of Central Georgia and Mercer University School of Medicine, Macon, GA 31208. Stage 24 chick limb bud mesenchymal cells, when plated at a density of 2×10^5 cells/cm^2, maintain their mesenchymal phenotype in culture. Treatment with insulin, in a serum-free defined media, results in a dose-dependent increase in chondrocytses and myotubes in the culture. Chondrogenesis was quantitated by histochemical staining for proteoglycans followed by the extraction of the stain while myogenesis was determined by a modified ELISA against myosin and DNA was measured by a standard fluorometric assay. Insulin stimulated chondrogenesis and myogenesis over a concentration range of 5-500 μg/ml. Myogenesis was maximal at 6 days in culture (4 days of treatment), while chondrogenesis was maximal at 10 days in culture (8 days of treatment). A time-course experiment established that both cartilage and myotubes began appearing after 24 hr. of treatment. Recombinant IGF-I (Amgen) also stimulated chondrogenesis and myogenesis, but did so at concentrations of 20-500 ng/ml. Thus, IGF-I is effective at molar concentrations of 50-1000 times less than insulin. It appears that insulin may be acting through the IGF receptors. The ability of IGF-I to stimulate cartilage and myotube formation in uncommitted mesenchymal cells may point to a role of the growth factor in repair of these tissues.

Q 119 MITOGENIC EFFECT OF CYTOKINES SECRETED BY ALVEOLAR MACROPHAGES ON LUNG FIBROBLASTS, Anna Ludwicka, Maria Trojanowska, Edwin Smith, E. Carwile LeRoy and Richard Silver. Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425.
The objective of this study was to determine how bronchoalveolar lavage (BAL)-derived fibroblasts from scleroderma patients differ from healthy lung fibroblasts obtained by autopsy of normal individuals, in terms of responsivness to cytokines produced by alveolar macrophages. Proliferative effects of cytokines [TGF-β, PDGF (AA, AB, BB), FGF, TNF, GM-CSF, IL-1, IL-6 and IL-8] were measured in serum free medium by DNA synthesis using ^{3}H thymidine incorporation. All tested cytokines were mitogenic for both BAL-derived and healthy lung fibroblasts when tested in early passages with significant differences between the level of responses. Most of the tested cytokines were highly mitogenic for BAL-derived fibroblasts, but only weakly mitogenic for healthy lung fibroblasts. Cytokines produced by scleroderma alveolar macrophages may stimulate and/or recruit lung fibroblasts with a high proliferative capacity. Levels of those cytokines secreted in vitro are currently under investigation.

Q 120 TGF-β PRIMES HUMAN PERIPHERAL BLOOD MONOCYTES TO SECONDARY STIMULI, Nancy McCartney-Francis, Diane Mizel, Sue Dougherty and Sharon Wahl, Laboratory of Immunology, National Institute of Dental Research, NIH, Bethesda, MD 20892
TGF-β is a multifunctional peptide which has been implicated as an important immunoregulatory molecule with proinflammatory as well as immunosuppressive activities. Whereas many of the proinflammatory activities have been described as early events, occurring within hours after exposure to TGF-β, in this study we describe the effect of prolonged exposure to TGF-β on monocyte function, specifically the responsiveness to secondary stimuli. Pretreatment of monocytes with TGF-β for 24-48 hours resulted in enhanced TNF-α mRNA levels within 30 minutes after exposure to lipopolysaccharide (LPS) as compared to untreated cells. This increase was reflected at the protein level within 3 hours, after which time the TNF levels returned to the baseline LPS response or less. Similar pretreatment with TGF-β also enhanced the LPS-induced transcription and translation of IL-1 and GM-CSF. In contrast, simultaneous addition of TGF-β and LPS resulted in a suppressed cytokine response. While LPS alone induces the expression of a variety of monokines, exposure to TGF-β appears to prime the cells to the effects of LPS, influencing not only the magnitude of the response but also the kinetics. TGF-β-primed monocytes may play an important role in the later phases of the inflammatory response and contribute to chronic inflammation.

Q 121 HIGH LEVEL EXPRESSION AND PURIFICATION OF THE PRO REGION OF TRANSFORMING GROWTH FACTOR-β1.

Grainne A. McMahon and Larry E. Gentry, Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43699

The pro region of transforming growth factor-β1, termed β1-latency associated peptide (β1-LAP) plays an important role in maintaining the mature TGF-β1 in a latent state. On proteolytic cleavage of the precursor, a non-covalent complex of β1-LAP and mature TGF-β1 forms, the β1-LAP regulating the biological activity of the mature TGF-β1. To enable further studies on this interaction, we have produced a purified source of β1-LAP. A 1 kb fragment of β1-LAP containing a mutated stop codon at Arg-278 was subcloned into the pSV2-dhfr mammalian expression vector and transfected into Chinese Hamster Ovary cells. Clones were amplified in increasing concentrations of methotrexate. A stable cell line was obtained in 2 μM methotrexate media, which secreted high levels of biologically active material, as determined by mink lung cell bioassays and its ability to crosslink with 125-I TGF-β1. A three stage purification scheme using DEAE-Sephadex, hydroxylapatite and gel filtration chromatography was developed and found to purify β1-LAP to homogeneity; the purified material was found to be biologically active. We are currently investigating the interaction of β1-LAP with mature TGF-β1 using circular dichroism studies.

Q 122 **BIOSYNTHESIS OF THE LARGE LATENT COMPLEX OF TGF-β1, Kohei Miyazono, Anita Morén, Anders Olofsson, Pascal Colosetti and Carl-Henrik Heldin,**

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TGF-β1 is synthesized as a high molecular weight latent complex (L-TGF-β1), composed of mature TGF-β1, the N-terminal remnant of the TGF-β1 precursor and the latent TGF-β1 binding protein (LTBP). The N-terminal remnant of the TGF-β1 precursor, denoted TGF-β1-latency associated peptide (β1-LAP), is sufficient for the latency of TGF-β1, whereas the functions of LTBP remains to be elucidated. In a human erythroleukemia cell line, HEL, the produciuton of L-TGF-β1 was induced more than 100-fold by phorbol ester. Analysis by Northern blot hybridization disclosed that both the TGF-β1 precursor and LTBP were induced in a coordinated fashion. The size of LTBP is 205 kDa in HEL cells, considerably larger than the platelet form (125-160 kDa). Tryptic digestion of LTBP in HEL cells produced similar proteins to the platelet form, indicating that the difference in molecular sizes involves cell-specific proteolysis. Pulse-chase analysis using antibodies to the TGF-β1 precursor and LTBP revealed that association between the TGF-β1 precursor and LTBP occurred very rapidly (within 15 min). Secretion of L-TGF-β1 was observed as early as 30 min after synthesis. In contrast, the TGF-β1 precursor, which did not associate with LTBP, remained inside the cells for a longer time period. These results suggest that LTBP may be important for assembly and secretion of L-TGF-β1. The physiological relevance of the production of L-TGF-β1 in the process of wound healing will be discussed.

Q 123 LOSS OF GROWTH INHIBITION BY TRANSFORMING GROWTH FACTOR-ß1 (TGF-ß1) AND ABERRANT EXPRESSION OF ALPHA 2 TYPE I COLLAGEN IN SPONTANEOUSLY TRANSFORMED AND VIRUS-TRANSFORMED NORMAL RAT KIDNEY CELLS,

Michael J. Newman, Matthew A. Nugent and Shue-Yuan Wang,
The Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

We have demonstrated that stimulation of type I collagen expression is required for TGF-ß1-mediated inhibition of the serum-free growth of normal rat kidney (NRK) fibroblasts (J. Biol. Chem. **264**, 18060-18067, 1989). Sensitivity to growth inhibition and stimulation of collagen RNA and protein expression by TGF-ß1 were examined in spontaneously transformed and Kirsten sarcoma virus-transformed NRK cells grown under serum-free conditions. Anchorage-dependent growth of the transformed cells was stimulated by TGF-ß1. Loss of sensitivity to growth inhibition by TGF-ß1 was associated with a specific defect in expression of the alpha 2 subunit of type I collagen in both transformed NRK cell lines. Lack of expression of alpha 2 type I collagen was observed both in the presence and absence of TGF-ß1. Normal expression of the alpha 1 subunit of type I collagen was observed in both transformed cell lines and expression of this collagen subunit was increased by TGF-ß1. The serum-free growth of both nontransformed and transformed NRK cells was inhibited by exogenous type I collagen, demonstrating that cell transformation did not result in loss of sensitivity to growth inhibition by exogenous collagen. These results provide additional support for the conclusion that NRK growth inhibition by TGF-ß1 is mediated by a stimulation of collagen expression. Our results suggest that induction of collagen expression by TGF-ß1 may play a role in the termination of the normal proliferative response during wound healing, and that the defects in extracellular matrix expression commonly associated with cell transformation and metastasis may allow cells to escape from the growth inhibitory effects of TGF-ß1.

Q 124 EFFECT OF FIBRIN GLUE AND aFGF-SUPPLEMENTED FIBRIN GLUE ON WOUND HEALING, Hernan A. Nunez, Reginald Kidd, Connie Tettenborn, Jeffery Taubenberger and William N. Drohan, American Red Cross, Holland Laboratory, Rockville MD 20855 and National Cancer Institute, Laboratory of Pathology, Bethesda MD 20892

Fibrin glue (FG) is a fibrinogen based hemostatic agent that forms a clot upon topical application. Because of this, FG is a potential vehicle to deliver wound healing enhancers to a wound site. To evaluate this potential in an animal model, two 6mm full-thickness skin biopsies on the dorsal part of genetically diabetic (db/db) mice were filled with FG to which 5 to 10ug of acidic fibroblast growth factor (aFGF) had been added. Untreated wounds and wounds treated with unsupplemented FG or aFGF alone were used as controls. At 9 or 12 days post-treatment, 5 to 20 animals per treatment group were sacrificed and histological preparations from each wound and its immediate surrounding skin were stained and analyzed. The extent of wound repair in each sample was evaluated by examining collagen deposition, reepithelialization, thickness of the granulation tissue and the density of inflammatory cells, fibroblasts and capillaries. Each sample was scored from 1 to 15, ranging from no repair (1) to maximum healing (15). The results indicate that the wounds treated with the supplemented FG generally scored higher than wounds treated with FG.

Q 125 TRANSFORMING GROWTH FACTOR-β1, β2 AND β3 INCREASE EXPRESSION OF TRANSFORMING GROWTH FACTOR-β2 mRNA, Michael A. O'Reilly, Anita B. Roberts and Michael B. Sporn, Laboratory of Chemoprevention, NCI, NIH, Bethesda, MD 20892.

The effect of transforming growth factor-β (TGF-β) proteins on expression of TGF-β2 mRNA was studied in the human pulmonary adenocarcinoma cell line A549. Northern blot analysis detected five distinct TGF-β2 mRNA transcripts of 5.8, 5.1, 4.0, 3.8 and 2.8 kb. Treatment of the cells with porcine TGF-β1, TGF-β2 or recombinant chicken TGF-β3 for 24 hours resulted in an increase in the 5.8, 4.0 and 3.8 kb transcripts, with little detectable change in abundance of the 5.1 and 2.8 kb transcripts. The effect of TGF-β proteins was dose-dependent; maximal induction of TGF-β2 mRNA was detected with 5ng/ml of protein. A similar dose-dependent increase in TGF-β1 mRNA was detected following treatment of the cells with TGF-β1, TGF-β2 or TGF-β3 protein. Increased expression of TGF-β2 mRNA in response to TGF-β1, TGF-β2 or TGF-β3 protein was blocked by co-treatment with actinomycin D. In the presence of actinomycin D, TGF-β2 mRNA was markedly decreased, suggesting that TGF-β2 mRNA is relatively unstable. These studies support the hypothesis that expression of TGF-β2 is regulated by members of the TGF-β family and that the TGF-β2 mRNA transcripts may be differently regulated.

Q 126 DOUBLE-STRANDED RNA SUPPRESSION OF PDGF-INDUCED MITOGENESIS IN HUMAN DIPLOID FIBROBLASTS IS NOT DEPENDENT ON INTERLEUKIN-6, Michael A. Perricone, Dallas M. Hyde, and Shri N. Giri, Departments of Anatomy and Veterinary Pharmacology and Toxicology, University of California, Davis, CA 95616

The double-stranded RNA (dsRNA) molecule, polyinosinic-polycytidylic acid (poly IC), inhibits the mitogenic activity of platelet-derived growth factor (PDGF) under certain culture conditions. Interleukin-6, a cytokine induced by dsRNAs, has been suggested as the inhibitory mediator in poly IC-suppressed mitogenesis based on evidence that antibodies against IL-6 neutralize the inhibitory activity of poly IC. The purpose of this study was to determine if exogenous IL-6 can inhibit the mitogenic action of PDGF in the absence of poly IC. Sub-confluent human diploid lung fibroblasts were synchronized in Go phase by serum-deprivation. Poly IC or IL-6 was added at different times prior to or after PDGF-induced mitogenesis. Serum-deprived fibroblasts did not secrete detectable levels of IL-6 into the media but both PDGF and poly IC elevated the IL-6 levels in the media of these cells to 100 U/ml and 1000 U/ml, respectively. Poly IC had no effect on [^{3}H]-thymidine uptake when added 0-24 hours after PDGF but it reduced [^{3}H]-thymidine uptake to 49% of the PDGF control group when added 8-48 hours before PDGF. Recombinant IL-6 (rIL-6) did not affect [^{3}H]-thymidine uptake when added 8-24 hours after PDGF; however, rIL-6 significantly enhanced PDGF-induced [^{3}H]-thymidine uptake by 120% of the PDGF control group when added at the same time or up to 48 hours prior to PDGF. These data suggest that exogenous IL-6 does not have the capacity to inhibit PDGF-induced mitogenesis and that the inhibitory action of poly IC on PDGF-induced mitogenesis appears to involve other factors.

Q 127 TGF-ß1: A POTENT CHEMOATTRACTANT FOR HUMAN NEUTROPHILS ACTS VIA A NOVEL MECHANISM, Joan Reibman, Steven M. Meixler, Kathleen A. Haines, Sharon A. Kolasinski, Bruce N. Cronstein, Trilok V. Parekh, Gerald Weissmann, Leslie I. Gold. Department of Medicine and Department of Pathology, New York University Medical Center, New York, NY 10016

We have previously demonstrated that TGF-ß1 is one of the most potent chemoattractants for human neutrophils (PMN). TGF-ß elicits chemotaxis but not degranulation or the generation of O_2^- at femtomolar concentrations (maximal response at 40 fM) whereas the classic chemoattractant fMLP acts at nanomolar levels (Gold et al. (1990) J. Cell. Biochem. 14C. 294). Further studies now show that TGF-ß3 elicited directed migration of PMN under agarose with a dose response similar to that of TGF-ß1. Chemotaxis elicited by TGF-ß1 involved protein synthesis since migration to TGF-ß1 was inhibited by cycloheximide (86.0±5.0% inhibition); actinomycin D inhibited by 61.6±20.3%. Classic chemoattractants act via a Pertussis toxin (PT)-sensitive G protein with subsequent elevations in $[Ca^{2+}]_i$. Whereas PT inhibited chemotaxis in response to fMLP, chemotaxis in response to TGF-ß1 was not inhibited (52.5±10.8% and 104.4±5.5% control respectively). Furthermore, GTPase activity was demonstrated in response to fMLP but not in response to TGF-ß1. Finally, increments in $[Ca^{2+}]_i$ were also not affected by TGF-ß1. Thus in contrast to fMLP, TGF-ß1 does not induce chemotaxis via a classic heterotrimeric G protein. Although the pathways of signal transduction differ, TGF-ß1, like fMLP, provoked the polymerization of actin (rhodamine-labeled phalloidin) in adherent PMN. The novel mechanism by which TGF-ß1 induces chemotaxis may be related to its potent and selective activity and thus its potential role as an early modulator of tissue inflammation and wound repair.

Q 128 TYPE II ALVEOLAR EPITHELIAL CELLS RESPOND TO AND PRODUCE TRANSFORMING GROWTH FACTOR BETA (TGF-β), Rita M. Ryan, Michelle M. Mineo-Kuhn, Christina M. Kramer, Jacob N. Finkelstein, Strong Children's Research Center, Department of Pediatrics (Neonatology) and EHS Center, University of Rochester, Rochester, New York, 14642.

In lung injury the type II alveolar epithelial cell has a critical function in the repair process serving to replace the entire damaged alveolar epithelium. While it is unknown what regulates type II cell proliferation, it is likely that polypeptide growth factors are involved. We have shown previously that TGF-β inhibits proliferation of adult and newborn type II epithelial cells in culture as measured by ^{3}H-thymidine (^{3}H-TdR) incorporation. Twenty hours after isolation and plating, cell counts were performed using a gridded eyepiece after which growth factor(s) and ^{3}H-TdR in serum-free media were added simultaneously to these same wells. After 24 hours, cell counts were repeated on these wells, cells harvested and ^{3}H-TdR incorporation determined. Control cell counts increased over the 24 hour period; TGF-β (10ng/ml) caused a decrease in cell counts compared to controls. These data confirm that changes in ^{3}H-TdR incorporation in newborn rabbit type II cells reflect actual changes in cell number. In addition to inhibition of basal type II cell proliferation, TGF-β was found to completely block the mitogenic effect of TGF-α, if added together. Type II cell conditioned media (CM) was collected from 24 hours to 48 hours after isolation and plating. This CM had a profound inhibitory effect on type II cell and lung fibroblast proliferation as measured by ^{3}H-TdR incorporation. This media, after acidification, was found to contain TGF-β as measured by radioreceptor assay. The quantity of TGF-β released by type II cells was greatly enhanced by irradiation *in vitro*. Doses as low as 2.5 Gy caused a more than 5 fold increase in detectable TGF-β. Also, after irradiation, type II cells were less responsive to TGF-α, a known mitogen. (Supported in part by HL 36543, HL 37388, and CA 27791)

Q 129 AUTOCRINE GROWTH OF HUMAN KERATINOCYTES IS MEDIATED BY TWO EGF-LIKE FACTORS: TGF-α AND KAF/AMPHIREGULIN. Gary D. Shipley, Paul W. Cook, Paul A. Mattox, Winifred W. Keeble, John P. Adelman and Mark R. Pittelkow*, Oregon Health Sciences University, Portland, OR 97201; and *The Mayo Clinic/Foundation, Rochester, MN 55905.

When normal human foreskin keratinocytes are cultured in defined medium at densities above 5 x 10^3/cells cm^2 the cells proliferate continuously and the addition of EGF, TGFα, bFGF, or aFGF does not significantly alter growth rate. Growth in the absence of EGF/TGFα or FGFs is inhibited by soluble heparin sulfate, TGFβ, or a monoclonal antibody which acts as an antagonist of the EGF receptor (EGF-R). The addition of EGF, TGFα, or aFGF reverses heparin-induced growth inhibition, while bFGF only partially negates this effect. An RIA of medium conditioned by keratinocytes at high density (CM) indicated the presence of TGFα peptide at a concentration of approximately 235 pg/ml. In contrast to high density growth, the initiation of clonal growth of keratinocytes requires the addition of EGF/TGFα or FGFs to the basal medium. CM replaces these factors in stimulating keratinocyte clonal growth, and CM-induced clonal growth is inhibited by heparin sulfate. A novel heparin-binding, keratinocyte-derived autocrine growth factor (KAF) was purified from CM. Heparin inhibited KAF mitogenic activity as well as the ability of KAF to compete with ^{125}I-EGF for cell surface binding. NH_2-terminal protein microsequencing revealed that this KAF preparation consisted of two overlapping polypeptides. Analysis of KAF peptide sequences revealed homology with the TGFα-like growth factor, amphiregulin (AR), and we subsequently cloned an AR-specific cDNA from human keratinocytes. Northern blot analysis using KAF/AR-specific cRNA revealed that cultured normal human keratinocytes express high levels KAF/AR mRNA, while this mRNA was not detected in cultured normal dermal fibroblasts or normal melanocytes. Thus, normal human keratinocytes grow in an autocrine manner, and this autocrine growth is mediated by at least two EGF-like growth factors, TGFα and KAF.

Q 130 ISOLATION AND CHARACTERISATION OF AN INHIBITOR OF TGFβ FROM HUMAN FEBRILE URINE. Anders Sundan and Terje Espevik, Institute of Cancer Research, University of Trondheim, Regionsykehuset, N-7006 Trondheim, Norway.

Antagonists of various cytokines, most notably IL-1 and TNFα, have recently been isolated from febrile urine. By screening urine from febrile leukemia patients we discovered at least two different urines which counteracted the TGFβ induced inhibition of DNA synthesis in the CCL-64 mink lung cell line. Furthermore, the most potent of these urines also inhibited the binding of iodinated TGFβ to CCL-64 cells. Attempts were made to purify this anti-TGF activity in a series of steps including urine ultrafiltration and concentration, ion exchange and Sepharose-blue chromatography and reversed phase chromatography. Preliminary evidence indicates that the anti-TGFβ activity may be due to a protein of approximately 23-30 kD. The inhibitor may act by binding to TGFβ itself, because pretreatment of the cells with the inhibitor and washing before TGFβ addition did not interfere with TGFβ action on the cells.

Q 131 **PDGF AND TGF-ß STIMULATE COLLAGEN GEL CONTRACTION VIA DIFFERENT MECHANISMS,** **Anders Tingström[1], Fredrik Rorsman[2], Carl-Henrik Heldin[3] and Kristofer Rubin[1], Dept of Medical and Physiological Chemistry[1], Dept of Pathology[2], Ludwig Institute for Cancer Research[3], University of Uppsala, Uppsala, Sweden.**

$ß_1$-integrin-mediated contraction of collagen type I gels by human dermal fibroblasts is strongly stimulated by PDGF. (Gullberg et al, Exp. Cell Res., Vol. 186, 264-272, 1990). Both PDGF-AA and PDGF-BB potentially stimulated collagen gel contraction implicating that at least the PDGF α-receptor can evoke this cellular response. The contraction stimulating effect of PDGF was compared to that of TGF-ß, IL-1α and TNF-α, factors that have been reported to induce PDGF-A-chain mRNA and/or PDGF-AA production. Apart from PDGF, only TGF-ß stimulated gel contraction, although weaker than PDGF, while IL-1α and TNF-α were inhibitory. The effect of TNF-α and IL-1α was at least partily due to the generation of active collagenase that eventually degrade the collagen gels. IgG directed towards PDGF-AA and PDGF-BB inhibited the effects of the corresponding factors while IgG derived from nonimmune serum was uneffective. None of these antibodies inhibited the effect of TGF-ß indicating that it can stimulate gel contraction via a PDGF-independent pathway. Anti human plasma fibronectin IgG inhibited the TGF-ß stimulated gel contraction but not the PDGF-stimulated. In conclusion our data indicate that PDGF and TGF-ß stimulate collagen gel contraction via different mechanisms and that the effect of TGF-ß at least partially is mediated by fibronectin.

Q 132 **WOUND-RELATED POLYPEPTIDE GROWTH FACTORS INDUCE ANCHORAGE-INDEPENDENCE IN V-*JUN* TRANSGENIC CELLS.** Luc Vanhamme, Glenn M. Marshall, Peter K. Vogt.
Department of Microbiology, USC School of Medicine, Los Angeles, CA 90033.

Full thickness skin wounding is a prerequisite for tumor formation in transgenic mice carrying the oncogene v-*jun* under the control of the $H2K^k$ promoter (murine MHC class I gene)[1]. Wound-induced sarcoma formation is accompanied by a 5-10 fold increase in v-*jun* transgene expression in tumor tissue. Wound healing is known to be associated with the local production of many polypeptide growth factors and extra-cellular matrix components which individually influence gene expression and cell growth.

We evaluated the ability of several wound-derived polypeptide growth factors to induce the transformation of v-*jun* transgene-containing cells in vitro and related this to transgene expression. TNFα, IL-1α, and TGFβ1 markedly increased the cloning efficiency in soft agarose of transgenic cell lines and transgenic embryo fibroblasts. This effect was not seen in either 3T3 cells or normal mouse embryo fibroblasts. We measured transgene mRNA expression and AP-1 activity in CAT assays following growth factor stimulation in the transgenic cells. Transfection of transgenic cells by a v-*jun* expression vector increased cloning efficiency by a similar magnitude to the growth factor effect. Our results identify three wound-related polypeptide growth factors as possible co-factors in the process of tumorigenesis in v-*jun* transgenic mice.

1. Schuh AC, Keating SJ, Monteclaro FS, Vogt PK, Breitman ML. *Nature* 1990; 346: 756- 760.

Q 133 TRANSIENT cAMP DECREASE ASSOCIATED WITH TGF-B INDUCED DELAY IN G2/M IN RABBIT ARTICULAR CHONDROCYTES: POSSIBLE INVOLVEMENT OF ADENYLATE CYCLASE ON THE REPLICATION PROCESS, Denis Vivien*, Philippe Galéra*, Emmanuel Lebrun**, Gérard Loyau* and Jean Pierre Pujol*. Laboratoire de Biochimie du Tissu Conjonctif*, Centre de Transfusion Sanguine**, CHU Caen Côte de Nacre,14033 CAEN, France.

We show that incubation of RAC for 24 h with TGF-ß (1 ng/ml) induced a recruitment of cells in late S phase (G2/M) either in actively- (10% FCS) or slowly-proliferating cultures (2% FCS) which disappeared between 24 and 48 h. Thus TGF-ß-induced G2/M delay resulted from an increase of the DNA replication rate. TGF-ß produced a transient cAMP decrease which could be associated with the TGF-ß-induced S delay. In addition both inhibition or transient stimulation of proliferation can be observed at 48 h as the result of TGF-ß-induced S phase delay depending on the presence of other serum growth factor(s). This finding contributes to the multifunctional properties of TGF-ß and may have general physiological significance in tissue repair and cancer.

Q 134 FsF-1, AN ACIDIC HEPARIN-BINDING FIBROBLAST GROWTH-STIMULATING LYMPHOKINE, David J. Wyler and Sadhana Prakash, Division of Geographic Medicine and Infectious Diseases, Tufts-New England Medical Center, Boston, MA 02111

Infection with the helminthic parasite Schistosoma mansoni can result in debilitating liver fibrosis. Results of studies that we conducted in a murine model suggest that hepatic fibrosis in this disease may be initiated by cytokines produced by the granulomas which form around helminth eggs deposited in hepatic portal tributaries. From serum-free culture supernatants of isolated hepatic granulomas, we purified a fibroblast growth factor (designated FsF-1) by gel filtration followed by heparin-affinity chromatography. FsF-1 is a protein that elutes from heparin with 1.5 M NaCl, has pI 6.2, and Mr 55-58 (reducing SDS-PAGE). It is a potent fibroblast mitogen, but lacks endothelial growth-stimulating activity. We prepared polyclonal anti-FsF-1 antibody which recognized purified FsF-1, but not acidic FGF or PDGF, by dot-blot ELISA or neutralization of biological activity. The amino acid content of FsF-1 is distinct from that of FGF. $CD4^+$ lymphocytes isolated from granulomas produce FsF-1, and a subpopulation of granuloma-derived $CD\ 4^+$ lymphocytes bear FsF-1 on their surface. Based on functional analyses, FsF-1 appears to be distinct from other lymphokines and fibroblast-stimulating cytokines identified to-date. We conclude that FsF-1 is a distinctive lymphokine produced in the setting of chronic inflammation and may play a key role in tissue fibrosis that complicates such inflammation.

Q 135 UPREGULATION OF PDGF α RECEPTORS IN SCLERODERMA FIBROBLASTS, Akio Yamakage, E. Carwile LeRoy and Maria Trojanowska. Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, Charleston SC 29425

TGF-β is mitogenic for human fibroblasts via induction of an autocrine PDGF loop. Also, modulation of PDGF α receptors by TGF β plays a critical role in this process (Ishikawa, et al., J Cell Physiol, 1990; Battegay, et al., Cell, 1990). We compared kinetics of expression of PDGF α receptors after TGF-β (0.01 - 10 ng/ml) stimulation in three human cell types: neonatal foreskin, healthy adult skin and scleroderma skin fibroblasts. All three cell types responded differently to TGF-β. In neonatal foreskin fibroblasts, the receptor number was decreased in a dose dependent manner by all TGF-β concentrations tested. In healthy adult skin fibroblast responses varied depending on TGF-β concentration and cell line: both slight suppression and stimulation of PDGF α receptor number were observed. Distinctive upregulation of PDGF α receptor numbers by TGF-β was a consistent characteristic of scleroderma fibroblasts. In contrast to healthy skin fibroblasts, low concentrations of TGF-β (0.1 - 1 ng/ml) were the most stimulatory. Increased responsiveness of scleroderma fibroblasts to TGF-β may result from previous exposure of these cells in vivo.

Q 136 CHANGES IN NUCLEAR PROTEIN EXPRESSION ACCOMPANY TGF-β1 INDUCED DIFFERENTIATION OF MACROPHAGES, Sue A. Yocum and Ronald A. Ignotz, Dept. of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01655.

Previously, we have shown that TGF-β1 treatment of promonocytic leukemia cell lines, THP-1, and U937, results in the differentiation of a population of these cells into macrophage-like cells. We extend those studies here to show that THP-1 cells which differentiate and become adherent following TGF-β1 treatment appear unable to resume proliferation when restimulated with fetal calf serum. c-myc expression is greatly reduced in these differentiated cells but remains elevated in the non-adherent population of cells. TGF-β1 induces the expression of a polyadenylated variant of histone H2b which has been reported to be expressed by growth inhibited and terminally differentiated cells. Also induced by TGF-β1 is statin, a 57 kDa nuclear protein expressed by growth arrested cells but not by proliferating cells. Detectable expression of statin is not observed in non-adherent cells even though they were exposed to TGF-β1. In addition, these cells are refractile to growth inhibitory effects of TGF-β1 when resupplied with fresh TGF-β1. These results further delineate the growth inhibitory mechanisms of TGF-β1 and suggests that changes in nuclear organization occur that may be incompatible with continued proliferation.

Extracellular Matrix Molecules in Wound Repair

Q 200 LYMPHOCYTES AND CONNECTIVE TISSUE COMPONENTS. EVIDENCE FOR A FIBRONECTIN DEGRADING LYMPHOCYTE FUNCTION, Sten-E. Bergström, Karl-G. Sundqvist. Department of Clinical Immunology and Pulmonary Medicine, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

Mononuclear cells change the pattern of secreted proteins synthesized by fibroblasts. This effect appears to reflect a direct action of the mononuclear cells on extracellular forms of newly synthesized fibroblast proteins, preferentially those with collagen-binding capacity. Lymphocytes, particularly when activated, mediate the effect which takes place with both autologous and allogeneic combinations of mononuclear cells and fibroblasts whereas monocytes rather act inhibitory. In the presence of mononuclear cells fibroblasts show disappearance of 250-500 kd extracellular protein(s) accompanied by appearance of proteins with lower molecular weight. The high molecular weight material (250-500 kd) comigrates with iodinated plasma fibronectin and reacts with anti-fibronectin antibodies. The disappearance of high molecular weight proteins as well as appearance of proteins of lower molecular weight require direct contact between the mononuclear cells and fibroblasts and conditioned medium from mononuclear cells or various cytokines (TNFα, IL-1, IL-4, TgFß, IL-2 and IFNγ) do not transfer the effect.

Q 201 ENHANCED EXPRESSION OF NEURAL CELL ADHESION MOLECULES (N-CAM) AND TENASCIN (CYTOTACTIN) DURING WOUND HEALING, Cheng-Ming Chuong and Hai-Ming Chen, Department of Pathology, USC School of Medicine, Los Angeles, CA 90033.
Both neural cell adhesion molecules (N-CAM) and tenascin (cytotactin) are important in embryonic morphogenesis but their expression are greatly reduced in adult. We asked whether they will be induced during wound healing. We compared the spatial and temporal expression patterns of these two and other adhesion molecules in the healing of skin, cartilage, and tendon. N-CAM, tenascin and fibronectin are all induced in the granulation tissue but the order of prevalence in fibronectin - tenascin - N-CAM. The order of appearance is N-CAM and fibronectin - tenascin. The order of disappearance is N-CAM - tenascin - fibronectin. L-CAM is present in the epidermis undergoing re-epithelization. Explant cultures showed that N-CAM and tenascin are synthesized by wound fibroblasts. These results suggest that N-CAM and tenascin, widely used in embryonic morphogenesis, are induced in a variety of connective tissues during wound healing.

Q 202 CHEMICAL AND MORPHOLOGIC ANALYSES OF PROTEOGLYCANS IN RABBIT CORNEAL SCARS, Charles Cintron, Claire L. Kublin, *John D. Gregory, *Shridar P. Damle, and Henry I. Covington. Eye Research Institute, Boston, MA 02114, and *The Rockefeller University, New York, NY 10021-6399.
Localization of proteoglycans (PGs) in sections of corneal scars were determined by cuprolinic blue dye staining and indirect immunofluorescence. Normal corneal stroma contains high-sulfated keratan sulfate PG (KSPG) and chondroitin 4-sulfate PG (C4SPG). Corneal scars contain C6SPG in addition to C4SPG throughout the scar tissue. Chondroitin, not evident in normal cornea, is present only along the edge of the scar. KSPG, primarily low-sulfated in scar, is distributed throughout the tissue. Some high-sulfated KSPG is present only in the anterior two-thirds of the scar. During the first week, normal PGs are lost from the cornea adjacent to the wound, but are soon replaced by a new pattern of PGs. The ratios of KSPG to CSPG in normal cornea, 2-week-old scar, and adjacent cornea are 2.3, 0.6, and 1.5, respectively. CSPGs from adjacent corneal tissue have a higher charge density than those from scar or normal cornea, and an unusually large CSPG is present only in scar tissue. We contend that PGs are not uniformally distributed in corneal scar tissue, and that the opacity of the tissue is due to the physical and chemical properties of PGs in scar.
This study was supported by NIH RO1 01199.

Q 203 DUCTUS ARTERIOSUS SMOOTH MUSCLE CELLS USE INTEGRIN RECEPTORS TO ADHERE TO AND MIGRATE OVER EXTRACELLULAR MATRIX (ECM) PROTEINS, Ronald I. Clyman, Randall H. Kramer, and Françoise Mauray. Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA 94143.

Permanent closure of the ductus arteriosus is accomplished by migration of smooth muscle cells (SMCs) through the surrounding extracellular matrix (ECM) to form intimal mounds. We used cultured fetal sheep ductus SMCs to identify the cell surface receptors that adhere to the ECM components (fibronectin [FN], laminin [LN], collagen I [Col I] and IV [Col IV], and vitronectin [VN]). Using ligand (FN, LN, Col I, Col IV, or VN)-affinity chromatography, immunoprecipitation, and SDS-PAGE, we isolated a series of receptors of the integrin superfamily: integrin complexes are classified by one of 7 β subunits, which combine with one of several α subunits. The receptors on the ductus SMCs that bind to the FN-affinity column are $\alpha5\beta1 > \alpha v\beta1 >> \alpha3\beta1 >> \alpha v\beta3$; to LN, $\alpha1\beta1 >> \alpha v\beta3$; to Col I, $\alpha1\beta1 = \alpha2\beta1$; to Col IV, $\alpha1\beta1$; and to VN, $\alpha v\beta3 > \alpha v\beta1$. Ductus SMCs adhere to surfaces coated with these ECM components. Consistent with the affinity chromatography data, a specific monoclonal antibody against the common $\beta1$ subunit blocks SMC adhesion to FN, LN, Col I, and Col IV by >90% but blocks adhesion to VN by 50%. Similarly, an antiserum against the $\alpha v\beta3$ complex blocks SMC adhesion to VN by 50% and has no effect on adhesion to LN or FN. The combination of the $\beta1$ and $\alpha v\beta3$ antibodies blocks adhesion to VN by >90%. In addition, ductus SMCs migrate over surfaces coated with these ECM components. Concentrations of the monoclonal antibody to the $\beta1$ subunit (that do not alter cell adhesion) significantly inhibit SMC migration on FN, LN, Col I, Col IV, and VN. TGF$\beta1$ alters the SMCs' integrin profile and inhibits their ability to migrate on the ECM. Thus, ductus SMCs express multiple integrin receptors that mediate cell attachment to and migration over the ECM and may regulate vessel remodeling after birth.

Q 204 INDUCTION OF SYNDECAN EXPRESSION IN HEALING WOUNDS. **Klaus Elenius, Seppo Vainio* and Markku Jalkanen. Department of Medical Biochemistry, University of Turku, SF-20520 Turku, Finland, and *Department of Orthodontics and Pedodontics, University of Helsinki, SF-00280 Helsinki, Finland.**

Cell surface matrix receptors are proposed to regulate cell proliferation, migration and differentiation during organogenesis, carcinogenesis and wound healing. One newly discovered matrix receptor is syndecan, an integral heparan sulfate proteoglycan, that binds via its extracellular glycosaminoglycans selectively to certain extracellular matrix molecules (e.g. collagen types I, III and V and fibronectin) but also to some growth factors, e.g. basic fibroblast growth factor (bFGF). We have used immunohistochemical and in situ hybridization techniques to detect the expression of syndecan in normal and wounded mouse skin. In normal skin, syndecan is expressed almost exclusively around epithelial cells of the epidermis and hair follicles. After wounding, the production of syndecan in migrating and proliferating epidermis and in the proliferating hair follicle cells at the wound edge is strongly enhanced. According to in situ hybridization experiments syndecan gene is activated also in the developing granulation tissue 3 to 4 days after full-thickness wounding and the expression is almost completely absent by the following 2 more days. The transcription of syndecan gene in granulation tissue is restricted to few scattered cells beneath and in front of the migrating epidermis. Our immunohistochemical data suggests that the cell types in which syndecan gene is activated during granulation tissue formation include also endothelial cells of growing capillaries. This allows us to speculate that activated synthesis of syndecan by the endothelial cells could cause binding of more bFGF molecules to the cell surface that could lead to stimulated angiogenesis. In conclusion, the expression of syndecan, both at protein and mRNA level, is significantly induced during cell proliferation and migration in healing cutaneous wounds and the pattern of enhanced expression is spatially and temporally highly restricted.

Q 205 GELATINASE EXPRESSION IN HEALING CORNEAL WOUNDS, M. Elizabeth Fini, Masao Matsubara, James D. Zieske, Charles Cintron, Marie T. Girard, and Claire L. Kublin, Eye Research Institute/ Harvard Medical School, Boston, MA 02114.

We have investigated events leading to expression of the gelatinolytic metalloproteinases, MMP-2 and MMP-9, in rabbit and rat cornea. MMP-2 in the proenzyme form, but not MMP-9, is synthesized by stromal cells of normal cornea. No change was found after epithelial scrape wounding. However, after thermal burn or keratectomy, wounds which damage both the epithelial and stromal layers of cornea, corneal cells also synthesize MMP-9. The enzyme is found primarily in the proenzyme form. It appears in both the epithelium and stroma, but is precisely localized to the regenerating area. Synthesis begins immediately after wounding and correlates with loss of the MMP-9 substrate, type VII collagen from the epithelial basement membrane zone after thermal burn. Also, enzyme disappears from the cornea within a few weeks after keratectomy, co-incident with reappearance of type VII collagen fibrils. MMP-2 expression is also increased after thermal burn or keratectomy, but unlike MMP-9, MMP-2 appears in both the pro- and activated forms. Also, the new enzyme is found only in the stromal layer of cornea, but like MMP-9, is precisely localized to the regenerating area. New MMP-2 appears later in the wound healing process than does MMP-9, paralleling entry of fibroblasts into the regenerating area. These changes in MMP-2 expression are still apparent in the corneal scar as long as 7 months after keratectomy. Our data suggest that each of the gelatinases has a different role in maintenance of the normal corneal structure. We hypothesize that MMP-2 performs a surveillance function in normal cornea, catalyzing degradation of collagen molecules that occasionally become damaged. MMP-9 appears to be important in the early stages of wound healing, and might help control re-synthesis of the epithelial basement membrane. During the later stages of wound healing, MMP-2 appears to assume again the dominant role, possibly participating in remodelling of the corneal scar over a prolonged period. *Supported by EY08408 (MEF), EY05665 (JDZ), and EY01199 (CC). MM was a fellow of Bausch and Lomb.*

Q 206 INTERLEUKIN-6 EXPRESSION CAN BE INDUCED BY A FIBRIN PEPTIDE, Gerald M. Fuller and Hernan E. Grenett, Dept of Cell Biology, University of Ala. Birmingham, AL. 35294

The formation of fibrin provides an insoluble protein polymer to aid in the processes of hemostasis. In addition, fibrin serves as a rich biomatrix for the binding, migration, and stimulation of cells involved in wound healing and tissue repair. Fibrin is slowly and systematically solubilized by the action of plasmin as a necessary part of tissue reformation. We investigated the potential biological role of the peptides derived during the solubilization of fibrin. We found that the fragment derived from the N-terminal region of fibrin (fragment E) induces IL-6 gene expression in macrophages and fibroblasts. Northern blot analyses showed that fragment E stimulates the expression of the IL-6 mRNA in a dose and time dependent manner. Additional exploration of the binding revealed the presence of a specific fragment E receptor with moderately high binding affinity. That one of the peptides of fibrin is involved in inducing the expression of IL-6 during a time when tissue reconstruction is occuring implicates IL-6 to be involved in the wound healing processes.

Q 207 FURTHER LOCALIZATION OF THE TWO BINDING SITES FOR FIBRIN WITHIN FIBRONECTIN, Leslie Gold and Agueda Rostagno, Department of Pathology, New York University Medical Center, New York, NY 10016

Fibronectin (Fn) is a high molecular weight glycoprotein (440 KDa dimer) composed of discrete domains that interact with various ligands, including fibrin, and surface receptors of various cells, such as platelets. Following enzymatic digestion of Fn with subtilisin, two fragments (domains) of 20 KDa and 11 KDa demonstrated fibrin binding activity by retention during fibrin affinity chromatography. The 20 KDa fragment demonstrated a lower affinity of binding to fibrin and by amino acid sequence determination was shown to commence at residue 18 (lysine) from the N-terminal of the intact Fn molecule. The 11 KDa fragment possessed a high affinity interaction since it required denaturing conditions (6.0M urea or 50% ethylene glycol) for its elution; this fragment begins at residue 2122 (glutamic acid) in the C-terminal of the Fn molecule. Both fibrin-binding polypeptide fragments are derived from type I repeat modules of Fn. A monoclonal antibody to the N-terminal of the Fn molecule and a polyclonal antiserum to the 11 KDa fragment inhibit the binding of Fn to fibrin using an ELISA thus, indicating that both fibrin binding sites are operable within the intact molecule; the C-terminal site contributes approximately 60% to the total fibrin binding interaction. With the use of synthetic peptides and/or genetically expressed Fn type I repeat modules, we plan to further delineate the primary structure that dictates fibrin binding specificity. Exploration of the structure function relationship of Fn binding to fibrin should elucidate mechanisms by which Fn plays a role in wound healing.

Q 208 EXTRACELLULAR MATRIX DEPOSITION IS REQUIRED FOR INJURY-INDUCED CELL MIGRATION ACROSS THE BASEMENT MEMBRANE, S.R. Gordon, B. Drabik, M.D. Sabet and C. Staley, Department of Biological Sciences, Oakland University, Rochester, MI 48309

Injury-induced cell migration of rat corneal endothelial cells across Descemet's membrane (the tissue's natural basement membrane) is dependent upon extracellular matrix (ECM) deposition. Tissue injury promotes cytoskeletal reorganization, fibronectin (FN) deposition, and cell migration. Using organ culture, we show that colchine treatment greatly retards cell movement, whereas cytochalasin B only slows down this process. Ultrastructural immunoperoxidase studies reveal that colchicine greatly reduces FN deposition while cytochalasin B has no effect, thus indicating that microtubule integrity is crucial for ECM deposition. Furthermore, exposure to 10^{-6}M monensin also retards cell movement, and results in the accumulation of FN positive vesicles within the cytoplasm. When injured endothelia are organ cultured in the presence of 10% anti-FN antisera, cell migration is greatly impeded. The inhibitory effect of the antisera is reversed when tissues are transferred back into medium containing 10% non-immune serum. Therefore, in the rat corneal endothelium, the deposition of ECM proteins, particularly FN, is important for injury-induced cell movement to occur across the natural basement membrane. (Supported by Grant EY-06435 from the NIH.)

Q 209 INTEGRIN MEDIATED INDUCTION OF CYTOKINE GENES IN MONOCYTES, Stephen Haskill, David F. Eierman and Sarah A. Sporn, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599-7295.
Adherence of monocytes to extracellular matrices (ECM) and/or connective tissue cells results in selective and rapid induction of important inflammatory mediators and growth factors. Several of these genes, including IL-1 and all three members of the GRO family, show little selectivity dictated by the ECM. In contrast the IL-1ra gene is tightly regulated by adherence interaction with fibronectin whereas monocyte adherence to collagen shows little induction. Other genes including the Act-2 and several novel adherence-induced genes we have recently described (J. Immunol. 1990, 144:4434-4441), are poorly expressed on fibronectin and show super-induction on collagen. In order to determine the degree to which these inductive events are dependent upon specific integrin interactions, we have studied the stimulation of non-adhered monocytes by various a-chain and b-chain-specific antibodies. Stimulation with anti-β1 resulted in similar levels of gene induction for all inflammatory genes studied regardless of the ECM specificities determined by adherence. Engagement of single VLA α-chain determinants with specific antibodies did not result in gene expression. Stimulation with anti-β2 antibody in the absence of a second signal (LPS) failed to stimulate gene expression. It is likely that interactions with components of the ECM are important in determining the host response during tissue repair.

Q 210 POSTOPERATIVE CHANGES IN COLLAGEN SYNTHESIS IN INTESTINAL ANASTOMOSES OF THE RAT, Thijs Hendriks and Michael F.W.C. Martens, Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Collagen synthesis is an essential feature of anastomotic healing in the intestine. We have studied postoperative collagen synthesis, measured in vitro as incorporation of (3H)proline into collagenase-digestible protein, in intestinal anastomoses from 3 hours to 28 days after operation. For this purpose, both an ileal and a colonic anastomosis were constructed within the same animal and the results in both intestinal segments were compared.
In ileum, collagen synthesis was already significantly increased, with respect to unoperated controls, 3 hours after operation. It remained elevated during the period of study, with a maximal 10-fold stimulation 4 days after operation, and had nearly returned to the preoperative level after 4 weeks. The general pattern was the same in colon, although quantitatively different: the increase in synthetic activity appeared delayed in comparison to ileum. Maximal stimulation was approximately 6-fold. In addition, we calculated for each rat the ratio between anastomotic collagen synthesis and the average value found in non-operated control animals. Postoperative stimulation in ileum was higher than in colon in almost every animal examined.
The results demonstrate that the ileum responds more quickly and strongly to wounding than the colon, at least as far as the production of new collagen is concerned. Possibly, this phenomenon contributes to the lower failure rate apparent for anastomoses in the small bowel.

Q 211 DIFFERENTIAL REGULATION OF EXTRACELLULAR MATRIX PROTEOGLYCAN GENE EXPRESSION BY TRANSFORMING GROWTH FACTOR-ß1. Veli-Matti Kähäri, Hannu Larjava* and Jouni Uitto. Dept. of Dermatology, Jefferson Medical College, Philadelphia, PA 19107 and *Dept. of Parodontology, University of Turku, Turku, Finland.
Proteoglycans participate in the formation of connective tissue by interacting with other extracellular matrix components and connective tissue cells. We have examined the effects of transforming growth factor-ß1 (TGF-ß1) on the expression of three distinct chondroitin/dermatan sulfate proteoglycans, decorin (PG II), biglycan (PG I) and versican (large fibroblast proteoglycan) in cultured human skin and gingival fibroblasts. Our results show that TGF-ß1 (5 ng/ml) markedly enhanced mRNA levels for biglycan (up to 24-fold) and versican (up to 6-fold), and the synthesis of the corresponding proteoglycan macromolecules in both types of fibroblasts. The increase in biglycan mRNA levels by TGF-ß1 was found to be coordinate with the increase in type I collagen mRNA levels up to 24 h of incubation, while the enhancement of versican mRNA levels was not coordinate with that of biglycan or type I collagen mRNA levels. Simultaneously, TGF-ß1 significantly decreased decorin mRNA levels and synthesis by up to 70% in the same cells. The reduction in the synthesis of decorin by TGF-ß1 was associated with an increase in the apparent molecular weight of decorin macromolecules due to synthesis of longer individual glycosaminoglycan side chains. The results suggest that these three proteoglycans have different roles in the assembly of the extracellular matrix in situations involving deposition of new connective tissue in response to TGF-ß1, such as wound healing and fibrotic conditions.

Q 212 NORMAL HUMAN MONOCYTES EXPRESS INSULIN-LIKE GROWTH FACTOR (IGF-I) IN RESPONSE TO MATRIX GLYCATION: ROLE IN TISSUE REMODELING, Martina Kirstein, Christopher Aston, Raymond L. Hintz and Helen Vlassara, Laboratory of Medical Biochemistry, The Rockefeller University, New York, NY 10021.

Normal tissue homeostasis requires the finely balanced interaction of cells, such as monocytes and macrophages which play an important role in senescence or local injury through the release of regulatory cytokines and growth factors. Tissue proteins undergo in vivo a series of nonenzymatic reactions with glucose over time to form advanced glycosylation endproducts (AGEs). Macrophages and monocytes possess receptors (a 60 kD and a 90 kD protein) specific for AGE-modified proteins mediating AGE turnover and clearance. Binding of AGE-proteins to monocytes/macrophages has been found to stimulate the synthesis and release of the multifunctional monokines TNF-α and IL-1ß, as well as PDGF, a growth factor with competence activity for mesenchymal cells.

Here we examined the synthesis and regulation of insulin-like growth factor I (IGF-I) which has progression-type properties and stimulates the proliferation of a variety of mesenchymal cells including fibroblasts and smooth muscle cells. Normal human monocytes express IGF-IA mRNA after incubation with in vitro glucose-modified bovine serum albumin (AGE-BSA) as well as when seeded onto in vitro glycosylated fibronectin. Lipopolysaccharide, when added together with AGEs abolishes the induction of IGF-I mRNA, and interferon-τ downregulates IGF-IA mRNA expression. Induction of IGF-IA mRNA may be mediated by AGE-induced IL-1ß since production of IGf-IA can be inhibited by the addition of anti-IL-1ß antibodies but not other antibodies. Studies showed that the AGE-induced IGF-I is the IGF-IA prohormone ($\approx$13-14 kD) and not the mature peptide.

These data support the hypothesis that ubiquitously present AGEs play a role in tissue regeneration and remodeling, but they may also contribute to the development of abnormal proliferative events, such as arterio- and atherosclerosis associated with age and diabetes, via the induction of growth factors with competence as well as progression-type properties in macrophages/monocytes.

Q 213 SPARC BINDS PDGF AB AND PDGF BB AND INHIBITS THE BINDING OF PDGF TO ITS RECEPTOR, Timothy F. Lane, Luisa Iruela-Arispe, Russell Ross, Helene Sage and Elaine W. Raines, Dept. of Biological Structure, Pathology & Biochemistry, Univ. of Washington, Seattle, 98195.

Extracellular matrix components are known to regulate the activity of several growth factors released following vascular injury. During a screen of extracellular matrix proteins, we observed that [^{125}I] PDGF AB and BB displayed specific binding to the extracellular glycoprotein SPARC. PDGF AB and PDGF BB, but not PDGF AA, bound to SPARC in a solid phase binding assay and were immunoprecipitated from solution by anti-SPARC antibodies only when SPARC was included in the incubation. Specificity was demonstrated by competition experiments with unlabeled PDGF. [^{125}I] PDGF BB bound to Western blots of SPARC, but not to blots containing laminin, type IV collagen, type VIII collagen, or a variety of other extracellular proteins. SPARC also inhibited binding of PDGF BB and PDGF AB, but not PDGF AA, to human dermal fibroblasts in a dose-dependent manner. These observations suggest that the availability of free PDGF might be regulated by specific extracellular proteins, and that this specificity is dependent on the dimeric form of PDGF. Since SPARC is secreted by a variety of injured cells *in vitro*, it is likely that increased levels of SPARC and PDGF are present in granulation tissue and in the vasculature following injury. The significance of these interactions is under investigation by immunolocalization of PDGF and SPARC in remodeling tissues.

Q 214 ATRIAL AND VENTRICULAR SIDES OF THE WOUNDED BOVINE MITRAL VALVE REPAIR DIFFERENTLY IN ORGAN CULTURE, Wanda M. Lester, Linda Marwood, and Earl D. Helwig, Departments of Pathology and Medicine (Cardiology), Foothills Hospital and The University of Calgary, Calgary, Canada, T2N 2T9

The mitral valve has atrial (A) and ventricular (V) surfaces, each of which is lined by a monolayer of endocardial cells. The valve stroma contains interstitial cells, collagen, glycosaminoglycans, and elastic tissue. In order to eliminate the effect of endocardium on in vitro wound repair in the mitral valve, 1.5 x 1 cm pieces of the anterior leaflet were scraped on both A and V sides to remove the endocardium. 15 preparations were cultured A surface uppermost for 6 days. By light microscopy (LM), 9 had a layer of surface cells on the V side while none had a surface cell layer on the A side. This difference was significant ($p<0.004$ by McNemar's 2-sided test). To rule out an effect of orientation in culture, 17 bilaterally denuded preparations were cultured V side uppermost. At 6 days, 1 had surface cells on the A side, while all 17 had surface cells on the V side ($p<0.002$). The results suggest that the A and V sides of the mitral valve do not respond to a severe denuding injury in the same fashion. Either the cell population of the valve stroma is not uniform throughout the valve, or extracellular matrix alters cellular responses in this tissue. The latter is possible because the mitral valve, by LM, is richer in glycosaminoglycan and elastin on the A side, and richer in collagen on the V side.

Q 215 PROTEOLYTIC PROCESSING OF A GELATIN-DEGRADING 66.000 DALTON PROTEIN BY UROKINASE AND OTHER PROTEOLYTIC ENZYMES. J. Lohi, A. Tuuttila, K. Tryggvason, T. Vartio and J. Keski-Oja, Departments of Virology, Pathology and Dermatology, University of Helsinki, Helsinki, and Department of Biochemistry, University of Oulu, Oulu, FINLAND.

Pericellular matrices of fibroblastic cells contain a 66 kDa protein that is susceptible to the action of a number of proteinases including plasmin, thrombin, tryptase, chymase and the plasminogen activator urokinase (u-PA). A similar polypeptide has been identified in the culture medium of a number of cultured cells as a 70 kDa (66 kDa) gelatin binding protein. The gelatin binding protein has been found to be a gelatin-degrading enzyme, which upon organomercurial activation behaves like a basement membrane collagenase. In the current work we have analyzed the susceptibility of the cell-derived collagenolytic enzyme to proteolytic degradation by u-PA. We find that like the matrix-associated 66 kDa protein the medium protein is degraded by u-PA, thrombin and tryptase to a 62 kDa form that retains its biological activity as a gelatinase as shown by gelatinolysis assays. The cleavage of the matrix and soluble forms of the protein by urokinase was inhibited by anti-urokinase antibodies but not by aprotinin. The cleavage of the 66 kDa protein by tryptase was partially inhibited by known inhibitors of tryptase but not by aprotinin, soybean trypsin inhibitor or EDTA. Tissue type PA was unable to bring about the cleavage under similar conditions. Direct degradation of pericellular matrices and activation of latent collagenolytic activity by u-PA producing cells is likely to contribute to the ability of the cells to migrate through basement membranes. Our results indicate that the plasminogen activator urokinase may have direct effects on the processing of basement membrane collagenolytic activity which property may be important for cells under different physiological and pathological invasion processes.

Q 216 EFFECT OF EXTRACELLULAR MATRIX (ECM) PROTEINS ON VASCULARIZATION IN WOUND GRANULOMA - A FUNCTIONAL AND HISTOLOGICAL ANALYSIS

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Neovascularization of a wound granuloma, induced by the s.c. implantation of a polyurethane sponge, has been monitored in Wistar rats by measuring the sequential changes in clearance of locally injected ^{133}Xe. Two days after sponge implantation the $t_{\frac{1}{2}}$ clearance value was 58 min., a figure representing passive diffusion, while by day 15 post sponge implantation this figure had fallen to 9.6 min. as a consequence of sponge vascularization. To explore the role of ECM proteins in facilitating the ingrowth of new vessels sponges were pre-soaked in fibrinogen, laminin, type IV collagen or bovine serum albumin (control) solutions prior to implantation. Both laminin and fibrinogen treatments enhanced ^{133}Xe clearance ($t_{\frac{1}{2}}$ at 9 days 13.5 min. and 11.8 min. respectively versus 28.3 min. for controls; $t_{\frac{1}{2}}$ at 12 days 6.4 min. and 8.9 min. respectively versus 14.0 min for control; $p < 0.01$). Histological analysis of sponges showed that these functional changes correlated well with the development of new capillaries. The assay allows quantitative assessment of factors capable of modulating angiogenesis during wound repair.

Q 217 INHIBITION OF ANGIOGENESIS IN VITRO BY Arg-Gly-Asp (RGD)-CONTAINING SYNTHETIC PEPTIDE, Roberto F. Nicosia and Elena Bonanno, Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129

The Arg-Gly-Asp (RGD) amino acid sequence is a cell recognition signal found in the attachment domain of several extracellular matrix (ECM) molecules. RGD-containing synthetic peptides interfere with the attachment of endothelial cells to substrates by competing with ECM molecules for cell membrane receptors. Adhesive interactions between endothelial cells and ECM molecules have been implicated in the regulation of angiogenesis but the function of the RGD sequence in this process has not been fully characterized. The present study was designed to evaluate the effect of the synthetic peptide Gly-Arg-Gly-Asp-Ser (GRGDS) on angiogenesis in vitro. We recently observed that serum-free collagen gel culture of rat aorta is a sensitive method for testing the effects of soluble or ECM factors on angiogenesis (Nicosia RF and Ottinetti A. Lab Invest 63:115-122,1990). Aortic explants embedded in gels of interstitial collagen were cultured in the presence or absence of GRGDS. Peptide-free control cultures gave rise to microvessels that sprouted from the resection ends of the explants. In contrast, cultures treated with GRGDS were markedly inhibited and produced only rare abortive microvessels that underwent early regression. Cultures treated with the control peptide GRGES, which lacks the RGD sequence, behaved as untreated controls generating numerous microvessels. The effect of GRGDS was reversible and depended on the availability of an adequate supply of peptide in the medium. Aortic explants previously inhibited with GRGDS generated microvessels if transferred to a GRGDS-free medium. The results of this study support the hypothesis that angiogenesis is an anchorage-dependent and RGD-sensitive process (Supported by NIH HL43392).

Q 218 RGD-SUPPORTED CORNEAL WOUND HEALING, Nancy J. Sipes, Janet A. Anderson*, Normand Richard*, Michael Rock*, Lisa B. Dreisbach, James W. Polarek and Michael Pierschbacher, Department of Wound Healing, Telios Pharmaceuticals, Inc., San Diego, CA 92121 and *Ophthalmology Research Laboratory, National Vision Research Institute, San Diego, CA 92110

We have developed a method for immobilizing short synthetic RGD containing peptides on the surface of damaged corneas for the purpose of providing support for accelerated reepithelialization. The method involves coupling the RGD peptide to chondroitin sulfate for delivery to the corneal surface. This peptide/chondroitin sulfate conjugate has been subjected to extensive in vitro and in vivo testing. Results will be presented demonstrating the effects of the conjugate in the following models: in vitro cell attachment, in vitro explant cultures of human corneas, and in vivo feline model for dry eye. Together these models provide evidence that the conjugate interacts with corneal epithelial cells via the RGD sequence, and thereby facilitates healing of the corneal surface.

Q 219 FIBROPLASIA IN RESPONSE TO A POROUS BLOWN MICROFIBER, V. Trinkaus-Randall, J. Capecchi, R. Banwatt, H.M. Leibowitz, C. Franzblau, Departments of Biochemistry and Ophthalmology, Boston University School of Medicine, Boston, MA, 02118.

We have demonstrated in vitro and in vivo that stromal fibroblasts penetrate and synthesize connective tissue proteins within a melt blown microfiber (polybutylene:polypropylene 80:20). This material is being evaluated for its use as the peripheral component in a synthetic cornea. To determine the optimal in vivo conditions for fibroplasia; untreated discs, discs treated with Type I collagen, discs preseeded with stromal fibroblasts for 4 days prior to insertion and discs coated and preseeded were placed into corneal interlamellar pockets. Each disc was evaluated clinically each week for a period up to 6 months and the rabbits were sacrificed and corneas were excised at 5 time points for histology, total collagen, total protein and histochemistry. Fibroblasts were detected entering the disc as early as 8 days and by the 42nd day the interstices of the discs were occupied with fibroblasts. The ratio of total collagen/total protein increased from 0.15 at 15 days to 0.49 at 42 days for untreated discs. Type VI has also been followed and is not detected in a normal distribution until the 35th day. The results indicate that the discs were retained in the cornea and that fibroplasia was enhanced with pretreatment. This model is also an excellent system for evaluating the remodelling of connective tissue proteins in response to wound healing.

Q 220 MACROPHAGES REGULATE THEIR PATTERN OF FIBRONECTIN EXPRESSION DURING WOUND HEALING, Livingston Van De Water, Lawrence Brown, Liz Lavigne, Dan Dubin and Timothy Murphy, Departments of Pathology, Harvard Medical School and Beth Israel Hospital, Boston, MA, 02215

Fibronectins (FN) comprise a family of adhesive glycoproteins important to wound healing. These proteins arise by alternative splicing of a single gene transcript at three sites, termed EIIIB, EIIIA, and V. Plasma FN, in which the EIIIB and EIIIA domains are excluded, is a prominent component of the provisional matrix at the outset of wound healing. We have recently observed that local synthesis of FN mRNA also occurs during wound repair including FN mRNAs containing the EIIIB and EIIIA domains. These latter FN mRNAs are expressed in a spatially restricted fashion suggesting that they may have roles in cell migration, division or differentitation. To determine the type of cells responsible for this local FN synthesis, we have performed in situ hybridization on sections of healing cutaneous wounds with probes for FN mRNAs and for fibroblast and macrophage-specific mRNAs. We find in this model that within the first few days macrophages at the wound margin express EIIIB- and EIIIA-containing forms of FN mRNA. After this time, fibroblasts are the predominate cell type expressing these forms of FN. Cells in adjacent dermis and muscle produce forms of FN largely excluding these domains. These results suggest that macrophages play an important role in the early stages of wound healing by producing functionally appropriate forms of FN and indicate that alternative splicing of FN is regulated by these cells in situ.

Q 221 EXTRACELLULAR MATRIX OF SKIN FROM PROGRESSIVE SYSTEMIC SCLEROSIS PATIENTS EXHIBITS WOUND HEALING CHARACTERISTICS

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Increased production of tenascin, a large oligomeric glycoprotein, has been associated with fetal development and wound healing in skin. Distribution of tenascin and other glycoproteins, including a new glycoprotein, designated stromalin, was analyzed in skin of patients with early and advanced progressive systemic sclerosis (PSS). Whereas in normal skin, tenascin was found to be present in a narrow zone at the dermal-epidermal junction, in PSS skin, tenascin was present deep into the dermis. Similar alterations in the distribution of stromalin were seen in PSS skin. Moreover, intensities of staining for stromalin and for type VI collagen were significantly higher in PSS skin. The punctate staining for type VII collagen extended deeper into the dermis of PSS skin than of normal. Ultrastructural studies indicated the presence of multilayered basal laminae, anchoring fibrils deeper into the dermis and wide-banded fibers resembling FLS collagen which surrounded many of the dermal capillaries in PSS skin. These changes observed in the dermis of PSS patients resembled the changes reported in skin during wound healing, and therefore, support the hypothesis that PSS is a condition resembling an extended wound healing.

Q 222 THE EXTRACELLULAR MATRIX IN FETAL WOUND HEALING. David J Whitby & Mark W J Ferguson Department of Cell and Structural Biology, University of Manchester, Manchester, England.

Fetal wound healing differs fundamentally from adult wound healing. Fetal wounds heal rapidly, without scar formation or inflammation and in a regenerative fashion, unlike adult wounds where scar formation limits tissue function and growth. Defining the mechanisms underlying the differences between adult and fetal wound healing may allow manipulation of the adult wound so that it heals in a fetal-like manner without scar formation. The spatial and temporal distribution of components of the ECM have been analyzed in upper lip wounds of 14, 16 and 18 d fetal mice, neonatal and adult mice from 1 h to 12 d post wounding. Collagen types I, III, IV, V and VI, fibronectin (Fn), tenascin (Tn), laminin, chondroitin and heparan sulphates were studied by immunostaining of cryosections. Staining for endogenous immunoglobulins was used as a marker for an inflammatory response. The distribution of Fn was similar in adult and fetal wounds. The pattern of Tn deposition was similar in adult and fetus but it was present in the fetal wounds from 1 h post wounding whilst in the adult it was first present at 24 h. Tn inhibits the integrin mediated cell adhesion effect of Fn and during development its appearance in the pathways of neural crest cell migration correlates with the initiation of cell migration. Tn was present in fetal and adult wounds prior to cell migration. Its early appearance in the fetal wounds may underlie their rapid epithelialization. All of the collagen types studied were present in fetal and adult wounds. Collagen was deposited from 24 h in the fetus and there was regeneration of a normal tissue pattern whilst in the adult collagen deposition was apparent from 72 h with the deposition of large parallel fibers disrupting the normal tissue architecture. The factors affecting collagen fibrillogenesis in fetus and adult are unknown but may relate to the absence of an inflammatory response in the fetus and differences in the glycosaminoglycans present in fetal and adult tissues.

Q 223 MOLECULAR COMPARISON OF THE INCORPORATION OF PLASMA AND CELLULAR FIBRONECTINS INTO FIBRIN CLOTS, Carole L. Wilson and Jean E. Schwarzbauer, Department of Molecular Biology, Princeton University, Princeton, NJ 08544

Plasma fibronectin (pFN) plays an integral role in early wound healing through its incorporation into blood clots via two fibrin-binding domains and a site for cross-linking to fibrin by coagulation factor XIIIa. Cellular FN (cFN) contains these same domains, but is distinguished from pFN by alternative splicing of three regions in the FN mRNA. One of these, the V region, is included in almost all cFN subunits but is absent from 40-50% of pFN subunits. Although cFN can be detected in healing wounds, it is pFN which is first cross-linked to fibrin when clotting is initiated *in vivo*. To determine if structural differences between the two forms contribute to functional differences in clotting, we have compared the cross-linking of cFN and pFN dimers to fibrin during clot formation. Using purified pFN and cFN in this assay, we have found that pFN is completely cross-linked to fibrin by 2 hours, whereas cFN subunits are never completely cross-linked, even with extended incubation (>20 hours). To determine if the variably spliced regions are responsible for the striking difference in cross-linking patterns, we have expressed cDNA-encoded versions of rat FN subunits which contain the fibrin-binding domains and either include (V+) or exclude (V0) the V region. The resulting recombinant V+/V0 dimers resemble pFN, and V+/V+ dimers resemble cFN. In the cross-linking assay, we find that V+/V0 dimers are incorporated into clots, while V+/V+ dimers are only partially cross-linked. This recapitulates our observations comparing the efficiency of incorporation of pFN and cFN and implicates the V region as an important element in modulating the interaction of FN with fibrin during clotting.

Cell Modulation During Wound Repair

Q 300 RESPONSE OF CULTURED HUMAN CORNEAS TO A THERMAL BURN: COMPARISON OF THE HEALING IN SERUM-FREE VS. HUMAN SERUM-CONTAINING MEDIA. Janet A. Anderson, H. Barry Collin*, Normand Richard, Perry S. Binder, National Vision Research Institute, Sharp Cabrillo Hospital, San Diego, CA 92110-5067, and *University of New South Wales, Kensington, New South Wales, Australia 2033.

The healing capacity of the cornea varies widely with the nature of the wound. Previous studies have indicated that the presence of blood factors enhances aspects of the healing process in this normally avascular tissue. The wound healing progress of organ-cultured human corneas was studied after application of a thermal burn penetrating 60 to 70% of the corneal thickness. Wound healing was calibrated by measuring epithelial cell influx into the wound after seven days in culture. Areas of epithelial cell influx were determined by digitized computer scanning of light micrographs. Human serum and human plasma were equally effective in stimulating epithelial cell entrance into the wound. Each was more active than serum-free medium. Immunohistochemical localization of bromodeoxyuridine incorporation at 1,2,3,4,and 7 days after wounding showed that DNA synthesis was stimulated in the epithelia of both wounded and paired nonwounded corneas by culture in human serum-containing medium. In addition, the wound itself stimulated epithelial cell DNA synthesis, first in the limbal region, followed by stimulation in the nonwounded portion of the cornea. The cultured human cornea offers a useful model for examining the factors which modulate the course of wound healing.

Q 301 CHARACTERIZATION OF CELL SURFACE GLYCOPROTEIN EXPRESSION OF MIGRATING ENDOTHELIAL CELLS. Hellmut G. Augustin-Voss and Bendicht U. Pauli, Cancer Biology Laboratories, Dept. of Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853

Directed migration of endothelial cells towards an angiogenic stimulus is one of the first cellular responses in the cascade of events that lead to the formation of new blood vessels. To examine the hypothesis that endothelial cells express a specific angiogenesis-associated phenotype, we analyzed the cell surface glycoprotein expression of migrating bovine aortic endothelial cells (BAEC). A silicon ring compartmentalization technique was used to analyze single cell migration and a circular scraping technique was employed to study BAEC migration as a population phenomenon. Cell surface glycoprotein expression was probed with a panel of lectins using a light microscopic cytochemical technique and an enzyme-linked lectin assay (ELLA). Both experimental approaches revealed a strong upregulation of binding intensities for some lectins during BAEC migration (Con A, WGA, PNA after neuraminidase treatment), whereas the expression of binding sites for other lectins remained unchanged (RCA-I, SBA after neuraminidase treatment). Based on these results, the expression of migration-associated cell surface glycoproteins was analyzed by SDS-PAGE. Several cell surface glycoproteins were specifically expressed on migrating BAEC (Con A-binding: 25, 48, 51, and 71 kD; RCA-I-binding: 25 kD; WGA-binding: 25 and 48 kD). Upon incubation with suramin, a growth factor antagonist that inhibits endothelial cell migration, BAEC express a Con A, RCA-I, and WGA-binding 28 kD cell surface glycoprotein. The changes in cell surface glycoprotein expression disappeared after the circularly scraped monolayer reached confluence. These results indicate that endothelial cells express a specific migration-associated phenotype. Probes against the migration-associated glycoproteins might be useful to target endothelial cells during angiogenesis. Supported by NIH-NCI grant CA 47668 and a fellowship from the Deutsche Forschungsgemeinschaft

Q 302 PROLIFERATION AND DIFFERENTIATION IN PRIMARY AND SECONDARY FIBROBLAST POPULATIONS, Klaus Bayreuther, Pal I. Francz, Katharina Maier, Raija Hommel, Klaus Dittmann, and Jochen Gogol. Institut für Genetik, Universität Hohenheim, 7000 Stuttgart 70, F.R.G.

Primary and secondary fibroblast populations of skin of prenatal and postnatal Valo-chicken, C3H-mice, BN-rats, and man are composed of fibroblasts which develop along a terminal differentiation sequence in the fibroblast stem cell system. In the fibroblast progenitor compartment the mitotic fibroblasts develop along the sequence MF I - MF II - MF III. When the mitotic capacity of the mitotic fibroblasts MF III is exhausted, MF III enters the fibroblast maturing compartment, in which the postmitotic fibroblasts differentiate along the lineage PMF IV - PMF V - PMF VI. PMF VI represents the terminally differentiated end cell of the cell system. In PMF VI the greatest number of genes are expressed. After a long period of high metabolic activity PMF VI either degenerates as PMF VII or transforms as PMF VIII. The different stages of the differentiation sequence are under the control of celltype-specific programs. The fibroblasts in dermis show a stratification with the MF I in the boundary zone between reticular dermis and fatty connective tissue and the PMF VI in the boundary zone between papillary dermis and the basal lamina. As a function of aging the mitotic fibroblasts are, different from species to species, partially to totally depleted.

K. Bayreuther et al. (1988) Proc. Natl. Acad. Sci. USA 85, 5112.
K. Bayreuther et al. (1988) J. Cell Sci. Suppl. 10, 115.

Q 303 EFFECTS OF GALLIUM NITRATE ON EPITHELIAL AND NON-EPITHELIAL CELLS FROM HUMAN SKIN, R.S. Bockman, L. Staiano-Coico, P.T Guidon, R. Salvatori and D. Semashko, Cornell University Medical College, New York, N.Y. 10021

Wound repair can be accelerated by increased production of protein matrix components and the modulation of epithelial and non-epithelial cell (keratinocyte and fibroblast) proliferation. We have previously shown that gallium nitrate can mimic the action of transforming growth factor-β1 (TGF-β1) in mesenchymally derived cells by increasing type I collagen expression and decreasing expression of those matrix elements important in bone resorption (P. Guidon & R. Bockman, J. Bone Min Res. 5:S91, 1990). We now describe the effects of gallium nitrate on cells derived from human skin. Primary human fibroblasts and keratinocytes were prepared as previously described (L. Staiano-Coico, J. Clin Invest. 77:369, 1986). Fibroblasts were cultured in the presence of gallium nitrate (0-100 uM) for 48 h; total RNA was isolated then separated on MOPS-Agarose gels, transferred to Nytran and hybridized with ^{32}P-labeled α_1(I)-procollagen or fibronectin cDNA. Cell proliferation was assessed by flow cytometry and ^{3}H-thymidine incorporation. The addition of gallium nitrate caused a dose-dependent increase in procollagen and fibronectin mRNA levels in fibroblasts. Fibroblast proliferation was decreased in a dose dependent manner after 72h of exposure to gallium nitrate. In contrast, a doubling in keratinocyte cell number was seen after seven days. We conclude that gallium nitrate mimics several of the effects of TGF-β1 on cells important for wound healing. The stimulating effect on keratinocyte proliferation appears to be unique to gallium nitrate.

Q 304 PHENOTYPIC FEATURES OF RADIATION FIBROSIS FIBROBLASTS FROM HUMAN BREAST, Danièle Brouty-Boyé, Hélène Raux, Véronique Magnien, Agnès Tamboise, *Bruno Azzarone, I.O.C.M.H., Université Paris-Nord, 93000-Bobigny; *U268, 94804-Villejuif, France.

To explore the mechanism of fibroplastic reactions, especially those implicated in breast, fibroblasts from post-radiation breast fibrosis (PRF) were compared to normal mammary gland fibroblasts for their karyotypes, proliferative activities, cytoskeleton and extracellular matrix composition and for their responses to growth factors. Despite multiple chromosomal anomalies, the proliferative activity of PRF fibroblasts did not significantly differ from that of normal cells : they did not survived longer, required serum to grow and they did not form colonies in soft agar. Type I, III,V collagen was similarly expressed in all types of fibroblasts. However, in contrast to normal fibroblasts, PRF cells produced high amount of oncofetal fibronectin and contained the α-actin isoform specific for smooth muscle cells. Response to EGF, FGF, PDGF and TGFα was lower in PRF fibroblasts than in normal fibroblasts and it was enhanced by TGFß, by itself poorly mitogenic. These results suggest the presence of atypical fibroblasts with fetal and myofibroblastic features in PRF and the implication of certain growth factors in their emergence *in vivo*.

Q 305 IN VIVO WOUND REPAIR AND IN VITRO PROLIFERATIVE CAPACITY OF DERMAL FIBROBLASTS AS A FUNCTION OF AGE, Sarah A. Bruce and Scott F. Deamond, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205.

A longitudinal study was performed to confirm the inverse relationship between the in vitro proliferative capacity of dermal fibroblasts and the in vivo age of the donor tissue previously observed in a cross sectional study, and to investigate the relationship between the growth of dermal fibroblasts in vitro and wound repair in vivo. Fibroblast cultures were generated from skin punch biopsies from 12 individual male Syrian hamsters (strain LVG) beginning at 1 month of age and at 6 month intervals thereafter until the natural death of the animal (median life span, 19.5 months; maximum life span, 36 months). All cultures from all individuals exhibited finite proliferative capacity, and an inverse relationship was observed between donor age and maximum in vitro proliferative capacity. In addition, a direct correlation between the in vitro proliferative capacity of the dermal fibroblasts in vitro and the repair efficiency of the biopsy site was observed. However, these changes in the in vitro proliferative capacity and in vivo wound repair efficiency were not progressive beyond 12-18 months of age and were not indicative at any age of an individual's ultimate life span. This study provides evidence that in vitro proliferative capacity of dermal fibroblasts and in vivo wound repair may be comparable phenomena that share common mechanisms. However, the non-progressive nature and the lack of correlation between these phenomena and the individual's ultimate life span indicate that their use as biological markers of aging is limited to animals younger than the median life span of the species. (Supported by NIH AG-03633 and AG-07875)

Q 306 CONSTITUTIVE MYC EXPRESSION IMPAIRS 'IN VITRO' HYPERTROPHY AND CALCIFICATION OF CARTILAGE, Rodolfo Quarto*, Beatrice Dozin*, Carlo Tacchetti*, Grazia Robino*, Martin Zenke+, Giuliano Campanile*, and Ranieri Cancedda*. *Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy and +Institute for Molecular Pathology, Wien, Austria.
Quail embryo chondrocytes (QEC) were infected with a retrovirus carrying the myc oncogene (pM5). In adherent culture, QEC-pM5 displayed a differentiated phenotype and synthesized type II collagen and Ch21 protein, while uninfected chondrocytes synthesized type I collagen. When cultured in suspension, in the constant presence of ascorbic acid, QEC-pM5 reconstituted a tissue histologically defined as cartilage. In these conditions these cells produced very low levels of alkaline phosphatase and no type X collagen (specific marker of hypertrophic chondrocytes). In addition no calcification of the reconstituted cartilage was found. Control uninfected QEC in the same conditions reconstituted calcifying hypertrophic cartilage with high levels of type X collagen and alkaline phosphatase activity. The cell density for surface unit was about 1.49 folds higher in QEC-pM5 reconstituted cartilage than in its normal counterpart. We conclude that the constitutive expression of the myc oncogene in quail embryo chondrocytes is compatible with a limited posssibility of differentiation to stage I, but it impairs the cells to reach the fully differentiated stage II chondrocytes.

Q 307 **EXPRESSION AND MODULATION BY HEPARIN AND γ-INTERFERON OF α-SMOOTH MUSCLE ACTIN IN CULTURED FIBROBLASTS**, A. Desmoulière, L. Rubbia-Brandt and G. Gabbiani, Dept. of Pathology, University of Geneva, 1, rue Michel Servet, 1211 Geneva 4, Switzerland.
Several observations suggest that fibroblastic cells represent an heterogeneous population during normal conditions *in vivo*. Furthermore, clinical and experimental investigations have shown that, during wound healing and fibrocontractive diseases, fibroblasts acquire more or less permanently morphological and biochemical features of smooth muscle (SM) cells including the expression of α-SM actin. Primary and passaged cultures of rat and human fibroblasts contain a sub-population of cells expressing α-SM actin. These cells could derive from local SM cells and/or pericytes, or represent fibroblasts modulated in culture. We have investigated the presence of α-SM actin in many fibroblast cultures and clones. We have also studied the action of heparin (Sigma type II, Sigma Chemical Co., St. Louis, MO) and γ-interferon (γ-IFN, rat, gift of Dr. P. van der Meide, TNO Primate Center, Rijswijk, NL, and human, gift of Boehringer Ingelheim AG, Basel, CH) on α-SM actin protein and mRNA expression in fibroblasts by immunofluorescence and Western blot with a specific monoclonal antibody and by Northern blot with an actin mRNA probe. The different fibroblastic populations studied showed always a proportion of α-SM actin expressing cells. Even after cloning, we never obtained populations negative for α-SM actin. We conclude that α-SM expression is not due to contaminant cells but is a feature of fibroblasts themselves which can develop when specific functional properties are required. Passaged fibroblasts treated for 7 days with 100 μg/ml of heparin or 1000 U/ml of γ-IFN showed a significant decrease of proliferation compared to untreated cells. α-SM actin protein and mRNA expression were increased by heparin and reduced by γ-IFN. Our results support the view that fibroblastic cells are an heterogeneous population. The expression of α-SM actin in these cells can be modulated by cellular factors or extracellular components.

Q 308 **KELOID TUMOR FIBROBLASTS EXHIBIT BIOLOGICAL VARIANCE IN VITRO: DNA CONTENT IS ASSOCIATED WITH RESPONSE TO EDF,** Jane S. Gibson, Russell R. Jaicks, Patricia B. Willoughby, and Charles A. Buerk. Department of Surgery, Orlando Regional Medical Center, Orlando FL 32806.
Keloids are benign tumors of dermal fibroblasts which result from an abnormal wound healing response and have considerable variability with respect to local extension. We have observed biological variability of keloid tumor fibroblasts in vitro with respect to DNA content and inhibition of fibroblast function by epidermal derived factors (EDF). Cell lines derived from keloid tumor fibroblasts (BKF, 134 M, FDK) and normal fibroblasts (HFF, 134 T, RDF) were evaluated for DNA content using flow cytometry. HFF, 134 T, and RDF exhibited diploid amounts of DNA, as did BKF keloid fibroblasts, while 134 M and FDF were found to have aneuploid and aneuploid (near tetraploid) cell components respectively. An involvement of EDF in promoting wound re-epithelization and minimizing scar formation has been proposed[1], therefore normal and keloid fibroblast function in the presence of EDF was evaluated[2]. EDF inhibited the ability of HFF, 134 T and RDF to contract collagen by 18%, 64%, and 29% respectively. BKF (diploid DNA content) was similarly affected (26% inhibition). 134 M (aneuploid DNA component) showed 0% inhibition, while the ability of FDK (near tetraploid DNA content) to contract collagen was actually enhanced (-46% inhibition). Results comparable to those of keloid fibroblasts were obtained with AMF-1, a malignant fibrous histiocytoma cell line (5%). These data suggest that heterogeneity exists among keloids with respect to DNA content, which is associated with susceptibility to EDF inhibition of fibroblast contraction. Furthermore, cell ploidy and responsiveness to growth factors (such as EDF) which regulate normal wound healing, may reflect behavior of these lesions in vivo.

[1] Eisinger, M., et al. 1988 Proc. Natl. Acad. Sci. USA 85: 1937-1941.

[2] Bell, E. et al. 1979. Proc. Natl. Acad. Sci. USA 76: 1274-1278.

Q 309 EPIDERMAL SIGNALLING MODULATED BY THE TRANSFORMING GROWTH FACTOR-ALPHA AND THE INFLAMMATORY PEPTIDE BRADYKININ, Raimund Kast, Gerhard Furstenberger, and Friedrich Marks, Institute of Biochemistry, German Cancer Research Center, D-6900 Heidelberg, F.R.G. The presence of TGFalpha mRNA and - protein in normal epidermis, its increased expression in hyperplastic epidermis and overexpression upon malignant transformation point to a growth-regulatory function of TGFalpha in skin. Accordingly exogenously added TGFalpha has been shown to induce-in an autocrine manner-TGFalpha expression and to initiate clonal replication of cultured keratinocytes. Mitogenic signalling has been found to strictly depend on the activation of the intrinsic tyrosine kinase activity of the epidermal growth factor receptor to which TGFalpha binds. Upon addition of TGFalpha to a mouse epidermal cell line responsive to the mitogenic effect of the growth factor we have observed enhanced biosynthesis of eicosanoids being predominantly the result of increased release of arachidonic acid from membrane phospholipids. The mechanism of this TGFalpha-dependent induction of arachidonic acid liberation is unknown. Here, we present evidence that TGFalpha stimulates the release of arachidonic acid in a dose-dependent manner by activating an endogenous phospholipase A_2 activity. The effect is abolished by pretreatment of the cells with tyrphostins, which inhibit the tyrosine kinase activity of the epidermal growth factor receptor. Moreover the stimulation of phospholipase A_2 activity apparently does not depend on the activation of the IP_3/DAG cascade, which is not activated on TGFalpha challenge. We compared the effects of TGFalpha with those of the inflammatory peptide bradykinin, which also activates an endogenous phospholipase A_2 activity in mouse keratinocytes. In contrast to TGFalpha, bradykinin-induced phospholipase A_2 activity is a G-protein mediated process. Presently, we do not know whether the observed phospholipase A_2 activities belong to identical or different enzymes.

Q 310 DIFFERENTIAL EXPRESSION OF THE CELLULAR ADHESION MOLECULES ICAM-1 AND ELAM-1 IN AN IN VITRO ENDOTHELIAL CELL (EC) WOUND MODEL. J. Ksiazek, R.W. Barton, R.N. Mascardo. Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT and Univ. of Connecticut Health Center, Farmington, CT.
ICAM-1 and ELAM-1 are cell surface glycoproteins that are at least partially responsible for CD18-dependent and CD18-independent, respectively, leukocyte adhesion to cellular substrates and migration into inflammatory sites. The expression of both molecules on EC is up-regulated by pro-inflammatory mediators with differing time courses of expression. To clarify the expression of both molecules induced by pro-inflammatory mediators on motile EC we have observed the changes in expression of ICAM-1 and ELAM-1 on confluent EC induced by an artificial wound stimulus in the presence of TNFα(50 units/ml). Human umbilical vein EC were cultured to confluency on glass cover slips, exposed to TNFα for 24 hrs, wounded by scraping off part of the monolayer, cultured for differing time periods, fixed and immunoperoxidase stained for ICAM-1 and ELAM-1 expression. In this model wounding induces EC polarization response and migration within 3 hrs after wounding. Cells at the border of the wound exhibited a progressive decrease in ICAM-1$^+$ cells (from 55% at 15 min to 25% at 3 hrs) whereas the cells in the confluent monolayer showed no change (55%). In contrast, ELAM-1 expression at the wound border was increased at 15-30 minutes post-wounding (65% vs. 25% at time 0) and decreased to 25% at 3 hrs. Monolayer cells maintained a constant ELAM-1 expression (25%). Thus, directed cell migration of TNF-stimulated EC was associated with a progressive decrease in ICAM-1 expression and a transient increase in ELAM-1 expression.

Q 311 **DEVELOPMENT OF AN ANTIBODY TO THE PRODUCT OF THE CHICKEN *GRO* GENE AND IMMUNOLOCALIZATION IN TISSUES OF NEWLY HATCHED CHICKS.** Martins-Green, M.*, Aotaki-Keen, A.+, Hjelmeland, L.+and Bissell, M. J.*. *Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, University of California Berkeley, CA 94720; + Department of Ophthalmology, University of California, Davis, CA 95616.

The chicken *gro* (9E3/CEF4), along with the human *gro*(MGSA), mouse *gro*(KC) and hamster *gro*, is a member of a family of genes that is evolutionarily conserved. The products of these genes have homologies with inflamatory mediators and may be involved in certain aspects of cell growth. We and others have shown that 9E3 is expressed in cells cultured under conditions that favor growth and is not expressed when cells are not growing. More recently, we have shown that this gene is expressed in normal connective tissue of newly hatched chicks, it is overexpressed in wounded tissues and is not expressed in RSV-induced tumors. Eventhough thse correlations have been made, a specific function for these genes has not yet been identified. In order to make progress in understanding the function of these genes, we have produced antibodies against the 9E3 protein. we have used a peptide sequence to produce polyclonal antibodies in rabbits. The titer and specificity of the antisera where determined by immunoblot analysis on nitrocellulose membranes with alkaline phosphatase-conjugated goat anti-rabbit IgG. Immunostaining of tissues shows that the protein is present in the cells and extracellular matrix of connective tissues, ti is not present in RSV-induced tumors but it is abundantly found in the granulation tissue of wounded wings. We are presently investigating the biochemical characteristics of this protein by immunoprecipitation with the antibody from the supernatant of cultured cells that are known to express the gene abundantly.

Q 312 TECHNOLOGICAL DEVELOPMENT OF LIPID MICROTUBULES FOR WOUND REPAIR, Alan S. Rudolph, Geoffrey E. Stillwell, Rod L. Monroy, and Florence Rollwagen, Center for Biomolecular Science and Engineering, Code 6090, Naval Research Laboratory, Washington, DC 20375-5000, and Naval Medical Research Institute, Bethesda, MD

We are developing lipid-based microtubules as control release vehicles and templates for cellular differentiation in wound repair. Lipid microtubules are hollow cylindrical microstructures (50-200 microns in length and .5 micron in diameter) that can be formed from chemically modified lecithins or galactocerebrosides. We have measured the release rate of a number of proteins from these structures. Metabolic toxicity of a histocytic monocyte cell line (U937) by pulsed tritiated thymidine incorporation and T-cell proliferation has been measured after exposure to increasing lipid microtubule dose. Percent phagocytosis of microtubules by an adherent monocyte population from peripheral blood was found to be reduced significantly by surface modification with 5-10 mole percent gangliosides. Subcutaneous implantation of ganglioside-coated lipid microtubules in agarose blocks in mice have been evaluated for recruitment of specific cell. Evidence of the ability of the microtubules to promote angiogenesis is shown by the presence of Factor VIII positive cells in the block.

Q 313 EFFECTS OF THE LIPIDIC PART OF A HEMODIALYSATE CLINICALLY USED TO IMPROVE WOUND HEALING ON FIBROBLAST POPULATED COLLAGEN LATTICE CONTRACTION, T. Schreier, A. Braakmann, C. Bobillier, W. Baschong, Biochemical Research and Development, Solco Basle Ltd, Switzerland.

Fibroblasts cultured in a three dimensional collagen gel (usually indicated as Fibroblast Populated Collagen Lattice (FPCL)) display a more in vivo like morphology as monolayer cultures. This "dermal" equivalent was used to study the influence of a low MW dialysate from calf blood (Solcoseryl, Solco Ltd., Basel) on the process of wound contraction. We observed, that the lipidic part of this hemodialysate (Folch extract) inhibits the contraction of collagen matrices populated by human skin fibroblasts similarly to retinol, retinoic acid or ascorbate. The Folch extract, Vitamin A, Vitamin A acid or Vitamin C seem to affect the rapid and the slow contraction phase, but not the state before the initiation of contraction (lag phase). Tocopherol (Vitamin E) and Oxypurinol, both radical scavengers, did not influence the contraction of collagen gels. We conclude that free radicals are not involved in the contraction process of FPCL. The implications of the modulation of collagen gel contraction in relationship to wound repair is discussed.

Q 314 UNIQUE INTERMEDIATE FILAMENT ASSOCIATED PROTEINS IN CORNEAL WOUND HEALING, Nirmala SundarRaj, Susan Anderson, Ophthalmology Department, The Eye and Ear Institute and University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

One of the features which is modulated in fibroblasts during wound healing is their cytoskeletal makeup. Cytoskeletal associated changes in the activated fibroblasts in corneal wound healing were analyzed in rabbit by employing specific monoclonal antibodies. Vimentin intermediate filaments (IF) are present both in the quiescent fibroblasts in the normal adult cornea and in the activated fibroblasts during wound healing. However, two unique intermediate filament associated proteins (IFAPs) expressed by fibroblasts in developing cornea and by cultured fibroblasts, were not detectable in the quiescent fibroblasts in normal adult cornea. These IFAPs, consisting of polypeptides with Mr of 130k, and 45k and 48k, respectively, were reexpressed by the corneal fibroblasts activated in wound healing but were absent when these cells reverted to a quiescent state. While the IFAP consisting of 45 and 48k Mr polypeptides was found to be localized at branching points along the IF in cultured fibroblasts, the 130 k IFAP was uniformly distributed along the IF. Therefore, the former IFAP may be associated with the organization of IF network in the fibroblasts. Transient expression of these IFAPs in the fibroblasts in active states of fibroblasts suggests that these IFAPs may be functionally important to the active state of the fibroblasts in developing and regenerating tissues. (Supported in part by NIH grant EY03263)

Clinical Studies

Q 400 THE SYSTEMIC ADMINISTRATION OF TGF-β1 ACCELERATES WOUND HEALING, Edward P. Amento, Leo DeGuzman, Wyne P. Lee, Yvette Xu, Lorrie L. McFatridge, and L. Steven Beck, Inflammation, Bone and Connective Tissue Research, Developmental Biology, Genentech, Inc., South San Francisco, CA 94080

Soft tissue wound healing is a complex but ordered process characterized by inflammation, angiogenesis, repair and remodeling. It is modulated at multiple levels by endocrine, paracrine and autocrine factors. At the local level a number of growth factors, including transforming growth factor β1 (TGF-β1), interact to provide the optimum cellular and biochemical response to produce a healed wound. Impairment of cellular responses directly influences extracellular matrix synthesis and ultimately the quality and strength of the healed wound. TGF-β1 enhances normal wound healing and returns impaired healing to normal conditions when applied topically to wounds. The effect of a single I.V. administration of TGF-β1 on the healing of incisional wounds in rats was examined. Administration of methylprednisolone to rats decreased breaking strength of incisional wounds to 50% of normal control. The I.V. administration of 10 μg/kg TGF-β1 to these healing-impaired rats increased the breaking strength of wounds compared to wounds from rats administered saline alone ($p<0.05$). The I.V. administration of 100 or 500 μg/kg TGF-β1 at the time of surgery increased breaking strength to that of normal-healing animals when measured at day 7. Indeed, the single systemic administration of TGF-β1 24 hrs. before or 4 hrs. after an incisional wound also accelerated wound healing. The breaking strength of incisional wounds from rats administered TGF-β1 (100 or 500 μg/kg) was greater than wounds from rats with impaired healing administered saline ($p<0.05$) and was similar to normal animals. Even though systemic clearance of TGF-β1 occurs in less than 5 minutes, a sustained stimulatory effect on extracellular matrix secretion is evident 24 hours after administration. These results indicate a potential role for the single I.V. administration of TGF-β1 in patients undergoing surgery with known impaired healing conditions.

Q 401 THE EFFECT OF THE PRIMARY AFFERENT NERVOUS SYSTEM ON WOUND HEALING IN AGED RATS, Paul V. Andrews, Mark E. Ardron*, and Robert D. Helme, National Research Institute of Gerontology and Geriatric Medicine, Mount Royal Hospital, Parkville 3052, Australia and *University Department of Geriatric Medicine, Selly Oak Hospital, Birmingham, England.

It has long been felt that ageing adversely affects wound healing rates, and recent studies suggest that an intact nociceptor system of primary afferent nerves are important for successful skin repair. One of the difficulties in this area has been to achieve a standardized experimental wound. We have developed such a technique and used it to measure healing rates in young adult rats (AR, aged 60–100 days), old rats (OR, aged 900 days). A previous study had demonstrated that OR heal at the same rate as rats treated as neonates with capsaicin (aged 60–100 days) to destroy their primary afferent nerves which contain the neuromodulator Substance P (SP).

The fur on the inter scapular region was removed with animal clippers and a depilatory agent. 24 Hours later the animals were anaesthetized with pentobarbitone and a CO_2 laser was used to induce a thermal injury to the area (power 16W, shutter speed 2secs, spot diameter 10mm). Animals were examined daily and the area of the wound was measured every 48 hours. OR healed more slowly than AR (20.13±0.99 days vs 14.50±0.34 days; mean±SD, $P<0.001$). OR had a significantly reduced level of SP in their dorsal root ganglia (57.97±13.62 fmol/mg compared to 82.96±19.15 for AR; $P<0.001$). Intradermal injection of SP (200μl of a 10μM solution 4 times a day for the first 5 days after injury) into the skin around the injury in OR caused the resolution of their injury at a similar rate to AR (15.40±0.58). This data suggests that an intact system of primary afferent nerves and their neuromodulators are important for optimum healing in aged rats.

Q 402 INDUCTION OF GRANULOCYTE - MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) mRNA IN mRNA IN THE EPIDERMIS OF MICE SENSITIVE OR RESISTANT TO THE CUTANEOUS EFFECTS OF PHORBOL DIESTERS. C. Stuart Baxter and Kersi B. Vasunia, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati,OH 45267-0056.

GM-CSF is a cytokine with reported autocrine function in keratinocytes, and in addition is a chemotactic agent for leukocytes and capable of inducing reactive oxygen metabolite production in these cells. These properties strongly imply a role for GM-CSF in dermal inflammation, hyperplasia and other aspects of wound healing and tumorigenesis. In order to further delineate this role, the presence of GM-CSF transcripts in epidermal RNA was determined in mouse strains sensitive (CD-1, SENCAR) and resistant (CBA/J) to the hyperplasiogenic, inflammatory, and tumor-promoting activities of 12-O-tetradecanoyl-13-acetate (TPA), an agent whose dermal effects bear many similarities to those induced by wounding. In all strains no GM-CSF transcripts were detected using a 1.1kb mouse GM-CSF cDNA probe in untreated animals. In the sensitive strains topical application of TPA (10 nmoles) resulted in detection of a predicted 1.2kb band, which appeared to be most intense 3-4 hr after TPA application, and was undetectable by 48 hr. In the resistant CBA strain no transcript was detectable under the same conditions. These findings suggest that induction of epidermal GM-CSF transcription may be an early event in the response of the skin to wounding and other toxic insult.

Q 403 TGF-β1 STIMULATES BONE FORMATION IN CALVARIAL NONUNION DEFECTS, L. Steven Beck*[1], Leo DeGuzman[1], Wyne P. Lee[1], Yvette Xu[1], Lorrie L. McFatridge[1], Nancy A. Gillett[2], and Edward P. Amento[1], [1]Inflammation, Bone and Connective Tissue Research, Developmental Biology, [2]Department of Safety Evaluation, Genentech, Inc., South San Francisco, CA 94080

Bone defects or fractures normally heal without an intervening fibrous scar. However, nonunion healing will result if the gap between the bone edges reaches a critical distance or there is inappropriate fracture fixation. Transforming growth factor β1 (TGF-β1) is known to regulate proliferation and differentiation of osteoblasts and mesenchymal precursor cells *in vitro* and stimulate bone formation *in vivo*. Based on the biological role of TGF-β1, the effect of a single topical application of recombinant human TGF-β1 was evaluated in a model of nonunion calvarial defects in rabbits. A single application at the time of surgery of 0.1, 0.4 or 2 μg TGF-β1 or vehicle alone to 12 mm calvarial defects induced dose-dependent bony closure by day 28. Bridging of the defects was characterized by intramembranous bone formation that contained well formed trabeculae of woven bone. Defects treated with vehicle alone (N=6) were reduced in size by approximately 45% and contained a dense fibrous connective tissue band between the bone margins which appeared quiescent at 28 days. The intermediate doses of 0.1 (N=7) and 0.4 μg (N=8) TGF-β1 reduced the size of the defects 63% and 71% respectively. Sites treated with 0.4 μg TGF-β1 contained islands of bone that appeared to be actively secreting matrix separated by loose connective tissue. The application of 2 μg TGF-β1 resulted in complete bridging in 5 out of 7 sites. The average reduction in this group was 94%. These observations support the hypothesis that TGF-β1 is a potent osteogenic growth factor *in vivo* and may be useful in the treatment of patients with bony defects or nonunion fractures.

Q 404 RECOMBINANT PROTEASE NEXIN I ENHANCES DERMAL WOUND HEALING IN GUINEA PIGS, Richard A. Berg*, David J. Foran*, Randy W. Scott[+] and Carol Ruppert*, Department of Biochemistry, *University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635 and [+]Invitron Corp., Redwood City, CA 94063

Protease Nexin I (PN-I) is a member of the serpin super family of proteinase inhibitors. Since PN-I has been observed to inhibit extracellular matrix breakdown in several systems, we hypothesized that PN-I may enhance wound healing. We tested this hypothesis by using recombinant PN-I on a guinea pig full thickness excision wound model. Guinea pigs were given a dose of 5.25 or 45 micrograms PN-I onto one wound bed. The second wound bed on the same animal served as a control. The guinea pigs were allowed to heal for either six or 12 days after which time the wound was evaluated by determining the hydroxyproline content per dry weight and the tensile-strength of the excised wounds. Histological sections of the wound beds stained with Mason's Trichrome were analyzed using a statistical image analysis system to quantitate the collagen and cells present in the wound bed. Comparing the experimental and control wounds, the results indicated that in six days the hydroxyproline content was increased significantly from 9.4 + 2.3 to 13.1 + 1.6 mg per gram dry wt.; however, the moduli of the stress-strain curves were not statistically different. After 12 days, the hydroxyproline content per gram dry wt. of PN-I-treated wounds was unchanged compared with the controls; however, the moduli of the stress-strain curves were increased from 0.64 + 0.15 MPa to 1.24 + 0.22 MPa. Histological analysis of the Mason's Trichrome stained sections indicated that the granulating beds of wounds treated with PN -I were more cellular at both 6 and 12 days and that there was more matrix deposition in sections of 12-day wounds treated with PN-I. There was also increased vascularization in the PN-I-treated wounds as compared with controls. The results indicated that a single dose of PN-I applied to a full thickness dermal wound at the site of granulation tissue formation improved matrix formation in six days and increased wound strength by 12 days. Our data also suggested that PN-I may enhance wound healing in part by stimulating new blood vessel formation.

Q 405 THE ROLE OF MACROPHAGES AND OXYGEN RADICAL FORMATION AROUND IMPLANTED TITANIUM FOR HEALING OF THE SURROUNDING TISSUES.
Lars M. Bjursten and Pentti Tengvall, Dept. of Experimental Research, Malmö General Hospital, S-214 01 Malmö and Department of Applied Physics, University of Linköping, S-581 83 , Linköping, Sweden

All implanted materials are surrounded with inflammatory cells, especially macrophages. These cells are known to produce hydrogen peroxide and oxygen radicals. When titanium is exposed to hydrogen peroxide the surface becomes more hydrophilic and hydrated titanium peroxy compounds are formed on the metal surface forming a titanium peroxy gel when the excess hydrogen peroxide has been consumed. In *in vitro* experiments this gel inhibits inflammatory cells and displays a selective ion and protein binding. Animal experiments show that titanium implant surfaces may be modified using hydrogen peroxide and titanium peroxy gels to elicit less inflammatory response and a more rapid healing.These differences are probably due to differences in macrophage activation as indicated by presently available histological analyses.

We propose that the interaction of oxygen radicals produced by macrophages oxygen radicals and hydrogen peroxide that is locally produced by for instance macrophages interact with implant surfaces. This has effects both on the implanted material and the organization of the surrounding tissue.

Q 406 **TRANSFECTION OF WOUNDS *IN VIVO* WITH TRANSFORMING GROWTH FACTOR-ß$_1$ ACCELERATES HEALING,** Kenneth N. Broadley and Jeffrey M. Davidson, Dept. of Pathology, Vanderbilt University School of Medicine and Veterans Affairs Medical Center, Nashville, Tennessee 37212
Administration of Transforming Growth Factor ß (TGFß) to a variety of wound models has been shown to accelerate healing. Here, we have introduced a plasmid containing a TGFß$_1$ cDNA driven by the simian virus 40 early promoter (pSV-TGFß$_1$, kindly provided by H.L. Moses, Vanderbilt University) into a model wound system to determine its effect on the rate of wound repair. Control transfections were carried out with pBR322, and the efficiency of transfection was determined using a plasmid expressing ß-galactosidase, also driven by the SV40 early promoter (pCH110, Pharmacia). Wounds were created by subcutaneously implanting polyvinyl alcohol sponges on the ventral surface of male Sprague-Dawley rats. These sponges fill with granulation tissue, and offer a discrete wound that can subsequently be removed from the rats and analyzed by histological, biochemical, and molecular biological techniques. Four days post-surgery, plasmids were introduced into the wound site by simply injecting 100µg of plasmid DNA in a 5 or 20% sucrose solution. Transfection of wounds with pSV-TGFß$_1$ resulted in an increase in the quantity of granulation tissue - a 60% increase collagen content and a 40% increase in cellularity (DNA) - fours days after treatment, over mock- or pBR322- transfected wounds. Plasmid DNA was recovered from wound homogenates, and DNA blotting indicated that there were approximately 20-40 copies of the plasmid per cell. ß-Galactosidase staining revealed a high degree of uptake and expression of the plasmid, with more than 30% of cells showing ß-galactosidase activity. The high efficiency of uptake may be due to the mitotically active wound environment. Histologically, ß-galactosidase activity was found mainly in fibroblast-like cells, although it was also expressed by other cell types. These reults indicate that the wound site is a favourable environment for plasmid uptake and expression, and is a suitable target for gene therapy. (Supported by the DVA & NIA AG06528).

Q 407 EFFECTS OF THROMBIN RECEPTOR ACTIVATING PEPTIDE (TRAP) ON CLOSURE OF FULL DERMAL EXCISIONS IN DIABETIC MICE. D. Carney, W. Redin, V. Paulley, K. Carraway and S. Warner, University of Texas Medical Branch, Galveston, TX 77550.
Previous studies using thrombin and thrombin receptor-activating peptides (TRAPs) in normal mouse and rat models have shown that TRAP-508 increases incisional breaking strength and accelerates closure of full-dermal excisions with a single, topical application post wounding. To examine the effects of TRAP-508 in an impaired model, we studied genetically diabetic C57BL/KsJ-db/db mice and their heterozygous (db/m) littermates. A 1.5 X 1.5 cm full dermal dorsal excision was made through the panniculus carnosus and a single application of synthetic peptide TRAP-508 in 30µl of saline, or control saline was added prior to covering the wound with an occlusive dressing. Wound closure was assessed at one to three day intervals for up to forty days by tracing the perimeter of the wounds onto a flexible matte acetate. The area of each wound was then determined by computer morphometric analysis. Closure of these wounds in untreated db/db mice requires from 30 to 40 days while the control db/m mouse was closed completely within 3 weeks. A single application of TRAP (200ng per wound) increases the total wound closure observed at 13 days in db/m from 83% observed in controls to 95%. TRAP also increases closure in db/db mice from 33% observed in controls to 55%. Thus, a single application of TRAP appears to accelerate closure in the diabetic mouse by up to 65% relative to controls. Much of this increased closure appears to be the result of new tissue growth rather than contraction. Studies are now underway to determine the mechanisms by which TRAP accelerates this process and its possible efficacy for clinical trials. (Supported by NIH-25807 and Texas Advanced Technology Program).

Q 408 A COMPARISON OF DRY AND MOIST WOUND HEALING USING NUDE IMMUNO-DEFICIENT MICE AND HUMAN SKIN TRANSPLANTED ONTO NUDE MICE, Graham R.Elliott, Marianne J.Fasbender, Arnold H.Hammer and Eric Claassen, Departments of Pharmacology and Immunology, Medical Biological Laboratory, T.N.O., P.O.B.45, 2280 AA, Rijswijk, The Netherlands.
We investigated the effect of an occlusive polyurethane film (Op-Site) ("moist wounds") on healing of full thickness punch wounds made in mouse skin and human skin transplanted onto nude C57/bl mice. Human breast skin was obtained, with the informed consent of patients, from mammary reductions. Re-epithelialization of THS and mouse wounds was followed over a 10 day period. Re-epithelialization of the wound surface was due to proliferation and migration of cells from the corresponding epidermis. The restored epidermis of THS, 10 days after wounding, showed a normal pattern of staining for human type IV collagen, HLA-ABC differentiation antigens and type 10 keratin (supra-basal epidermal cells). Both mouse and THS "dry" wounds (without Op-Site) re-epithelialized more slowly than "moist" wounds. The surface of mouse wounds was covered more quickly by epidermal cells (4-5 days after wounding) than that of THS wounds (7-8 days after wounding). The quicker re-epithelialization of mouse wounds was possibly due to the greater degree of wound contraction associated with repair of mouse wounds (day 6 wounds, wound diameter, mouse = 1.49mm ± 0.38, THS = 2.40mm ± 0.43; n = 6, mean ± S.D., $P<0.005$, Mann-Whitney U test). There were no differences in either the number or composition of granulation tissue cells (macrophages, neutrophils and fibroblasts) found at the site of mouse or THS 6 day wounds ("moist" and "dry") indicating that mouse inflammatory cells reacted to damaged mouse and human tissue in a similar manner. We conclude that the THS/nude mouse model is suitable for studying early events associated with wound healing in human skin in vivo.

Q 409 THE STIMULATORY EFFECTS OF TRANSFORMING GROWTH FACTOR-β_1 ON EPIDERMAL HEALING IN PORCINE SKIN WOUND MODELS, P. A. Hebda, Depts of Dermatol and Neurobiol, Anat and Cell Sci, U of Pittsburgh Sch of Med, Pittsburgh, PA 15261

Transforming Growth Factor-β (TGFβ) is a pleiotrophic regulator of epidermal keratinocytes, promoting migration and hypertrophic maturation while inhibiting mitosis. It is present in epidermis in the intracellular form and is rapidly converted to the extracellular form following injury (See Abst by Kane et al.) In normal healing an endogenous supply of TGFβ is rapidly mobilized within the tissue such that exogenous TGFβ will not further stimulate epidermal healing. However, in wounds with delayed healing, such as burns, active TGFβ may be suboptimal such that exogenous TGFβ will speed healing. To test this hypothesis, partial thickness burns were made in porcine skin then treated with TGFβ_1 (50-75ng/wd) at different times after injury. Epidermal healing was measured by a macroscopic evaluation of daily excised wounds. Histologic examination confirmed the macroscopic assessment and provided information on dermal healing. In this study TGFβ_1 accelerated epidermal and dermal healing. Tissue response was dependent on time of treatment. Least response was achieved in wounds treated immediately (day 0). A moderate response occurred in wounds treated on day 3. Maximal response was achieved in wounds treated twice, on days 3 and 6. TGFβ stimulation of healing in burn wounds but not excisional wounds suggests that clinical application of TGFβ may be beneficial in chronic or slow healing wounds but not in acute or normal healing wounds.

Q 410 INTERFERON AND INTERFERON INDUCER (POLY I:C) ENHANCE WOUND HEALING IN MICE AND RATS, Deepa Bhartiya, Jerold W. Sklarsh, and Radha K. Maheshwari. Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

In-vivo treatment with interferon α/β in mice (40,000 units/day) and polyinosinic-polycytydylic acid, a ds-RNA and a potent inducer of interferon (Poly I:C) (1 mg/kg body weight) injected one day prior to creation of wounds, in both mice and rats result in faster restoration of tissue integrity in both full skin punch biopsy as well as skin incision wound model. This could be evidenced by faster and enhanced closure of wounds as compared to untreated controls on day 7 (wound area measured on Macintosh II CX using NIH image 1.30u programme), increased migration of dermal fibroblasts in the wound bed, complete re-epithelialization evidenced by routine histology and scanning electron microscopic procedures; and increased collagen synthesis which correlate to greater tensile strength. In addition dermal fibroblasts synthesize much more laminin whereas no effect is observed on fibronectin synthesis by classical immunoflorescence procedures using frozen sections.

Q 411 CHARGED BEADS: EFFICACY IN IMPAIRED WOUND HEALING MODELS AND NEW INSIGHT INTO MECHANISM OF ACTION, Jyung RW, Porras-Reyes BH, Krukowski M, and Mustoe TA, Department of Otolaryngology-Head and Neck Surgery, Department of Cell Biology, and Division of Plastic Surgery, Washington University School of Medicine, St. Louis, MO 63110

Our previous work has demonstrated that Sephadex beads (40-120 μ) with positive surface charge enhance incisional wound healing in rats beyond that of a non-specific foreign body reaction. In order to further define the mechanism of action of the positively-charged beads, we have tested their efficacy in models of impaired healing, using total body- and surface-irradiated rats with paired dorsal incisional wounds. In normal animals, the mean breaking strength (MBS) of positive bead-implanted wounds measured at 10 days post-treatment was 26% greater than that of control wounds implanted with neutral beads (672 gm ± 36 vs. 535 gm ± 31, $p < 0.01$). In contrast, in animals with markedly depleted peripheral monocyte counts secondary to total-body radiation, the magnitude of this difference was diminished by approximately 50% and statistical significance was lost. The increase in MBS of positive bead-treated wounds was still evident, however (508 gm ± 24 vs. 445 ± 26, $p = 0.07$). In surface-radiated rats, there was no difference in MBS between positive and neutral bead-treated companion wounds; however, the MBS of wounds with positive beads was 41% greater than control wounds treated with buffer alone (296 gm ± 32 vs. 210 gm ± 19, $p = 0.02$). Immunohistochemical results clearly show greater numbers of wound macrophages associated with positive beads than with neutral bead controls. We conclude that beads with positive surface charge augment wound healing by a charge-specific mechanism that is macrophage-dependent.

Q 412 CHANGES IN TRANSFORMING GROWTH FACTOR-ß1 SPATIAL AND TEMPORAL IMMUNOREACTIVITY CORRELATE WITH PHYSIOLOGICAL CHANGES DURING INITIATION, PROGRESSION AND RESOLUTION OF CUTANEOUS WOUND HEALING. Cynthia J.M. Kane, Patricia A. Hebda* and Philip C. Hanawalt. Department of Biological Sciences, Stanford University, Stanford, CA 94305 and *Department of Dermatology, University of Pittsburgh, Pittsburgh, PA 15261.

The expression of transforming growth factor [TGF]-ß1 protein in human and porcine skin following cutaneous injury has been analyzed by immunohistochemistry with two polyclonal antibodies, anti-CC and anti-LC. TGF-ß1 may play a role in wound healing as suggested by its effect on multiple cell types *in vitro* and its acceleration of wound repair in animals. We have evaluated the natural appearance and localization of TGF-ß1 protein *in situ* in two models of cutaneous injury: suction blister in human skin and partial thickness excision of porcine skin. Anti-CC reactive TGF-ß1 is absent in normal skin but is rapidly and progressively induced at the site of injury. The induction reflects a structural or conformational change in TGF-ß1 protein that can be blocked by the protease inhibitor leupeptin, suggesting a change in TGF-ß1 activity. Immediately after injury anti-CC reactive TGF-ß1 is present in all epidermal keratinocytes adjacent to the wound, including basal cells, corresponding to a transient block in the basal keratinocyte mitotic burst following epithelial injury. TGF-ß1 is excluded from the rapidly proliferating basal cells at the peak of the wound healing response and its extracellular association with suprabasal keratinocytes may reflect physiological compartmentation of TGF-ß1 activity. Migrating keratinocytes within the wound exhibit anti-CC reactivity. Anti-CC reactive TGF-ß1 disappears as the wound healing response resolves. The constitutive anti-LC reactivity observed in suprabasal keratinocytes in normal skin is neither relocalized nor abolished in the epidermis adjacent to the injury, but anti-LC staining is absent in the keratinocytes migrating within the wound. Thus, changes in the structure or conformation of TGF-ß1 protein, its localization, and perhaps its activity, occur following cutaneous injury. A model will be presented to correlate the physiological changes observed during the initiation, progression and resolution of the adult wound healing response with changes in the spatial and temporal expression of TGF-ß1 protein. [Supported by NIH AR07422 (CJMK), GM36617 (PAH), and CA44349 (PCH)]

Q 413 FULL THICKNESS WOUND REPAIR FOLLOWING SINGLE, MULTIPLE AND DELAYED ADMINISTRATION OF bFGF. Corine K. Klingbeil, Sudha Nagarajan, and S. Anne Pletcher, California Biotechnology Inc., Mountain View, CA 94043

We have previously reported that recombinant human basic fibroblast growth factor (rhbFGF) accelerates wound repair when applied as a single dose on day 0 to full-thickness excisional wounds. A dose of 1 $\mu g/cm^2$ on day 0 accelerates closure by 10% in normal mice and by 30-40% in healing impaired diabetic (db/db), obese (ob/ob), and steroid-treated hr/hr mice. Multiple dosing, however, significantly reduces the cumulative dose required to stimulate the maximum rate of healing. Dosing at 0.1 $\mu g/cm^2$ for a total of 1, 3, or 5 days significantly increased the rate of healing at cumulative doses of 0.3 and 0.5 $\mu g/cm^2$. The effect of 5 days of dosing at 0.1 $\mu g/cm^2$/day was equivalent to a single dose of 10 $\mu g/cm^2$ on day 0. Thus a 20-fold lesser dose was required when delivered daily. A recent study has shown that multiple doses as low as 1 ng/cm^2/day can significantly accelerate wound closure.

In other experiments, administration of rhbFGF was delayed and dosing occurred after the acute inflammatory phase (days 0-3) or after the greatest amount of wound contraction had occurred (day 10). Regardless of the day of administration and the stage of wound healing, a single dose of rhbFGF accelerated closure when given on days 0, 3, 6, or 10 (1 $\mu g/cm^2$). These results indicate that various dosing regimens and dose concentrations can elicit significant biological effects. The choice of a therapeutically effective dose of bFGF in the clinic may depend on factors such as the indication, the route of administration and the condition of the wound.

Q 414 HISTIOLOGIC CHARACTERISTICS OF OVERUSE TENDON INJURY IN SPORTS: Wayne B. Leadbetter, M.D.Shady Grove Center for Sports Medicine & Rehabilitation, 9711 Medical Center Drive, Rockville, MD 20850

It is estimated that 30% to 50% of all sports injuries are caused by soft tissue overuse as diagnosed by the symptoms of inflammation. The term "tendonitis" is frequently utilized to justify a therapeutic regimen. However, many tendon injuries may represent aborted healing responses or overload/overuse induced matrix degeneration. At the crux of the clinical dilemma is the question of whether inflammation is truely present in all forms of the pathology. A series of ten human tendon biopsy specimens from common sites of sport-induced tendon injury were obtained at the time of surgical treatment. These were analyzed for histiologic evidence of inflammation versus degeneration and cell atrophy responses. In all cases, there was a paucity of inflammatory cell activity. Characteristic findings included: mucoid degeneration, metaplasia, neo-vascularization, and collagen fibril disorganization. Histologic observations support a new scheme for the pathobiology of sports-induced tendon injury. Observations imply that currently prescribed therapeutic modifiers of inflammation have little valid application to the clinical complaints of "tendinitis". Attention is directed to defining more effective clinical use of cellular mediators.

Q 415 REGULATION OF FETAL WOUND HEALING Michael T. Longaker, Karyn S. Bouhana, Michael R. Harrison, Michael J. Banda, Laboratory of Radiobiology & Environmental Health and Dept. of Surgery, UCSF, San Francisco, CA 94143-0750

Fetal skin heals without a scar during the first and second trimester. Third trimester fetal skin heals with a scar. In the adult, inflammatory macrophages are essential for normal healing with scar formation. Therefore, two questions were addressed. Are macrophages recruited to fetal wounds and can growth factor transcripts be detected in fetal and adult wound macrophages? Simulated wounds were created by subcutaneously implanting wire mesh cylinders into 100 day and 120 day gestation fetal lambs (term=145 days) and maternal ewes. Wound fluid was aspirated at 3, 7, 14, or 21 days post implantation. Macrophages were isolated by adherence and identified by phagocytosis of Dil-acetylated LDL. Total macrophage RNA was extracted, purified, reverse transcribed, and amplified by specifically primed PCR using primers to TNF-a, TGF-ß1, and TGF-ß2 and visualized on agarose gels by ethidium bromide staining. Transcripts for TNF-a were detected in both second and third trimester fetal and adult wound macrophages from every wound fluid collection. Conversely, TGF-ß1 appears at 14 days post implantation in second trimester fetal wound macrophages while from third trimester fetal and adult wound macrophages transcript is detected as early as 3 days post implantation. TGF ß2 is detected in both fetal and adult wound fluid macrophages. These results demonstrate that there is recruitment of macrophages to the fetal wound site suggesting there is inflammation in scarless fetal wound healing and that fetal wound macrophages contain transcripts for growth factors. Therefore, fetal macrophages may function as regulatory cells because they have the potential to synthesize growth factors that have been associated with adult wound healing. If these factors contribute to scar formation in adult wound healing, then the fetal environment may limit or alter their effectiveness. Supported by DOE-OHER (DE-AC03-76SF01012) and NIH (HD-25505).

Q 416 THE TRIPEPTIDE-COPPER COMPLEX GLYCYL-L-HISTIDYL-L-LYSINE-Cu^{++} (GHK-Cu) STIMULATES WOUND HEALING IN VIVO. François-Xavier Maquart, Janusz Wegrowski, Georges Bellon, Philippe Gillery, Jacques-Paul Borel, CNRS URA 610, Lab. Biochemistry, Faculty of Medicine, 51095 Reims Cedex, France, and Leonard M. Patt, Ron E. Trachy, Loren Pickart, Procyte Corporation, Kirkland, WA 98034-6900.

GHK-Cu is a tripeptide-copper complex which was shown to possess many properties of a potential activator of wound healing in vitro : chemotactic for monocytes/macrophages, mast cells and endothelial cells, stimulator of angiogenesis, neurogenesis and fibroblast metabolism. We investigated the effects of GHK-Cu on the wound healing process in vivo, using the wound chamber model described by Schilling et al. (Surgery, 1959, 46, 702). Wound chambers made of stainless steel wire mesh cylinders were implanted subcutaneously on the back of rats. The animals received serial injections of various amounts of GHK-Cu dissolved in normal saline, either directly in the wound chamber or intramuscularly. Control rats received the injection of the same volume of saline. Wound chambers were collected after one month and their content analyzed. When injected into wound chambers, GHK-Cu increased the accumulation of collagen (+ 117 %, $p<0.001$), glycosaminoglycans (+ 77 %, $p<0.05$) and elastin (+ 42 %, $p<0.05$) but not fibronectin. The distribution of glycosaminoglycans was markedly modified : In control wound chambers, the dermatan sulfate/heparan sulfate/hyaluronic acid ratio was 53/18/29 vs 70/13/17 in the GHK-Cu-treated ones. Intramuscular injection of GHK-Cu was also able to promote the accumulation of collagen in the chambers.

Q 417 WOUND-RELATED TUMORS IN CHICKENS INDUCED BY AVIAN SARCOMA VIRUS 17 (V-*JUN*) INOCULATION. Glenn M. Marshall, Luc Vanhamme, Wing-Yen Wong, Heyun Su, Peter K. Vogt. Department of Microbiology, USC School of Medicine, Los Angeles, CA 90033.

Avian sarcoma virus 17 (ASV17) carries the retroviral oncogene v-*jun*, induces fibrosarcomas in chickens and transforms chicken embryo fibroblasts in vitro. The proto-oncogene c-*jun* is one of a family of genes which encode transactivating proteins which bind DNA via homo- or heterodimeric complexes at the AP-1 consensus sequence. The transforming ability of the v-*jun* oncoprotein relates to its transactivating properties. Transgenic mice carrying v-*jun* under the control of the $H2K^k$ promoter form sarcomas only in the context of a full thickness skin wound. In these tumors v-*jun* expression is increased 5-10 fold.

We investigated whether wound-related tumors (WRT) occurred in chickens injected with ASV17 as has been reported in the case of Rous sarcoma virus. Fibrosarcomas arose in 4/4 birds at the site of a metal clip inserted in the wing opposite to the site of ASV17 inoculation (0.8-2.0 x 10^6 ffu). A tumor at the site of virus injection was seen at 3-4 weeks after inoculation while WRTs were evident at 4-5 weeks and grew to 30-50% of the injection site tumor. Much smaller tumor nodules were seen in liver and lung tissue. WRTs were not seen in birds wounded but not inoculated with virus, in birds wounded and injected with the non-transforming helper virus RAV-3, nor in birds injected with ASV17 but not wounded. No spontaneous wing tumors were seen in healthy chickens. Equivalent concentrations of infectious virus were isolated from tumors at the sites of injection and wounding, but not from normal liver or kidney, nor tumor nodules from liver and lung. V-*jun* expression and proviral integration pattern were analysed in normal and tumor tissue. We conclude that in ASV17 inoculated chickens wounding or wound healing collaborates with viral infection to promote tumorigenesis.

Q 418 THE EFFECTS OF MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) ON WOUND HEALING IN NORMAL AND GENETICALLY DIABETIC (db/db) MICE, Scot C. Middleton and Sharon Lea Aukerman, Department of Pharmacology, Cetus Corporation, 1400 53rd Street Emeryville, CA 94608
The effects of M-CSF on wound healing were assessed using an excisional model of dermal wound repair. Full-thickness circular wounds were made in the dorsal skin of healing-impaired female diabetic C57BL/KsJ db/db mice and normal (db/+) littermates, and wound area was measured every 2 days. In untreated mice, wound closure was impaired in the diabetic (db/db) mice compared to normal (db/+) littermates. Wounds in diabetic mice required 23-25 days to heal completely, while wounds in normal mice were completely healed within 14 days. To test the effect of M-CSF on wound healing, M-CSF was administered intravenously once a day for 7 days beginning 4 hours after wounding. Healing in normal mice was slightly enhanced by M-CSF treatment at a dose of 5.0 mg/kg, such that wounds in those animals reached half their initial area approximately 2 days earlier than controls, yet the time required for 100% closure to occur was not different. In diabetic (db/db) mice, wound closure was significantly improved by M-CSF treatment at doses ranging from 0.5 to 5.0 mg/kg/day. Wounds in M-CSF treated db/db mice reached both half-maximal and 100% closure an average of 4 days earlier than wounds from non-treated db/db mice. Diabetic (db/db) mice were shown to have elevated blood glucose and cholesterol levels compared to normal (db/+) mice. Three daily intravenous doses of M-CSF at 1.0 mg/kg had no effect on blood glucose in db/db mice, but reduced plasma cholesterol by 24% ($p<.005$) to a level which approached that of normal mice. Histological evaluation of wounds was supportive of these findings, suggesting that M-CSF may be useful in the treatment of patients with deficient wound repair.

Q 419 NEW IN VITRO MODEL OF WOUND HEALING WHERE PDGF-BB IS EFFECTIVE: Mustoe, TA, Kumar SB, Porras-Reyes BH, Gramates P,. Division of Plastic Surgery, Washington University School of Medicine, St Louis MO 63110.
PDGF-BB has been demonstrated to accelerate healing in both incisional and open wounds. In impaired incision model, its effects are dependent on the presence of macrophages. However, in cell culture PDGF mediates fibroblast proliferation and chemotaxis. We demonstrate the activity of PDGF in a new in vitro wound healing model in the absence of macrophages. Via a midline laparotomy incision, the mesentery windows between vascular walls were wounded and left in vivo, or excised, wounded and placed in varying serum concentration with MCDB-402 media in vitro. The results show that percentage healing of perforation in vivo was 82% and 71% in 10% serum in vitro. Utilizing immunohistochemical technique showed abundant macrophage influx by day 5 in vivo, where in vitro there was a virtual absence of macrophages. Collagen production was demonstrated in vitro and in vivo with a procollagen type I antibody. PDGF in low serum (2%) was able to achieved a 50% healing while 2% serum alone demonstrated 10% healing or less. We concluded that PDGF is able to promote healing in a new in vitro model of wound healing in the absence of macrophages.

Q 420 TGFα IS A POTENTIAL MEDIATOR OF ESTROGEN ACTION IN THE MOUSE UTERUS, K.Nelson, T.Takahashi, D.Lee*, N.Luetteke*, N.Bossert, B.Eitzman, and J.McLachlan, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, *Lineberger Comprehensive Cancer Center and Dept. Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599-7295.
The quest for the isolation, characterization, and mechanism of action of factors involved in steroid hormone mediated growth and differentiation of the reproductive tract has focused on an expanding number of polypeptide growth factors. Several peptide factors (IGF1, TGFβs, PDGF, EGF, CSF1, thrombin, plasminogen activator, complement factors, tissue factor, lactoferrin) involved in wound healing have also recently been shown to be associated with steroid hormone regulation of the rodent reproductive tract. In this study, the effects of estrogen on the expression of mouse uterine transforming growth factor alpha (TGFα), another peptide associated with wound healing, was investigated. Estrogen induced the expression of uterine TGFα mRNA, predominantly in epithelial cells. High levels of immunoreactive TGFα protein, as measured by immunoblot analysis and radioimmunoassay, was secreted into mouse uterine luminal fluid following estrogen treatment. Induction of uterine TGFα was specific to estrogen; nonestrogenic steroids did not induce expression. Furthermore, antibody specific to TGFα significantly reduced estrogen-mediated growth which suggests that TGFα may act as an in vivo mitogen for the reproductive tract. Analysis of TGFα/EGF receptors indicated that functional uterine receptors necessary to transduce the growth factor signal were present. Thus, the mitogenic response to estrogen is partly dependent on the local production of TGFα in mouse uterus and supports a physiological role for TGFα and its receptor pathway in the female mouse reproductive tract. In addition, our data and that of others provide support for the notion that estrogen may initiate a cascade of events involving multiple peptide factors similar to that seen in wound healing, the end result being stimulation of DNA synthesis.

Q 421 ASSESSMENT OF ENHANCED CLOSURE OF FULL-THICKNESS WOUNDS IN RATS TREATED WITH TRAP-508 PEPTIDE: CONTRACTION VS RE-EPITHELIZATION. S. D. Pernia, W. R. Redin & D. H. Carney, Dept. of Biochemistry, Univ. of Texas Medical Branch, Galveston, TX 77550

Synthetic thrombin receptor-activating peptide, TRAP-508, has been shown to enhance closure of full-thickness wounds in rats. This study was preformed to assess the contribution of contraction versus new tissue growth and re-epithelization to the wound's closure. Under anesthesia, 2.0 cm dia. circles were tattooed on the denuded dorsal skin of male Sprague-Dawley rats, and a 1.5 cm dia. circle was surgically excised concentric to the tattoo to form a full-thickness wound. Tattoo and wound perimeters were traced onto acetate film, and areas were determined by computer planimetry. At Day 8, TRAP-508 wounds had closed by ~43%, while control wounds had only closed ~30%, relative to their respective areas on Day 2. This represents about a 40% enhancement of closure of TRAP wounds relative to controls. In contrast, the reduction in areas of tattoes (approximately 22%) was statistically equal for both TRAP and Control animals. These results indicate that the enhancement of wound closure observed with TRAP peptide treatment is largely due to increased new tissue growth and epithelization rather than an increase in contraction. This tattoo technique may be useful to assess the contribution of contraction to wound closure in other systems.
(Supported by Texas Advanced Technology Program and NIH grant AM25807)

Q 422 ENHANCEMENT OF WOUND HEALING BY A NEW DESCRIBED ALKALOID: TASPINE. Porras-Reyes BH, Lewis W, Roman J, and Mustoe TA. Division of Plastic Surgery, Departments of Biology and Medicine. Washington University School of Medicine, Saint Louis MO 63110.

Substances that accelerate wound repair are scarce. Taspine (369 MW) is an alkaloid extracted from the sap of trees from the family *Euphorbiaceae* of the Amazon river valley that has been used by natives to accelerate wound repair. We purified Taspine to test its healing properties by using different topical concentrations in the paired rat surgical incision model. Samples harvested at days 5, 7, and 12 post-wounding were examined for maximum breaking strength (MBS) and histology. Samples treated with 250 μg of Taspine at the time of wounding but not with 50 μg or 10 μg had significant higher values for MBS than the paired control (26% $p<0.005$ and 30% $p<0.001$ by days 5 and 7 respectively). Taspine did not modify MBS in samples obtained at day 12. Samples treated with 250 μg of Taspine had significantly higher cellular infiltration and granulation tissue at days 5 and 7 but not at day 12 post-wounding. Fibroblast proliferation, measured by cell counting and thymidine incorporation and fibronectin formation, measured by a non-quantitative assay of immunofluorescence fibronectin staining, were not different in samples treated or not with Taspine ruling out its role in fibronectin synthesis or matrix assembly. The data demonstrate promotion of wound healing by Taspine in a dose-dependent manner in the early phase of the healing process with no substantial modification thereafter. Its mechanism of action is related to increased cellularity in the initial phases of the healing process and is not mediated by changes in extracellular matrix.

Q 423 REDUCTION OF SCAR TISSUE FORMATION IN ADULT RODENT WOUND HEALING BY MANIPULATION OF THE GROWTH FACTOR PROFILE, Mamta Shah, David Foreman and Mark Ferguson, Department of Cell & Structural Biology, University of Manchester, Coupland III Building, Manchester M13 9PL, England.

Fetal wound healing is characterised by absence of scar tissue formation and a markedly reduced inflammatory infiltrate and growth factor profile within the healing wound. We therefore investigated whether experimental reduction of the growth factor profile of healing adult wounds would lead to an alteration of scar tissue formation. Scarring is a major problem in nearly all forms of surgery leading to deleterious function, growth and aesthetics. Adult male Sprague Dawley rats were anaesthetised by inhalation of halothane nitrous oxide and oxygen. Four linear full thickness incisions, 10mm in length, were made on the dorsal skin equidistant from the midline and adjacent to the four limbs. In each animal one wound was unmanipulated (control), one was injected with an irrelevant antibody (sham control), one was injected with either TGFβ$_1$, PDGF, EGF, acidic or basic FGF (positive control) and one was injected with a neutralising antibody to the listed growth factors (experimental wound). Wounds were injected daily for either the first three days or the first seven days. At least two animals in each group were killed on postwounding day 7, 14, 28 and 42, the wounds removed, frozen and processed for immunocytochemical staining using antibodies to collagens I, III, IV, laminin, fibronectin or processed for routine histological examination using a variety of connective tissue stains or freeze dried for biochemical analysis. There were no differences between the control and sham control wounds at any timepoints indicating absence of major effects of introduced foreign proteins. Further no wounds showed impaired healing and the rate of epithelialisation was similar in all treatments. However, major effects were seen with the neutralising antibodies to transforming growth factor beta 1 and PDGF. These experimental wounds contained much less collagen I and III compared to the other three wounds in the same animal at any timepoint. There was much greater spacing between the collagen fibrils whose orientation was almost identical to that of normal skin. Indeed in the neutralising antibody treated wounds it was often difficult to detect the latter (except for the loss of ectodermal hair follicles). This was in sharp contrast to the other wounds which showed a distinct scar with vertically orientated parallel and densely packed collagen fibrils. These effects were most marked in the papillary dermis. Wounds treated with neutralising antibodies to TGFB$_1$ or PDGF also showed a marked reduction in fibronectin, particularly in the reticular dermis. Although fibronectin staining was markedly reduced throughout the wound, it was still brightest at the dermal epidermal junction. Treatment with neutralising antibodies to TGFB$_1$ and PDGF also decreased the number of blood vessels within the healing wound. By contrast the positive control wounds treated with TGFB or PDGF showed a marked increase in: extracellular matrix accumulation, the density of extracellular matrix packing and the number of blood vessels. Scarring was more prominent in these growth factor treated wounds compared to controls. These results demonstrate the ability of neutralising antibodies to selected growth factors to markedly reduce scar tissue formation in adult dermal wound healing. Importantly this advantageous effect was not accompanied by attendant problems of delayed wound healing or delayed epithelialisation. This suggests a major new therapeutic approach to the manipulation of scarring and fibrosis during adult wound healing.
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Q 424 ROLE OF UROGASTRONE IN SMALL INTESTINAL WOUND HEALING, J.G. Sharp, S.K. Saxena, S.S. Joshi and J.S. Thompson, Departments of Anatomy and Surgery, University of Nebraska Medical Center, Omaha, NE 68198-6395.

We have employed a surgical model of small intestinal wound healing in which the perimeter of a longitudinal incision in the antimesenteric aspect of the ileum of rats or rabbits was sutured to the serosal surface of the cecum to create an oval defect. The progress of epithelialization of ileal mucosa on the cecal serosa was followed employing a battery of morphological, immunohistochemical, cell kinetic and biochemical techniques. Recombinant human urogastrone (kindly provided by Chiron Corporation) accelerated the repair of these defects so that, after 7 days of intravenous infusion of 1.5 μg/kg/hr, 61±4% of wounds had epithelialized in treated rabbits versus 36±3% in saline treated controls. Early in the course of repair, from days 2 to 4, there was increased binding of radiolabelled urogastrone to the regenerating and adjacent epithelium. Originally, we proposed that epithelial cell proliferation stimulated by the urogastrone was responsible for accelerated repair. The crypt cell production rate was 16.0±1.7 cells/crypt/hr in urogastrone treated rabbits versus 8.6±0.7 in saline treated controls. However, a temporal analysis showed that increased cell migration onto the wounded area preceded proliferation in adjacent crypts and correlated more closely with urogastrone binding. Also evident and a likely consequence of the migration of cells onto the surface of the defect was a reduction in the size of villi adjacent to the wound. Potentially therefore the increased cell proliferation is an indirect consequence of the reduction in levels of an inhibitor (TGF-beta?) present in adjacent villi and this would account for the temporal delay in proliferation in surrounding crypt cells. Overall, our data indicated that intestinal wound healing had temporally sequential components: increased cell migration onto the wounded area following by increased cell proliferation in the adjacent area. Urogastrone, both directly and indirectly, enhanced these effects.

Q 425 PHARMACOKINETICS OF rPDGF-BB IN RODENTS AFTER ADMINISTRATION BY DIFFERENT ROUTES, Katie Sprugel, DeeAnn Curtis, Michelle Thompson and Megan Lantry, ZymoGenetics, Inc., Seattle, WA 98105

In the development of rPDGF-BB for therapeutic use, its absorption, distribution, and excretion *in vivo* have been examined after administration in pharmacologic doses. rPGDF-BB was rapidly cleared from serum after i.v. bolus injections in rats. 20 μg or 500 μg rPGDF-BB spiked with ^{125}I-rPDGF-BB was injected i.v. and blood samples were taken at various times after injection (0.5-100 min) and analyzed for TCA precipitable radioactivity and PDGF immunologic reactivity (ELISA). The clearance of rPDGF-BB from serum was biphasic with an initial $t_{1/2}$ = 1.2 min while the second phase was slower ($t_{1/2}$ = 25 min). Absorption and distribution of ^{125}I-rPDGF-BB were comparable after s.c. injection into rats and after topical application to full thickness skin excisions in Balb/c mice. Animals were killed 2-72 hrs after injection. By 2 hrs, 45% of the TCA precipitable ^{125}I was gone from the injection site. At 24 hrs, 15% of the protein associated ^{125}I remained in the application site and by 72 hrs only 3% remained. Most of the radioactivity is excreted in the urine and feces but is not TCA precipitable. Circulating levels of protein associated ^{125}I reached a maximum at 4 hrs after s.c. injection (0.19% of injected dose). Liver, kidney, and gut showed modest, time dependent accumulations of radioactivity, peaking at 4 hours. These studies suggest that rPDGF-BB can be absorbed from wounds and subcutaneous sites but that the resultant serum and tissue levels are low, presumably due to rapid metabolism and excretion.

Q 426 **PROLIFERATION AND REPAIR AFTER CHEMICAL DAMAGE TO RAT KIDNEY PROXIMAL TUBULE EPITHELIUM (RPTE). J.L. Stevens, Q. Chen, K. Yu, G. Zhang and A. Wallin.** The W. Alton Jones Cell Science Center, Lake Placid, NY 12946.

We examined the nephrogenic repair response after chemical damage of RPTE by nephrotoxic cysteine conjugates (NCC). [^{35}S]NCC are metabolized to reactive ^{35}S-containing chemical species which covalently bind to macromolecules and kill RPTE. The initiation, damage and repair responses in RPTE in vivo were mapped using covalent binding of ^{35}S-label, BrdU labelling and vimentin staining for regenerative tubules. Proliferation after NCC damage has two distinct component, 1) a burst upstream from the wound site which occurs early, and 2) a later burst at the wound site concommitant with appearance of regenerative (vimentin-positive) RPTE. This biphasic response could be explained if the toxin directly induced phase 1 while phase 2 is prompted by loss of cell mass at the wound site. NCC directly induced c-fos and c-myc expression in vitro suggesting that the toxin itself might induce a GO to G1 transition, perhaps accounting for the early phase of proliferation. This is consistent with other data showing that peroxide renders 3T3 cells competent (Shibanuma et al. Oncogene 3,17, 1988)

To determine the nature of the growth factors which might regulate the phase 2 proliferative response, we investigated RPTE growth in culture. α-FGF is a potent growth factor for RPTE cells. Whole rat kidney and proximal tubules contain α-FGF as determined by western blot and bioassay. RPTE also synthesize α-FGF in culture and have mRNA for α-FGF. The data suggest that FGFs may be an important autocrine mediators of proliferations during nephrogenic repair of the proximal tubule epithelium.

Q 427 THE EFFECT OF AGING ON BONE AND CARTILAGE FORMATION AFTER SUBPERIOSTEAL TGF-B2 INJECTIONS, Darryl A. Tannenbaum, Michael B. Sporn, Anita B. Roberts, and Mark E. Bolander, NIAMS, NIH, and Laboratory of Chemoprevention, NCI, NIH, Bethesda, MD 20892.

TGF-B2 is a 25 kDa homodimeric peptide that belongs to a larger class of proteins involved in cell growth and differentiation. Previous work has revealed that TGF-Bs 1 and 2 were able to induce osteogenesis and chondrogenesis in newborn rats following subperiosteal injection into uninjured femurs. We initiated experiments to determine the effect of animal age on this response. Two hundred ng of TGF-B2 were injected subperiosteally into the mid-diaphyseal region of the femur daily for 14 days. Animals were harvested at 15 days. Five different ages, from newborn to retired breeder, were studied and we observed a greater mass in the younger animals both in relation to the size of the femur and in absolute terms. Masses contained cartilage as well as intramembranous and endochondral bone in all age groups. A quantitative analysis of the composition is currently underway. Clearly, there is a difference in the degree to which animals of different ages repond to TGF-B2. Determining whether it is a decrease in the absolute number of undifferentiated primitive mesenchymal cells or a waning in individual cell response with age is an important question essential in understanding the molecular mechanisms of fracture healing.

Q 428 IDENTIFICATION OF THE MAJOR FIBROBLAST GROWTH FACTORS SPONTANEOUSLY RELEASED IN INFLAMMATORY ARTHRITIS AS PDGF AND TNF ALPHA. Stephen C Thornton, Ronald Penny and Samuel N Breit. Centre for Immunology, St Vincent's Hospital and University of NSW, Sydney, Australia.

Rheumatoid arthritis (RA) is characterized by chronic inflammation and proliferation of a number of important elements within the joint including the synovial fibroblasts. Elevated levels of a number of cytokines such as IL-1, IL-2, IL-6, γ-IFN, TGF-β and TNF-α have been detected in the synovial fluid of patients with RA and other inflammatory arthritides. It seems quite likely that local release of such mediators may be responsible for the proliferation and overgrowth of connective tissue elements in these disorders.

In order to ascertain whether there was local production of fibroblast growth factors in the joint in inflammatory arthritis, and to determine their identity, cells were obtained from the synovial fluid of 15 patients with chronic inflammatory arthritides. All subjects' synovial fluid cells, spontaneously released growth factor activity for fibroblasts. This was present in large amounts, being detectable in culture supernatants diluted to a titre of at least 1/625. By a series of depletion experiments using solid phase bound antibodies to cytokines, it was possible to demonstrate that this activity was due to TNF-α and PDGF. Both of these cytokines appeared to contribute in approximately equal amounts to this fibroblast growth factor activity, and their effect in combination was much greater than either on its own. This study provides evidence for the local production of these two cytokines and strongly suggests that together they are the dominant factors in fibroblast proliferation within the synovial cavity.

Q 429 EFFECTIVENESS OF FETAL LIVER LYSATE IN THE TREATMENT OF CHRONIC ACTIVE HEPATITIS B WITH HEPATOCIRRHOSIS, Qiang Tu, Cnutse Wu, Kai R. Zhang, Xu Z. Wang, Institute of Radiation Medicine, Beijing100850, P.R.China

Fetal liver lysate was demonstrated to be an effective measure in the treatment of a group of 12 patients suffering from chronic active hepatitis with hepatocirrhosis. In comparing with a group of control, none of them died after receiving the transfusion of fetal liver lysate. Meanwhile the obvious advantages of fetal liver lysate in promoting the recovery of abnormal serum indications including glutamic-pyruvic transaminase activity total bilirubin level, prothrombin time and its activity were observed. The mechanism of fetal liver lysate treatment in hepatic failure is mainly due to the presence of a bioactive hepatocyte growth factor which plays an important role in repairing or repopulating the deteriorated liver tissue.

Q 430 RGDS-PROTEINS AND INTEGRINS IN PROLIFERATIVE INTRAOCULAR DISORDERS, Peter Wiedemann, Michael Weller, Peter Esser, and Klaus Heimann, Department of Vitreoretinal Surgery, University Eye Hospital Cologne, Köln, Germany

RGDS-proteins exert their biological actions which include cellular adhesion, spreading, and proliferation, by means of a special aminoacid sequence (RGDS) recognized by cell surface receptors, the integrins. Traction membranes from patients with idiopathic and posttraumatic proliferative vitreoretinopathy and proliferative diabetic retinopathy were examined for the distribution of the RGDS-proteins fibronectin, vitronection, thrombospondin, fibrinogen, and factor VIII- related antigen, as well as two corresponding integrins (fibronectin receptor and vitronectin receptor), using double label immunofluorescene, phosphatase, and peroxidase immunolabels. Due to the multitude of proteins participating in cell RGDS interactions, the selective pharmacological modification of cellular responses to RGDS remains a difficult task.

Late Abstracts

THE LIVING SKIN EQUIVALENT AS A MODEL SYSTEM FOR THE STUDY OF WOUND REPAIR, **Eugene Bell and Sumi Scott, Organogenesis Inc., 83 Rogers Street, Cambridge, MA 02142.**

A Living Skin Equivalent (LSE) model system reconstituted with cultured human keratinocytes and dermal fibroblasts in a tissue matrix has been adapted for studying wound repair. The LSE, in a well measuring 24mm in diameter, consists of an epidermis exposed to air with a functional stratum corneum and elements of a basal lamina. The epidermis, organized into basal, suprabasal, spinous and granular layers exhibits the principal biochemical markers of differentiation; its substrate is a dermal equivalent (DE) populated by fibroblasts that contract, organize and enrich the matrix in which they are cast. The DE is cast on an acellular collagen lattice, separated from a nutrient pool below by a polycarbonate membrane having pores 3.0 micrometers in diameter. An 8.0mm biopsy punch is used to remove a central disk of the LSE down to the level of the acellular collagen pad. The "wound bed" thus created, can then be filled with a matrix or cell matrix mixture of the same formulation as that of the DE or of other formulations containing factors and cells that may retard, accelerate or modify would repair. In addition, the composition of this nutrient medium can also be manipulated. Essentially what is provided is a substrate for epidermal over growth that itself enters into the repair process and undergoes remodeling. When the wound bed filler is of the same design as that of the original dermal equivalent, wound closure, occurring radially, takes about 6 days. Refilling the wound bed with a collagen containing cell-matrix mixture initiates events typical of early repair, namely contraction or condensation of newly made collagen matrix by connective tissue cells. As the replaced dermal equivalent tissue contracts, mainly in the thickness mode, the epidermis from the wound edge begins its migration inward. In this first study with the LSE model system rates of epidermal migration have been measured as a function of the number of dermal fibroblasts and of other cell types such as mast cells added to the replacement mix used to fill the wound bed. The LSE system offers significant advantages over other models because of its mechanistic similarity to actual skin and because of its standardization as a manufactured product.

HEPATOCYTE GROWTH FACTOR: TARGET CELL SURFACE INTERACTIONS. Donald P. Bottaro, Jeffrey S. Rubin, Donna L. Faletto*, Andrew M.-L. Chan, Thomas E. Kmiecik*, George Vande Woude*, and Stuart A. Aaronson. LCMB, NCI, Bethesda, MD and *ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD.

We have isolated a heparin-binding mitogen from conditioned medium of human lung fibroblasts that acts on epithelial and endothelial cells, as well as melanocytes. cDNA cloning revealed that it was a variant of hepatocyte growth factor (HGF), a plasminogen-like humoral mitogen thought to be involved in liver regeneration. The broad spectrum of target cell types and its normal expression in fibroblasts suggests that HGF may also act in a paracrine mode during tissue growth, differentiation and repair. HGF specifically stimulates the rapid tyrosine phosphorylation of a 145 kD protein in target cells, suggesting that a tyrosine kinase receptor mediates its mitogenic signal. These characteristics are similar to other heparin-binding peptide mitogens (such as the FGFs), which bind with relatively low affinity to cell surface proteoglycans, but stimulate mitogenesis via high affinity cell-specific tyrosine kinase receptors. Identification and characterization of the high affinity receptor for HGF will be discussed.

PLATELETS EXPRESS AN ALTERNATIVE CYTOPLASIC DOMAIN IN THE β_1 INTEGRIN SUBUNIT, Lucia R. Languino and Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, California 92037

Platelet adhesion to the subendothelial matrix plays a crucial role in the pathophysiology of vascular obstruction, atherogenesis and inflammation. The adhesion of platelets to vascular tissues and their spreading on such substrates appears to be regulated by the specificity of platelet integrins for various adhesive proteins, including fibronectin and laminin. Platelets adhere to fibronectin and laminin, but they are able to spread only on fibronectin; in contrast, other cells can spread on both substrates. We have observed that the β_1 subunit of the platelet integrin that mediates the adhesion of platelets to laminin is immunochemically different from the β_1 subunit expressed in other cells. Polyclonal antibodies directed against the cytoplasmic domain of the previously reported β_1 subunit fail to react with the platelet integrin counterpart while monoclonal anti-β_1 antibodies do recognize it. We have isolated a cDNA encoding a new cytoplasmic domain of the β_1 subunit from a human leukemia cell cDNA library. This β_1 variant (β_1') contains an alternatively spliced insert of 116 bp which produces a frameshift in the previously reported 3' end of this subunit and generates a unique 48 aa COOH-terminal sequence. A synthetic peptide corresponding to the unique sequence reacts in an ELISA assay with polyclonal antibodies prepared against either a platelet extract or a platelet β_1 integrin. These results show that a variant β_1 subunit containing an alternative cytoplasmic domain is expressed by platelets. We propose that such structural variations of integrins might modulate intracellular signalling and adhesion-dependent cytoskeletal reorganization.