FGF, ENDOTHELIAL CELL GROWTH FACTORS AND ANGIOGENESIS

Organizers: Michael Klagsbrun and Ken Thomas

April 1-7, 1991

Plenary Sessions Page

Poster Sessions

Keynote Addresses (joint with Wound Repair meeting)

CF 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 Wound Repair meeting)

CF 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.

CF 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.

FGF Family Members

CF 004 EXPRESSION OF K-FGF AND INTERACTION WITH ITS RECEPTORS ,

Claudio Basilico, Department of Microbiology, New York University School of Medicine, New York, N.Y. 10016.

The K-*fgf*/*hst* protooncogene encodes a growth factor of the FGF family whose expression is detectable only during the very early stages of development or in undifferentiated embryonal carcinoma (EC) cells. Inappropriate expression of K-FGF leads to transformation through an autocrine mechanism, which requires secretion of the growth factor and interaction with its receptor(s) in extracellular compartments. The DNA regulatory elements which are responsible for the specific activation of the K-*fgf* gene in undifferentiated EC cells reside within a DNA fragment which is part of the non-coding 3' region of the K-*fgf* third exon, in comparable locations in both the human and murine K-*fgf* genes These elements are able to stimulate transcription only in undifferentiated EC cells but not in HeLa, NIH3T3 cells or differentiated EC cells, and thus they behave as developmental specific enhancers. Analysis of the smallest human and murine K-*fgf* sequences with full enhancer activity revealed the presence of two octamer motifs, similar to those involved in the binding of the Oct 1, 2, and 3 transcription factors. The contribution of specific octamer binding proteins as well as of other generalized transcription factors to the control of the expression of the K-*fgf* protooncogene will be discussed. To investigate the interaction of K-FGF and of other members of the FGF family with their receptor(s), we have cloned three murine cDNAs encoding distinct tyrosine kinase receptors with about 80% similarity to the chicken bFGF receptor and to each other. When transfected into receptor negative cells, these receptors can bind various members of the FGF family, although with different affinities. The receptors are phosphorylated upon stimulation with the growth factors, leading to DNA synthesis and cell proliferation. Expression of these cDNAs in hematopoietic FGFr-negative cells revealed unsuspected requirements for FGFs stimulation of their receptors. The significance of these findings vis a vis the physiological and pathological role of FGF receptors will be discussed.

CF 005 EXPRESSION AND POSSIBLE FUNCTIONS OF MOUSE FGF-5 FROM EGG CYLINDER TO ADULT, Mitchell Goldfarb, Olivia Haub, Brian Bates, Beverly Drucker, Vera de Costa Soares, David Clements, and Jen-Kuei Wang, Department of Biochemistry and Molecular Biophysics, Columbia University, College of Physicians and Surgeons, 630 W. 168 st., New York, NY 10032

The expression of murine fibroblast growth factor-5 (FGF-5) RNA is highly restricted in developmental time and space, as revealed by Northern blot and in situ hybridization analysis of embryonic and adult tissues (ref. 1,2). At least eight discrete time-restricted sites of FGF-5 expression have been identified: early post-implantation epiblast (Embryonic days 5.3-7.5), lateral splanchnic mesoderm near hepatic bud (E 9.5-10.5), lateral somatic mesoderm extending rostro-caudally from the bulbus arteriosis to the liver primordium (E 10.5-12.5), myotomes (E 10.5-12.5), mastication muscle cell lineage (E 11.5-14.5), a non-muscle, non-cartilagenous hindlimb mesenchyme patch (E 12.5-14.5), the acoustical ganglion (E 13.5-14.5), and the adult brain (8 week old). Brain expression is broadly distributed at very low levels, but several sites of higher expression have been found: the dentate gyrus, presubiculum, and CA3 pyramidal neurons of the hippocampus, the reticular thalamic nucleus, and several layers of the cerebral cortex. The FGF-5 expression profile demonstrates complexity of regulation which is both tissue- and region-specific, suggesting the influence of pattern-forming genes either directly or indirectly. The profile also points to a range of biological functions for FGF-5, which may include roles in vasculogenesis, inhibition of skeletal muscle terminal differentiation, hepatogenesis, limb formation, and CNS neuronal or glial function.

Several approaches are being employed to identify FGF-5's regulation, functions and mechanisms of action. These include the generation of chimeric and transgenic mice, the use of purified FGF-5 protein in biological and receptor-binding assays, and the testing of known FGF receptors for interaction with FGF-5. Progress in these areas will be presented.

1) Haub, O., Drucker, D., and Goldfarb, M. (1990) Proc. Nat. Acad. Sci. USA 87: 8022
2) Haub, O., Drucker, D., and Goldfarb, M. (1991), manuscript submitted December,1990

CF 006 THE INT-2 GENE AND ITS PRODUCTS, Gordon Peters, Piers Acland, Paul Kiefer, Frances Fuller-Pace, David McAllan and Clive Dickson, Imperial Cancer Research Fund, PO Box 123, London WC2A 3PX, United Kingdom.

Int-2 is classed as a member of the FGF family, based on sequence homology and genome organization, and qualifies as a proto-oncogene for several reasons. The gene is frequently activated by proviral integration in tumors induced by mouse mammary tumor virus (MMTV); transgenic mice expressing int-2 sequences under the control of an MMTV promoter develop pregnancy-dependent mammary neoplasms and other hyperplastic disorders; and mouse int-2 cDNAs expressed from appropriate promoters can induce morphological transformation of NIH3T3 cells. However, the mouse int-2 gene encodes two primary translation products depending on the choice of initiation codon, raising the possibility that it has more than one function. The 28.5 kd product, beginning at the most upstream AUG codon, has an amino-terminal signal sequence that directs it to the secretory pathway, presumably for export as a paracrine or autocrine effector molecule. The true targets for these effects have yet to be defined but are likely to include specialized embryonic cell types, given the tightly regulated pattern of int-2 expression in the developing mouse embryo. Paradoxically, while int-2 cDNA, optimized for expression of the AUG-initiated product, is capable of causing morphological transformation, the in vitro translation product is not detectably mitogenic for fibroblasts. We are currently assessing whether these anomalies reflect low affinity for FGF receptors on these cells or are compounded by interactions between int-2 and extracellular matrix. In contrast, the function of the second int-2 product, initiated at a CUG codon, must be sought in the cell nucleus. The addition of 29 residues to the amino terminus appears to compromise the action of the signal peptide so that around 50% of this extended product is found in the nucleus rather than the secretory pathway. The sequences responsible for nuclear localization must reside in the body of the protein since truncated forms lacking both the amino-terminal extension and the signal peptide are exclusively nuclear. Recent attempts to identify residues that determine the fate and function of int-2 will be discussed.

TGF-Beta (joint with Wound Repair meeting)

CF 007 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.

CF 008 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.

CF 009 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.

FGF Receptors

CF 010 REGULATION OF CELL BEHAVIOR BY SYNDECAN, A bFGF-BINDING PROTEOGLYCAN by Merton Bernfield, M.D., Joint Program in Neonatology, Harvard Medical School, Boston, MA

Heparan sulfate (HS) is at the surface of all adherent cells where it can bind and immobilize basic FGF and various extracellular components. This HS is part of an integral membrane proteoglycan (PG) that can be shed from the cell surface, sequestered in various membrane domains, or directed to various sub-cellular compartments. Of several such HSPGs, syndecan, first isolated from murine epithelia, represents a family of PGs that shows homologous sequences in their transmembrane and cytoplasmic domains, but differs in their extracellular domains except for conserved protease-susceptible sites near the plasma membrane and putative GAG attachment sites.

Via its heparan sulfate chains, syndecan binds epithelial cells to the interstitial matrix proteins fibronectin, types I, III and V collagen and thrombospondin, and to bFGF. The significance of the bFGF binding is unclear, but syndecan may alter the conformation, degradation or presentation of bFGF, modifying its interaction with the high affinity bFGF receptor. Binding of matrix proteins leads to crosslinking of syndecan in the plane of the membrane and to association of its cytoplasmic domain with the actin-containing cytoskeleton. Suspending cells leads to rapid shedding of the ectodomain and syndecan does not reappear at the cell surface while the cells are suspended. Thus, syndecan acts as a receptor which can reversibly link the cytoskeleton to the matrix.

Syndecan shows tissue-specific differences in localization and structure. In epithelia, syndecan contains smaller HS chains and is predominantly at basolateral cell surfaces where it colocalizes with cortical actin bundles. In mesenchymal cells, its HS chains are much larger and it is predominantly intracellular. Indeed, mammary epithelial cells made syndecan-deficient by stable transfection with antisense cDNA show reduced expression of other epithelial adhesion molecules and become mesenchymal-like in shape and organization. Thus, reduced syndecan at the cell surface correlates with a loss of epithelial cell behavior.

Syndecan also changes in amount, location and structure during development. It appears initially on 4-cell embryos, becomes restricted in preimplantation embryos to the cells that will form the embryo proper and changes its expression during epthelial-mesenchymal interactions: it is lost transiently when epithelia change form and permanently upon terminal differentiation, but is expressed transiently when mesenchymal cells condense.

Syndecan appears to influence cell behavior by virtue of its association with various heparan sulfate-binding ligands, potentially both extracellular matrix molecules and bFGF.

(Supported by HD-06763, CA-28735 and the Lucille P. Markey Charitable Trust).

CF 011 HEPARAN SULFATE IS REQUIRED FOR bFGF HIGH AFFINITY BINDING AND BIOLOGICAL ACTIVITY, Bradley B. Olwin, Laura W. Burrus, Arthur J. Kudla, Alan Rapraeger*, and Michael E. Zuber. Departments of Biochemistry and Pathology*, University of Wisconsin, Madison, WI 53706.

FGF represses myogenic differentiation *in vitro* and is implicated in regulating myogenesis *in vivo*. We have previously demonstrated that aFGF and bFGF repress acquisition of several differentiated skeletal muscle phenotypes independently of cellular proliferation. Myogenic phenotypes repressed by FGF include irreversible withdrawal from the cell cycle, induction of skeletal muscle gene expression, and fusion of individual cells into multinucleated myotubes. Furthermore, we have shown that a loss of FGF binding sites, which may play a role in myogenesis, occurs during terminal differentiation and is correlated with establishment of a permanent post-mitotic phenotype. We have examined further the loss of high affinity FGF binding sites and now demonstrate that treatment of cell cultures with heparatinase or inhibition of heparan sulfate proteoglycan(HSPG) sulfation by treatment of cells with chlorate results in the loss of high affinity as well as low affinity bFGF binding sites. Inhibition of HSPG sulfation by chlorate is dose-dependent and reversed by concomitant addition of sulfate to the medium. High and low affinity FGF binding is also restored by simultaneous treatment with chlorate and sulfate. These binding and crosslinking analyses suggest that a MM14 bFGF receptor (*flg*) requires HSPG for bFGF binding. Consistent with the loss of bFGF binding, treatment of MM14 cells with chlorate causes terminal differentiation in the presence of bFGF concentrations that repress differentiation. Addition of sulfate reverses chlorate inhibition of bFGF biological activity. HSPG is thus essential for bFGF-mediated repression of myogenesis in skeletal muscle cells. Candidate FGF-binding proteins that mediate repression of myogenesis were identified in proliferating and differentiated MM14 cells by Northern blot analysis of FGF receptors and FGF-binding HSPG. Undifferentiated MM14 skeletal muscle cells express mRNA for at least two FGF binding proteins, the *flg* gene, a FGF receptor protein tyrosine kinase, and syndecan, a heparan sulfate proteoglycan. These mRNAs are not expressed or expressed at very low levels in terminally differentiated cultures, suggesting the loss of FGF binding sites is due to the loss of the flg protein and/or syndecan. We have also identified a non-tyrosine kinase FGF-binding protein that is not a heparan sulfate proteoglycan, which may function as a FGF receptor. Sequencing of cDNAs has identified an open reading frame encoding a 1142 amino acid protein containing 16 unique cysteine repeats, a signal peptide, and membrane-spanning domains. Northern blots identify a ~8.5-10 kb mRNA for the non-tyrosine kinase putative FGF receptor. Thus, three distinct FGF-binding proteins have been identified that may play important roles in regulation of skeletal muscle differentiation.

CF 012 CLONING AND EXPRESSION OF TWO DISTINCT HIGH-AFFINITY RECEPTORS CROSS-REACTING WITH ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS, Joseph Schlessinger, New York University Medical Center, Department of Pharmacology, 550 First Avenue, New York, NY 10016.

The fibroblast growth factor (FGF) family consist of at least seven closely related polypeptide mitogenes which exert their activities by binding and activation of specific cell surface receptors. Unanswered questions have been whether there are multiple FGF receptors and what factors determine binding specificity and biological response. Two distinct receptors encoded by two distinct genes were cloned. One gene product previously termed flg (1-3) and a second one was termed bek (4). These genes encode two similar but distinct cell surface receptors comprised of an extracellular domain with three immunoglobulin-like regions, a single transmembrane domain, and a cytoplasmic portion containing a tyrosine kinase domain with a typical insert. The expression of these two cDNAs in transfected NIH 3T3 cells led to the biosynthesis of proteins of 150 kd and 135 kd for flg and bek, respectively. Direct binding experiments with radiolabeled acidic FGF (aFGF) or basic FGF (bFGF), inhibition of binding with native growth factors, and Scatchard analysis of the binding data indicated that bek and flg bind either aFGF or bFGF with dissociation constants of (2↔15) x 10^{-11}M(5). The high affinity binding of two distinct growth factors to each of two different receptors represents a unique double redundancy without precedence among polypeptide growth factor-receptor interactions.

References:

1) Ruta, M., Howk, R., Ricca, G., Drohan, W., Zabelshansky, M., Laureys, G., Barton, D.E., Francke, U., Schlessinger, J. and Givol D. (1988) Oncogene, 3, 9-15

2) Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger, N., Kaplow, J., Crumley, G., Dionne, C., Jaye, M. and Schlessinger J. (1989) Proc. Natl Acad. Sci. USA, 86, 8722-8726.

3) Lee, P.L., Johnson, D.E. Cousens, L.J., Fried, V.A. and Williams, L.T. (1989) Science, 245, 57-60.

4) Kornbluth, S., Paulson, K.E. and Hanafusa, H. (1988) Mol. Cell. Biol., 8, 5541-5544.

5) Dionne, C.A., Crumly, G., Bellot, F., Kaplow, J.M., Searfoss, G., Ruta, M., Burgess, W.H., Jaye, M. and Schlessinger, J. (1990) EMBO J. 9, 2685-2692.

Biological Actvities of FGF

CF 013 BASIC FGF RECEPTOR: A PORTAL OF CELLULAR ENTRY FOR HERPES SIMPLEX VIRUS. David P. Hajjar*, Robert J. Kaner*, Robert Z. Florkiewicz+, Pamela A. Maher+, Alka Mansukhani, Claudio Basilico and Andrew Baird, Department of Biochemistry, Cornell University Medical College*, New York, N.Y. 10021. Department of Molecular and Cellular Biology, The Whittier Institute for Diabetes+, La Jolla, C.A. 92037, and Department of Microbiology, New York University School of Medicine, New York, N.Y. 10010.

Herpes simplex virus type-1 (HSV-1) is a ubiquitous pathogen responsible for considerable morbidity in the general population. Our results establish the basic fibroblast growth factor (FGF) receptor as a pathway for its entry into vertebrate cells. Inhibitors of basic FGF binding to its receptor and competitive polypeptide antagonists of basic FGF prevented HSV-1 uptake. Chinese Hamster Ovary (CHO) cells that do not express FGF receptors were resistant to HSV-1 entry; however, HSV-1 uptake was dramatically increased in CHO cells transfected with a cDNA encoding a basic FGF receptor (flg).

Experiments were also designed to define the mechanism by which the virion recognizes and binds to the basic FGF receptor. We found that: 1) an immunoreactive basic FGF-like protein was associated with the viral particle and that this association is responsible for viral uptake; 2) HSV-1 infection of Swiss 3T3 cells stimulates the tyrosine phosphorylation of the 90-kDa substrate that characterizes the initial cellular response to basic FGF; 3) antibodies to basic FGF prevent this phosphorylation and inhibit HSV-1 uptake. Because no significant basic FGF sequence is found in the HSV-1 genome, a model for the infection for some target cells is presented whereby the viral particle utilizes host cell-derived basic FGF to ensure subsequent infectivity of newly replicated virus.

We believe that the distribution of the basic FGF receptor in vivo may explain the tissue and cell tropism of HSV-1.

CF 014 THE ROLE OF FIBROBLAST GROWTH FACTORS IN EARLY DEVELOPMENT ON XENOPUS LAEVIS, J.M.W. Slack, S.F. Godsave, L.L. Gillespie, H.V. Isaacs, G.D. Paterno and J. Thompson. Developmental Biology Unit, Imperial Cancer Research Fund, Dept. Zoology, University of Oxford, Oxford OX1 3PS, UK.

All members of the FGF family can induce mesodermal tissues from isolated animal caps of Xenopus embryos. This occurs without effect on the cell cycle, and also works on isolated animal cap cells in vitro. The period of sensitivity extends through the blastula stages and closely parallels the level of FGF receptor on the cell surface. The effect of bFGF is inhibited by heparin, while that of aFGF or KFGF is not.

Low levels of bFGF mRNA and protein are found in blastulae but their regional distribution is still unknown. Originally it was thought that bFGF was secreted from cells of the vegetal hemisphere, since heparin can inhibit mesoderm induction in a transfilter apparatus. However neutralizing antibodies are without effect and it may be that bFGF has a role at some later step of the process. Uniform overexpression of bFGF from injected synthetic mRNA causes some anterior defects and some autoinduction of animal caps. KFGF appears to have stronger effects, perhaps because it is secreted more efficiently.

CF 015 FGF AND TGF-ß ISOFORMS IN THE NERVOUS SYSTEM: DISTRIBUTION AND FUNCTIONAL ROLES, K. Unsicker, C. Grothe, G. Lüdecke, D. Otto R. Westermann, K. Flanders, B. Lafyatis, A. Roberts, M. Sporn, Dept. Anatomy and Cell Biology, University of Marburg, Germany; Lab. Chemoprevention, NCI, NIH, Bethesda, MD 20892, USA

FGFs and TGF-ßs are multifunctional growth factors with specific distributions and functions in the nervous system. Basic FGF is located in several areas of the isocortex, hippocampus, brain stem and in peripheral ganglia. It rescues in vitro and in vivo, neurons from lesion-induced death, and also affects glial cells. TGF-ßs 2 and 3, but not ß1, and their mRNAs are located in several neurons and glial population in the central and peripheral nervous system. In vitro TGF-ßs regulate neuron survival and glial cell performances in combination with other growth factors, including basic FGF. Overlapping localization of TGF-ßs and basic FGF in several brain areas suggests that combinatory actions of these two families of growth factors may also occur in vivo.

Supported by grant Un 34/13 from German Research Foundation.

Endothelial Cell Growth Factors/Angiogenesis Factors

CF 016 MOLECULAR CHARACTERIZATION AND DISTRIBUTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR

Napoleone Ferrara, David W. Leung*, Heidi S. Phillips, Lyn Jakeman, Departments of Developmental Biology and Molecular Biology*, Genentech, South San Francisco, Ca 94080.

Vascular Endothelial Growth factor (VEGF) is a heparin-binding growth factor with a target cell selectivity apparently limited to vascular endothelial cells derived from small or large vessels and is able to induce angiogenesis *in vivo*. VEGF is a dimeric protein with a molecular mass of 45,000 composed of two subunits of identical molecular mass and has a unique NH_2-terminal amino acid sequence. Complementary DNA clones encoding bovine and human VEGF were isolated from cDNA libraries derived from pituitary folliculo-stellate cells and HL60 leukemia cells, respectively. VEGF is structurally related to the A and B chains of platelet-derived growth factor (PDGF) and, by alternative splicing of mRNA, may exist in at least three different molecular species. The mature VEGF sequence is preceded by a typical signal peptide, indicating thet VEGF is a secreted protein, unlike other endothelial cell mitogens such as acidic or basic fibroblast growth factors and platelet-derived endothelial cell growth factor. VEGF has similar amino acid sequence to a protein called vascular permeability factor (VPF) which was identified on the basis of its ability to induce vascular leakage and protein extravasation. We studied the distribution of VEGF mRNA in normal adult rat by *in situ* hybridization. VEGF mRNA is highly expressed in a variety of richly vascularized tissues and organs where no active angiogenesis is taking place, such as the adenohypophysis, the neurohypophysis, several brain areas, the Bowman's capsule in the kidney. We also detected expression in the ovary. In this organ, a temporal relation exists between VEGF mRNA expression and growth of capillary vessels, since high hybridization signal was detected in the highly vascularized corpus luteum but not in the avascular granulosa cells. We also studied the distribution of VEGF binding sites in the adult rat by receptor-binding autoradiography, using ^{125}I-recombinant human VEGF as a ligand. Specific binding sites were widely distributed and their localization apparently corresponded to that of vascular endothelial cells. These findings suggest that VEGF may be involved both in the development and in the maintainance of the differentiated state of the vasculature in a variety of tissues and organs.

CF 017 STRUCTURAL AND FUNCTIONAL PROPERTIES OF PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR, Carl-Henrik Heldin, Kensuke Usuki and Kohei Miyazono, Ludwig Institute for Cancer Research, Box 595, S-751 24 Uppsala, Sweden.

Platelet-derived endothelial cell growth factor (PD-ECGF) is a single chain polypeptide, which stimulates growth and chemotaxis of endothelial cells *in vitro*, and angiogenesis *in vivo*. Structural analysis of purified PD-ECGF and its cDNA, revealed that PD-ECGF is a novel type of angiogenic factor without sequence homology to other known proteins. PD-ECGF is present in human platelets as well as in the placenta; N-terminal amino acid sequencing revealed that PD-ECGF purified from human platelets is processed at amino acid number 11, in contrast to PD-ECGF purified from human placenta which is processed at amino acid 6. Analysis of cultured cells revealed that PD-ECGF is produced by normal fibroblasts and vascular smooth muscle cells, as well as by certain transformed cell lines. PD-ECGF lacks a hydrophobic signal sequence, and it is released from the producer cell only very slowly. The *in vivo* function of PD-ECGF remains to be elucidated. Various kinds of endothelial cells respond to PD-ECGF, as do NRK cells and a choriocarcinoma cell line. The tissue distribution and target cell specificity of PD-ECGF suggest roles in angiogenesis, e.g. in the developing placenta and during wound healing, as well as in the maintenance of the endothelial lining of large vessels.

CF 018 THE STRUCTURE AND FUNCTION OF ANGIOGENIN, Robert Shapiro and Bert L. Vallee, Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, MA 02115. Human angiogenin, a 14.1-kDa monomeric protein, induces neovascularization on the chick embryo chorioallantoic membrane (CAM) and in rabbit meniscal cartilage, and activates phospholipases C and A_2 in cultured endothelial cells. It displays extensive sequence homology to the pancreatic RNases and is in fact a ribonucleolytic enzyme, although its specificity and potency differ markedly from those of pancreatic RNase. Abolition of angiogenin's enzymatic activity by chemical modification, mutagenic replacement of the active site residues His-13, Lys-40, or His-114, or binding of placental RNase inhibitor substantially diminishes angiogenic activity. Conversely, mutation of Asp-116 to histidine increases both activities by 1-2 orders of magnitude. These results clearly indicate that the ribonucleolytic activity of angiogenin is <u>necessary</u> for biological activity. It is not, however, <u>sufficient</u>: a segment of angiogenin lying outside the enzymatic active site is also required. Structural alterations within this segment - including proteolytic cleavage, deamidation, site-specific mutation, and replacement by the corresponding segment of RNase A - virtually abolish angiogenic activity without impairing catalytic efficiency. Moreover, while the enzymatically inactive H13A and H114A mutants effectively inhibit angiogenin-induced angiogenesis on the CAM, derivatives modified in this non-active-site region of the protein do not. These findings suggest that the biological effects of angiogenin are mediated through a cell-surface receptor and that this region forms part of the receptor binding site. Further support for this hypothesis is provided by the isolation of an angiogenin-binding protein from endothelial cell plasma membranes. This protein binds angiogenin tightly (K_d < 1 nM) and specifically and can be readily cross-linked. Angiogenin derivatives mutated at their active sites strongly compete with unmodified angiogenin for binding to this protein, whereas derivatives modified in the putative receptor binding region are much less effective. Studies are also in progress exploring pharmacological applications of angiogenin and angiogenin inhibitors, e.g., for wound healing, cartilage repair, and anti-tumor therapy.

Regulation of Endothelial Cell Growth - I

CF 019 ENDOTHELIAL CELLS MODULATE VASCULAR TONE BY TRANSCRIPTIONAL CONTROL OF GENES ENCODING VASOCONSTRICTORS. S. Kourembanas, P. Marsden, and D. V. Faller. Dana-Farber Cancer Institute, The Children's Hospital, Brigham and Womens' Hospital, and Harvard Medical School, Boston, MA. 02115

Hypoxia is the most potent physiologic inducer of local vasoconstriction known, and chronic hypoxia is associated with increased blood vessel muscularity as well as constriction of certain vasculature beds. The molecular mechanisms underlying hypoxia-induced vasoconstriction have not been previously elucidated. The endothelium is in a unique position to mediate the effects of oxygen tension via the potential release of vasoactive substances regulating smooth muscle tone in a paracrine manner. We have found that physiologic levels of hypoxia initiate the transcriptional activation of specific genes in human vascular endothelial cells, resulting in the elaboration of potent protein products able to induce vasoconstriction and smooth muscle hyperplasia. PDGF and endothelin are the most potent peptide vasoconstrictors known. PDGF-B chain mRNA levels increase more than 8-fold and 12-fold in endothelial cells grown in 40 torr and 20 torr oxygen atmospheres, respectively (relative to transcript levels present when these cells are grown at ambient oxygen tensions). The accumulation of PDGF mRNA is progressively enhanced when endothelial cells are exposed to oxygen tensions of 120, 60, 40 and 20 torr. The transcript levels of endothelin mRNA are increased by 3-5 fold within 1 hour of exposure 60 torr oxygen atmospheres. Endothelin gene transcription is thus induced more rapidly than PDGF-B, and at less severe degrees of hypoxia. Physiologic levels of hypoxia increased endothelin secretion from cultured endothelial cells five-fold above the secretion rate observed at ambient oxygen tension. The stimulatory effect of hypoxia on the production of vasoconstrictive peptides is mediated at the level of gene transcription, and is reversible upon re-exposure to normoxic environments. The endothelial cell oxygen sensor mediating some or all of these effects on gene transcription has been partially characterized and is a heme-containing protein. We propose that the sensitive and rapid regulation of endothelin expression in response to environmental oxygen make it a likely candidate as one of the important regulators of regional blood flow in response to acute changes in oxygen tension. PDGF is not only a potent vasoconstrictor, but is also a powerful growth factor for vascular smooth muscle. Chronic production of PDGF by endothelium in hypoxic vascular beds could modulate the smooth muscle hypertrophy and architectural remodeling observed to result from chronic hypoxia.

CF 020 MODULATION OF ENDOTHELIAL CELL (EC) FUNCTION IN HYPOXIA: ALTERATIONS IN CELL GROWTH AND THE RESPONSE TO MONOCYTE-DERIVED MITOGENIC FACTORS. S. Ogawa, J. Leavy, M. Clauss, S. Koga, R. Shreeniwas, J. Joseph-Silverstein, M. Furie and D. Stern. Columbia University College of Physicians and Surgeons, New York, NY 10032, St. John's University, NY, and SUNY-Stonybrook, NY.

Bovine aortic and capillary ECs exposed to hypoxia (pO_2 ≈14 mm Hg) maintained their viability, but cell growth was slowed. One mechanism underyling these findings was lower levels of FGF expression by hypoxic EC cultures concomitant with an increase of ≈3-fold in the number of high affinity FGF receptors. Consistent with this interpretation, addition of bFGF prevented, at least in part, hypoxia-mediated suppression of EC growth, and enhanced wound repair in hypoxia, stimulating both motility and cell division. These observations suggested that hypoxic ECs could be stimulated to proliferate and migrate by mitogenic factors potentially released by other cells in the presence of low levels of oxygen.

Exposure of human monocyte-derived macrophages (M) to hypoxia led to elaboration into the supernatant of mitogenic activity for both hypoxic bovine aortic and capillary ECs, whereas conditioned media from normoxic M had no effect. When M were placed in hypoxia, mitogenic activity was released in a time-dependent manner, and was most evident as the pO_2 fell to levels <30 mm Hg. Throughout exposure to hypoxia, monocytes remained viable and release of mitogens was not a consequence of general loss of intracellular contents. Chromatography of conditioned medium from hypoxic M on a heparin column demonstrated three peaks of mitogenic activity for ECs, eluting at NaCl concentrations of 0.5-0.8 M, 1.5 M and 2.0 M, with the major activity in the last peak. Mitogenic activity for hypoxic ECs in the 2.0 M eluate was blocked by an antibody to bFGF. In addition, elevated levels of bFGF were demonstrated in the supernatants of hypoxic M by immunoprecipitation.

These results indicate that under hypoxic conditions, endothelial proliferation and migration is slowed, but ECs maintain the capacity to respond to a mitogen such as bFGF. Monocyte-derived macrophages on exposure to hypoxia release several types of mitogens for hypoxic ECs, especially bFGF. This suggests a model in which the growth and migration of ECs under hypoxic conditions, as would exist in a wound or other locus in which oxygen delivery is suboptimal, can be directed by mitogens whose production and release by other cell types is stimulated in response to oxygen deprivation.

CF 021 **SEQUESTRATION AND RELEASE OF BASIC FIBROBLAST GROWTH FACTOR,** Israel Vlodavsky, Rivka Ishai-Michaeli, Pnina Bashkin, Adriana Friedman and Zvi Fuks, Department of Oncology, Hadassah-Hebrew University Hospital, Jerusalem 91120, ISRAEL, and Department of Radiation Oncology, Memorial Sloan-kettering Cancer Center, New York, NY 10021

Our studies on the control of cell proliferation and differentiation by its local environment focus on the interaction of cells with the extracellular matrix (ECM) produced by cultured endothelial cells (EC). This ECM resembles the subendothelium in vivo, contains bFGF and promotes EC proliferation. The distribution of bFGF was studied immunohistochemically in fresh frozen sections of normal human tissues. Expression of bFGF was ubiquitously detected in basement membranes of all size blood vessels. Whereas homogeneous immunoreactivity was observed in large and intermediate size blood vessels, heterogeneity of expression was found in capillaries, with the most intense staining in the anastamosing sites of branching capillaries. It appears that bFGF binds specifically to heparan sulfate (HS) in the ECM since about 90% of the bound growth factor was displaced by heparin, HS, or HS-degrading enzymes, but not by unrelated GAGs or GAG degrading enzymes. Our studies suggest that bFGF is sequestered from its site of action by binding to HS in the ECM. It is thus protected and reserved for induction of localized EC proliferation and neovascularization during wound repair, inflammation and tumor development. Exposure of ECM to heparanase, but not to chondroitinase ABC, resulted in release of the ECM-bound bFGF. Moreover, heparanase activity expressed by intact cells (platelets, neutrophils, lymphoma cells) was found to be involved in release of active bFGF from ECM and Descemet's membrane of bovine corneas. Heparanase may thus function in both cell migration and neovascularization. Basic FGF has also been found to be associated with HS on the cell surface. Some of the cell surface-, but not ECM- bound bFGF was released by treatment with phosphatidylinositol-specific phospholipase C (PI-PLC), suggesting association with glycosyl-PI anchored HS. The PI-PLC released mitogenic activity was inhibited by neutralizing anti-bFGF antibodies. Moreover, addition of PI-PLC to sparsely seeded EC resulted in induction of cell proliferation. We suggest that bFGF associated with HS in the cell surface and ECM, may act in an autocrine manner provided that it is released as a complex with HS or HS-degradation fragments and is properly presented to high affinity cell surface receptor sites. Apart from the cell surface- and ECM- associated bFGF, intracellular bFGF may be liberated under pathological conditions such as in response to radiation damage. In fact, radiation was found to induce in EC an increased expression of the bFGF gene and protein and the secreted bFGF led to recovery of EC from potentially lethal radiation damage.

Regulation of Endothelial Cell Growth - II (joint with Wound Repair meeting)

CF 022 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)

CF 023 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

CF 024 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.

Angiogenesis in Pathological Processes

CF 025 GROWTH FACTOR CONTROL OF NORMAL AND MALIGNANT MAMMARY CELL GROWTH. M.E. Lippman, A. Wellstein, R. Lupu, R.B. Dickson, Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC 20007.

The mechanisms by which physiologic concentrations of estrogen control the growth of hormone dependent breast cancer (BC) are unclear as are the means by which hormone dependent BC progresses to hormone independence. Our studies suggest that hormone dependent BC cells secrete growth factors which are under estrogenic control. These include an insulin-like growth factor I activity, insulin-like growth factor II, transforming growth factor alpha, platelet derived growth factor, one or more secreted members of the fgf family, and in some cases erbB2 ligands. The erbB2 ligands have molecular weights of 30 and 75k. The former also binds with lower affinity to EGF receptor, the latter exclusively to erbB2. They both stimulate receptor phosphorylation and clonogenic growth of tumor cell lines which overexpress erbB2 and reverse the effects of anti-erbB2 blocking antibodies and soluble extracellular domain. Secreted fgf molecules participate in both autocrine and paracrine loops which appear pathogenetically significant in tumor progression. Secretion is stimulated by estradiol. Antiestrogens act by decreasing the secretion of these growth factors. A variety of strategies which block either the secretion or action of these growth factors in vitro can interfere with the growth of human BC cells. These include antigrowth factor antibodies; antigrowth factor receptor anti-bodies; drugs which interfere with the ligand receptor interaction, treatment with antisense oligonucleotides for specific growth factors, and synthetic peptides which function as growth factor antagonists. Hormone independent BC secretes a similar array of growth factor activities. Conversion to the hormone independent phenotype is accompanied by constitutive growth factor production. Hormone dependent human BC cells invariably require estrogen for in vivo tumori-genesis in athymic nude mice. We have been able to mimic the effects of estrogen supplementation by continuous infusion of concentrated conditioned medium from hormone dependent cells cultured in vitro. Taken together, these data support the pathophysiologic role of secreted trophic substances in neoplastic progression of human BC.

Inhibitors of Angiogenesis

CF 026 TUMOR SUPPRESSOR GENE CONTROL OF ANGIOGENESIS. Bouck, N., Polverini, P. J., Tolsma, S. S., Frazier, W. A., and Good, D. J., Departments of Microbiology-Immunology and Pathology, Northwestern University Medical School, Chicago, IL, 60611 and Department of Biochemistry and Molecular Biophysics, Washington University at St. Louis Medical School, St. Louis, MO, 63110.

Successful solid tumors develop the ability to attract the new vessels upon which they depend by increasing production of angiogenic factors and/or by decreasing production of inhibitors of angiogenesis. In an immortal but non-tumorigenic line of hamster cells, BHK21/cl13, an angiogenic phenotype can be induced concomitantly with a tumorigenic phenotype by either mutagenic inactivation of a tumor suppressor gene or by addition of the viral oncogene, polyoma virus middle T. These cells become angiogenic as a result of decreased production of a protein inhibitor of angiogenesis that was recently identified as a truncated thrombospondin molecule (TSP). In vivo, TSP (i) blocks neovascularization in response to a wide range of compounds in the rat cornea assay and (ii) inhibits both tumor growth and tumor-induced angiogenesis when implanted into the nude mouse in a sponge along with pieces of solid tumor derived from the B16 mouse melanoma. In vitro, TSP interferes with endothelial cell migration and mitogenesis. Neither the amino-terminal nor the carboxy-terminal domain of the TSP protein is essential for the inhibition of angiogenesis. The control of angiogenesis by a tumor suppressor gene first seen in hamster cells can also be observed in a human tumor cell. Conditioned media from cultured osteosarcoma cells is potently angiogenic in vitro and in vivo yet media collected from osteosarcoma cells expressing the retinoblastoma suppressor gene cDNA is not angiogenic.

CF 027 A METALLOPROTEINASE INHIBITOR AS AN INHIBITOR OF NEOVASCULARIZATION, Marsha A. Moses, Judith Sudhalter and Robert S. Langer, Department of Surgical Research, The Children's Hospital and Harvard Medical School, Boston, MA 02115

Metalloproteinases and their endogenous inhibitors are key components of an enzyme system which is important in a number of important biochemical and cellular processes. Our recent work has focused on the role of a particular metalloproteinase, collagenase, and the role of an endogenous inhibitor of this enzyme, in the control of neovascularization. The proteolytic degradation of extracellular matrix components by capillary endothelial cells (EC) has been shown to be one of the key prerequisites of the angiogenic process. One of our interests has been in determining the effect(s) of the inhibition of collagenase on neovascularization. We have recently reported the purification, characterization and partial NH_2-terminal sequence of a cartilage-derived inhibitor (CDI) of angiogenesis in vivo and in vitro. This protein, Mr 27,650 +/- 500, is a specific inhibitor of mammalian collagenase as well as a potent inhibitor of growth factor-stimulated capillary endothelial cell (EC) proliferation (72% inhibition at [96 nM]) and capillary EC migration (IC_{50} =16 nM). We have also found that partially purified CDI can inhibit capillary tube formation in vitro. CDI is a powerful inhibitor of angiogenesis in vivo, inhibiting both embryonic and tumor-induced neovascularization. Picomole quantities of CDI tested in the chick chorioallantoic membrane assay (CAM) caused large avascular zones on the CAM surface (1). We have now tested CDI for its ability to inhibit neovascularization induced by V_2 carcinoma using the rabbit corneal pocket assay. When assayed in this in vivo model, blood vessel growth towards the tumor was significantly inhibited by 40 ug of highly purified CDI. We have also purified a metalloproteinase inhibitor from the conditioned media of scapular chondrocytes established and maintained in serum free culture and have found that it shares the same anti-collagenase and anti-angiogenic activities as its tissue-derived counterpart. These results demonstrate an important role for metalloproteinases in the process of neovascularization and suggest that one means of controlling the deregulated vascular growth characteristic of a number of "angiogenic diseases" may be at the level of the control of metalloproteinase activity. (Supported by NIH EY705333).

1. Science 248:1408-1410, 1990.

Structure and Function of FGF Family Membranes

CF 100 THE SUB-CELLULAR FATE OF THE INT-2 PROTEINS IS DETERMINED BY CHOICE OF INITIATION CODON, Piers Acland, Frances Fuller-Pace, Gordon Peters and Clive Dickson Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

The Int-2 gene is a member of the FGF-family of growth factors that is expressed in early embryogenesis and is frequently transcriptionally activated in tumours induced by mouse mamary tumour virus. Int-2 has been difficult to detect in natural settings so we have used an SV40-based expression vector to investigate the synthesis and processing of this oncoprotein in COS-1 monkey cells. Int-2 proteins ranging in size from 27.5kd to 34.5kd have been detected that arise by the post-translational modification of two primary products initiated at either an AUG or CUG codon. Initiation at the predicted AUG generates a 28.5kd product which bears a signal sequence directing entry into the secretory pathway. However initiation of translation at an upstream CUG codon provides a 29 amino acid N-terminal extension which appears to compromise the properties of the signal pepide since at least 50% of CUG-initiated products are directed to the nucleus. Two motifs of basic residues have been identified in the first exon with superficial resemblance to documented nuclear localization signals and whose deletion restores efficient reticular transport to CUG-initiated Int-2. We are currently testing the ability of these motifs to act as functional nuclear loaclization sequences when fused to heterologous cytoplasmic proteins.

CF 101 NUCLEAR TARGETS OF bFGF IN ENDOTHELIAL CELLS, F. Amalric, V. Baldin, P. Belenguer, I. Bosc-Bierne, G. Bouche, A.M. Roman.
C.R.B.G.C. du CNRS - 118 route de Narbonne - 314062 TOULOUSE Cédex.

Basic fibroblast growth factor (bFGF) is a potent mitogen for a large variety of cell types of mesodermal and neuroectodermal origin. Primary culture of aortic endothelial cells required the addition of bFGF to the medium to proliferate. Intracellular uptake of bFGF occured at define steps of the cell cycle. bFGF accumulated into the cytoplasm throughout the cell cycle and is translocated to the nucleus specifically at the transition G1-S. Nuclei prepared from growing cell were cross linked by Cl-Pt treatment. After digestion of nuclei by restriction enzyme (Sau 3A), bFGF was purified by heparin sepharose column, then was immunoprecipitated. bFGF was digested with proteinase K and associated DNA was purified. This DNA was then inserted into vectors and cloned. 14 different clones were isolated and sequenced some of them contained repetitive sequences (B1 like). The inserts bind bFGF in vitro, as was shown by band shift and filter retention assays. In another set of experiments, we have shown that in confluent cells bFGF is no longer in the nucleus. In parallel the transcription of several genes and in particular rDNA is highly decrease. In vitro the sole addition of bFGF to nuclei isolated from confluent cells increases the level of transcription by a factor 10. This activation is mediated by the stimulation of protein kinase CKII that directly controls the transcription of rDNA.

CF 102 A FIBROBLAST GROWTH FACTOR-LIKE ACTIVITY SECRETED BY A DENDRITIC B-CELL LINE, Rudolf Berger, Martin Purtscher, Reinhard Gillitzer, Dan Tong, Klaus Wolff, and Georg Stingl, Div. Cut. Immunobiol., Dept. Dermatol. I, Univ. of Vienna Medical School, Vienna, Austria

We have recently established an Epstein-Barr virus (EBV)-positive B cell line (R594-4) which acts as a potent stimulator of the allogeneic mixed leukocyte reaction. We have recently observed that culture supernatants of R594-4 cells support the long-term growth of cell lines derived from Kaposi's sarcoma lesions.

In further studies, cell lines of mesenchymal origin (MRC-5, 711-3, T-24) were now cultured in the presence of conditioned medium (CM) derived from R594-4 cells. Using ^{3}H-thymidine incorporation assays as well as a monoclonal antibody against a proliferation-associated antigen (Ki-67), we found that cell lines cultured in CM-supplemented medium exhibited a severalfold higher proliferation rate than cell lines kept in normal tissue culture medium.

Further experiments showed that incubation of CM with heparin-sepharose led to an abolishment of its growth-promoting activity. This indicates that the factor(s) responsible for this activity is (are) closely related to members of the fibroblast growth factor (FGF) family. Neutralizing antibodies to IL-1α, IL-1β, TNF-α, aFGF and bFGF did not block proliferation suggesting that the factor(s) in R594-4CM differ(s) from these cytokines. Analysis by gel filtration showed that the active component in R594-4CM had a molecular size of approximately 52 kd.

Further functional and biochemical studies are currently underway to delineate the nature of this (these) factor(s).

CF 103 bFGF MODULATES THE BEHAVIOUR OF THE PERINATAL O-2A PROGENITOR CELL, BOTH ON ITS OWN AND IN COMBINATION WITH PDGF.
Oliver Bögler, Damian Wren and Mark Noble, Ludwig Institute for Cancer Research, London W1P 8BT, England.

Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells give rise to oligodendrocytes and type-2 astrocytes in cultures of rat optic nerve. In the presence of platelet derived growth factor (PDGF) bipolar, motile O-2A progenitors undergo limited clonal expansion before cells become mitotically unresponsive to PDGF and differentiate into oligodendrocytes with a timing similar to that seen *in vivo*. This behaviour is based on the intrinsic ability of O-2A progenitors to measure time. In contrast to PDGF, basic fibroblast growth factor (bFGF) elicits a different behaviour: O-2A progenitors rapidly become multipolar, non-motile, and divide as they differentiate into oligodendrocytes[1]. If both PDGF and bFGF are present. oligodendrocytic differentiation is prevented, while O-2A progenitors continue to divide[2]. Recent work has concentrated on the role that these growth factors play in controlling developmental plasticity in the O-2A lineage.

1. Noble, M., Wolswijk, G. and Wren, D. (1989) *Progr. Gr. Fact. Res.* **1**: 179-194. (review)
2. Bögler, O., Wren, D., Barnett, S.C., Land, H. and Noble, M. *Proc. Natl.Acad. Sci. USA* **87**: 6368-6372.

CF 104 X-RAY CRYSTALLOGRAPHIC STUDIES OF A MODIFIED BASIC FIBROBLAST GROWTH FACTOR, Daniel M. Bollag[1], James C. Myslik[1], Andrew P. Seddon[2], Peter Böhlen[2], Yakov Gluzman[2], and Clarence E. Schutt[1], [1]Chemistry Department, Princeton University, Princeton, N.J. 08544 and [2]Medical Research Division, American Cyanamid Company, Pearl River, N.Y. 10965

We are interested in determining the high resolution three-dimensional structure of human basic fibroblast growth factor (bFGF) by X-ray crystallography. A modified form of human bFGF has been crystallized. The bacterially expressed bFGF displays wild type mitogenic activity, and the resulting crystals diffract to high resolution. When polyethylene glycol is used as the precipitant, parallelogram-shaped plates form (triclinic space group P1) with unit cell parameters a = 31.0 Å, b = 33.6 Å, c = 34.7 Å, $\alpha = 88°$, $\beta = 85°$, $\gamma = 76°$. Using an Enraf-Nonius FAST TV area detector, we have completed collecting data to 2.8Å from native bFGF crystals as well as from heavy atom derivatized crystals containing mercury. We are currently searching for more heavy metal derivatives with the goal of solving the three-dimensional structure of bFGF using the method of multiple isomorphous replacement. A high resolution crystallographic structure of bFGF should provide important information about heparin and receptor binding regions of the protein.

CF 105 BASIC FIBROBLAST GROWTH FACTOR BINDS TO A HEPARAN SULFATE PROTEOGLYCAN OF HUMAN BONE MARROW AND IS RELEASED BY PLASMIN, Georg Brunner, Dan B. Rifkin and E. Lynette Wilson, Department of Cell Biology, NYU Medical Center, New York, NY 10016.

Basic fibroblast growth factor (bFGF) is known to be a potent mitogen for human bone marrow stromal cells and also stimulates hematopoiesis. We therefore studied the binding of bFGF to primary human bone marrow cultures as well as the mechanism of its release from these cells. bFGF binding occurred predominantly to a 200-kDa heparan sulfate proteoglycan (HSPG) localized on the cell surface and, to a lesser extent, to a 600-kDa HSPG found in the stromal cell matrix. Whereas heparinase treatment of the bone marrow cultures resulted in the disappearance of the bFGF binding sites, plasmin released complexes of bFGF and the 200-kDa HSPG from the cell surface. The bFGF present in these complexes was biologically active as shown by its ability to induce plasminogen activator in bovine aortic endothelial cells. We conclude that HSPGs from human bone marrow serve as a reservoir for bFGF, from which it can be released in a biologically active form via the proteolytic cascade of plasminogen activation. The release of active bFGF could subsequently result in a stimulation of hematopoiesis.

CF 106 **THE bFGF NATIVE FORMS: SUBCELLULAR LOCALIZATION AND ASSOCIATED PHENOTYPES WHEN EXPRESSED IN ENDOTHELIAL CELLS,** Béatrix Bugler, Bettina Couderc, Véronique Patry, Catherine Zanibellato, Hervé Prats and François Amalric.
Laboratoire d'Endocrinologie Expérimentale, CHU Rangueil, Bât L3 , 31054 Toulouse, FRANCE .

The subcellular localization of three forms of bFGF initiated at an AUG (bFGF 155aa) and two CUG (210 and 195aa) initiation codons was investigated directly or by using chimeric genes constructed by in frame fusion of bFGF cDNA and the chloramphenicol acetyl transferase. The AUG initiated protein was cytoplasmic while the CUG initiated forms where nuclear. The nuclear targeting signal is comprised between aminoacids 15-57 (bFGF 210 or 195aa) and mutagenesis studies allowed to point out arginine residues involved in the nuclear translocation or in the control of the bFGFs localization.
In a way to understand the biological significance of such a differencial localisation we have constructed retroviruses expressing one, two or the three natives bFGFs forms. Constitutive expression of these constructs in bovine aortic endothelial cells (ABAE) induced two different phenotypes: independant growth anchorage was found associated with the expression of the AUG initiated form, while the cells synthesizing CUG1 or [CUG1+ CUG2] initiated forms were immortalized with a dependant growth anchorage. These phenotypes seem to be related to constitutive expression of bFGF more than high levels of the factor. We also report the analysis of the infected cells sensibility to exogenous bFGF and TGFß and the effects of suramin on the phenotypes.

Alternative initiation of translation regulates the subcellular localization of bFGF and probably modulates its role in cell growth and differenciation control.

CF 107 EXPRESSION OF HUMAN ACIDIC FGF IN MAMMALIAN CELLS BY USING A VACCINIA VIRUS SYSTEM. Yihai Cao and Ralf F. Pettersson, Ludwig Institute for Cancer Research, Stockholm Branch, Box 60202, S-10401 Stockholm, Sweden.

HBGF I (acidic FGF) and II (basic FGF) belong to a growing family of proteins lacking an N-terminal hydrophobic signal sequence that mediates translocation through the membrane of the endoplasmic reticulum. Such proteins have to be externalized either by leakage from damaged cells, or directly through the plasma membrane by a mechanism distinct from the classical exocytic pathway. Recent evidence suggests that release of interleukin 1β and a 14 kDa lectin is highly regulated.

We have recently shown that less than 1% of aFGF overexpressed in insect cells by using a baculovirus expression system was released into the medium. To be able to study the biosynthesis and release of FGF in mammalian cells, we have now expressed human aFGF to high levels by using a T7 RNA polymerase-driven vaccinia virus system. The kinetics, level of expression, and release were characterized in HeLa cells by pulse-chase labeling, Western blotting and immunofluorescence. Despite the high expression level, no FGF was found in the medium, confirming earlier observations that FGF remains cell-bound under culture conditions. We are currently extending the expression studies to a wide range of different cell types to find out whether release might be inducible or regulated during differentiation.

CF 108 HUMAN ACIDIC FIBROBLAST GROWTH FACTOR GENE IN $5q^-$ CHROMOSOMES, Ing-Ming Chiu, Wen-Pin Wang, Samuel W. Needleman, Donald E. Thornton, Karl Theil and Robert Payson, Department of Internal medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210

Acidic fibroblast growth factor (aFGF), also known as heparin-binding growth factor 1, is a mitogen for a variety of mesoderm-and ectoderm-derived cells. We have previously reported several different sizes of aFGF mRNA species and different aFGF cDNA clones resulting from alternative splicing. These results suggest that the gene structure and regulatory mechanism for gene expression of aFGF are complex. As a first step toward understanding aFGF gene structure, we have isolated nine contiguous genomic DNA clones spanning 54 kbp and determined the complete DNA sequences of all three coding exons. Comparison of the nucleotide sequences between the human and bovine DNA showed the sequence similarity extended 2400 bp downstream from the coding region. We have also isolated another 40 kbp of contiguous DNA sequences containing the upstream untranslated exons. These two clusters do not overlap suggesting that the aFGF locus is more than 100 kbp in size. Cloning of the aFGF gene allowed us to characterize this locus in acute nonlymphocytic leukemia (ANLL) patients. A fraction of ANLL patients (10-20%) have a deletion in the long arm of chromosome 5, whose distal breakpoint overlaps the aFGF locus and multiple genes of hematopoietic importance. We have characterized this region in a therapy-related ANLL patient by pulse-field gel electrophoresis and Southern blotting analysis. The results suggested that one chromosome has a large deletion involving PDGF-R, c-fms and aFGF loci, which is consistent with the cytogenetic analysis of the patient. In contrast, the other chromosome 5, which appeared normal cytogenetically, was shown to have a smaller deletion in proximity to but not involving any of these three genes. Characterization of breakpoints in the $5q^-$ chromosomes is likely to lead to identification of the presumptive tumor suppression gene. The loss of such a gene might give rise to its profound dysregulation of cellular proliferation and differentiation in ANLL patients.

CF 109 Characterization of FGF6 recombinant protein expressed in E. coli.

François Coulier, Sandrine Pizette, Michèle Batoz and Daniel Birnbaum
INSERM U119, 27 bd. Leï Roure, 13009 Marseille, France

The Fibroblast Growth Factor (FGF) genes family comprises to date 7 members, and has been implicated in a wide range of physiological and biological processes including mitogenesis, angiogenesis, morphogenesis or tumorigenesis. Their actions appear to be relayed through specific interaction with at least two types of transmembrane receptors with different affinities.

We have previously isolated the *FGF6* gene on the basis of sequence similarity with *K-FGF/HST*. *FGF6* is able to transform NIH 3T3 fibroblasts with high efficiency, and conditionned media from culture of *FGF6*-transformed cells are mitogenic.

In order to study more thoroughly the activities of FGF6, we have constructed a bacterial expression vector by inserting *FGF6* cDNA sequences into the T7 RNA polymerase-based pET3a vector. The resulting construct is able to drive the expression of high amount of FGF6 protein in *E. coli*, in the form of inclusion bodies. The solubilized protein is further purified through heparin-sepharose chromatography and high salt elution. The heparin-sepharose- purified FGF6 protein displays a strong mitogenic activity (half maximal dose in the ng/ml range) and is able to morphologically transform Balb/c 3T3 fibroblasts.

CF 110 PURIFICATION AND CHARACTERIZATION OF A NEW ENDOTHELIAL CELL GROWTH FACTOR NAMED HARP (HEPARIN AFFIN REGULATORY PEPTIDE).

José COURTY, Marie-Claude DAUCHEL, Danielle CARUELLE,
Thi Thé NGUYEN and Denis BARRITAULT : Laboratoire de Biotechnologie des Cellules Eucaryotes, Université Paris Val de Marne, Avenue du Général De Gaulle, 94010 Créteil, FRANCE.

Heparin affin regulatory peptide (HARP), is a 18 Kd growth factor for endothelial cell, isolated from neutral extract of adult bovine brain. Purification of this polypeptide was achieved by a three step procedure including cationic exchange chromatography, heparin-Sepharose affinity chromatography and Mono S cation-exchange chromatography. 370 µg of purified HARP can be obtained per kilogram of wet weight brain. In vitro, the purified growth factor displayed a maximal mitogenic effect on bovine brain capillary cells at the concentration of 100 pM. *In vivo*, in the rabbit cornea assay, it was also angiogenic in the same range of magnitude. Despite similar features between HARP and acidic or basic FGF, HARP was shown to be different from FGF molecules by its peptidic sequence and by its immunological properties. However, the 25 NH_2-terminal amino-acids are identical to those reported either for heparin-binding growth-associated molecule (HB-GAM) a neuronal maturation protein (Rauvala, H. (1989) *EMBO J.* 8, 2933-2941) or for HBGF8 (Milner, P. et al. (1989) *Biochem. Biophys. Res. Commun.* 165, 1096-1103). The exact structural and

CF 111 INTERLEUKIN-1 REGULATES HEPARIN-BINDING GROWTH FACTOR-2 GENE EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS

Cyril G. Gay[1] and Jeffrey A. Winkles[2], [1]Biotechnology, Biologics, and Environmental Protection, United States Department of Agriculture, 6505 Belcrest Road, Hyattsville, MD 20782; [2] Laboratory of Molecular Biology, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.

The angiogenic polypeptide class II heparin-binding growth factor (HBGF-2), also known as basic fibroblast growth factor, is a mitogen for vascular smooth muscle cells *in vitro* and *in vivo*. Since these cells can also express HBGF-2, it may function in both an autocrine and paracrine manner to regulate their proliferation *in vivo*. We have observed that the inflammatory cytokines interleukin (IL)-1α and IL-1β can induce HBGF-2 gene expression in human saphenous vein smooth muscle cells. Maximal HBGF-2 mRNA levels are detected 2-4 h post-IL-1 stimulation; induction may require *de novo* protein synthesis and does not occur if transcription is inhibited. Immunoprecipitation analysis indicates that IL-1-stimulated cells also express an increased amount of HBGF-2 protein. Interferon-gamma and glucocorticoids, inhibitors of smooth muscle cell proliferation, suppress the induction of HBGF-2 mRNA expression by IL-1. IL-1 induction of HBGF-2 mRNA expression also occurs in aortic smooth muscle cells and fibroblasts, but not in endothelial cells. In addition, antibody neutralization experiments indicate that IL-1-stimulated smooth muscle cell growth is, at least in part, mediated by HBGF-2. These results indicate that cytokines released at sites of vascular injury or inflammation may regulate HBGF-2 production by smooth muscle cells *in vivo*. Increased HBGF-2 levels within the vessel wall could play a role in both smooth muscle cell proliferation and the neovascularization associated with the development of atherosclerotic lesions.

CF 112 ENDOTHELIAL AND FIBROBLASTIC ACTIVATION IN SCLERODERMA, Ralph Giorno, Henry N. Claman and James R. Seibold, University of Colorado School of Medicine, Denver, CO 80262, and R. W. Johnson Medical School, New Brunswick, NJ 08903

Scleroderma may be an autoimmune condition where tissue fibrosis is the result of processes originating in T cell, mast cell and endothelial activation. We studied the location and extent of endothelial activation by staining skin biopsies with an antibody to endothelial-leukocyte adhesion molecule-1 (ELAM-1), a marker of endothelial activation. We determined the sites of newly formed collagen using an antibody to the amino-terminal part of the propeptide of type I collagen (procollagen-1, PC-1). We studied 15 control skin biopsies and 20 pairs of skin biopsies from patients with scleroderma. Each pair consisted of clinically affected skin and skin judged to be "uninvolved." These sites were usually forearm and deltoid areas, respectively. ELAM-1 was seen in 3/15 controls, 14/20 affected biopsies and 14/20 unaffected biopsies. PC-1 in the papillary dermis was seen in 15/15 controls (1^+ intensity), in 19/19 affected biopsies (2^+-3^+), and 20/20 nonaffected samples (2^+-3^+). PC-1 in the interstitium was seen in 0/15 controls, 11/19 affected and 11/20 nonaffected samples. We conclude that there is increased evidence of endothelial and fibroblastic activation in scleroderma skin compared to control skin. As there was no difference between "affected" and "uninvolved" skin, and as many patients were getting worse clinically, it seems that endothelial and fibroblastic activation precedes clinical fibrosis.

CF 113 STRUCTURE AND FUNCTION OF THE aFGF/HBGF1 GENE: ROLE OF EARLY GROWTH RESPONSE GENES IN aFGF/HBGF1 REGULATION. Harris S.E., †Hall J.A., †Rong Z.X., *Chang C., Harris M.A. Univ of Texas Health Science Center, Division of Endocrinology, San Antonio, TX, 78284-7877. †W. Alton Jones Cell Science Center, Lake Placid, NY 12946, *Dept of Surgery/Urology, Univ of Chicago, Chicago, IL 60637.

In the human prostate cancer cell, LNCAP, and the hamster ductus deferrens tumor cell, DDT1, androgens stimulates accumulation of acidic FGF/HBGF1 mRNA and protein. The kinetics of induction of aFGF/HBGF 1 mRNA by androgens and the structure of the 5' promoter of the FGF/HBGFI gene, suggest a variety of early growth response genes may be involved in androgen-stimulated growth. The 5'-flanking region of one of the aFGF promoters, isolated from the hamster genome, contains several potential AP-1, and Egr-1 DNA binding sites. Androgens stimulate TR3/Nur 77 and Egr 1 mRNA by 5 hours in LNCAP cells. TR3/Nur 77 mRNA continues to increase up to 24 hours, but Egr 1 mRNA decreases after 5 hours. Nuclear run-on experiments indicate androgens indirectly increase the transcription efficiency of the TR3/Nur 77 gene within 6-12 hours. By co-transfection of 5'-flanking aFGF/HBGF 1-CAT construction with CMV-Egr 1 and CMV-TR3/Nur 77 expressions vectors, we are attempting to map the functional TR3/Nur 77 and Egr-1 binding sites in the aFGF/HBGF 1 promoter. We have also identified a novel 5' non-coding exon of aFGF gene utilized in human LNCAP prostate cancer cells. We are presently isolating this 5'-flanking region using the LNCAP specific 5' non-coding exon DNA sequence as probe.

CF 114 EFFECT OF ENDOTHELIAL CELL GROWTH FACTOR ON AN ENDOTHELIAL SPECIFIC SURFACE ANTIGEN. S. Hasthorpe, J. Rogerson and A. Fournier. Cell Biology, Peter MacCallum Cancer Institute, 481 Little Lonsdale Street, Melbourne, 3000.

An endothelial specific surface antigen (ESSA) is expressed on murine endothelial cells in in vivo (vena cava, aorta and bone marrow sinus endothelium) and in vitro explants of marrow cultured for up to 12 weeks. Long term cultures of marrow develop an adherent stromal layer of cells which comprises endothelial cells, macrophages and fibroblasts. It is known that endothelial cells secrete a growth factor for granulocyte macrophages (GM-CSF) and have an important role in hematopoiesis. Expression of ESSA has been studied in a somatic cell hybrid (H5Scl1.4.4; marrow x A9) and a stromal cell line (U2). ESSA does not appear to be expressed on a wide variety of cell lines. Endothelial cell growth factor (ECGF, acidic fibroblast growth factor) appears to reduce the expression of ESSA. Using a radio-labelled binding assay with a monoclonal antibody (H513E3) it was found that a 16-38% decrease in ESSA binding sites per cell occurred over a 24 to 48 hr exposure to ECGF. Change in the level of ESSA expression was dependent on the duration and dose of ECGF and required heparin. ECGF did not appear to bind directly to ESSA at 4°C, to a significant degree. The mechanism by which ECGF reduces H513E3 binding sites is not yet understood. The effect of ECGF could be due to an indirect action on the production of ESSA. However, interleukin-1, gamma interferon, lipopolysaccharide and tumour-promoting phorbol diester (TPA) did not effect ESSA expression suggesting that a secondary effect by other mitogens or agents is not responsible. Study of ESSA may provide further information on the biological action of ECGF.

CF 115 STIMULATION OF SYNDECAN GENE EXPRESSION IN MESENCHYMAL CELLS BY bFGF AND TGFß. **Markku Jalkanen, Arto Määttä and Klaus Elenius, Department of Medical Biochemistry, University of Turku, SF-20520 Turku, Finland**

Syndecan - an integral membrane proteoglycan - binds selectively both extracellular matrix molecules (interstitial collagens, fibronectin, trombospondin, tenascin) but also some growth factors (bFGF). In normal adult tissues syndecan is expressed almost exclusively at epithelial cell surfaces but during embryogenesis its synthesis is transiently activated also in the developing mesenchymes of several tissues. This induction has been suggested to be a result of reciprocal interactions between epithelium and mesenchyme but the actual factors mediating the effect have not been described. We have studied the effect of bFGF, transforming growth factor beta (TGFß) and their combination on the transcription of syndecan gene, production of syndecan molecule to the cell surface and shedding of syndecan from cell surface into the culture medium in 3T3 fibroblast cultures. Both bFGF and TGFß, when applied separately, had only weak stimulatory effect on syndecan gene expression and increased only sligthly syndecan amounts at the cell surface. Activation of syndecan expression was, however, much more prominent when bFGF and TGFß were added simultaneously into 3T3 cultures. This several fold-stimulation was observed both in syndecan mRNA-levels and in syndecan amounts at the cell surface of 3T3 cells. However, the shedding of syndecan ectodomain into culture medium was not significantly enhanced. These findings suggest that growth facors, like bFGF and TGFß could be potential regulators of syndecan expression in vivo, too. They can also act coordinately and very locally, and explain the very precise temporary and spatial expression of syndecan which follows morphogenetic rather than histological boundaries during tissue formation and organogenesis.

CF 116 Expression of aFGF or aFGF coupled to a heterologous signal peptide induces invasive potential in transfected epithelial cells. Jacqueline Jouanneau*, Ginette Moens*, Danielle Caruelle° and Jean-Paul Thiery*. *Laboratoire de Physiopathologie du Developpement, CNRS URA 1337, E N S 46, rue d'Ulm, 75005 Paris, France and °Laboratoire de Biotechnologie des Cellules Eucaryotes, Université Paris XII. Avenue du Général de Gaulle, 94010 Créteil, France.

In order to approach the cellular and molecular mechanisms involved in the very early steps of cancer cell invasion and metastatic spreading, the potential role of growth factors of the FGF family have been studied. It has been previously reported that addition of exogeneous aFGF to the epithelial carcinoma NBTII cells results in morphological transformation towards fibroblast-like motile cells°. In an attempt to study the potential autocrine or intracrine role of aFGF in the critical step of carcinoma dissociation and the acquisition of motile invasive potentially metastatic properties at the single cell level, we have generated clones of NBTII cells expressing either human aFGF or an aFGF-signal peptide hybrid growth factor by transfection. Most of the growth factor producing cells are converted to motile fibroblast-like cells and are induced to secrete endogeneous metalloprotease activities. The aFGF is not secreted into the medium but in high producers it can be immunologically detected in the extracellular matrix; in contrast, cells producing the chimeric signal peptide-aFGF secreted these growth factor in the culture medium. Results will be discussed in view of acquisition of invasive potentialities together with the expression of the growth factor.

°*A.M. Valles et al.,1990, Proc. Natl. Acad. Sci. US. 87 1124-1128*

CF 117 ENDOGENOUS EXPRESSION OF THE FGF GENE FAMILY IN MPTP LESIONED MOUSE BRAIN, Sherry Leonard, Judith Logel, Desiree Luthman, Johann Luthman, and Barry Hoffer, Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado, 80262.

The best characterized growth factor, nerve growth factor, stimulates growth and regeneration of central cholinergic neurons, but has little effect on the central dopaminergic pathway. Substantial evidence suggests that exogenous fibroblast growth factor may influence the survival of dopaminergic neurons both in vitro and in vivo. Endogenous response of the FGFs to chemical lesion in dopaminergic systems has not been measured. The fibroblast growth factor family of genes, related by sequence homology and function, now comprises at least four unique gene sequences. To study endogenous gene expression in dopaminergic neurons, we have treated Swiss-Webster mice with 3 X 30 mg/kg of the neurotoxin 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTP) on successive days. This treatment lowers dopamine levels by 95%, but by five weeks post treatment dopamine levels return to pre-treatment values. After one week and five weeks, animals were sacrificed and the brains were removed for dissection of the substantia nigra and caudate. mRNA was isolated from a pool of each of these tissues for each time point. Quantitation of mRNA levels for aFGF, bFGF, and the FGF receptor by quantitative multiplex PCR will be presented.

CF 118 A PUTATIVE NUCLEAR TRANSLOCATION SEQUENCE OF HBGF-1 IS REQUIRED FOR IN VIVO TRANSFORMATION. M. MacPhee, X. Zhan#, R. Forough#, S. Friedman#, T. Wiltrout*, R. Wiltrout, K. McCormick*, T. Maciag# & T. Sayers*, NCI-BRMP & *BCDP PRI/DynCorp NCI-FCRDC, Frederick, MD 21701 & #American Red Cross, LMB, J. H. Holland Lab Rockville MD 20855.

Constitutive expression of heparin-binding growth factor (HBGF-1) may confer upon fibroblasts a transformed phenotype in vivo, either by release of HBGF-1 (which lacks a signal sequence) into the environment by an unknown pathway, (possibly via cell death) or HBGF-1 may function as an intracellular mitogen using the putative nuclear translocation signal(NTS), NYKKPKL, as described in Science, 249:1567(1990). To gain insight into the mechanism of HBGF-1-induced in vivo transformation, the signal sequence (ss) for secretion from the hst/KS3 oncogene was ligated to HBGF-1β, and its biologically active des 1-20 form, HBGF-1α. To determine the importance of the NTS to the activity of HBGF-1, the deletion mutant, HBGF-1U, lacking the putative NTS, was also constructed. NIH 3T3 cells were independently transfected with vectors containing HBGF-1α, ss-HBGF-1α, ss-HBGF-1β, or HBGF-1U. Irradiated Athymic mice were injected subcutaneously with either 10^5 or $5x10^5$ cells and mean tumor diameter measured on Day 10. Constitutive expression of HBGF-1 resulted in highly vascularized tumors (4.2 and 9.2mm). The signal sequence linked to HBGF-1α and β produced similar tumors (10^5 cells: 3.5 and 7.5 mm respectively). In contrast, transfection with HBGF-1U resulted in no tumor formation. These results correlated with in vitro tests for transformation (data not shown). Thus a signal sequence is not required for HBGF induced tumor formation, but a putative NTS may be necessary. Attempts to interfere with tumor growth and development utilizing HBGF-1U are underway.

CF 119 ANALYSIS OF FIBROBLAST GROWTH FACTORS PRODUCED BY A HUMAN GLIOBLASTOMA CELL LINE: EVIDENCE FOR MULTIPLE bFGF PROTEINS. Joseph F. Megyesi, Michael Klagsbrun, Judah Folkman, Rosalind A. Rosenthal and David R. Brigstock, Departments of Surgery, Biological Chemistry, and Anatomy and Cellular Biology, Children's Hospital and Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115

Glioblastoma multiforme, a type of brain tumor, is among the fastest growing and most vascularized neoplasms in humans. In order to determine which growth factors may be contributing to this rapid growth rate and high degree of vascularity, a human glioblastoma cell line was established in culture. Extracts of these cells were subjected to Biorex 70 cation exchange chromatography followed by heparin-affinity FPLC. Growth factor activity was monitored by the ability of column fractions to stimulate DNA synthesis in bovine capillary endothelial cells or Balb/c 3T3 cells. Growth factor activity that did not bind to Biorex columns was found to bind to heparin-affinity columns and to be eluted as a single peak by 0.9M NaCl. This activity peak contained a protein of M_r 16,000 which co-migrated with recombinant aFGF and was detected on Western blots by antisera to aFGF. In addition to this aFGF-like mitogen, glioblastoma cells were found to contain growth factor activity which bound to Biorex columns and required 0.8M NaCl for its elution. This cationic factor bound to heparin-affinity columns and was eluted as a single peak of activity by 1.3M NaCl. Western blot analysis of this activity peak demonstrated the presence of proteins of M_r 18,000, 22,500 and 24,000 which were detected by conventional antisera to bFGF. An antiserum that specifically recognizes the *N*-terminal extension sequences of human high molecular weight bFGF proteins was used to confirm that the 22.5-24kDa proteins were authentic forms of bFGF. These results suggest that glioblastoma cells synthesize both aFGF and bFGF. Moreover these cells naturally express multiple molecular weight forms of bFGF, a feature that, in human cells, has been reported only in one other cell line, namely SK Hep-1 hepatoma cells. Since aFGF and bFGF stimulate angiogenesis *in vivo* and *in vitro*, the presence of these two mitogens in glioblastoma may contribute to vascularization of this tumor. However in the absence of evidence for FGF secretion, the production of secreted angiogenic factors by glioblastoma cells and the functional significance of endogenous intracelluar FGFs in these cells requires further study.

CF 120 ACIDIC FIBROBLAST GROWTH FACTOR ACCELERATES RODENT DERMAL WOUND HEALING, Theodore N. Mellin, Robert D. Busch, Joanna Capparella, Mary Lou James, Robert J. Mennie, Jerry DiSalvo, Christine Huber, Matthew J. Van Zwieten, John Frank, Illya Fagin, David Linemeyer and Kenneth A. Thomas, Department of Biochemistry, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

Acidic fibroblast growth factor (aFGF) is a potent vascular endothelial cell mitogen in culture and induces blood vessel growth in vivo. In addition, it stimulates the mitotic activity and synthetic functions of fibroblasts. These properties suggest that aFGF may play an important role in the wound healing process. aFGF was found to accelerate the rate of closure of 6 mm diameter full-thickness punch biopsies wounds in rats and to reduce the number of days to complete closure. Histomorphometric studies indicate that both granulation tissue and capillary density are substantially increased in aFGF treated wounds. Dose-response studies identified a minimum effective dose of 1.0 μg/wound/day (3.5 $\mu g/cm^2$/day). Dose frequency studies revealed that intermittent dosing was approximately as effective as daily dosing, whereas a single dose was less effective.

CF 121 PURIFICATION AND N-TERMINAL AMINO ACID SEQUENCE OF A NOVEL 17 kD GROWTH FACTOR. P.G. Milner, Y.-S. Li, R.M. Hoffman, C.M. Kodner, N.R. Siegel, and T.F. Deuel. Department of Medicine, Jewish Hospital, Washington University, and Monsanto Corporation, St. Louis, MO.

Since uterus is important in supporting embryo development we sought to characterize the growth factors expressed in this tissue. Substantial mitogenic activity was observed in bovine uterine extracts. One activity appeared to bind more tightly to cation exchange column than did basic fibroblast growth factor (bFGF). We have purified to homogeneity a novel 17 kD growth factor from bovine uterus which we designated heparin-binding growth factor-8 (HBGF-8). The growth factor binds tightly to cation exchange resins and to Heparin-Sepharose and is stable to acetone precipitation and labile in acid. Based upon total activity in acetone extracts of bovine uterus stimulating ^{3}H-thymidine incorporation into DNA of serum-starved NIH 3T3 cells, a 6940 fold purification was achieved with an overall yield of HBGF-8 activity of 0.4%, using extraction of acetone powders and chromatographic separations at neutral pH. Approximately 18 μg protein was obtained from 1.2 kg wet weight of tissue. HBGF-8 was clearly separated from bovine uterus bFGF by purification and the 25 amino acid N-terminal sequence was unique. HBGF-8 was as active as acidic fibroblast growth factor (aFGF, 4090 dpm/ng) and slightly less active than bFGF (9000 dpm/ng) in the mouse NIH 3T3 fibroblast mitogenic assay system with an intrinsic specific activity of 5000 dpm/ng under standard assay conditions. This protein appears to be closely related, or identical to a recently described 18 kD protein from developing rat brain which stimulated dendrite outgrowth.

CF 122 ROLE OF ENDOGENOUS bFGF IN 3T3 FIBROBLASTS PROBED BY ANTISENSE INHIBITION OF BASIC FIBROBLAST GROWTH FACTOR. Joachim Sasse, Avner Yayon*, Michael Klagsbrun**, and Georgeann Smale. Shriners Hospital for Crippled Children, Tampa, Fl, *Weizmann Institute of Science, Rehovot, Israel, **and Department for Biological Chemistry, Childrens Hospital and Harvard Medical School, Boston, MA.

Basic fibroblast growth factor stimulates growth in a variety of cell types of mesodermal and neuroectodermal origin. Despite its abundant distribution most of these tissues are not undergoing active growth, suggesting that basic fibroblast growth factor activity must be tightly regulated in order to prevent autostimulation of cell and organ growth. In order to address the question of the role of bFGF in autocrine growth regulation we have transfected the gene for bovine bFGF into mouse 3T3 cells and developed the "B35" cell line (NATURE, 1988, Vol 331, p 173-175).

Here we report the effect of an antisense bFGF oligomer on cell phenotype and growth regulation of the transfected B35 cells. The 25 bp anti-sense S-oligodeoxynucleotide was synthesized corresponding to the translation start site (AUG codon) of bFGF and surrounding nucleotides. As a control, a 25 bp S-oligonucleotide was prepared displaying an unrelated sequence. B35 cells in normal medium or cells treated with 1 - 15 micromolar of control oligomer showed a very rapid cell growth and displayed a partially "transformed" phenotype as characterized by the loss of contact inhibition. However, B35 cells treated with 1-15 micromolar of basic FGF antisense oligomer exhibited a progressive reversion to the normal 3T3 cell phenotype as characterized by the re-establishment of contact inhibition and display of the normal cell morphology. Accordingly, cellular growth was largely reduced by the bFGF antisense oligomer but not by the control oligomer.

It is remarkable that regulation of cell growth and reversion of the transformed phenotype is achieved by bFGF antisense oligomers in this model system even though the transfected B35 cells do not secrete the bFGF due to the lack of a leader sequence. This raises questions about the mode of intracellular bFGF action and trafficking and makes antisense bFGF oligomers a powerful tool in investigating the mechanism of bFGF auto- and paracrine action.

CF 123 SYNERGISTIC INTERACTIONS BETWEEN GROWTH FACTORS: BASIC FGF UPREGULATES THE EXPRESSION OF THE PDGF α-TYPE RECEPTOR IN VASCULAR SMOOTH MUSCLE CELLS. Claudia Schöllmann, Renate Grugel, Dieter Marmé and Herbert A. Weich, Inst. Mol. Cell Biol. University of Freiburg, 7800 Freiburg, FRG. The growth factor combination bFGF together with PDGF has a highly synergistic effect on the incorporation of ^{3}H-thymidine in bovine aortic smooth muscle cells [Weich and Folkman, J. Cell. Biochem. 13B, 148 (1989)]. This phenomenon was analysed in more detail. Although PDGF-AA is a poor mitogen for smooth muscle cells, all PDGF isoforms (AA, AB, BB) together with bFGF act strongly synergistic on DNA synthesis; the same effect was found in proliferation experiments over 6 days. Preincubation of cells with bFGF over 12 or 24 h indicated that the mitogenic effect of all PDGF isoforms increased: the mitogenicity of PDGF-AB and -BB increased 2.3-2.4-fold, whereas the effect of PDGF-AA increased more than 6-fold. Based on these data we assumed, that bFGF may influence the distribution of the two PDGF receptor subtypes. Using Northern blot analysis we found, that preincubation with bFGF selectively increased the expression of the α-type but not of the β-type receptor. The increase was maximal after 12-18 h of preincubation (app. 7.5 to 10-fold) and remained substantially unchanged during 36 h of incubation. Dose-response experiments indicated that a bFGF concentration of 0.75 ng/ml already maximized the expression of the α-type receptor. Currently we are investigating the receptor numbers for the PDGF isoforms and the change of the tyrosine kinase activity of the α-type receptor after preincubation with bFGF. Our data suggest, that the overproportional increase of PDGF-AA mitogenicity in relation to PDGF-AB or -BB is due to the increased expression of the PDGF α-receptor subtype. We suppose, that more PDGF receptors may dimerize to the αα-conformation, whereas under normal culture conditions αβ- and ββ-conformations may dominate. This finding is even more relevant, because in many cell types the secretion of PDGF activity correlates with the expression of the A-chain but not of the B-chain and only the α-type receptor is able to bind PDGF-AA with high affinity.

CF 124 CYTOTOXICITY OF aFGF-*PSEUDOMONAS* TOXIN FUSION PROTEIN ON HUMAN TUMOR CELLS, Clay B. Siegall, Stephen E. Epstein*, Tim Hla+, Sadatoshi Biro*, Ya-Min Fu*, Edith Spier*, David J. FitzGerald, Tom Maciag+, and Ira Pastan, Laboratory of Molecular Biology, DCBDC, NCI, NIH, *Cardiology Branch, NHLBI, NIH, Bethesda, MD 20892, and +LMB, Jerome Holland Laboratories, The American Red Cross, Rockville, MD 20855.
We have constructed chimeric proteins composed of acidic fibroblast growth factor (aFGF) and different forms of *Pseudomonas* exotoxin (PE) which have been mutated so that they cannot bind to PE receptors. These gene fusions have been expressed in *Escherichia coli* and purified to homogeneity using heparin-affinity columns. The aFGF-PE fusion proteins were cytotoxic to human tumor cells which bear FGF receptors including prostatic, colon, and hepatocellular carcinomas. In addition, aFGF-PE was cytotoxic against rapidly proliferating rat smooth muscle cells. Specific cytotoxicity of the chimeric toxin through FGF receptors was demonstrated by blockage of its cell-killing effect upon addition of excess aFGF. aFGF fused to a defective mutant version of PE was not toxic to any of the cell lines tested indicating that the cell-killing effects of aFGF-PE were toxin-mediated. These results indicate that aFGF-PE is a molecule that should be further evaluated *in vivo* as a potential antitumor agent and an agent that might be useful in inhibiting growth of certain non-transformed cells including activated smooth muscle cells following vascular injury.

CF 125 IDENTIFICATION OF MULTIPLE FORMS OF aFGF mRNA GENERATED BY ALTERNATIVE SPLICING AT THE 5' END. Kathleen A. Sullivan and Thomas M. Palisi, Department of Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, N.J. 07065.
Little is known about how transcription of aFGF is initiated and regulated. In an effort to characterize the aFGF transcriptional unit we used RT-PCR to clone novel cDNAs from human foreskin fibroblast and fetal heart mRNA. These cDNA's differ in the exon sequences spliced upstream of the first coding exon and are distinct from those previously described from human neonatal brainstem libraries [Chiu *et al.* Oncogene 5:755 (1990); Crumley *et al.* BBRC 171:7 (1990)]. This brings to five the total number of variant 5' untranslated regions for aFGF mRNA. This complexity might reflect tissue specific alternative splicing and/or usage of alternative promoters. To address this question we have isolated and are characterizing a 14 Kb genomic clone corresponding to the 5' untranslated sequence derived from human foreskin fibroblast mRNA.

CF 126 STIMULATION OF MIGRATION AND PLASMINOGEN ACTIVATOR ACTIVITY OF NORMAL HUMAN KERATINOCYTES BY KERATINOCYTE GROWTH FACTOR(KGF). R. Tsuboi, T. Sato, Y. Kurita, J. Rubin# and H. Ogawa. Department of Dermatology, Juntendo University, School of Medicine, Tokyo, # Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD.
Keratinocyte growth factor(KGF), originally isolated from human embrionic lung fibroblasts, is mitogenic only for epithelial cells. Although its activity as a paracrine factor suggests its role in wound healing, the biological activities of this molecule on human keratinocytes have not been well documented. The following experiments were performed to examine the effects of KGF on migration and plasminogen activator(PA) activities. Human adult and foreskin keratinocytes were cultivated in KGM medium (modified MCDB 153, Kurabo Corp.) with 0.08 mM Ca^{++}. Half of the plate's subconfluent monolayers of keratinocytes were removed with a razor blade, and the remaining keratinocytes were incubated in KBM medium in the presence of growth factors. Migration was quantitated by counting the number of the cells in successive sections from the wound edge. KGF at a concentration of 1 nM stimulated migration to a similar level as TGFα, but more strongly than bFGF. PA activities of cell extracts were assayed with a synthetic substrate. KGF stimulated PA activities of keratinocytes to a similar level as TGFα, but more strongly than bFGF. Stimulatory activities were not observed in the cell extracts of bovine endothelial cells. These results suggest that KGF may have important roles in wound healing and inflammatory diseases in vivo.

CF 127 STUDY OF NUCLEUS LOCALIZATION OF HBGF-1 X. Zhan, S. Friedman, T. Imamura*, K. Engleka, X. Hu, R. Forough and T. Maciag, Lab. Mol. Biol., J.H.Holland for the Biomed. Sci., American Red Cross, Rockville, MD 20855. * Cell Sci. and Tech. Div., Fermen. Res. Ins., Ministry of International Trade and Industry, Higashi 1-1-3, Tsukuba, Ibaraki 305 Japan.

We have genetically manipulated a putative nuclear translocation signal (NTS) which is composed of a NYKKPK sequence in heparin-binding growth factor-1 (HBGF-1). This sequence is capable of translocating the reporter protein bacterial β-gal into the nucleus. One truncated mutant (HBGF-1U) that lacks this NTS is 100-fold less mitogenic than other truncated forms containing this NTS, even though HBGF-1U is able to stimulate receptor tyrosine phosphorylation and induce expression of c-fos and c-jun proto-oncogenes. The reduced mitogenic activity of HBGF-1U was fully recovered when a NTS from the yeast histone 2B protein was fused to the N-terminal of HBGF-1U. In co-transfection experiments, HBGF-1U appeared to partially inhibit the efficiency of transformation by a secreted mutant of HBGF-1 in which the signal sequence from HBGF-4 (hst/KS3) was fused to HBGF-1α, suggesting that intracellular HBGF-1U can disturb the function of a secreted form of HBGF-1. We also generated a series of point mutants which converted residues of lys in the NTS to either gly or thr. The preliminary data has shown that these proteins have less activity than HBGF-1α in the absence of heparin. Since these proteins are readily ^{125}I-labeled, they should allow us to examine in more detail the relationship between HBGF-1 function and nuclear localization.

FGF Receptors/TGF Beta

CF 200 A novel member of the FGFR gene family with a distinct fetal expression pattern

Kari Alitalo, Juha Partanen, Tomi P. Mäkelä, Elina Eerola, Jaana Korhonen, Harri Hirvonen and Kay Huebner

Cancer Biology Laboratory, Departments of Virology and Pathology, University of Helsinki, Haartmanink. 3, 00290 Helsinki 29, FINLAND, Department of Medical Biochemistry,University of Turku, 20520 Turku, The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140, USA

We have cloned from a human leukemia cell cDNA library a novel member to the FGFR family. This gene, which we call FGFR-4 has been chromosomally mapped and is expressed as an apparently single mRNA species in several human tumor cell lines and specific human fetal tissues. FGFR-4 is expected to encode a protein with three immunoglobulin motifs and six potential glycosylation sites in its extracellular domain. The expression pattern of FGFR-4 is distinct from that of *flg* and *bek*, the two previously characterized FGF receptors. Ligand binding properties of FGFR-4 will be reported.

CF 201 BASIC FGF AND FGF RECEPTOR EXPRESSION IN HUMAN BLADDER CARCINOMA CELL LINES WITH DIFFERENT INVASIVE POTENTIALS, *Lynn E. Allen and Pamela A. Maher, The Whittier Institute for Diabetes and Endocrinology, 9894 Genesee Avenue, La Jolla, CA 92037*

The ability of tumor cells to invade and metastasize is dependent on changes in a number of different cellular functions. Tumor cells must be able to grow at low density in a foreign environment and then, for continued growth, to develop their own blood supply to sequester nutrients from the host. Basic fibroblast growth factor (bFGF) has been demonstrated to have both mitogenic and angiogenic activities, both of which are important in tumor progression. To ascertain whether bFGF plays a role in the development of invasive potential, cells derived from a low grade, slightly invasive human bladder carcinoma (RT4), were compared with cells derived from a high grade, highly invasive human bladder carcinoma (EJ) for the presence of bFGF and its receptor.

When RT4 and EJ cells were examined for the production of bFGF, Western blot analysis of whole cell lysates revealed that the EJ cells produced three distinct molecular weight species of bFGF whereas no immunoreactive bFGF species were detected in the lysates of the RT4 cells. Further, EJ cells which had been injected transurethrally into nude mice and had subsequently metastasized to the lung showed a pattern of bFGF production similar to that of the parental cells.

To determine if either the EJ or RT4 cells could respond to bFGF, they were tested for bFGF binding. Cross-linking studies showed that the EJ cells bound significantly more bFGF than the RT4 cells. In addition, Northern blot analysis indicated that the EJ cells expressed higher levels of an FGF receptor transcript than the RT4 cells.

These results show that the cells derived from the more invasive bladder carcinoma produced more bFGF and were able to bind bFGF to a greater extent than the cells derived from the less invasive carcinoma. The bFGF produced by the EJ cells may act both as an autocrine growth factor through their FGF receptor and as an angiogenic factor to vascularize the tumor mass.

CF 202 EVIDENCE FOR TWO PUTATIVE BASIC FIBROBLAST GROWTH FACTOR RECEPTORS IN NORMAL AND TRANSFORMED HUMAN CELL LINES.

K. E. Eidsvoog, R. A. Horlick, W. F. Herblin and J. L. Gross Medical Products, The DuPont Merck Pharmaceutical Co., Wilmington DE

Two putative receptors for basic fibroblast growth factor (bFGF) have been identified and their relative distribution studied. Using autoradiography, high levels of binding to [125I]-human recombinant human bFGF (hr-bFGF) were observed in adult rat lung tissue sections, as compared to other normal organs, and in a human endometrial carcinoma (A159) grown in nude mice. Cultured A159 cells also expressed significant levels of high affinity receptors (20,000 sites/cell; Kd , 75-147pM) for hr-bFGF. Because of abundant levels of bFGF binding proteins in rat lung and A159 cells, degenerate olionucleotides, made to the published amino acid sequence of the chicken FGF receptor gene, were used to isolate cDNA to determine whether these bFGF binding proteins were unique. The receptor-like gene isolated from lung represented a closely related and novel potential bFGF receptor subtype, whereas the human tumor receptor was 99.2% identical to *flg*. Oligonucleotide primers were made to sequences unique for each cDNA to amplify and probe poly A(+) mRNAs from a panel of tumor cell lines, a nontransformed cell line and normal tissue using reverse transcriptase-PCR. The putative bFGF receptor subtype isolated from lung was present in all cell lines as demonstrated by Southern analysis of PCR amplified products, but message to human tumor receptor was not present in all lines. The presence of message to human tumor *flg* correlated with high affinity bFGF binding to cell lines in culture. This demonstrates differentical expression of two members of the bFGF receptor family in normal tissue and tumor cell lines.

CF 203 GENE EXPRESSION OF ACIDIC FGF AND ITS RECEPTOR(S) BY CARDIAC MYOCYTE CELL LINES: TOOLS FOR ANALYSIS OF HEART DEVELOPMENT.
Gary L. Engelmann Loyola University, Department of Medicine, Maywood, IL, 60153

Ventricular myocyte development in the mammalian fetus is characterized by rapid, near-terminal proliferative events and initiation of cellular maturation. The results are such that the neonatal heart contains a finite number of post-mitotic cardiomyocytes adaptive to hypertrophic growth stimuli. Analysis of the growth and maturation of the embryonic and fetal cardiomyocyte has been limited due to the lack of a suitable cell line which reflects the cardiac muscle lineage. To that end, primary cultures of 16-day gestation fetal cardiomyocytes were infected with v-*myc* or v-H-*ras* containing retroviruses, which resulted in the formation of three separate cardiomyocyte cell lines (CLEM-1102C, BWEM-1102A, and ELEM-658HH). The CLEM and BWEM cell lines were from separate v-*myc* infected cultures; whereas the ELEM cell line was from a v-H-*ras* infection. Both of the v-*myc* transformed cell lines express high levels of flg and bek transcripts. In contrast, the ELEM (v-*ras*) cell line only expressed the bek transcripts. Whole heart RNA from fetal ventricles only contained flg transcripts. Immunohistochemical staining with anti-flg/bek antisera confirmed the molecular analsis. Crosslinking studies and cell culture analysis of their proliferative response to FGFs and their expression of differentiated cellular characteristics after FGF treatment by the cell lines are underway. For the first time, cell lines of cardiomyocytes have been generated which will facilitate the analysis of the growth and differentiation of this unique cell type of the cardiovascular system, particularily in response to exogenous growth factors such as the heparin-binding growth factor family.

CF 204 ISOLATION OF A NEW FIBROBLAST GROWTH FACTOR RECEPTOR-LIKE MOLECULE FROM MOUSE FIBROBLASTS.
Nicolas J. Faselº, Michel Bernardº, Nicole Déglonº, Roselyn Eisenberg+, Claude Bronº and Gary C. Cohen*. ºInstitute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland, +Department of Pathobiology, School of Veterinary Medicine and *Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Structural definition of the receptors for neurotropic and angiogenic modulators such as fibroblast growth factors (FGF) and FGF-related polypeptides could yield insight into the mechanisms that control early development, embryogenesis, organogenesis, wound repair and neovessel formation. Isolation of the different FGF and FGF-related receptors (FGF-R) forms and characterization of their ligand affinity is essential for the understanding of the mode of action of these modulators. For this reason, we isolated murine cDNAs encoding different binding domains of these receptors. Comparison of these ectoplasmic portions showed that two of the forms corresponded to previously described murine molecules whereas the third one had a different ectoplasmic portion generated by specific deletions in two regions. Interestingly, expression of this third form seems to be restricted in its tissue distribution. Such modifications could influence the ligand specificity of the different receptors and/or their binding affinity.

CF 205 α_2-MACROGLOBULIN-TGFß$_1$ COMPLEXES ARE BIOLOGICALLY ACTIVE ON ADRENOCORTICAL CELLS,
Jean-Jacques Feige, Michelle Keramidas and Edmond M. Chambaz, Unité INSERM 244, Fédération des Laboratoires de Biologie, DBMS/BRCE, Centre d'Etudes Nucléaires de Grenoble, FRANCE

Among the pleiotypic effects of TGFß$_1$ an important one is its strong potency to inhibit adrenocortical steroidogenesis (J.J. Feige et al., 1987, J. Biol. Chem., 262, 13491-13495). We observed recently that the TGFß$_1$ protein is widely distributed in the bovine adrenal cortex tissue whereas it is undetectable in the adrenal medulla and the capsule. Bovine adrenocortical cells in culture also synthesize and secrete a TGFß-like activity under a latent form. Moreover, we have identified the most abundant protein secreted by these cells as α_2-macroglobulin (α_2M), a major serum protease inhibitor that is also a growth factor binding protein. (D.L. Shi et al., 1990, J. Biol. Chem. 265, 2881-2887). In this study, we characterized the effects of α_2-M on TGFß$_1$ actions in adrenocortical cells. We observed that bovine α_2M (S form) binds ^{125}I-TGFß$_1$ in a non-covalent complex. α_2M inhibits the binding at 4°C of ^{125}I-TGFß$_1$ to its receptors (type I and type III) on adrenocortical cells (50% inhibition for 0.1 mg/ml). However, the same concentrations of α_2M that inhibit the binding at 4°C do not reverse (and even amplify) the inhibition of hormone-induced steroid production triggered by a 24 h TGFß$_1$ treatment at 37°C. The association of TGFß$_1$ to adrenocortical cells at 37°C (binding + internalization) appears to be inhibited by α_2M during the first 2 hours whereas after 15 hours it is increased 4 fold. The possibility that TGFß$_1$-α_2M complexes may induce TGFß-like biological effects through binding to α_2M receptors is under current investigation.

CF 206 EFFECTS OF TGF-ß GENE EXPRESSION DURING MULTISTAGE CARCINOGENESIS IN MOUSE SKIN, Sabine Feil, Gerhard Fürstenberger and Peter Krieg, Departments of Virus Research and Biochemistry, German Cancer Research Center, Heidelberg, FRG

A critical role of TGF-ß in epidermal growth regulation and in epidermal tumor development has been discussed. This idea is supported by several findings showing that TGF-ß might contribute to the process of tumor promotion and progression of multistage carcinogenesis in mouse skin (initiation-promotion protocol). Further evidence for a role of TGF-ß in epidermal tumorigenesis gives our finding that steady state levels of TGF-ß mRNA are very high in tumorigenic mouse keratinocytes compared to their non-tumorigenic counterparts. In addition, a constitutive overexpression of TGF-ß mRNA in malignant mouse skin tumors was observed, indicating an association with malignant progression. Being interested in elucidating the function and mechanism of this tumor-specific overexpression of TGF-ß mRNA we will describe here the effects of TGF-ß on the expression of several cellular genes in malignant versus non-malignant mouse keratinocytes. Discussed will be the ability of TGF-ß to influence the expression of genes by altering their equipments of transacting factors, inducing or inhibiting the transcription of the corresponding genes.

CF 207 TGF-β GROWTH STIMULATION OF H-ras TRANSFORMED FIBROBLASTS IS LINKED TO THE INDUCTION OF RIBONUCLEOTIDE REDUCTASE. Robert A.R. Hurta, Arnold H. Greenberg and Jim A. Wright, Department of Biochemistry and Molecular Biology, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0V9.

Ribonucleotide reductase (R.Rase) is a rate-limiting enzyme in the synthesis of deoxyribonucleotide precursors of DNA. Its catalytic activity requires both its large (M1) and small (M2) subunits which are noncoordinately expressed. While the M1 subunit is constitutively expressed by proliferating cells, M2 expression is cell cycle dependent with a tight correlation with S-phase. Metastatic cell lines were obtained by transformation of 10T½ fibroblasts using transfection with T-24 H-ras. Earlier studies have shown that highly metastatic H-ras transformed 10T½ cells were growth stimulated by TGF-β_1 and β_2 while parental 10T½ cells were growth inhibited (Schwarz et. al., Cancer Res., 48, 6999, 1988). Under the experimental conditions used, TGF-β_1 had no detectable effect on the expression of M1 and M2 genes in 10T½ cells. However, the gene expression of R.Rase M1 and M2 subunits was increased in cells of high metastatic ability. Early induction of M1 and M2 gene expression was observed, occurring prior to S-phase. It is hypothesized that this modulation of gene expression of R.Rase by TGF-β_1 is a direct result of unique changes in highly malignant cells. (Funded by N.C.I. of Canada).

CF 208 PATTERN OF EXPRESSION OF TRANSFORMING GROWTH FACTOR-ß4 IN THE DEVELOPING CHICKEN EMBRYO, Sonia B. Jakowlew, Jeremy Cubert, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892. cDNA probes and antibodies for TGF-ß4 were used to study expression of TGF-ß4 in the developing chicken. TGF-ß4 mRNA was detected by day 4 of incubation and increased with developmental age until day 12. TGF-ß4 mRNA was detected by RNA Northern blot analysis in every embryonic tissue examined, with expression being highest in smooth muscle and lowest in the kidney. At later stages of development, the level of expression of TGF-ß4 mRNA remained elevated in neonatal and adult chicken striated muscle, smooth muscle and cardiac muscle, while it decreased in other tissues. Immunohistochemical staining techniques were used to detect TGF-ß4 protein in many embryonic chicken tissues; significant expression was detected by the fourth day of incubation in specialized cells of the brain, heart, kidney and intestine, suggesting unique roles for TGF-ß4 within the specific tissue. The level of TGF-ß4 immunohistochemical staining decreased in most adult tissues except in spleen. Immunohistochemical staining of spleens of developing chickens showed increasing levels of immunoreactive TGF-ß4 with increasing developmental age. Paralleling the increase in the level of TGF-ß4 expression in spleen with age, the level of expression of TGF-ß4 mRNA also increased significantly with developmental age in chicken spleen, with expression of TGF-ß4 mRNA in the adult spleen being greater than that in the embryonic spleen.

CF 209 TRANSFORMING POTENTIAL OF OVEREXPRESSED FGF RECEPTORS, *FLG* AND *BEK*, IN NIH 3T3 CELLS, June M. Kaplow, Françoise Bellot, Gregg Crumley, Joseph Schlessinger*, Michael Jaye and Craig A. Dionne, Rhône Poulenc Rorer, King of Prussia, PA 19406, * New York University Medical Center, New York, NY 10016

The human FGF receptors, *flg* and *bek*, were separately overexpressed in NIH 3T3 cells. These transfected cells displayed a partial transformed phenotype *in vitro* and a transformed phenotype *in vivo*. Both *flg* and *bek* overexpressing cell lines promoted tumor formation in nude mice. The time of onset of these well vascularized large fibrosarcomas correlated with the level of overexpressed receptors. The genomic DNA from the tumor mass was analyzed by Southern blot using bek or flg specific cDNA probes, and revealed hybridization bands that were identical to those exhibited by the injected cells. The overexpression of FGF receptors also correlated with cell growth in serum free media; only bek and flg overexpressing cell lines were capable of sustained growth under these conditions. This growth ability was most likely ligand dependent, the ligand being supplied by the endogenous expression of FGF's, and the presence of both ligand and enhanced levels of receptor established a possible autocrine loop. The NIH 3T3 cells which naturally express low levels of mouse *flg*, did not grow in serum free conditions unless exogenous ligand (aFGF) was provided. *Bek* and *flg* overexpressing cell lines also displayed an enhanced expression of metalloproteases. The expression of these enzymes is amplified in cancer and their presence has been attributed to the invasiveness and metastatic capabilities of tumorigenic cells. These studies suggest that the overexpression of FGF receptors confer a partially transformed phenotype and that enhanced FGF receptor expression may participate in cell transformation resulting in tumorigenesis *in vivo*.

CF 210 **Inhibition of adrenocortical steroidogenesis by α_2-macroglobulin is caused by associated transforming growth factor ß.,** Michelle Keramidas and Jean-Jacques Feige, Unité INSERM 244, Fédération des Laboratoires de Biologie, DBMS/BRCE, Centre d'Etudes Nucléaires de Grenoble, FRANCE.

We reported previously that transforming growth factor $ß_1$ ($TGFß_1$) is a very potent inhibitor of adrenocortical steroidogenesis (1). It can be considered as an autocrine regulator of adrenocortical functions since bovine adrenocortical cells (BAC cells) synthesize $TGFß_1$ under a latent form and possess $TGFß_1$ receptors. BAC cells also secrete large amounts of α_2 macroglobulin (α_2-M), an abundant serum protease inhibitor which is also a TGFß-binding protein (2).

We here report that purified α_2-M inhibits ACTH- as well as angiotensin II-induced BAC cell cortisol production. The inhibition is dose-dependent (1/2 inhibition = 1.5 mg/ml = 2mM) and time-dependent (1/2 inhibition = 20 h). It is still observed when α_2-M is heated (95°C, 2 min) or acid-treated (transient acidification at pH 2.0), suggesting that it may be caused by an associated heat- and acid-stable protein. Polyclonal antibodies raised againt the peptide anti-$TGFß_1$ [91-102] could reverse the inhibition of steroid production induced by α_2-M. Taken together, these data indicate that commercial α_2-M preparations contain associated TGFß and demonstrate that the α_2-M-TGFß complex is biologically active.

1. Feige J.J., Cochet C. and Chambaz E.M. (1986) Biochem. Biophys. Res. Commun. 139, 693-700.
2. Shi D.L.,et al. (1990) J. Biol. Chem. 265, 2881-2887.

CF 211 ENHANCED PRODUCTION OF PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN AND MURINE KERATINOCYTES BY TRANSFORMING GROWTH FACTOR-β1, Katri Koli and Jorma Keski-Oja, Departments of Virology and of Dermatology, University of Helsinki, SF-00290 Helsinki, Finland.

TGFβ is the most potent known inhibitor of keratinocyte growth. Pericellular proteolytic activity is usually high in proliferating and malignant cells and decreased in resting or growth arrested cells. We have therefore analyzed the effects of TGFβ1 on the production of plasminogen activator activity by normal human keratinocytes and by a mouse keratinocyte cell line (BALB/MK-2) under serum-free conditions. The plasminogen activation activity of the culture medium was analyzed using caseinolysis-in-agar assays, zymography and reverse zymography assays and Northern hybridization assays for the PAs and PAI-1. Alterations of radiolabeled polypeptides were observed in autoradiograms of SDS-gels. It was found that like in human epidermoid carcinoma cells picomolar concentrations of TGFβ1 (0.2-20 ng/ml) enhanced total plasminogen activator activity in both keratinocyte cell systems in a dose-dependent manner. Zymographic analyses indicated that the activator was of the urokinase type (u-PA). Immunoprecipitation of the conditioned medium of human keratinocytes with monoclonal antibodies against u-PA indicated enhanced synthesis of u-PA in TGFβ treated cultures. Immunoprecipitation and concanavalin A affinity chromatograpy of the culture medium indicated that the cells also started to produce the plasminogen activator inhibitor PAI-1. Analysis of the pericellular matrices of the keratinocytes showed pericellular deposition of PAI-1. Northern hybridization analysis of human keratinocytes indicated that TGFβ1 rapidly elevated both u-PA and PAI-1 mRNA levels. Comparison of the temporal induction profiles indicated that the mRNA for u-PA increased more slowly but was more persistent than that of PAI-1. Actinomycin D but not cycloheximide inhibited the induction of both u-PA and PAI-1 mRNAs, suggesting that the induction was due to increased transcription. The results indicate that enhanced plasminogen activator activity can be associated with growth inhibition also in cultured nonmalignant epithelial cells like human or murine keratinocytes.

CF 212 EXOGENOUS FGF INHIBITS GROWTH OF A HUMAN BREAST CARCINOMA CELL LINE EXPRESSING ELEVATED LEVELS OF flg mRNA. Sandra W. McLeskey, Marc E. Lippman, and Francis G. Kern, Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, D.C. 20007. We have found that the breast carcinoma cell line, MDA-MB 134, contains elevated levels of mRNA for the fibroblast growth factor (FGF) receptor (flg). However, when grown on plastic, these cells do not respond to exogenously added basic fibroblast growth factor (bFGF) with increased growth. In fact, at high levels of exogenously added bFGF (>10 ng/ml), MDA-MB 134 cell growth is inhibited in a dose-dependent fashion. Moreover, when these cells are grown in soft agar, colony formation is inhibited by added bFGF with an EC_{50} of about 1 ng/ml. Both estrogen and fetal calf serum produce growth stimulation of these cells. When grown in the presence of fetal calf serum (10%) and varying doses of the putative FGF antagonist, pentosan polysulfate, cell growth was stimulated in a dose-dependent fashion by the pentosan polysulfate. We believe these data support the hypothesis that MDA-MB 134 cells are secreting an FGF-like material which is inhibitory to their growth.

CF 213 MAST CELL HEPARIN DISPLACES AND COMPLEXES WITH BASIC FIBROBLAST GROWTH FACTOR FROM ENDOTHELIAL CELL MATRIX, Cynthia J. Meininger, Sidney J. Sherwood and James R. Hawker, Jr., Microcirculation Research Institute and Department of Medical Physiology, Texas A&M University College of Medicine, College Station, TX 77843.

Basic fibroblast growth factor (bFGF) is produced by endothelial cells and stored bound to heparin in their matrix. The mechanism for release of this growth factor is not known. We have previously shown that mast cells or mast cell lysates can release radiolabeled bFGF bound to coronary venular endothelial cell (CVEC) matrix. This released bFGF retains its biological activity and co-migrates with bFGF standards in SDS polyacrylamide gels. When mast cell lysates are pre-treated with heparinase (and then heat-treated to inactivate the heparinase), their ability to release bound bFGF is significantly reduced. The addition of protease inhibitors did not affect growth factor release, suggesting that it is heparin within mast cells that is responsible for displacing the growth factor. When bFGF released by mast cell lysates is heat-treated (3 minutes at 80°C) or protease-treated (0.025% trypsin for 4 hours at 37°C), its ability to stimulate proliferation of untreated CVEC is only slightly diminished. However, bFGF standards subjected to the same treatments showed significantly reduced proliferative capabilities. These data suggest that the mast cell heparin not only competitively displaces bFGF from low affinity binding sites in the matrix but also complexes with released bFGF to protect it from heat or proteolytic degradation. (Supported by American Heart Assoc.,Tx. Affiliate #89G-029 and N.I.H. B.R.S.G. #RR05814).

CF 214 TYROSINE PHOSPHORYLATION OF GAP BY THE PDGF-β-RECEPTOR CORRELATES WITH THE ACTIVATION OF $p21^{c\text{-}RAS}$: EVIDENCE FOR A DIRECT ROLE OF $p21^{c\text{-}RAS}$ IN MITOGENIC SIGNALLING BY ACTIVATED TYROSINE KINASES. Christopher J. Molloy[1,2], Timothy P. Fleming[1], Donald P. Bottaro[1], Antonio Cuadrado[1], Michael Pangelinan[1] and Stuart A. Aaronson[1], [1]LCMB, National Cancer Institute, Building 37, Room 1E24, Bethesda, MD 20892; [2]present address, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543. In intact, quiescent NIH3T3 cells, PDGF-BB rapidly induced the tyrosine phosphorylation of up to 40-50% of cellular GAP molecules. Anti-GAP immunoprecipitates were found to contain activated PDGF-receptors, as well as another tyrosine phosphorylated protein(s), p64/62, which was localized predominantly in membrane fractions. GAP was not found to specifically associate with either PLC-γ, Raf-1, or $p21^{c\text{-}ras}$. Tyrosine phosphorylation of GAP was not required for transformation of NIH3T3 cells by activated v-H^{ras} or overexpression of c-H^{ras}. Concomitant with PDGF stimulation of NIH3T3 cells or cells overexpressing c-H^{ras}, we observed an increase in the ratio of p21-GTP versus p21-GDP. Taken together, our results support a direct role for tyrosine phosphorylation of GAP in the activation of $p21^{c\text{-}ras}$, and directly link functional $p21^{c\text{-}ras}$ in the mitogenic signalling cascade induced by certain tyrosine kinases, including the PDGF-receptors.

CF 215 CHARACTERIZATION OF THE RECEPTORS FOR VASCULAR ENDOTHELIAL GROWTH FACTOR, Nora Vaisman*, Denis Gospodarowicz# and Gera Neufeld*, Department of Biology Technion, Israel Institute of Technology, Technion City, Haifa, 32000, ISRAEL* and the Cancer Research Institute, University of California Medical Center, San-Francisco, CA, 94143

We have characterized the vascular endothelial growth factor (VEGF) receptors of endothelial cells. Analysis of binding experiments revealed two types of high affinity binding sites on the cell surface of bovine endothelial cells. One has a dissociation constant of 10^{-12} M, and is present at a density of $3x10^3$ receptors/cell. The other has a dissociation constant of 10^{-11} M, with $4x10^4$ receptors/cell. A high molecular weight complex of 225 kDa containing ^{125}I-vEGF is formed when ^{125}I-vEGF is cross-linked to bovine endothelial cells. When the complex is reduced two new bands of 170 and 195 kDa appear, suggesting that these complexes are derived from the 225 kDa complex by reduction of disulfide bonds. The labeling of the receptors was inhibited by unlabeled vEGF, but not by several other growth factors. Suramin and protamine, as well as several species of lectins, inhibited the binding. The expression of functional vEGF receptors was inhibited when the cells were pre-incubated with tunicamycin. Pre-treatment with swainsonine on the other hand, did not prevent ^{125}I-VEGF cross-linking to the receptor. However, the mass of the 225 kDa complex is decreased by 20 kDa when ^{125}I-vEGF is cross-linked to swainsonine treated endothelial cells. The VEGF receptor therefore appears to be a glycoprotein.

CF 216 Expression of FGF receptor (FLG) in embryonal rhabdomyosarcoma cells and glioblastomas: potential involvement in autocrine stimulation, Martin Ruta, Nancy Neiger, Laurie Pollock, Jeffrey Winkles, and J. Epstein, Laboratory of Retrovirology, FDA 8800 Wisconsin Ave., Bethesda, Md. 90852

We have previously reported the identification of a receptor (FLG) that binds both acidic and basic fibroblast growth factors. The FLG receptor is expressed at high mRNA levels in the A204 rhabdomyosarcoma cell line, but at lower levels in other rhabdomyosarcoma cell lines. The overexpression of FLG in the A204 cell line is not due to gene amplification. We have confirmed previous studies reporting the expression of basic FGF in the A204 cell line. Our results suggest that overexpression of the FLG receptor coupled with b-FGF expression may play a role in autocrine stimulation of this cancer. Similarly, we also detected FLG expression in a series of tumor cell lines of glial origin, some of which have been shown to express high levels of acidic FGF mRNA and biologically active FGF. These results suggest that autocrine stimulation may occur through simultaneous expression of either acidic FGF, basic FGF, or both FGFs and be mediated through the FLG receptor.

CF 217 METALLOTHIONEIN-REGULATED TGF-β_1 SECRETION IN MALIGNANT FIBROSARCOMAS ENHANCES EXPRESSION OF PROTEASE AND PROLIFERATION-LINKED GENES. Shanti K. Samuel[1], Robert A.R. Hurta[1], Jackie Damen[1], Paturu Kondaiah[2], Anita Roberts[2], Jim A. Wright[1], and Arnold H. Greenberg[1], [1]Manitoba Institute of Cell Biology, 100 Olivia Street, Winnipeg, Manitoba, R3E 0V9, [2]Laboratory of Chemoprevention, National Cancer Institute, NIH, Bethesda, MD, 20892.

We have proposed that highly malignant fibrosarcomas use TGF-β as an autocrine or paracrine factor to promote invasion and establishment of malignant cells. To test this hypothesis, a plasmid (pPK9A) containing the complete coding region of porcine TGF-β_1 was inserted between the metallothionein promoter and human growth hormone polyadenylation signal, and transfected into a ras-transformed 10T½ fibroblast cell line (C1) which secreted only low levels of TGF-β_1. The TGF-β_1 cDNA was mutated replacing cysteines 223 and 225 with serines which allowed secretion of mature TGF-β_1. Of 50 discrete drug resistant colonies that were screened for overexpression of the TGF-β_1 transcript in the presence of zinc sulphate, two candidate clones were identified that increased TGF-β_1 mRNA expression 5-12 fold. Initial characterization indicated that these clones also markedly increased TGF-β_1 secretion, showed increased expression of the protease genes, MEP and collagenase IV, as well as enhanced expression of ribonucleotide reductase M2 and ornithine decarboxylase, genes which are linked to proliferative responses. These responses suggest that TGF-β_1 autocrine stimulation may activate genes that are important for invasion and proliferation of metastatic tumors. (Supported by the NCI of Canada).

CF 218 TGF-β BINDING SITES ON ENDOTHELIAL CELLS. J.D. Sato, Y. Myoken, M. Kan, W.L.McKeehan, and G.H. Sato. W. Alton Jones Cell Science Center, 10 Old Barn Rd., Lake Placid, NY 12946

TGF-β modulates HBGF-1-stimulated proliferation of endothelial cells in a concentration-dependent bifunctional manner. We have examined the relationship between cell growth and the expression of binding sites for TGF-β and HBGF-1 on fetal bovine heart endothelial cells (FBHEC) and human umbilical vein endothelial cells (HUVEC). TGF-β at low concentrations stimulated the growth of FBHEC while higher concentrations were inhibitory. These cells expressed two affinity classes of binding sites for TGF-β with K_ds of 24 pM (6,300 sites/cell) and 900 pM (12,000 sites/cell). By contrast, HUVEC which were slightly growth stimulated by TGF-β, expressed a single class of high affinity binding sites (K_d=45 pM; 4,500 sites/cell). By affinity cross-linking, FBHEC possessed low molecular weight TGF-β binding sites of 85 kDa and 58 kDa while HUVEC expressed only 85kDa binding sites. Preincubation of FBHEC with high but not low concentrations of TGF-β down modulated the expression of high affinity HBGF-1 receptors on those cells. A simialr response to TGF-β by HBGF-1 receptors on HUVEC was not observed. These results suggested that the ability of TGF-β to stimulate or inhibit endothelial cell proliferation correlated with the expression of specific types of TGF-β binding sites and involved the transmodulation of HBGF-1 receptors.

CF 219 PLATELET FACTOR 4 BLOCKS THE BINDING OF BASIC FIBROBLAST GROWTH FACTOR TO THE PLASMA MEMBRANE RECEPTOR AND INHIBITS THE SPONTANEOUS MIGRATION OF VASCULAR ENDOTHELIAL CELLS. Sato, Y., Abe, M., and Takaki, R. First Department of Medicine, Medical College of Oita, Hazama-cho, Oita-gun, Oita 879-56, JAPAN.

Platelet factor 4 (PF-4) is a platelet alpha granule protein and has a high affinity fot heparin. When platelets are aggregated, PF-4 is released from the alpha granule. Although the physiological role of PF-4 was originally proposed to be related to the coagulation system, increasing evidence supports that PF-4 has various biological effects including inhibition of angiogenesis. Angiogenesis is a complex phenomenon which includes protease production, migration, proliferation, and tube formation of vascular endothelial cells. Among various angiogenic factors reported, basic fibroblast growth factor (bFGF) is produced by the vascular endothelial cell itself, and regulates migration, plasminogen activator synthesis, and DNA synthesis as an autocrine factor (Sato and Rifkin, 1988). We report here that PF-4 blocks the binding of bFGF to the plasma membrane receptor. Five ug/ml of PF-4 completely blocks the specific binding of bFGF to the receptor. Forethermore, PF-4 inhibits the spontaneous migration of bovine aortic endothelial cells in a dose dependent and reversible manner. The inhibition reachs maximum at 5 ug/ml of PF-4, where the binding of bFGF to the receptor is completely blocked.

CF 220 FGF MEDIATION OF CORONARY ANGIOGENESIS, Margaret E. Schelling, Program in Genetics & Cell Biology, Washington State University, Pullman, WA 99164-4234.

We have found that FGF modulates coronary endothelial cell proliferation and differentiation. FGF therefore possesses therapeutic potential for the increase of coronary angiogenesis (including collateral formation) following myocardial infarction. We isolated coronary venular endothelial cells (CVECs) for the study of coronary angiogenesis, blood vessel formation in the heart (Am. J. Physiol. 254:H1211). I have previously reported the characterization of the 110 K_D Acidic and Basic Fibroblast Growth Factor receptor from CVECs (J. Cell Biol. 105:110a), heparin modulation of the binding of A and B FGF to the receptor, associated FGF-induced endothelial cell proliferation (FASEB J. 2(6):A1714), and some of the molecular controls involved in coronary in vitro angiogenesis (Fed. Proc. 46:1535). Additionally, we have prepared anti-idiotypic and other monoclonal antibodies against the CVEC A and B FGF receptor(FASEB J. 4(3):A487) to use as probes in studying receptor structure/function and signal transduction. Heparin fragment length and level of sulfation regulate binding of a and bFGF to the CVEC FGF receptor.

CF 221 **TRANSFORMING GROWTH FACTOR BETA INCREASES BASIC FIBROBLAST GROWTH FACTOR LEVELS AND INHIBITS PROLIFERATION OF FIBROBLASTS DERIVED FROM HUMAN PROSTATE,** **Michael T. Story, Departments of Urology & Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226**

Basic fibroblast growth factor (bFGF) has been isolated from the human prostate. Basic FGF levels are elevated in benign prostatic hyperplasia (BPH) and prostate cancer suggesting that bFGF may be important in these diseases of the prostate. Basic FGF stimulates the growth of cultured fibroblasts-derived from the prostate (PF). These cells also synthesize bFGF. Thus, it is possible that growth regulation of PF is under autocrine control. The current studies were undertaken to determine if other polypeptide growth factors influence bFGF production by PF. The growth of PF in vitro is dependent on supplementation of the culture medium with serum. Basic FGF could substitute for serum. Transforming growth factor beta-1 (TGFβ) inhibited PF proliferation. The inhibition by TGFβ could be overcome by the exogenous addition of bFGF, but not by platelet-derived growth factor, epidermal growth factor or insulin. TGFβ increased immunoreactive bFGF levels in cultures of PF in a dose-and-time-dependent fashion. None of the other growth factors studied influenced PF bFGF levels. Studies are in progress to determine if the affect of TGFβ is at the transcriptional level. Interestingly, PF also express TGFβ-mRNA. These observations suggest that factors that regulate bFGF and TGFβ gene expression may be important modulators of autocrine regulated prostatic fibroblast cell growth. (Supported by NIH grant DK31063).

CF 222 MOLECULAR CLONING OF HUMAN FGF-RECEPTOR RELATED cDNA AND ITS EXPRESSION IN VARIOUS HUMAN TUMORS, Klaus Strebhardt, Uwe Holtrich, Andreas Bräuninger and Helga Rübsamen - Waigmann, Georg-Speyer-Haus, Paul-Ehrlich-Str. 42-44, 6000 Frankfurt 70, FRG

The Fibroblast Growth Factor (FGF)-family consists of at least 7 closely related polypeptide mitogens which exhibit their activities by activation of specific cell receptors. Since protein tyrosine phosphorylation plays an important role in the transmission of growth and differentiation signals we analyzed protein tyrosine kinases expressed in lung tumors. Characterization of 160 PCR-amplified cDNA clones resulted in the identification of nine different protein-tyrosine kinase (PTK) - related sequences. Six of these were identical to known PTKs: yes, fgr, lyn, hck, CSF-R and PDGF-R. Two clones were similar but not identical to sequences of the FGF-receptor family. The kinase domain of the new receptor gene shows a 77,7 % homology to the corresponding domain of the FGF-receptor. Within this family especially the length of the kinase insert region is highly conserved as well as in the new member of this group. The expression pattern of the new genes in normal and malignant tissues will be discussed.

CF 223 **IDENTIFICATION OF A NOVEL HUMAN ENDOTHELIAL CELL RECEPTOR TYROSINE KINASE,** Bruce I. Terman and Miguel Carrion, Lederle Laboratories, Building 205/ 227, Pearl River, NY 10965

A novel growth factor receptor tyrosine kinase gene has been identified from a human umbilical chord endothelial cell library. The gene was identified using the polymerase chain reaction (PCR) and degenerate oligonucleotide primers complementary to conserved tyrosine kinase domains that flank at insert domains characteristic of known type III receptor tyrosine kinases. A specific oligonucleotide probe designed from the DNA sequence of the PCR product was used to identify a cDNA clone of the receptor from the original endothelial cell library. DNA sequencing of the clone revealed a unique DNA sequence that encoded a protein with multiple characteristics typical of a type III receptor tyrosine kinase. Analysis of RNA from endothelial cells reveals that the novel gene is expressed as a 7.0 kb mRNA. The receptor locos was mapped to human chromosome 4 by Southern blot analysis of DNA from human x mouse somatic cell hybrids.

CF 224 POSSIBLE IMPLICATION OF TGF-β1 IN TUMOR-ASSOCIATED ANGIOGENESIS IN VIVO. Noboru Ueki,Masaru Nakazato,Toshihisa Ohkawa,*Tathuhiko Ikeda,Yoshiki Amuro,Toshikazu Hada and Kazuya Higashino, The Third Department of Internal Medicine, Hyogo College of Medicine, Hyogo 663, *King Brewing Co.,LTD. Central Institute, Hyogo 675-01, Japan
Angiogenesis, the growth and formation of new capillary blood vessels, is a characteristic phenomenon common to most solid tumors. Following establishment of an adequate blood supply, tumor cells will not only grow but also acquire metastatic potential to distant tissues or organs. Since many tumor cells secrete TGF-ß, a family of pleiotropic growth factor, but have the ability to respond to this peptide, it has been postulated that TGF-ß might stimulate tumor growth indirectly via paracrine effects including angiogenesis. In the present study, using the transfected Chinese hamster ovary (CHO) cell line overproducing recombinant TGF-β1, we examined its possible role in the growth and angiogenesis of these cell line xenograft in a nude mouse model. The CHO cells overproducing TGF-β1 grew more rapidly when injected subcutaneously into nude mice than control CHO cells. Histologically, the nude mice tumors derived from the TGF-β1-overproducing cell line disclosed a prominent tumor-associated angiogenesis but not fibrous stroma whereas the control cell line produced tumor lacking angiogenetic reactions. In addition, the rabbit IgG polyclonal antibody, having neutralizing property against TGF-β1 in vitro, was able to inhibit both the growth and angiogenesis in the tumors in animals injected with TGF-β1-overproducing CHO cells. These results indicate that TGF-ß, secreted by tumor cells which escaped from direct growth inhibitory effect by this factor, can augment tumor growth indirectly by the enhancement of tumor-associated angiogenesis in vivo.

CF 225 CONSTITUTIVE AND INDUCIBLE bFGF LIGAND-BINDING ACTIVITY IN CULTURED RAT AORTIC SMOOTH MUSCLE CELLS,
Johan W. van Neck, John J. Medina*, Henri P. J. Bloemers, Stephen M. Schwartz*, Department of Biochemistry, University of Nijmegen, the Netherlands and *Department of Pathology, University of Washington, Seattle WA 98195.

Basic fibroblast growth factor (bFGF) stimulates the growth of aortic smooth muscle cells (SMC) when added to cultures derived from adult rat aortas. Cultures from newborn rats do not respond. The expression of the bFGF gene is not induced by bFGF addition to SMC. However, expression of the myogenic determination gene MyoD1, after infection with a MyoD1-containing retroviral construct (MDSN), creates a nuclear environment in which bFGF addition does induce the synthesis of its own gene in both newborn and adult SMC.

bFGF ligand binding studies show the presence of a 130 kD and a 160 kD bFGF-receptor complex in MDSN-infected and uninfected adult SMC. In uninfected newborn SMC, the ligand binding studies do not show any signal. However, MyoD1 expression in newborn SMC induces a 145 kD bFGF receptor complex.

These results show that bFGF can play a mitogenic role in adult SMC and that the effect caused by MyoD1 depends on the nuclear context in which this protein is working. Newborn SMC differ from adult SMC in morphology, gene expression and, as shown here, in the differential effect on bFGF binding. Therfore these cultures provide more insight in the differences in growth regulation and growth stimulation in proliferating and non-proliferating SMC as a model for the study of human atherosclerosis.

CF 226 EXPRESSION OF DIVERSE FORMS OF FGF-RECEPTORS 1 AND 2 IN VARIOUS TISSUES AND CELL LINES, Sabine Werner, Kevin Peters, Pauline L. Lee and Lewis T. Williams, Department of Medicine, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143, USA
We and others have identified 3 different receptors for basic and acidic fibroblast growth factors. The cDNAs of FGF Receptors I and II are the full length forms of the flg and bek cDNAs, respectively, and FGF Receptor III cDNA was isolated using a v-sea probe (Keegan et al.,in press). In this study we have investigated the expression of FGF Receptors I and II in various mouse tissues and cell lines. FGF-receptor I was found to be expressed in all tested mouse tissues with the exception of liver. In most tissues, we detected two FGF receptor I transcripts which encode receptors with 2 or 3 immunoglobulinlike domains. In contrast, only mRNA encoding the FGF receptor form with 3 immunoglobulinlike domains was found in brain and all tested embryonic cell lines. In addition to membrane-spanning receptors, we also detected the mRNA expression of a putative secreted form of FGF Receptor I. The expression pattern of FGF Receptor II differed significantly from that of FGF-receptor I. This receptor was expressed at high levels in liver and lung, while intermediate levels were found in brain, kidney, stomach and testis. No FGF Receptor II mRNA was detected in heart and skeletal muscle. These results indicate a differential regulation of expression of more than one FGF receptor gene and suggest unique biological functions of the diverse receptor forms.

Developmental and Neurotrophic Activities of FGF/VEGF

CF 300 ALTERNATIVE FORMS OF GLIOMA-DERIVED VASCULAR ENDOTHELIAL CELL GROWTH FACTOR, Marvin L. Bayne, Greg Conn, Perry Kwok, Prashant Trivedi, Denis Soderman and Kenneth A. Thomas, Department of Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, N.J. 07065.

The rat glioma cell line GS-9L synthesizes a number of growth factors active on vascular endothelial cells (Conn *et al. PNAS*, 87: 1323-1327, 1990). One of these growth factors has been purified and shown to be a dimer of identical 164 amino acid subunits (Conn *et al. PNAS*, 87: 2628-2632, 1990). Analysis of the mRNA from GS-9L cells by RT-PCR reveals three species that can be amplified using PCR primers directed to the extreme ends of the protein coding region. Subsequent cloning and sequencing of these cDNAs have characterized two alternative forms of GD-VEGF mRNA. One clone contains a 72 bp insert between Asn114 and His115. This insert codes for the 24 amino acid sequence KKSVRGKGKGQKRKRKKSRFKSWSV, a putative nuclear targeting sequence highly homologous to that found in human VEGF. The second alternative form contains a 132 bp deletion between Asn114 and Arg159. This cDNA thus encodes a 120 amino acid form of VEGF. These alternative messages probably arise from differential slicing of the mRNA. Direct protein sequencing of purified GD-VEGF demonstrates the presence of the 164 amino acid subunit. The relationship of the alternative forms of VEGF mRNA to the other growth factors in the GS-9L conditioned media is currently under investigation.

CF 301 INTERLEUKIN-4 IS A MITOGEN FOR CAPILLARY ENDOTHELIAL CELLS, Roy Bicknell and Masakazu Toi, Imperial Cancer Research Fund, Institute of Molecular Medicine, University of Oxford, Oxford, UK

Interleukin-4 (IL-4) is a strong mitogen for microvascular (human adrenal capillary, HACE) endothelial cells. Maximal stimulation occurred with 1 nM IL-4. Growth promotion was confirmed by both ^{3}H-thymidine incorporation and quantitation of cell number. The mitogenic effect of IL-4 on HACE cells was 50% of that in response to the potent mitogen FGF. The presence of IL-4 receptors on HACE cells was confirmed by specific binding of iodinated IL-4. Scatchard analysis showed a tight binding receptor with Kd = 80 pM and 358 receptors per cell.

CF 302 TUMOR GRADING IN CHONDROSARCOMA COULD BE RELATED TO aFGF CONTENT

Danièle CARUELLE, Béatrice GROUX-MUSCATELLI, Marie-Catherine VOISIN*, Gérard DELEPINE*, Denis BARRITAULT and Jean-Pierre CARUELLE.:
Laboratoire de Biotechnologie des Cellules Eucaryotes, *Service d'Anatomopathologie, Université Paris Val de Marne, Avenue du Général de Gaulle, 94010 Créteil, FRANCE.

A heparin binding growth factor extracted from various normal and pathological cartilaginous tissues could immunologically, biochemically and biologically be related to Acidic Fibroblast Growth Factor (aFGF). Quantitative enzyme immunoassays and immunohistochemical studies performed on normal cartilage and on different grading of chondrosarcoma revealed that tumor grading could be related to the aFGF content. The aFGF content increased with the degree of undifferentiation of the tumor. Values were ranged from 8 ng of aFGF immunoreactive material per gram of normal cartilage to 20 ng/g in grade II and 50 ng/g in grade IV chondrosarcoma. In the same biopsies, the bFGF content (60ng/g) remained constant and no EGF was detected. Interestingly after thermotherapy treatment, aFGF content dropped to 3 ng/g while bFGF content remained at the same value.
Immunohistochemical studies allowed to specify the aFGF tissular localization. A quite exclusive label was mostly observed at the membrane level in chondrocytes. A specific granular immunostaining could be only located in chondrosarcoma myxoïd matrix. Demonstration of the presence of aFGF and its quantitative variations in pathological cartilages suggested that this growth factor could be implicated in cartilage physiology and could be used as a marker by anatomopathologists.

CF 303 TGF-ß and bFGF MODULATION OF ENDOTHELIAL MONOLAYER REPAIR IN VITRO, Brenda L. Coomber, Department of Biomedical Sciences, OVC, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Cytoskeletal alterations are involved in replacement of partially denuded endothelial monolayers in vitro. This study examines interactions between actin microfilament, and microtubule changes and cell proliferation in regenerating large vessel endothelial monolayers treated with bFGF, TGF-ß, and both factors. As previously described by others, monolayer regeneration in this study is enhanced by bFGF treatment and reduced by TGF-ß treatment. Centrosome reorientation in wound edge cells and microtubule staining patterns are not altered by either factor. Cell proliferation at the wound edge, as indicated by bromodeoxyuridine incorporation, is not affected by bFGF but is inhibited by TGF-ß. Endothelial cell morphology is also altered by TGF-ß treatment. Cells loose their cobblestone appearance and assume a pleomorphic shape. As well, actin microfilament staining is modified in both intact and regenerating TGF-ß treated monolayers. There is a loss of dense peripheral band staining and an enhancement in number and staining intensity of cytoplasmic stress fibres. No such alterations are seen in bFGF treated cultures. Endothelial monolayers co-cultured with TGF-ß and bFGF respond as those cultured only with TGF-ß for all parameters measured. Results of this study suggest that the mitostatic effects of TGF-ß on regenerating endothelial monolayers are not due to a lack of cell migration, but may be mediated via actin microfilament alterations.

CF 304 IDENTIFICATION OF UPSTREAM DNA SEQUENCES THAT CONFER FIBROBLAST GROWTH FACTOR INDUCIBILITY OF ngf1a IN PC12 CELLS. Deborah H. Damon, Christopher L. DeFranco*, Mitsutoshi Endoh*, and John A. Wagner*. Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7764 and *Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

NGF1A is a putative transcriptional activator that may play a role in cell proliferation and/or differentiation. In PC12 cells the transcription of ngf1a is increased in response to fibroblast growth factors (FGFs). To determine the ngf1a upstream DNA sequences that confer FGF transcriptional inducibility we generated nested deletions of -1400, -380, -257, -125, -91 and -45 base pairs. These deletions were placed upstream of a CAT construct and tested for FGF inducibility. FGF induced CAT constructs containing -1400 to -125 base pairs of ngf1a upstream DNA 3-6-fold. The CAT constructs containing -91 and -45 base pairs were induced less than 2-fold. The coaddition of FGF with serum, nerve growth factor, activators of protein kinase C or dbcAMP induced -1400 to -125 CAT constructs 7-10-fold. Inductions of -91 and -45 CAT constructs were still less than 2-fold. These data indicate that FGF inducibility is conferred by -125 base pairs of ngf1a upstream DNA. These data also demonstrate that FGF can interact with other growth factors and signal transduction systems to cooperatively activate transcription of ngf1a.

CF 305 IN VIVO ANGIOGENIC ACTIVITY OF PLATELET DERIVED GROWTH FACTOR-BB, Vance D. Fiegel, Barbara G. Penner, Robert C. Wohl, and David R. Knighton, Department of Surgery, University of Minnesota, Minneapolis, MN 55455 and Curative Technologies, Inc., Setauket, NY 11733.

Angiogenesis involves the stimulation of endothelial cell (EC) protease activity, chemotaxis, proliferation, and differentiation. Studies have provided little evidence that platelet derived growth factor (PDGF) plays a role in controlling these EC functions. Utilizing rabbit wound capillary endothelial cells (RWCEC) isolated from subcutaneous sponge implants we have demonstrated the ability of PDGF-BB to induce dose dependent chemotaxis in these wound derived EC. In addition, receptor studies using ^{125}I-PDGF-BB have demonstrated the presence of high affinity binding sites for PDGF-BB on RWCEC. To further investigate the angiogenic activity of PDGF-BB we have examined its in vivo angiogenic potential in the rabbit cornea. Recombinant, human PDGF-BB at doses from 10-500 ng/implant were mixed with Hydron and implanted into rabbit corneas. Control implants contained 1.0 mg/ml human serum albumin in phosphate buffered saline which was also the diluent for the PDGF-BB. The diluent and PDGF-BB were free of detectable endotoxin as determined by the limulus amoebocyte lysate assay. The corneas were examined visually and scored on a 0 to +4 scale with 0 representing no angiogenesis and +4 representing a maximal response. Corneas with scores of +1 or greater were considered positive. Control implants were uniformly negative with 0/8 positive corneas. Corneas containing PDGF-BB demonstrated a dose dependent response: 10 ng/imp (1/4 positive, avg. score=0.25), 50 ng/imp. (4/6 positive, avg. score=1.5), 100 ng/imp. (5/6 positive, avg. score=2.5) and 500 ng/imp. (4/4 positive, avg. score=4). Routine histology of the 500 ng/imp. corneas demonstrated the presence of an inflammatory infiltrate 2 days post-implant which was diminished, but still present at day 7. The remaining doses of PDGF-BB did not produce any histologically evident inflammation at days 2 or 7. These results demonstrate that PDGF-BB can induce angiogenesis in vivo and when combined with the results of the in vitro studies provide evidence of a potential role for PDGF-BB in wound healing angiogenesis.

CF 306 TRANSCRIPTIONAL ACTIVITY OF THE α1(I) COLLAGEN PROMOTER IS CORRELATED WITH THE ANGIOGENIC PHENOTYPE IN CULTURED ENDOTHELIAL CELLS, Laurie Fouser, Luisa Iruela-Arispe, Paul Bornstein, and Helene Sage, Departments of Biological Structure, Pediatrics, and Biochemistry, University of Washington, Seattle, WA 98195.

Bovine aortic endothelial cells (BAEC) exhibiting spontaneous angiogenesis *in vitro* were transfected with the chloramphenicol acetyl transferase (CAT) gene driven by 430 bp of the human α1(I) collagen promoter (pCOL-CAT) or by the simian virus-40 early promoter (pSV2CAT). Immunostaining for CAT in BAEC transfected with PSV2CAT displayed broad reactivity in cells scattered throughout both endothelial monolayers and cord structures. In contrast, immunostaining of cultures transfected with pCOL-CAT demonstrated that collagen promoter activity was selective for BAEC involved in the formation of endothelial cords. This pattern of immunolocalization was also observed when sprouting BAEC were immunostained with antibodies to type I procollagen. When compared with undifferentiated monolayers, the transcriptional activity of pCOL-CAT increased by 4.6-fold in cultures undergoing angiogenesis *in vitro*. Transcription of the construct pCOL-hGH, containing 804 bp of promoter, the first exon, and 1230 bp of the first intron of the α1(I) collagen gene, increased by nearly 20-fold in cultures with sprouts and cords. The correlation between α1(I) collagen promoter activity and development of an angiogenic phenotype suggests an intrinsic role for type I collagen in BAEC morphogenesis and offers a unique approach to the study of cellular mechanisms that regulate collagen gene expression during differentiation.

CF 307 REVC: A RABBIT ENDOTHELIAL CELL LINE WITHOUT SUBSTRATA OR SUPPLEMENT REQUIREMENTS, Corey K. Goldman and G. Yancey Gillespie, Brain Tumor Research Laboratories, Division of Neurosurgery, University of Alabama at Birmingham, Birmingham, AL 35294

Endothelial cells (EC) are fastidious in their growth requirements *in vitro* and won't survive extensive passages. Thus, repetitive studies require fresh preparations of EC. We have partially characterized a continuous cell line established in culture from NZW rabbit *vena cava* endothelium (REVC). REVC cells behave like typical EC, but remain hardy when grown in uncoated plastic culture flasks with DMEM/F12 + 10% FBS. REVC cells have typical cobblestone appearance, are contact-inhibited in monolayers and express Factor VIII-related antigen and TIMP. Proliferation assays utilizing REVC cells grown in 2% FBS on plastic demonstrate dose-dependent increases in ^{3}[H]-thymidine uptake in response to acidic FGF (10-100 ng/mL), basic FGF (3-100 ng/mL), EGF (10-50 ng/mL), and ECGS (10-100 μg/mL). In these cells, heparin (5-100 μg/mL) potentiates proliferation induced by aFGF and lowered the aFGF ED_{50}. Enhanced proliferation of REVC cells was induced by Tumor Necrosis Factor-α, but only in the presence of IFNγ and 10% FBS. Transforming growth factors β_1 and β_2 profoundly inhibited thymidine uptake in REVC cells at ID_{50} doses as low as 25 and 8 pg/mL, respectively and this inhibition was blocked by anti-TGFβ antibodies. When REVC cells were plated on EHS tumor extracellular matrix (Matrigel), proliferation ceased and the cells underwent morphological changes with elongated cytoplasmic extensions reminiscent of differentiation. This continuous cell line is undergoing further characterization and may be of use in investigating many aspects of endothelial cell behavior *in vitro*.

CF 308 GANGLIOSIDES AS MODULATORS OF THE ANGIOGENIC RESPONSE IN THE ADULT ORGANISM, Pietro M. Gullino*, Giulio Alessandri*, Marina Ziche**,
* Department of Biomedical Sciences, Turin University, Italy
**Department of Pharmacology, Florence University, Italy

Neovascularization of the rabbit cornea was utilized as the experimental model. PGE1 and bFGF were utilized as angiogenesis triggers delivered by a slow-release, vinyl copolymer pellet implanted in the cornea. By the end of day 3 after pellet implantation endothelial buds appeared on the limbal vessels. At this time, before capillaries had invaded the cornea, the fragment of tissue to be colonized by capillaries was removed. Total ganglioside content of the corneal fragment was about twice that of the contralateral cornea treated with a pellet without angiogenic factor. Increment of the 3 major corneal gangliosides was not uniform, GM3 increased about 30%, GM2 about doubled and GD3 about tripled. Bovine capillary endothelium cultured in DMEM + 1% CS + bFGF (50 ng/ml) about tripled the number of cells in 72 h. Addition of a GM3 + GM2 + GD3 mixture in proportions mimicking that of the normal cornea produced growth arrest, addition of a mixture mimicking the proportions in the cornea stimulated by the angiogenesis trigger, permitted normal growth.

The change in relative proportions of the 3 corneal gangliosides appear to modulate the growth rate of microvascular endothelium.

CF 309 AUTOCRINE EFFECTS OF FGF IN THE REPAIR OF RADIATION DAMAGE IN ENDOTHELIAL CELLS,Adriana Haimovitz-Friedman, Israel Vlodavsky,Zvi Fuks,Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021
This study demonstrates that basic fibroblast growth factor (bFGF) serves as a specific inducer of radiation damage repair in bovine aortic endothelial cells (BAEC). When plateau phase BAEC were seeded after irradiation on top of the bFGF-enriched natural basement membrane-like extracellular matrix HR9-bFGF/ECM, they exhibited an enhanced repair capacity of radiation damage as compared to cells plated on top of the bFGF-free isotype of this extracellular matrix (the HR9/ECM). While the slopes of the dose survival curves did not differ significantly (the Do on the HR9/ECM was 107±6.8 cGy, compared to 112±1.3 cGy on the HR9-bFGF/ECM), there was a nearly complete elimination of the threshold shoulder in the curves generated on the FGF-free HR9/ECM (Dq of 29 ±19 cGy, compared to 174 ±22 cGy in the cells plated on the HR9-bFGF/ECM; $p < 0.05$). When the cells were withheld after irradiation on top of the bFGF-free HR9/ECM in bFGF-free medium, they exhibited significant potentially lethal damage repair (PLDR), which was inhibited by a neutralizing monoclonal antibody against bFGF. This experiment indicated the presence of bFGF in the cultures despite the lack of exogenously added bFGF, presumably secreted by the irradiated cells themselves. Indeed, Northern blot hybridization experiments showed a 5.6 fold increase of the 3.7 kb species and a 4.7 fold increase of the 7.0 kb species of the bFGF transcriptional mRNA within 6 hours after delivery of a single dose of 400 cGy. The study thus suggested that radiation induced in BAEC a complete cycle of an autoregulated damage-repair pathway. This cycle was initiated by radiation-induced damage to cellular DNA resulting in a "stress response" type of gene activation with transcriptional and post-transcriptional expressions of the bFGF gene, continued with secretion of the newly synthesized bFGF into the culture medium and with induction of the PLDR pathway by the bFGF acting via an extracellular autocrine loop, and was completed with recovery of the damaged cell and with restoration of its clonogenic capacity.

CF 310 POTENTIATION OF BASIC FIBROBLAST GROWTH FACTOR-PROMOTED NEURITE OUTGROWTH IN PC 12 CELLS BY DIBUTYRYL cAMP AND FORSKOLIN,
Paulo L. Ho and Isaias Raw, Biotechnology Center, Butantan Institute, São Paulo, Brazil, CP 65, Cep 05504.

Basic Fibroblast Growth Factor (bFGF) is a well known mitogen which has also been demonstrated to possess neurotrophic effects *in vitro* and *in vivo* (Annu. Rev. Biochem., *58*: 575, 1989). Using PC 12 cells as a model system for the study of neuronal differentiation, we investigated if neurite outgrowth elicited by bFGF can be positively modulated by dibutyryl cAMP or Forskolin, a drug that increases intracelular cAMP through adenylate cyclase activation. Our results show that bFGF-elicited neurite outgrowth is potentiated by dibutyryl cAMP or Forskolin. The combination of such factors with bFGF not only increased the number of cells with neuritic processes, but also induced a larger branching effect. This property was also described for Nerve Growth Factor (NGF)(J. Cell Biol., *102*: 821, 1986), suggesting that both NGF and bFGF may share common intracellular events leading to neurite outgrowth and sinergism with dibutyryl cAMP and Forskolin.

This work was supported by FAPESP and CNPq Brazilian grants to P.L.H. and FINEP to I.R..

CF 311 ENDOTHELIAL-SELECTIVE ATTACHMENT SUBSTRATES OBTAINED BY SURFACE-GRAFTED ARG-GLU-ASP-VAL, Jeffrey A. Hubbell, Stephen P. Massia, Neil P. Desai, and Paul D. Drumheller, Department of Chemical Engineering, University of Texas, Austin, TX 78712-1062
Cell adhesion substrates are an important factor in angiogenesis, and in particular the control of angiogenesis in biomedical applications. We show herein that human umbilical vein endothelial cells (HUVECs) express a surface cell adhesion receptor for the Arg-Glu-Asp-Val (REDV) domain in the alternately spliced type III connecting segment of plasma fibronectin. By contrast, human foreskin fibroblasts, human vascular smooth muscle cells, and human blood platelets do not possess a receptor for the REDV peptide. Peptide binding assays revealed approximately 5×10^6 receptors per cell on the HUVEC with a dissociation constant of approximately 3×10^{-5} M. When the peptide GREDVY was immobilized on otherwise cell nonadhesive ceramic and polymeric substrates by the N-terminal primary amine, the substrates so formed exhibited adhesion toward the HUVECs but not the other blood vessel wall and blood cells mentioned. Reflection interference contrast microscopy revealed focal contact formation, and cytoskeletal staining with rhodamine-conjugated phalloidin revealed the formation of f-acting filaments. Cell spreading and peptide binding studies indicated that the receptor-ligand affinity was not dependent upon calcium ions, as is the case for many other adhesion receptors. When HUVECs were cultured on REDV substrates and exposed to fluid shear stresses of 100 dynes/cm^2 (in excess of arterial shear stresses), approximately 80% of the cells remained after 24 hours. When these monolayers were exposed to flowing blood, blood platelets did not attach to nonwounded monolayers; when the monolayers were wounded, platelets and polymorphonuclear neutrophil leukocytes did attach to the periphery of the wound site. These endothelial-selective materials may be useful in the clinical exploitation and spatial control of angiogenesis.

CF 312 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) mRNA EXPRESSION IN THE RAT OVARY. Robert D. Koos and C. Erik Olson. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

VEGF is a specific endothelial cell mitogen related to PDGF. Unlike the fibroblast growth factors, VEGF appears to be to be freely secreted. To determine if VEGF could play a role in the development of the ovarian follicular and luteal vascular networks, we examined VEGF mRNA expression in the rat ovary using reverse transcription-polymerase chain reaction (RT-PCR). When RT-PCR was performed on 1 μg of total RNA from preovulatory rat ovaries using VEGF-specific primers, a single DNA product of the predicted size of 320 bp was obtained. To confirm that this product corresponded to the rat VEGF sequence, restriction enzyme analysis was carried out; treatment with Alu I, Ava II, and Rsa I yielded fragments of the expected size. It was concluded, therefore, that the RT-PCR product was the target sequence of rat VEGF. We next determined if granulosa cells, the cells of the inner, avascular compartment of the follicle, are a site of VEGF expression. RNA from granulosa cells from either small antral follicles of immature, untreated rats or from preovulatory follicles of immature, gonadotropin-stimulated rats was analyzed. All samples yielded the VEGF product. Residual ovary RNA also yielded the VEGF product. Thus, VEGF is expressed by granulosa cells, and possibly by other cell types in the stromal/thecal compartment of the ovary. In contrast to VEGF, a bFGF product was obtained only from residual ovary RNA. VEGF expression by granulosa cells was maintained when the cells were cultured for 24 or 48 h. A strong VEGF signal was also obtained from RNA of corpora lutea isolated on day 1 or day 9 of pseudopregnancy. In summary, VEGF is expressed by granulosa cells of growing follicles and expression continues in the corpus luteum. Thus, VEGF of granulosa cell origin may mediate growth of first the thecal and later the luteal vascular networks (supported by NIH grant CA45055).

CF 313 IN VIVO EFFECTS OF GROWTH FACTOR AGONISTS AND ANTAGONISTS ON MAMMALIAN CNS INJURY RESPONSES, Ann Logan, #Sally A. Frautschey, #Andrew Baird, *Katherine Flanders and *Michael Sporn, Department of Clinical Chemistry, University of Birmingham, Birmingham B15 2TT, UK, # Department of Molecular and Cellular Growth Biology, The Whittier Institute, La Jolla, CA 92037 and *NIH, Bethesda, MD 20892.

Penetrating injury of the CNS results in dense permanant scarring which mitigates the regeneration of severed axons. We have infused basic FGF, TGF B1 or their respective blocking antibodies via cannulae into the lateral ventricles of lesioned rat brains to examine their influence on the post-injury cellular response. Subcutaneously implanted Alzet mini pumps supplied vehicle alone, basic FGF, TGF B1, anti-basic FGF, anti-TGF B1 or non-immune serum for 14 days. Brains were processed for immunocytochemistry using antibodies for astrocytic GFAP, laminin, macrophage/microglial ED 1 and regeneration markers (GAP 43, RT 97). In control animals the lesion margins were clearly defined by an astrocytic limiting membrane, lined with laminin, which surrounded a condensed fibrotic core. Few macrophages or microglia were evident. No regenerating axons crossed the scar tissue. Animals that were infused with basic FGF or its blocking antibody showed no difference in astrocytic scar production or regeneration. In contrast, all animals infused with TGF B1 showed increased scar formation, as evidenced by increased laminin deposition and a more widespread and numerous microglial population. Two of three animals infused with TGF B1 blocking antibodies showed a reduced fibrotic scar of limited depth. The other animal showed superficial gliosis only. Experiments are underway to rigorously establish this response but these results suggest that manipulation of TGF B1 and not basic FGF may affect CNS injury repair.

CF 314 DEVELOPMENT OF A HUMAN *IN VITRO* BLOOD BRAIN BARRIER (BBB): EFFECTS OF CULTURE CONDITIONS, Laura A. McCarroll, Lisa A. Curry, Patricia M. Watson and Susan R. Doctrow, Alkermes, Inc., Cambridge, MA 02139

The BBB protects the brain from insult due to infection or fluctuations in physiological status by restricting the transport and diffusion of solutes between the blood and the brain parenchyma. Specific transport systems and complex tight junctions of the brain endothelium are active components of the BBB. For the development of an *in vitro* BBB, endothelial cells were cultured from human cerebral microvessels. The identity and homogeneity of the cultures were confirmed by expression of Factor VIII associated antigen, uptake of acetylated LDL, morphology and presence of non-thrombogenic surfaces. Cells were cultured on permeable membranes to assess barrier integrity. This involved assaying the diffusion of two BBB-excluded molecules, sucrose and dextran (ave. M.W. 70KD), on successive days in culture. With an initial plating density of 20%, the cellular monolayers showed the slowest diffusion of both solutes after 15 days in culture. When Endothelial Growth Mitogen (EGM) was omitted from the culture medium after the cells reached confluency (6 days), there was a significant reduction in the sucrose diffusion by day 17. The monolayers became more permeable to sucrose and dextran if EGM was added back to the cultures at day 15. The BBB is induced by the brain environment including astrocytes that form foot processes on the ablumenal surface of the endothelial cells. The effect of astrocyte factors on the permeability of the monolayers was tested by coculture with C6 glioma cells or addition of medium conditioned by them (C6CM). The addition of C6CM resulted in significantly reduced permeability to dextran while coculture had no effect.

CF 315 ORGANOID NEOVASCULAR STRUCTURE : EFFECTS OF VARIOUS MATRIX AND ANGIOGENIC FACTORS. Philippe Moullier, Olivier Danos and Jean-Michel Heard. Laboratoire Rétrovirus et Transfert Génétique, Institut Pasteur, Paris 75015, France.
Restoration of a genetic defect can be achieved by gene transfer into cultivated cells. Organoid vascular structures could facilitate reintroduction of cells expressing secreted transgene products in the organism. However, one major drawback is the constant inflammatory background that occurs as the organoid develops. Chronic inflammation must be prevented as fibrosis may ultimately impair their use as transgenic cell receptacles. We studied the influence of various matrix and growth factors on early inflammatory response to expanded PTFE fibers (Gore & Associates, Inc., Flagstaff, AZ.) as a synthetic backbone. Collagen type I and basement membrane (Matrigel, CRI, MA) were used as coating agents and aFGF, bFGF or aFGF peptide as angiogenic factors bound to the matrix. Coated fibers were surgically implanted in the peritoneal cavity of C3H/He mice. After a 3 to 4 week period, organoids were removed, fixed and hematoxylin-stained. Blood vessels could be observed macroscopically within the neo-organ, whereas control fibers without matrix and angiogenic factors did not induce neovascularization. In all cases, histological examination showed giant cells surrounding the fibers. However, basement membrane coated fibers with either FGFs, presented significantly less lympho-plasmocytes and polynuclear infiltrates (15.4 + 3.8% of the total cells) than collagen-FGFs coated fibers (34.1 + 6%). In addition, the basement membrane coating resulted in wider vascularized areas of loose connective tissue within the fibers, providing easier and possibly more efficient conditions for cell reimplantation.

CF 316 A NOVEL HUMAN ANGIOGENIC FACTOR: PlGF
M. Graziella Persico, Giuseppe Viglietto, Valente Guerriero and Domenico Maglione, International Institute of Genetics and Biophysics, CNR, Naples, Italy.
We have isolated and characterized a cDNA clone from a human term placenta library coding for a 149-aminoacid-protein, named PlGF, which shares very strong similarity (49%) with the PDGF-like region of the vascular permeability factor VPF. The 149-aminoacid-long precursor is processed and exported to the media of COS cells transfected with a recombinant plasmid containing the coding portion of the cDNA under the SV40 promoter control. A computer search for secondary structures in the PlGF mRNA has revealed a stem-loop structure preceding the starting AUG codon. Only after removal of the first 304 nucleotides at the 5' untranslated region can the PlGF-mRNA, obtained by T7 RNA polymerase transcription, be translated at a good level in the reticulocyte lysate translational system. We are now studying the probable translational regulation of PlGF-mRNA in vivo using either COS cells transfected with different PlGF constructs or various cell lines (HepG2, JEG-3) which express the PlGF gene. PlGF can be detected by immunoprecipitation assays with the specific antibodies we have produced in chicken and/or rabbit.

CF 317 A PARTIALLY PURIFIED EXTRACT FROM SKELETAL MUSCLE STIMULATES ANGIOGENESIS IN VIVO AND IN VITRO, Gregg D. Phillips, Lewis A. Schilb, Vance D. Fiegel and David R. Knighton, Department of Surgery, University of Minnesota, Minneapolis, MN 55455
When muscle fibers are injured they degenerate and subsequently regenerate. Spontaneous revascularization occurs in concert with this process, and is one of the limiting factors in successful regeneration of skeletal muscle. The stimulus for the revascularization is soluble factors released from the damaged tissue. The object of this study was to purify the factors responsible for the observed angiogenesis in regenerating muscle and determine their mechanism of action. Skeletal muscle from the hind limbs of rabbits was minced and extracted with a combination of HCl and ethanol. The resulting extract was fractionated on a heparin-TSK column eluted with a continuous gradient of NaCl. Fractions were collected every 4.5 minutes and tested for angiogenesis activity in the rabbit cornea, and for rabbit wound derived endothelial cell chemotaxis and peripheral blood monocyte chemotaxis activity in modified Boyden chamber assays. Unfractionated extract stimulated inflammation associated angiogenesis plus endothelial cell and monocyte chemotaxis in vitro. Fraction 3 (23 ug of protein), eluted at 0.8M NaCl stimulated (+4) angiogenesis in vivo, without any visible inflammation. Fraction 3 (450 ug of protein/ml) also demonstrated significant endothelial cell chemotaxis (488 +/- 61 cells/high powered field (HPF) vs 12 +/- 9 cells/HPF for the negative control). Monocyte chemotaxis activity was not observed in any of the fractions, but was recovered from a peak in the column load (600ug of protein/ml; 71.0 +/- 7.2 cells/HPF vs 14.0 +/- 3.8 cells/HPF for the negative control). Therefore, acid/ethanol extraction of skeletal muscle followed by fractionation on a heparin-TSK column separates factors capable of stimulating endothelial cell chemotaxis and monocyte chemotaxis. These results suggest that damaged muscle releases factors capable of eliciting angiogenesis by acting directly on endothelial cells and by recruiting macrophages which in turn release compounds capable of acting on endothelial cells.

CF 318 SYNTHESIS OF VASCULOTROPIN / VEGF BY CULTURED CELLS : A PARACRINE GROWTH FACTOR.
Jean Plouët, Hafida Moukadiri and Catherine Favard*. ATIPE CNRS- CRBGC-118 route de Narbonne- 31062-TOULOUSE and *U 86 INSERM- 2 place du Parvis Notre-Dame-75004- Paris.

A growth factor mitogenic for vascular endothelial cells was recently purified to homogeneity. The cloning of the gene showed that it is structurally related to the PDGF-sis family. Since its bioactivity seemed to be restricted in vitro to vascular endothelial cells,this growth factor was provisionaly named vasculotropin (VAS) or Vascular Endothelial Growth Factor (VEGF). In vitro, its potency on the proliferation was lower than that of basic Fibroblast Growth Factor (bFGF), and better than bFGF on the migration of vascular endothelial cells. In vivo VAS elicited blood capillary neoformation in rabbit corneas as well as bFGF.

Since VAS is secreted and might control the normal angiogenesis, it was looked on its synthesis and the presence of specific receptors on various cells. Among 8 normal cells, only the vascular smooth muscle cells synthetized VAS; by contrast almost all the 12 tumoral cells examined expressed VAS. Two VAS receptors - 180 kDa and 110 kDa- were characterized : only the vascular-derived endothelial cells hah both, whereas only the 110 kDa was identified on some non responsive cells such as corneal endothelial or lens epithelial. The comparison between the production and the binding of VAS, leaded to the observation that whether a cell secretes VAS and does not bind it or a cell bind (irrespectively to its sensitivity to the mitogenic effect) VAS and does not synthetize VAS.

Taken together these results suggest that VAS is a promotor of angiogenesis acting through a paracrine pathway.

CF 319 DIFFERENTIAL EXPRESSION OF ACIDIC FIBROBLAST GROWTH FACTOR mRNA IN HYPEROXIC-INJURED LUNGS IN ADULT AND NEONATAL MICE. M. Powers, SR Planck, J. Liebler, JT Rosenbaum, Depts of Pediatrics, Medicine, and Cell Biology, Oregon Health Sciences University, Portland, Oregon.

Hyperoxia-induced lung injury in mice has been shown to be age-related with neonatal animals having a relatively higher tolerance to oxygen toxicity. Adult animals have evidence of injury to the capillary endothelium and develop interstitial inflammation with as little as 2-3 days of 80% oxygen exposure. Comparatively, neonatal animals do not develop cellular injury and interstitial inflammation until 1-2 weeks of 80% oxygen exposure. We evaluated the adult (8-10 weeks) and neonatal mouse lung for the presence of aFGF mRNA after exposure to 80% oxygen. Total mRNA was extracted from whole lung lysates of adult mice after 1, 2, 3, and 4 days of hyperoxia exposure. Total mRNA was extracted from pooled whole lung lysates of neonatal mice after 1, 4, 7, and 14 days of hyperoxia exposure. Northern blot analysis of the whole lung RNA was performed using a riboprobe synthesized from human aFGF cDNA. By densitometric measurements standardized to a constitutively expressed message for cyclophilin, the expression of mRNA for aFGF in the adult lung is gradually reduced on each day of hyperoxia exposure, with day 4 being 30% of room air control. In comparision, there are minimal changes in mRNA expression for another growth factor, TGF-β1, in the adult lung after 4 days of hyperoxia. The expression of mRNA for aFGF in the neonatal animals was present in all days of hyperoxia exposure and the expression was unaltered from room air controls. These results show that aFGF gene expression is down regulated in the adult mouse lung after exposure to 80% oxygen. We speculate that this down regulation of aFGF mRNA may contribute to lung injury in the adult animals, and the unaltered expression of aFGF mRNA in the neonatal animals may possibly protect against oxidant injury.

CF 320 EXPRESSION OF ANGIOGENIC GROWTH FACTORS IN THE COLLATERALIZED SWINE MYOCARDIUM
Hari S. Sharma, M. Wünsch, M. Schmidt, R. J. Schott, R. Kandolf* and W. Schaper Max-Planck Institute, Dept. of Experimental Cardiology, Bad Nauheim, *Max-Planck Institute for Biochemistry, Martinsried, FRG.

Poly-peptide growth factors contribute in the regulation of new blood vessel growth, a phenomenon called angiogenesis. We have observed that during a progressive coronary artery stenosis, growth of new blood vessels and enlargement of existing ones (sprouting and non-sprouting angiogenesis) occur in pig hearts. Employing polymerase chain reaction (PCR) technique, We examined the expression of vascular endothelial growth factor (VEGF), a dimeric peptide mitogen of 45 kd; transforming growth factor-ß1(TGF-ß1), a 25 kd homodimeric protein; and endothelial cell growth factor-ß (ß-ECGF) an anionic peptide mitogen of 20 kd in the collateralized (Coll) and normal (N) swine myocardium. Pigs were implanted with a hygroscopic ameroid constrictor around the left circumflex coronary artery (Cx) and collateralization was verified angiographically. RNA was extracted from Coll and N, reverse transcribed and amplified by PCR using specific VEGF, TGF-ß1 and ß-ECGF oligo-nucleotide primers. RNA from Coll yielded dominant bands of VEGF, TGF-ß1 and ß-ECGF DNA fragments corresponding in size spanned by the two primers. Using radiolabeled PCR products as well as human cDNA probes in Northern hybridization, we observed enhanced expression of TGF-ß1 and ß-ECGF in Coll as compared to N. Furthermore, ß-ECGF transcripts were mainly seen in developing collateral vessels, whereas TGF-ß1 mRNA was localized in cardiac myocytes by *in situ* hybridization. Thus, the expression of ß-ECGF, TGF-ß1 and VEGF may have important implications in the regulation of myocardial angiogenesis in response to ischemia.

CF 321 COVALENT ASSOCIATION OF NUCLEOTIDES WITH PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR, Kensuke Usuki, Kohei Miyazono, Jorge Gonez and Carl-Henrik Heldin, Ludwig Institute for Cancer Research, Uppsala Branch, Box 595, S-751 24 Uppsala, Sweden.

Platelet-derived endothelial cell growth factor (PD-ECGF) is a 45 kDa peptide endothelial cell mitogen which has angiogenic activity *in vivo*. cDNA cloning revealed that PD-ECGF lacks signal sequence, consistent with the observation that it is secreted by the producer cells only very slowly. We report here that nucleotides are covalently bound to PD-ECGF through a phosphate bond. Incubation of pure recombinant PD-ECGF with [2,8^{3}H], [2,5',8^{3}H], [α^{32}P], or [γ^{32}P]ATP in the presence of Mg^{2+} resulted in labeling of the protein as demonstrated by the appearance of a 45 kDa band in SDS-PAGE. ATP and GTP were the preferred nucleotide substrates out of a dozen nucleotides tested *in vitro*. After incubation of *in vitro* phosphorylated PD-ECGF in 6 M HCl at 110°C for 1 h, O-[^{32}P]phosphoserine and O-[^{32}P]tyrosine were released. PD-ECGF was also labeled with [^{32}P]orthophosphate in a human epidermal cancer cell line *in vivo* and phosphoamino acid analysis revealed that serine residues were phosphorylated. Snake venom phosphodiesterase or piperidine treatment of *in vitro* or *in vivo* labeled PD-ECGF resulted in the liberation of ^{32}P-radioactivity in the form of nucleotides. Furthermore, phosphorylated PD-ECGF that was secreted from cells was resistant to phosphodiesterase treatment while it in cell lysate was hydrolysed, indicating that denucleotidylation may occur during secretion. (PD-ECGF is a kind gift from Dr. Arlen Thomason Amgen, CA)

CF 322 SUBSTANCE P STIMULATES POLIFERATION OF BOVINE AORTIC ENDOTHELIAL CELLS IN SERUM-FREE CULTURE, Amparo C. Villablanca, Ted W. Reid,Department of Internal Medicine and Opthalmology, University of California, Davis - Davis, CA 95616

The effects of the vasoactive perivascular neuropeptides Substance P (SP), Neurokinin A (NK-A), Neurokinin B (NK-B), and calcitonin gene-related peptide (CGRP) were studied on the proliferation of cultured bovine aortic endothelial cells (BAEC) in serum-free culture conditions with cells quiescent in the G_o-G_1 phase of the cell cycle. SP increased DNA synthesis 1,200% as determined by tritiated thymidine DNA incorporation and increase mitochondrial activity as determined by MTT assay. The effects were time and dose dependent with SP being a more potent mitogen than CGRP. NK-A and NK-B were ineffective. However, Nor-leucine SP, which exhibits more stability but weaker binding than SP, showed the most stimulatory effects. The mitogenic effects of SP were enhanced by CGRP, Insulin (10μg/ml), fetal bovine serum, (0.5%) and platelet poor plasma (5%) but were unaffected by fatty acid-free bovine serum albumin. Hydroxyurea (1 mM), and inhibitor of scheduled DNA synthesis, abolished the effects of SP and CGRP alone or in combination with insulin. Enkephalinase (1-10μg/ml), which cleaves SP, also abolished the SP effects. These studies are the first demonstration of mitogenic effects of SP and CGRP on BAEC. The results indicate that SP stimulates growth arrested cells to enter the DNA synthesis phase of the cell cycle and progress through mitosis. The mitogenic response shows synergism with CGRP and Insulin. Thus, we suggest that neuropeptides may play an important role in regulating cell growth and possibly endothelial cell responses to the pathophysiologic events of atherosclerosis, ischemia, inflammation and would healing.

CF 323 DIFFERENT SPLICING FORMS OF THE VEGF mRNAS ARE SYNTHESIZED IN AIDS-KS CELLS: MOLECULAR CLONING BY PCR, Karin Weindel, Dieter Marmé, Herbert A. Weich, Inst. Mol. Cell Biol., University of Freiburg, 7800 Freiburg, FRG.

Kaposi's sarcoma (KS) are highly vascularized lesions of the skin and often found in patients with HIV infection. Cells from long-term culture have differentiation markers specific for vascular smooth muscle cells. It was demonstrated that these cells secrete several growth factors which are mitogenic for vascular endothelial cells and have angiogenic activity *in vivo* as demonstrated by the CAM-assay and after transplantation of AIDS-KS cells into nude mice. Recently, a new growth factor of the PDGF-growth factor family was isolated from different tumor cells. This factor, named vascular endothelial growth factor (VEGF) is specific for vascular endothelial cells and promotes angiogenesis *in vivo*. The aminoterminal sequence also demonstrates, that the growth factor is identical to vascular permeability factor (VPF), a potent factor from cultured tumor cells that increases the fluid permeability of blood vessels. The *in vivo* function of VEGF is still unknown, but it is speculated that it could act during wound repair and tumor vascularization. To address the question if VEGF is also expressed in KS-cells, we isolated RNA from AIDS-KS-3 cells and used the RT-PCR technique for specific amplification of the cDNA.We could demonstrate that KS-cells transcribe two different splicing forms of the VEGF gene, termed VEGF-β and VEGF-γ. Northern blot analysis using the VEGF-β cDNA demonstrates, that two cell lines (KS-3 and KS-4) express high levels of the VEGF mRNA in relation to other tumor cells and primary cells. Our data suggest, that isolated cells from Kaposi's sarcoma are able to synthesize a potent mitogen specific for endothelial cell growth and that this factor is also identical with the former described VPF. We suppose that VEGF secreted by KS-cells is responsible for increased endothelial proliferation and for the high vascularization of the KS lesions. The vascular permeability activity of this factor may explain the increase of the fluid permeability of blood vessels characteristic for the edema associated with the lesions in AIDS patients.

CF 324 ROLE OF DIRECT ANGIOGENIC FACTORS IN TGFβ1-INDUCED ANGIOGENESIS. Edmund Y. Yang, Agnieszka Gorska, and Harold L. Moses, Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

TGFβ1 is a potent angiogenic factor *in vivo* (Yang, E.Y., and Moses, H.L., *J. Cell Biol.* 111:731-741, 1990), and yet it is also a potent inhibitor of endothelial cell growth *in vitro*. We thus hypothesize that TGFβ1 may function indirectly *in vivo*, via an intermediate cell type, to induce angiogenesis. When TGFβ1 is applied to the chicken chorioallantoic membrane (CAM) fibroblast accumulation, growth inhibition, and extracellular matrix synthesis occur at the point of TGFβ1 delivery and prior to the appearance of angiogenesis. Since bFGF is a direct-acting angiogenic factor, produced by fibroblasts, and secreted into the ECM in a heparan-glycosaminoglycan (GAG) bound form, we have investigated whether bFGF might function as an intermediary signal in TGFβ1-induced angiogenesis. Using a partial chicken cDNA for bFGF, *in situ* hybridization was performed on control, 16 hour, and 24 hour TGFβ1-treated CAM's. Initial results did not demonstrate increased CAM fibroblast bFGF expression in regions of microvascular angiogenesis. RNase protection experiments performed on RNA's purified from TGFβ1-treated CAM fibroblast cultures also did not reveal an increase in bFGF mRNA within the first 24 hours of TGFβ1 treatment. Finally, TGFβ1-treated CAM's were metabolically labelled with $^{35}SO_4$ and analyzed for increased heparan-GAG production. Although sulfated-GAG production was significantly induced by TGFβ1, treatment of sections with heparinase III did not diminish the autoradiographic $^{35}SO_4$ signal, suggesting that heparan-GAG's did not contribute significantly to the ECM of the CAM. These results collectively suggest that bFGF does not function as a paracrine angiogenic factor induced by TGFβ1 during angiogenesis. The possibility that TGFβ1 may regulate other direct angiogenic factors is being investigated.

Endothelial Cell Growth Control and Angiogenesis

CF 400 ANTI-THROMBOSPONDIN ANTIBODIES ENHANCE TUBE FORMATION BY ENDOTHELIAL CELLS *IN VITRO*, Luisa Iruela-Arispe, Paul Bornstein and Helene Sage, Departments of Biological Structure and Biochemistry, University of Washington, Seattle, WA, 98195

The formation of capillaries from pre-existing vessels (angiogenesis) is known to be modulated by growth factors and the extracellular microenvironment. The extracellular matrix protein, thrombospondin (TSP), has been shown to inhibit endothelial cell proliferation, decrease focal contacts, and inhibit angiogenesis in a rat corneal assay. To assess directly the effect of TSP on angiogenesis *in vitro*, we have analyzed protein and mRNA levels during the spontaneous formation of cord-like structures by endothelial cells from both the microvasculature (rat brain capillary endothelial cells) and macrovasculature (bovine aortic endothelial cells). There was a significant decrease in secreted TSP in cord-containing cultures, compared to subconfluent cultures, of both aortic and microvascular cells. Consistent with this trend, TSP mRNA was reduced 16-fold in aortic and 60-fold in microvascular endothelial cells. TSP was present as fine extracellular fibrillar arrays delimiting endothelial cords; this distribution was not observed, however, in subconfluent or confluent endothelial cell cultures that lacked cords. When anti-TSP IgG was added to cord-containing cultures, there was a 33-50% increase in the number of cords after 2 d. No significant changes were observed in control experiments using purified normal rabbit IgG and an ammonium sulfate precipitate of normal rabbit serum. These results suggest that TSP is an inhibitor of angiogenesis in vitro and are consistent with its proposed roles as a destabilizer of focal contacts and as an inhibitor of endothelial cell proliferation.

CF 401 NORMAL AND TRANSFORMED ENDOTHELIAL CELLS DIFFER IN THEIR INTERACTIONS WITH EXTRACELLULAR MATRIX COMPONENTS.
Monique Aumailley, Rupert Timpl, Werner Risau, Max-Planck Intitute for Biochemistry and Psychiatry, 8033 Martinsried, Germany.

Adhesion patterns to laminin and its fragements, fibronectin and collagens were determined for microvascular and large vessel normal endothelial cells and for endothelial cells expressing the polyoma middle T oncogene. The lattest have previously been shown to induce hemangioma formation in vivo. The normal endothelial cells displayed a low adhesion to laminin and its long arm fragment 8 while a considerably stronger adhesion, RGD dependent and followed by spreading, was observed on laminin fragment 1 known to be cryptic in the native molecule. They also attached well to collagen IV. In contrast, transformed endothelial cells had a low adhesion to laminin fragment 1 and collagen IV and a much stronger adhesion to laminin and its fragment 8 mediated by integrin ß1a6 . In addition these cells also interacted with laminin fragment 3 known as the heparin-binding domain. All cells could bind fibronectin however with presumably different interactions, since fibronectin binding was RGD dependent for normal cells and independent for the transformed cells. These results suggest that changes in the interactions between endothelial cells and extracellular matrix components may be involved in the formation of hemangiomas.

CF 402 POSSIBLE ROLE OF METAL IONS IN THE REGULATION OF ANGIOGENIN BINDING TO ENDOTHELIAL CELLS AND TO RIBONUCLEASE INHIBITOR, Josette BADET, Fabrice SONCIN and Denis BARRITAULT, Laboratoire de Biotechnologie des Cellules Eucaryotes - Université Paris XII - 94010 Créteil, France.

Angiogenin, a potent blood vessel-inducing polypeptide, has no known direct effect on cell proliferation but a unique ribonucleolytic activity (1). A functional enzymic active site appears necessary for its angiogenic property and placental ribonuclease inhibitor abolishes both activities (2). Furthermore, angiogenin induces intracellular events such as activation of phospholipases in endothelial and smooth muscle cells (3).

Using recombinant human ANG produced by Rhône-Poulenc Santé and shown to be angiogenic *in vivo* both in the chick chorioallantoic membrane and the rabbit cornea, we have demonstrated the presence of high- and low-affinity binding sites on endothelial cells from calf pulmonary artery (CPAE), bovine aorta, cornea and adrenal cortex capillary (4). Divalent copper, a modulator of angiogenesis, and Zinc metal ions were shown to induce a severalfold increase in specific cell-bound radioactivity without affecting, significantly, the apparent dissociation constants of the binding sites. Placental ribonuclease inhibitor, a tight binding inhibitor of both ribonucleolytic and angiogenic activities of angiogenin abolished its binding to the CPAE cells. However, Cu(II) and Zn(II) blocked this inhibition and their action could be reversed by the addition of a metal chelator, the diethyldithiocarbamate. The effects of Cu(II) and Zn(II) on the binding of angiogenin to its receptors and to ribonuclease inhibitor could suggest a tight regulation of angiogenin activation in the process of angiogenesis.

(1) Vallee B.L. & Riordan J.F., Adv. Exp. Med. Biol. (1988) 234: 41. (2) Riordan J.F. & Vallee B.L., Br. Cancer (1988) 57: 587. (3) Bicknell R. & Vallee B. L., Proc. Natl. Acad. Sci. USA (1988) 85: 5961; (1989) 86: 1573. Moore F. & Riordan J.F., Biochemistry (1990) 29: 228. (4) Badet J. et al. Proc. Natl. Acad. Sci. USA, 1989, 86: 8427.

CF 403 ANGIOGENESIS, AN EARLY AND ESSENTIAL STEP OF EPIDERMAL TUMOR CELL INVASION: AN IN VIVO MODEL TO STUDY PREINVASIVE STAGES, Petra Boukamp, Pascal Tomakidi and Norbert E. Fusenig
Division of Carcinogenesis and Differentiation in vitro German Cancer Research Center, Institute of Biochemistry, Im Neuenheimer Feld 280, 6900 Heidelberg (Germany)

One of the most important steps during epidermal tumor cell invasion is the induction of angiogenesis in the surrounding connective tissue. The mechanism by which the formation of small blood vessels is mediated and its role in tumor invasion is still unclear. In conventional tumor transplantation assays it is difficult to study such mechanisms. We have therefore developed a transplantation assay where human epidermal cells, precultivated on a collagen gel (type I) in vitro (organotypic culture) are transplanted onto the muscle fascia of nude mice. Since the epidermal cells are clearly separated from the mouse mesenchyme by the thick collagen layer for at least one week, this sytem allows to study phases prior to the actual infiltration of the epidermal cells into the host tissue, namely the attraction of mouse fibroblasts, migrating into the collagen gel and the formation of small blood vessels just underneath the collagen. We furthermore have established clonal populations of epidermal cells with different tumorigenic potential following Ha-ras oncogene transfection (Boukamp et al. 1990, Cancer Res.). When these cells were transplanted only the malignant cells rapidly degraded the collagen gel and invaded the host mesenchyme (1 to 2 weeeks after transplantation). Angiogenesis was, however, already visible at about day 4 and was predominant in transplants of malignant and not benign tumor cells. Moreover, since this process was induced before the tumor cells got into cell-cell contact with the host mesenchyme, diffusible factors are required which mediate the feedback mechanisms involved in the process of invasion.

Thus, with these cells and the transplantation assay we have now models at hand to study gene expression of the epidermal and mesenchymal cells involved in the induction of angiogenesis.

CF 404 RETINOID-INDUCED CHANGES IN CAPILLARY ENDOTHELIAL CELL (EC) GROWTH, SHAPE AND PRODUCTION OF COLLAGENASE AND COLLAGENASE INHIBITORS, S. J. Braunhut and M. A. Moses, Departments of Ophthalmology and Surgical Research, Harvard Medical School and Childrens Hospital, Boston MA 02115

Retinoids have been shown to effect morphology, proliferation and secretion of collagenase and collagenase inhibitors in other cell systems. We investigated the effects of retinol (R) and retinoic acid (RA) (1uM) on these parameters in cultures of bovine capillary EC. Proliferation studies revealed that treatment with R, but not RA, inhibited the growth of EC, by 33% in the first 72 hr, and by 100% in a subsequent 72 hr period. Computer image analysis of EC demonstrated that R-treated cells occupied a smaller cellular area than control ($p < .002$) or RA-treated EC ($p < .001$). In contrast, RA-treated EC occupied a larger cellular area by 24 hr of treatment ($p < .03$), and spread at a faster rate than control or R-treated EC ($n > 100$). EC grown for 6 days with or w/o retinoids were washed and used to condition media (CM) for 24 hr. The CMs were concentrated 25-fold, chromatographed on a Sephacryl S-200 column and fractions analyzed for latent and active collagenase and for collagenase inhibitors using a quantitative [^{14}C] collagen film collagenase assay. In concentrated CM, there was more total collagenase activity following either R- or RA-treatment compared to control CM. The samples also contained collagenase inhibitors. One species, Mr=19,000-22,000, was detected in all treatment groups in varying amounts; a higher molecular weight form(s) of inhibitor, Mr=28,000-32,000, was detected in CM derived from R- and RA-treated EC. Retinoids appear to effect the expression of EC metalloproteinases as well as endogenous metalloproteinases inhibitors. We hypothesize that these shifts may contribute to a process of remodeling of the underlying extracellular matrix that is involved in the altered growth kinetics and shape of EC following retinoid treatment. (Supported by NIH EY06726 and EY05333).

CF 405 DIFFERENTIAL EFFECTS OF LEUKEMIA INHIBITORY FACTOR (LIF) AND ONCOSTATIN M (ONCO M) ON ENDOTHELIAL CELL (EC) PHYSIOLOGY. T. Joseph Brown and Mohammed Shoyab, Oncogen (Bristol-Myers Squibb Pharmaceutical Research Institute), Seattle, Washington 98121.

Although histological examination reveals fibroblasts in close proximity to the endothelial lining in the tunica intima of the elastic arteries (e.g. aorta), the role of stromal fibroblasts in regulating EC physiology is poorly understood. A potent growth inhibitory polypeptide for cultured bovine aortic EC (BAEC) was isolated from the culture medium of the human diploid fibroblast cell line, HEPM, following a 40,000-fold purification procedure. Amino acid sequence analysis of this polypeptide indicated identity with the differentiation inducing factor, LIF. HEPM-derived LIF inhibited the growth of BAEC with the same potency as human, recombinant LIF. Thus, LIF shares the functional property of inhibiting the growth of BAEC along with several cytokines, including Onco M. Onco M is produced by activated T lymphocytes and monocytes, whereas LIF is constitutively produced by fibroblasts. Combined treatment of BAEC with suboptimal doses of LIF and Onco M resulted in synergistic anti-proliferative effects, suggesting possible cooperativity between stromal and hemopoietic elements in inhibiting EC growth. By contrast, Onco M, but not LIF, stimulated plasminogen activator (PA) activity in BAEC. Therefore, the differential effects of fibroblast-derived LIF and leukocyte-derived Onco M on PA activity may indicate distinct roles for these cytokines in regulating EC physiology during non-inflammatory versus inflammatory states.

CF 406 *IN VITRO* PHAGOCYTOSIS BY ENDOTHELIAL CELL LINES OF DIFFERENT ORIGINS, Todd C. Case, Geronimo Ramirez, Dennis L. Way, Marlys H. Witte and Charles L. Witte, Department of Surgery, The University of Arizona College of Medicine, Tucson, AZ

Despite lineage of endothelium from the reticuloendothelial system, the ability of divergent endothelial cells to phagocytose bacteria, lipids and other particulates has not been sufficiently addressed. Using standard tissue culture techniques, endothelial cell lines derived from human omentum, cardiac blood vessels, cavernous lymphangioma and rat hepatic sinusoids were grown to confluence as monolayers in multiwell plates. The endothelial nature of the cultured cell lines was confirmed by typical cobblestone morphology and positive staining for Factor VIII-associated antigen and Ulex europaeus ligand. Fluoresbrite carboxylate microspheres ($2.5x10^8$; 1.34μ diameter, Polysciences) were added to well plates for each cell line and incubated for 2, 6, 24 and 48 hours prior to trypsin-EDTA cell harvest. The number of captured beads per cell was estimated (for 10,000 cells per well), displayed logarithmically and analyzed using flow cytometry and 4CYTE (H. Shapiro) and Sigma Plot (Jandel Scientific) software. Of the endothelial cell lines, heart vasculature had the greatest proportion of rapidly phagocytosing cells (>80% and >95% containing 10^1-10^2 microbeads at 2 and 48 hours, respectively). By comparison, lymphangioma derived endothelium was barely phagocytic with only 10% of cells containing 10^1-10^2 microbeads even at 48 hours. Human omental and rat sinusoidal cells showed intermediate values of >50% and ~30% phagocytosing cells respectively and had a high number (10^2-10^3) of microbeads per cell by 6 hours. These data demonstrate that endothelial cells at least *in vitro* exhibit remarkable phagocytosis, and accordingly, may play a heretofore unrecognized role in the phagocytic component of host defense and immunoreactivity. In view of the array of receptors on the endothelial cell surface, the influence of hormones, cytokines, and other immunomodulators in regulating the magnitude and kinetics of phagocytosis by endothelial cells of different origins needs further elucidation.

CF 407 TRANSGENIC MOUSE MODEL FOR TUMOR PROGRESSION AND ANGIOGENESIS.

Gerhard Christofori, François Radvanyi, Mark Lacey, and Douglas Hanahan. Hormone Research Institute, University of California San Francisco, San Francisco, CA 94143-0534.

Transgenic mice (RIP1Tag2) carrying the simian virus 40 large T antigen under the control of a rat insulin promotor develop pancreatic ß cell tumors (insulinomas) in a predictable manner. Distinct stages of tumor progression are clearly recognizable, thereby providing the means to investigate in detail the different steps involved in the transition from normal pancreatic ß cells to insulinomas. Virtually all ß cells express SV 40 T antigen, yet only about 50% acquire a hyperplastic state. Eventually, 1 to 2% of the islets in the pancreas develop into solid, highly vascularized tumors, indicating that additional genetic or epigenetic changes occur during the stepwise progression of tumorigenesis. For example, two populations of hyperplastic islets can be distinguished: most of the hyperplastic islets are in a prevascular state, whereas a small proportion of the hyperplastic islets secrete angiogenic factors which are able to induce chemotactic migration and proliferation of bovine capillary endothelial cells. Currently, we are attempting to isolate cDNAs for these angiogenic factors by direct expression cloning in cos cells. In addition, we seek to identify other genes that might play an important role in the multiple steps of tumor development and tumor angiogenesis using differential screening of tumor cell cDNA libraries.

CF 408 ADVANCED GLYCOSYLATION ENDPRODUCT (AGE)-MODIFIED PROTEINS STIMULATE ENDOTHELIAL CELL GROWTH, Maria Torcia, Clara Crescioli, Silvia Fabiani, Maria Lucibello, David M. Stern, Federico Cozzolino, IV Department of Internal Medicine, University of Firenze, I-50139 Italy; Department of Physiology and Cellular Biophysics, Columbia University, New York, NY 10032

Endothelial Cells (EC) express surface receptors for AGE-modified proteins, that are specific for the sugar moiety of the molecule; these receptors trigger several proinflammatory events, such as tissue factor expression and permeability to macromolecules. We describe that AGE-Bovine Serum Albumin (BSA), as well as other AGE-modified proteins (transferrin, hemoglobin, ribonuclease) also stimulate EC growth in a dose-dependent fashion, with half-maximal activity at concentrations close to the Kd of the specific surface receptor (≈ 100 nM). A time-course analysis showed that stimulation was maximal at 48 hours. EC cultures set up with various amounts of bFGF and AGE-BSA revealed that bFGF-induced proliferation was augmented at any concentration tested; however, maximal activity of AGE-BSA was observed with sub-optimal doses of bFGF, indicating that it enhanced growth factor utilization by EC. This hypothesis was reinforced by the observations that neutralizing antibodies to bFGF affected the activity of AGE-BSA alone on EC growth, and that the affinity of FGF receptor increased in AGE-BSA-stimulated EC. These findings suggest that AGE-modified proteins influence the FGF/FGF receptor system in EC; they may also bear relevance for the molecular understanding of several pathological events in diabetes and ageing.

CF 409 IMMUNOSTIMULATION AND HIV-1 INFECTION HAVE COMPLEMENTARY EFFECTS IN THE INDUCTION AND PROGRESSION OF AIDS-ASSOCIATED KAPOSI'S SARCOMA VIA RELEASE OF CYTOKINES AND TAT (IN VITRO STUDIES), Barbara Ensoli, Giovanni Barillari, Luigi Buonaguro, Hsiao-Kuey Ghang and Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892

Kaposi's sarcoma is a vascular tumor of mesenchymal origin particularly frequent in HIV-1 infected homosexual individuals (AIDS-KS). In previous work we have shown that cells derived from KS lesions of these patients (AIDS-KS cells) proliferate in response to the HIV-1 tat gene product, which is released during acute infection of T cells by HIV-1. However, normal mesenchymal cells did not proliferate in response to Tat, suggesting that other stimuli are necessary to induce Tat responsiveness by normal cells, which are the possible progenitors of the spindle cells of the KS lesions. Here we show that treatment of normal mesenchymal cells (endothelial and smooth muscle cells) with conditioned media (CM) from activated T cells induce normal mesenchymal cells to become Tat responsive. Furthermore, the same CM or extracellular Tat protein are also capable of activating HIV-1 gene expression and consequently Tat release. Therefore, supported by clinical-epidemiological data, we conclude that immunostimulation, particularly frequent in homosexuals, acts as a predisposing factor in the induction and progression of KS in HIV-1 infected susceptible individuals.

CF 410 VASCULAR ENDOTHELIAL GROWH FACTOR IS EXPRESSED IN SCLEROTIC HUMAN CORONARY ARTERY. I. Gemperlein, Ch. Grund, S. Wellershof, H. Graf. Research Laboratories, Schering AG, Müllerstr. 170-178, 1000 Berlin 65, FRG.

Vascular endothelial growth factor (VEGF) has been reported to induce angiogenesis and to increase vascular permeability. Therefore VEGF is considered to be an important factor during inflammation, wound healing and tumor angiogenesis. Biosynthesis of VEGF has been shown to occur in tumor cell lines, in bovine pituitary follicular stellate cells and in U937 cells. The protein shows sequence homology to PDGF A and B. In contrast to other endothelial growth factors (FGF or PDGF), VEGF is a secreted mitogen.

Here we demonstrate by in situ-hybridization using a synthetic oligonucleotide probe that VEGF-mRNA is also present in human sclerotic coronary arteries. The most intense signal was localized in the luminal region of the vasculature. In contrast TGFa - another endothelial mitogen - was predominantly expressed in cells of the sclerotic plaque. EGF-receptor mRNA was most prominent in the intima and the adventitia. It was not found in smooth muscle cells of the media. TGFß was found both in the plaque region and the media. In an attempt to identify cells expressing the variuos growth factors serial sections were processed by immunocytochemistry using antibodies selective for endothelial cells, smooth muscle cells, filamenteous proteins, LDL-receptor, fibronectin receptor and EGF-receptor. These results suggest, that VEGF is synthesized within cells of the luminal region of the sclerotic human artery and stimulates endothelial growth and permeabilty in an autocrine or paracrine manner. TGFa is an important mitogen increasing intimal proliferation. However, it seems not to be involved in medial hyperplasia.

CF 411 HIGH mRNA LEVELS OF MACROPHAGE CHEMOTACTIC PROTEIN 1 AND INTERLEUKIN-6 IN LESIONS OF KAPOSI`S SARCOMA IN SITU. Reinhard Gillitzer[1] and Rudolf Berger[2]. [1]Dept. of Dermatol., University of Würzburg, D-8700 Würzburg, [2]Dept. of Dermatol.I, University of Vienna, A-1090 Vienna.

Two cell types, KS cells themselves and dermal macrophages/dendrocytes (DD) are the main constituents of KS tumors. We applied RNA-RNA in situ hybridization techniques to elucidate the cytokine profile of KS cells and DD in vivo. Using radioactive antisense probes for basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF) and tumor necrosis factor α (TNF-α) sparce signals were observed. However, high levels of interleukin 6 (IL-6) mRNA transcripts were detected in cells corresponding DD in their distribution pattern. The high abundance of DD prompted us to investigate the production of macrophage chemotactic cytokines. Our results showed that KS cells expressed high levels of macrophage chemotactic protein 1 (MCP-1) mRNA. Therefore, we postulate that KS cell derived MCP-1 may attract DD, and in addition may stimulate them to secrete angiogenic cytokines (IL-6, bFGF, PD-ECGF and TNF-α). This may lead to a self-amplifying circle of paracrine stimulation causing KS development and hyperplasia.

FGF, Endothelial Cell Growth Factors and Angiogenesis

CF 412 SK HEP-1: A MODEL FOR ANGIOGENESIS, Sue C. Heffelfinger and Gretchen Darlington, Department of Pathology, Baylor College of Medicine, Houston, Texas 77030

SK HEP-1 is a polyclonal, immortal cell line of endothelial origin. Individual clones vary in their ability to undergo a morphologic change in vitro. from monolayer to tubular structures, depending on the culture substrate. In some clones the addition of exogenous extracellular matrix is not required for this morphologic change. One clone not only forms tubules, but exhibits extensive sprouting and invasion into underlying extracellular matrix substrates, forming a three-dimensional tubular network. Another clone is unable to form tubules even with a complete basement membrane substrate. Protein kinase system agonists allow this clone to form tubules in vitro. The clonal variation provides a unique system for dissecting the various steps involved in the interaction of extracellular matrix and intracellular signals during this morphologic change.

CF 413 OXYGEN REGULATES BASIC FIBROBLAST GROWTH FACTOR mRNA EXPRESSION IN CULTURED Y79 RETINOBLASTOMA CELLS. Rosemary D. Higgins, M.D., Dale L. Phelps, M.D., and Stuart Horowitz, Ph.D. Departments of Pediatrics and Ophthalmology, University of Rochester, Rochester NY 14642.

Retinopathy of Prematurity is a vasoproliferative disorder whose pathophysiology is poorly understood. The *in vivo* kitten model of oxygen induced retinopathy shows that chronic hypoxia during the recovery phase exaggerates the vasoproliferation and that mild hyperoxia during the recovery phase improves the retinopathy. Angiogenesis in the eye may be regulated, in part, by bFGF, a known angiogenic growth factor found in the retina and other tissues. Oxygen tension in the eye may regulate bFGF expression, and thus affect retinal vasoproliferation. To test the hypothesis that oxygen regulates bFGF, Y79 human retinoblastoma cells were used as a neural retinal model. Y79 cells were grown under standard conditions (95% air, 5% CO_2) and varying oxygen (2-50% O_2, 5% CO_2, remainder as N_2), for 24 hours, counted, lysed, and RNA was isolated. RNA dot blots were hybridized to labeled cDNA Probes encoding human bFGF and the housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) whose mRNA is constitutively expressed. Quantitative analysis of hybridizations by soft laser densitometry indicated an inverse correlation between bFGF mRNA abundance (normalized to GAPDH mRNA) and O_2 tension. There was a greater than 40-fold difference between bFGF mRNA levels at 2% O_2 versus 50% O_2 after 24 hours in culture. Cell counts were unaffected from 2 to 40% O_2. We conclude that oxygen regulates bFGF mRNA expression in cultured Y79 retinoblastoma cells in a dose dependent manner.

CF 414 REGULATION OF ANGIOGENESIS BY ANTIBODIES TO THE β_1 INTEGRINS, P. Kaur, J.R. Gamble, L. Matthias and M.A. Vadas, Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, SOUTH AUSTRALIA, 5000.

The process of angiogenesis was investigated using a three dimensional collagen gel assay. Using this system and with the addition of PMA, human umbilical vein endothelial cells undergo a morphological change from a flat cobblestone monolayer to a network of branching tubes or sprouts within 24 hours and this process is thought to be a measure of angiogenesis. Some monoclonal antibodies to the β_1 integrins (VLA) are able to alter the extent of tube formation while other antibodies have no effect. In addition, an antibody to an extracellular matrix component inhibits tube formation. Since the β_1 integrins are involved in the attachment of endothelial cells to their extracellular matrix we would hypothesise that endothelial cell contact with the surrounding matrix appears critical in the regulation of angiogenesis. The mechanism by which the integrins may be important in the regulation of angiogenesis will be discussed.

CF 415 A NEW PROCEDURE FOR RECORDING AND MEASURING ANGIOGENESIS *IN VIVO*.
T. J. Conrad, D. B. Chandler, J. M. Corless and G. K. Klintworth, Departments of Ophthalmology, Pathology, Cell Biology and Neurobiology, Duke University Medical Center, Durham, NC 27710 USA

Accurate quantitation of corneal angiogenesis is useful for evaluating the response to corneal injuries and topical therapies, and for documenting the effects of both putative angiogenic factors and inhibitors of angiogenesis. Previously we have measured angiogenesis by computerized image analysis of cut and flattened corneas, excised after perfusion with India ink *in situ*. Although accurate, the procedure is tedious and costly, susceptible to incomplete perfusion and differential tissue shrinkage, and each animal yields data for only one time point. To overcome some of these inherent difficulties and to reduce the number of animals needed per study, we have developed a non-invasive method for recording corneal neovascularization *in vivo*. The device has 3 major components. (1) A stereotactic device is used to hold, align and move the anesthetized animal, so that the visual axis is oriented vertically. (2) A Questar long working distance (56 cm) microscope is aligned with its optic axis normal to the peripheral cornea. The microscope is coupled to a video camera, monitor and video recorder. Rotation of the animal (eye) permits sequential recording of adjacent corneal segments. The resolution of the lens system is ~ 3 μm, allowing capillaries to be imaged. (3) Contrast between blood vessels and corneal stroma is optimized by using a filtered light source centered on the Soret band absorption maximum of hemoglobin. Recorded images are digitized later from the video tape. Overlapping regions are identified by statistical cross-correlation or common feature coordinates, and eliminated. Non-overlapping image segments are then remapped to form a smaller number of montaged frames, which are processed to remove noise and enhance vessel contrast. Vessel area is calculated by pixel counting after establishing the density range for vessel identification. Support: NIH - EY04922 and EY00146.

CF 416 A NOVEL ENDOTHELIAL CELL RESTRICTED ANTIGEN LOCATED AT CELL TO CELL CONTACTS. Maria Grazia Lampugnani§,Rod Piggott', Massimo Resnati §, Luigi Ruco*, PierCarlo Marchisio *, Elisabetta Dejana §, § Istituto Mario Negri , Milano. 'Università di Roma, *Università di Torino. ITALY, 'British Biotechnology Limited, Oxford, UK.

The integrity and the selective permeability of the endothelium is maintained by molecules regulating cell to cell contacts. Monoclonal antibodies (mab) to human umbilical vein endothelial cells (EC) were raised and screeened for their ability to recognize in immunofluorescence microscopy antigens located at the EC contacts. A mab called 7B4 was found to stain the cell borders at the sites of cell to cell contacts in monolayer of cultured EC. In addition the staining pattern differed from that observed with mabs to CD31or to $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrins or endoglin that are the other molecules found at cell to cell contacts in EC. 7B4 antigen was exclusively present in EC at variance with CD31 which is expressed also in platelets, granulocytes and monocytes. 7B4 mab precipitated from ^{35}S-methionine labelled EC a protein of 140 kd apparent molecular weight which was different from CD31 (m.w. 130 kd) and endoglin (m.w. 180 kd) In histological sections of many different organs 7B4 antigen specifically recognized EC of large and small vessels and capillaries with restricted positivity at the cell to cell contacts. Treatment of EC monolayers with substances known to increase EC permeability like thrombin or to affect the monolayer continuity like citokines (IL-1β or TNF and γIFN) induced disorganization of 7B4 immunofluorescence pattern. This suggests that 7B4 antigen might be involved in the regulation of EC monolayer integrity.

CF 417 MONOCLONAL ANTIBODIES TO RECOMBINANT HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR (rHuVEGF) Bing Li, Jane Winer, Napoleone Ferrara and K. Jin Kim. Depts of Medicinal Analytical Chemistry and Cardiovascular Research, Genentech Inc., South San Francisco, CA 94080

VEGF has been reported as a specific growth factor for vascular endothelial cells which can induce angiogenesis in vivo. To further understand the role of VEGF on vascular endothelial cells, we have generated monoclonal antibodies (mAbs) specific for the 165 amino acid species of rHuVEGF. Balb/c mouse spleen cells immunized with rHuVEGF conjugated to keyhole limpet hemocyanin were fused with P3/X63-Ag8U1. Five strong positive mAbs (A2.6.2, A3.13.1, A4.6.1, B2.6.2 and B4.3.1) were selected by ELISA. MAb A2.6.2 is a IgG2b isotype and the rest are IgG1. These mAbs had no cross reactivity to other growth factors such as endothelial cell growth factor, platelet derived growth factor, basic fibroblast growth factor and nerve growth factor. All these mAbs bind to the rHuVEGF blotted on nitrocellulose paper, however, A2.6.1 and A3.13.1 bind only to native rHuVEGF while A4.6.1, B4.3.1 and B2.6.2 bind to both native as well as denatured rHuVEGF. At present we are screening neutralizing monoclonal antibodies using the proliferative assay. There was a significant increase in the 3H-thymidine uptake on day 4 after bovine adrenal cortex capillary endothelial cells were treated with as little as 2.5 ng of rHuVEGF. Further, we are investigating the effects of other known cytokines on the proliferative response of rHuVEGF on these endothelical cells.

CF 418 BASIC FGF IN THE VESSEL WALL AND ITS ROLE IN SMOOTH MUSCLE CELL PROLIFERATION AFTER VASCULAR INJURY, Volkhard Lindner, Michael A. Reidy, University of Washington, Department of Pathology, Seattle, WA 98195

An antibody raised against human recombinant bFGF was used to localize bFGF in adult rat arteries. Immunocytochemistry showed predominantly nuclear staining of both endothelial and medial smooth muscle cells (SMC) and no apparent staining of the extracellular matrix. Basic FGF is thought to be released from injured cells and therefore arterial injury is likely to expose vascular cells to this mitogen. We investigated whether bFGF in rat arteries is responsible for the rapid SMC proliferation induced by balloon catheter denudation which is accompanied by substantial cell death. A neutralizing antibody against bFGF or nonimmune IgG (10 mg/rat protein G purified IgG) was administered intravenously prior to balloon catheterization of the carotid artery. Determination of SMC replication after ^{3}H-thymidine injection (24, 32, and 40 hours after injury) and autoradiography showed an approximate 80% reduction in ^{3}H-thymidine index (1.5% vs. 7.6% in controls). The intimal thickening that developed within 2 weeks after balloon catheter denudation, however, was not significantly reduced. These results demonstrate that SMC proliferation after arterial injury is largely due to release of bFGF from damaged cells in the vessel wall. (Supported by NIH grants HL 03174-35 and 1P50HL 42270-01).

CF 419 QUANTITATION OF ENDOTHELIAL CELLS WITHIN B16 MELANOMAS IN VIVO. **M.E. Neville. Medical Products, Dupont Merck pharmaceutical Glenolden, PA 19036.** **Endothelial cells (EC) have a high number of surface membrane receptors for acetylated (AC) low density lipoprotein (LDL). The uptake of fluorescent-labeled 1,1'-dioctadecyl-1-3,3,3',3'-tetramethyl-indo-carbocyanine perchlorate (DiI) -AC-LDL by these receptors has been used to identify and quantitate EC within mixed primary fibroblast-like cell cultures in vitro using either a fluorescence microscope or FACS analyzer. A method to quantitate the number of EC within B16 melanomas in vivo has been developed using the same rationale. DiI-AC-LDL was injected intratumorally 1 hr before the mice, bearing B16 tumors, were euthanized. The tumors were removed, weighed, and homogenized. The total number of cells (excludingRBC) per mg of tumor was determined. After washing, the % of fluorescent cells in each homogenate was quantitated with a fluorescence microscope or by FACS analyzer. The number of fluorescent cells per mg of tumor was calculated. This method can quantitate EC within B 16 tumors since 1) intratumorally injections of DiI-AC-LDL resulted in even distribution of DiI-AC-LDL within the tumor: 2) uptake of DiI-AC-LDL was only apparent in EC and not in B16 melanoma cells ,fibroblasts or smooth muscle cells and 3) the number of EC per mg of tumor remained consant over a wide range of tumor weights (200-1300 mg). Since EC are required for angiogenesis and tumor growth, this method may be used to quantitate the inhibition of angiogenesis after treatment with anti-angiogenic compounds.**

CF 420 BASAL MEMBRANE AND VASCULAR LESIONS ASSOCIATED WITH LICHEN SCLEROSIS

J.H. Pavlovitch[2], M. Lejbovitch[1], and D. Garcia Plata[1]
[1]Hôpital Tarnier ; [2]CNRS URA 583, Hôpital Necker, Paris.

Recent findings suggest that epidermoid carcinoma associated with lichen sclerosis (LS), a rather frequent vulvar disease, has higher risk of invasiveness than that associated with Bowen disease. The latter has a benign evolution or may produce a noninvasive epithelial disorder (carcinoma in situ). Since the failure to maintain a complete basal membrane and an increase in angiogenesis may be the critical step in the convertion from non-invasive to invasive lesions biopsies taken from sites with plane LS, hyperplasic LS and LS associated with epidermal carcinoma, were analysed for basal membrane integrity and vascularisation by indirect immunofluorescence with antilaminin, anti-fibronectine and antifactor VIII, and were compared with biopsies of normal vulvar skin, chronic inflamation lesions and hyperplastic or noninvasive neoplastic (carcinoma in situ) ones. The results show discontinuities of the basal membrane exclusively in the lesions associated with LS. Alterations consisting with insufficient mass and desorganization of both laminine and fibronectine were present in the early lesions of LS and in LS associated carcinoma. The basal membranes of the vascular structures also appear abnormal : in biopsies taken from LS, hyperplasistic LS or LS associated with cancer capillaries of the superficial dermis are either absent or abnormally thin and irregular in shape and distribution. They appeared normally structurated in other lesions, including chronic inflamation and hyperplasia or carcinoma in situ. The degree of angiogenesis was, however, highly coupled with the degree of epithelial hyperplasia, and independent of LS lesions : fewer vessels were present in atrophic LS lesions than in normal vulvar skin, whereas both LS associated carcinoma and carcinoma in situ were highly vascularized. These findings suggest that higher invasiveness of cancer associated with LS is rather due to basal membrane and or vascular walls alterations than to angiogenic capacity.

CF 421 CYTOTOXICITY OF ACTIVATED MONOCYTES ON ENDOTHELIAL CELLS: ROLE OF β2 INTEGRINS, Giuseppe Peri, Francesca Chiaffarino, Sergio Bernasconi, Ines Martin Padura and Alberto Mantovani, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Unstimulated human monocytes (Mo) did not express appreciable levels of cytotoxicity on normal human umbelical vein endothelial cells (EC) in a 24 - 48 hr TdR release assay. Or activation with IFN-g and LPS, Mo had appreciable cytotoxicity on EC. Mo cytotoxicity on EC was not dependent on the presence of contaminating lymphoid cells. Recombinant TNF, IL-1, and IL-6 as well as monocyte supernatants did not exert a citotoxic effect on EC. Antibodies against the ß-chain (CD18) of leukocyte integrins inhibited the adhesion and cytotoxicity of activated Mo on EC. Pretreatment of EC with IL -1 augmented the adhesion of Mo on EC. Normal Mo were not cytotoxic on IL -1- pretreated EC and IL -1 treatment did not increase the susceptibility of EC to activated Mo. Thus adhesion is necessary but not sufficient for Mo killing of EC. Anti- αL (LFA-1) antibodies markedly reduced monocyte cytotoxicity of EC, although anti - αX (p150) antibodies had only a modest effect. Anti - αM (Mac - 1/CR_3) antibodies were intermediate inhibitors of EC killing by activated Mo. Thus, αL,β2 (LFA-1), and, to a lesser extent,αM,β2(Mac-1/ CR_3) and αX,β2 (p150,95) integrins are the main adhesive structures involved in the cytotoxicity interaction of activated Mo with EC. Monocytes-mediated damage of EC could play a role as a mechanism of tissue injury under conditions of local or systemic activation of mononuclear phagocytes.

CF 422 **EXPRESSION AND CHARACTERIZATION OF BASIC FGF-SAPORIN IN *E. COLI.***
Ignacio Prieto, Douglas A. Lappi, Michael Ong, Risë Matsunami and Andrew Baird. Department of Molecular and Cellular Growth Biology, The Whittier Institute, 9894 Genesee Ave., La Jolla, California, 92037

We have chemically conjugated saporin (SAP), a ribosome-inactivating protein from the plant *Saponaria officinalis*, to basic fibroblast growth factor (basic FGF). This conjugate is cytotoxic to cells that express functional bFGF receptors. Accordingly, we have constructed a gene that encodes for a fusion protein between bFGF and SAP and expressed it in *E. coli*. A cDNA encoding SAP (Benatti et al., 1989, Eur. J. Biochem. *183*, 465-470) was inserted into a plasmid (pFC80) containing a human bFGF gene. The SAP coding sequence was ligated to the 3' end of the bFGF gene and expression of the fusion protein regulated by the trp promoter. Western blotting of the bacterial extracts that contain this new plasmid were performed using anti-SAP and anti-bFGF antibodies. SDS-PAGE and immunoblotting reveals the presence of a band at the expected molecular weight for the bFGF-SAP fusion protein (45kD). The purified product is subsequently characterized for heparin binding, FGF receptor binding, ribosomal inhibiting activity and mitotoxic effects on FGF target cells. We are now purifying the fusion protein to characterize these biological properties and constructing alternative bFGF-SAP genes to enhance expression. We are also constructing plasmids to express SAP in *E. coli* in order to produce SAP-bFGF fusion proteins.

CF 423 ENDOTHELIAL PHENOTYPIC HETEROGENEITY IN BOTH AIDS-KAPOSI SARCOMA LESIONS AND CULTURED CELLS, Geronimo Ramirez, Dennis L. Way, Marlys Witte, Ray Nagle, Milan Fiala, Michael Bernas, Charles Witte and Michael Milligan, Depts. of Surgery and Pathology, University of Arizona College of Medicine, Tucson, AZ and Depts. of Medicine and Pathology, Eisenhower Medical Center, Palm Springs, CA.

The nature of Kaposi sarcoma (KS) (malignant neoplasm vs multicentric angiogenic response to infection) and the lineage of the KS cell remain controversial. Although vascular and specifically lymphatic endothelium has been favored as the progenitor cell, recent reports call into question an endothelial origin of KS based on morphology and immunohistochemical staining of cultured cells derived from AIDS-KS pulmonary lesions and pleural effusions and propagated in HTLV-II conditioned medium. Yet blood vascular and lymphatic endothelium from different sources and under varying conditions is itself known to display phenotypic heterogeneity both *in vivo* and *in vitro*. Therefore, we prepared enzyme isolate cultures of excised KS lesions from 7 HIV seropositive patients with AIDS and over 14-24 months subjected them to modified enzymatic dissociation, medium depletion, short pulse trypsinization, and increased $C0_2$ in the absence of HTLV-II condition medium. Endothelial marker positivity for Factor VIII associated antigen (F8+), endothelial antigen (EA+), and Ulex europaeus ligand (UL+) was determined by flow cytometry (10,000 cell count) and compared to original paraffin sections (immunoperoxidase method). Whereas the proportion of vascular lumina and spindle cells on tissue section varied from patient to patient, large thick-walled channels were consistently strongly F8+ and UL+, smaller vascular slits and thin-walled lakes were variably and weakly + for both markers, and the less prominent spindle cell component was almost uniformly negative. Each AIDS-KS culture yielded a heterogenous population of spindle to stellate and flattened endothelial-like cells. Similar to control endothelium (lymphatic and omental microvasculature) which showed 40-90% F8+ and UL+ cells, KS cultures displayed 51.2±16.0% (mean±SD) F8+, 60.4±18.4% EA+, and 71.0±13.9% UL+ cells. Thus, AIDS-KS cells retain the predominant morphologic characteristics and staining patterns of endothelium over successive generations *in vitro*, thereby mirroring original tissue sections and also exhibiting the phenotypic heterogeneity of lymphatic and microvascular endothelium.

CF 424 DEVELOPMENTAL EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR mRNA IN THE RAT CENTRAL NERVOUS SYSTEM, Marco A. Riva and Italo Mocchetti, Department of Anatomy and Cell Biology, Georgetown University, School of Medicine, Washington, D.C. 20007.

Although several studies have demonstrated that basic fibroblast growth factor (bFGF) possess a trophic activity on different neuronal/glial cell populations, little is known about the regulation of bFGF in the brain. Using RNase protection assay, we determined the levels of bFGF mRNA in several brain structures of adult rats. bFGF mRNA was found to be widely distributed throughout the brain with the highest expression in the cerebral cortex, hippocampus and spinal cord, while it was virtually absent in the cerebellum. These data suggest that bFGF might be a growth factor for different CNS cell populations. In order to determine whether bFGF mRNA expression changes during development, we measured bFGF mRNA content during brain development. We found that in most of the brain regions, bFGF mRNA content is low at birth, but increases progressively reaching a plateau between 3 and 4 weeks after birth. This finding could be in line with the neurotrophic role of bFGF. However, in the brain of 3 month old rats, bFGF mRNA and bFGF protein content, unlike other neurotrophic factors, is equal to that of 4 weeks old rats. This result suggests that bFGF might have a potential role in neuronal maintenance. This hypothesis will be tested by determining whether bFGF mRNA expression declines in the brain of aged rats (supported by Farmitalia-Carlo Erba, Milan, as part of a join project with Center of Neuropharmacology, University of Milan, Italy).

CF 425 SPARC MODULATES CELL CYCLE PROGRESSION IN BOVINE AORTIC ENDOTHELIAL CELLS, H. Sage and S. Funk, Department of Biological Structure, University of Washington, Seattle, WA 98195.

SPARC (Secreted Protein, Acidic and Rich in Cysteine) is an extracellular, Ca^{+2}-binding protein associated with active cellular populations undergoing migration, proliferation, and/or differentiation. Active preparations of SPARC bind to specific components of the extracellular matrix and cause mesenchymal cells to assume a rounded phenotype (Sage, H. et al., (1989) JCB, **109**, 341). SPARC also modulates the progression of bovine aortic endothelial cells through the cell cycle. At a concentration of 20g/ml, SPARC inhibited the incorporation of [^{3}H] thymidine into newly-synthesized DNA by approximately 70%, as compared to control cultures, within 24 h after release from G_0. The effect was dose-dependent and reached greater than 90% inhibition at 30 µg SPARC/ml after 24 h. A 20-residue synthetic peptide (termed 2.1) from a non-Ca^{+2}-binding, disulfide-rich domain of SPARC also exhibited a dose-dependent inhibition of [^{3}H] thymidine uptake in endothelial cells within 24 h after release from G_0. An inhibition of 50% was seen with peptide 2.1 at a concentration of 0.4 mM. Peptides from other regions of the SPARC protein did not produce this effect. Maximum inhibition of [^{3}H] thymidine uptake by SPARC and peptide 2.1 occurred during the early-to-mid G_1 phase of the endothelial cell cycle. From 0-12 h after release from G_0, cells exhibited delayed entry into S-phase, which normally occured at 24 ± 2 h. These results were further corroborated by flow cytometry. In the presence of 20 µg/ml SPARC, there were 72% fewer cells in S-phase after a 24 h period; a similar but less marked reduction was seen with peptide 2.1. Peptide 2.1 did not cause cell rounding, while peptide 1.1, a highly efficient inhibitor of endothelial cell spreading, exhibited essentially no activity with respect to cell cycle progression. It therefore appears that the transient, inhibitory effect of SPARC on the entry of endothelial cells into S-phase does not depend on the overt changes in cell shape mediated through cytoskeletal rearrangement.

CF 426 LONG-TERM CULTURED AND FRESHLY ISOLATED KAPOSI'S SARCOMA CELLS EXPRESS ACTIVITY INDUCING VASCULAR PERMEABILITY. Shinsaku Sakurada, Shuji Nakamura, S. Zaki Salahuddin and Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892 and University of Southern California School of Medicine, Department of Hematoncology, Norris Hospital, 1441 Eastlake Avenue, Los Angeles, CA 90033

In patients with AIDS-associated Kaposi's sarcoma (AIDS-KS), edema, pleural effusion and continuous diarrhea are sometimes observed. The mechanism of this vascular hyperpermeability is not clear. We reported a novel system for long-term culture of AIDS-KS derived cells with the aid of factor(s) from activated CD4+ T-cell and the induction of angiogenesis by these cells in a nude mouse model system. Using this system, we tested the induction of vascular permeability. Cultured AIDS-KS cells induced a biphasic vascular permeability response with early histamine dependent phase (15 min) and late histamine independent phase (12 hr). This late phase was resistant to indomethacin, but sensitive to dexamethasone at high dose (10mg/kg). Concentrated conditioned medium derived from AIDS-KS cells also induced a similar biphasic vascular permeability response. Histamine independent late phase (1 hr) was appeared in a dose dependent manner and did not show prophylaxis. AIDS-KS cells freshly isolated from patient samples also induced angiogenesis as well as vascular permeability response. This indicated that AIDS-KS cells have already been activated in vivo. Data presented here suggest that soluble mediator(s) released by AIDS-KS cells may be involved in the edematous feature of KS lesions, and also add further support to our hypothesis that AIDS-KS cells release biologically active molecules which induce KS lesions in humans.

CF 427 MODULATION OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) ACTIVITIES ON ENDOTHELIAL CELLS BY THROMBOSPONDIN, Giulia Taraboletti, Dorina Belotti and Raffaella Giavazzi, Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, 24100, Italy

Platelet thrombospondin (TSP) is a high molecular weight multifunctional glycoprotein, found in plasma, associated to the cell surface and embedded in the extracellular matrix. It is released and interacts with platelets and a variety of cells, including endothelial and tumor cells. TSP induces adhesion and motility of endothelial cells as well as of tumor cells. Our study shows that soluble TSP (25 to 100μg/ml) inhibits serum- or bFGF-induced endothelial cells proliferation. TSP also modulates endothelial cells chemotactic response to bFGF. Infact TSP addition to bFGF in the lower compartment of the Boyden chamber at the concentration of 0.1μg/ml inhibits chemotaxis, while higher concentrations of TSP do not affect motility response induced by bFGF. The mechanism of the regulatory activity of TSP on bFGF and endothelial cells will be addressed.

CF 428 PENTOSANPOLYSULFATE INHIBITS KAPOSI-FGF DEPENDENT TUMOR GROWTH, Anton Wellstein, Gerhard Zugmaier, Joseph A. Califano III, Soonmyoung Paik and Marc E. Lippman, Lombardi Cancer Center, Georgetown University, Washington DC 20007

A neoangiogenic response is critical for the unrestricted growth of solid tumors beyond a few millimeters in diameter. Release of adequate growth stimulating activity from tumor cells is obviously required for the stimulation of blood vessel growth and blockade of such stimulatory activity should repress tumor growth at microscopical level. To test this hypothesis and to study appropriate inhibitors, we used a cell line (SW-13) engineered to secrete Kaposi's Sarcoma FGF (SW-13/K-fgf) and thus grows into highly vascularized subcutaneous tumors in animals (WELLSTEIN et al. (1990): Cell Growth and Diff 1:63-71). Different polysulfates were studied for their selective inhibition of K-FGF induced versus growth factor-independent proliferation. Suramin and detransulfate showed slightly selective inhibition (3- and 5-fold respectively), whereas heparin was inactive. The heparin analogue pentosanpolysulfate (PPS), however, was > 1,000-fold selective and PPS growth inhibitory effects on SW-13/K-fgf as well as endothelial cells were fully reversible by an excess of added FGF. Daily i.p. injections of PPS were tolerated well by athymic nude mice and prevented growth of subcutaneous SW-13/K-fgf tumor xenografts. In contrast, heparin treatment of the animals showed no effect on tumor growth. PPS will be a useful tool to elucidate effects of FGFs *in vitro* and *in vivo* and appears to be a prototype for the development of tumoricidal therapy based on growth factor targeted tumor therapy.

CF 429 THE C-ETS-1 PROTOONCOGENE IS EXPRESSED IN THE ENDOTHELIUM DURING ANGIOGENESIS, UNDER NORMAL AND PATHOLOGICAL CONDITIONS, Nicolas Wernert, Bernard Vandenbunder, Marie-Berthe Raes, Bernard Gosselin and Dominique Stéhelin, Unité d'Oncologie moléculaire, INSERM U 186, CNRS URA 1160, Institut Pasteur, 59019 Lille Cedex, Service d'anatomie et de cytologie pathologique C, CHU de Lille, 59037 Lille Cedex, France

We have shown recently (Nature, July 1990, 346, 191-193) that the c-ets proteins are transcriptional activators which bind to a PEA3 motif found in the polyoma enhancer.We examined the expression of c-ets-1 by *in situ* hybridization in angiogenesis under different conditions in man. c-ets-1 is expressed in the endothelia of developing vessels of 8-10 weeks old embryos while mature vessels in adult tissues practically never express c-ets-1. An expression is likewise seen in the developing capillaries of granulation tissue as well as in the stromal capillaries during the vascularization of different begin and malignant tumors. In culture, endothelial cells of bovine brain capillaries express c-ets-1 when they are in a preconfluent state. After confluency the expression diminishes for about 10-fold. Therefore c-ets-1 might regulate migration and/or proliferation of endothelial cells. Alternatively secretion of proteinases could be controlled as a PEA3-motif plays a role in the stimulation of transcription of the u-plasminogen-activator and the collagenase gene.

CF 430 CAPILLARY ENDOTHELIAL-CELL CHEMOTACTIC FACTORS IN PLATELET-DERIVED WOUND-HEALING FORMULA (PDWHF, CT102-APST), Robert C. Wohl and Ronald Duff, Curative Technologies, Inc., Setauket, NY 11733, and Vance D. Fiegel, Department of Surgery, University of Minnesota, Minneapolis, MN 55455.
Capillary Endothelial-Cell migration is critical to the angiogenesis process and consequently to wound healing. We routinely use the Boyden chamber chemotaxis assay to measure the specific effects of growth factors on Rabbit Wound Capillary Endothelial Cells (RWCEC). In order to define the role of growth factors in PDWHF for stimulation of chemotactic activity of RWCEC, we tested EGF, TGFa, FGFa, FGFb, TGFb, BTG, CTAPIII, NAP-2 and thrombin, of which TGFb and CTAPIII are known to be present in PDWHF. None of these factors cause RWCEC chemotaxis. These factors were also ineffective as inhibitors of chemotaxis. PF4 did induce 50% inhibition of chemotaxis at concentrations of 25-125 μg/mL. Commercially obtained Heparin does not affect chemotaxis by itself, or in combination with any growth factors. About 90% of the chemotactic activity in PDWHF is induced by PDGF, specifically, by its B chain. The rest of the chemotactic response in PDWHF is caused by a platelet-derived angiogenesis factor which has not yet been fully characterized. Our results indicate that directed endothelial cell migration is regulated by multiple growth factors and possibly, by as yet unspecified inhibitors.

CF 431 A NOVEL PROTEIN KINASE INHIBITOR BLOCKS IN VITRO ANGIOGENESIS, Paul S. Wright, Doreen Cross-Doersen and Alan J. Bitonti, Merrell Dow Research Institute, Cincinnati, OH 45215
Tube formation on basement membrane preparations, Matrigel, has been used as an in vitro model of angiogenesis because the endothelial cells migrate through the matrix and differentiate into capillary-like structures. Inhibitors of cellular kinases prevented capillary-like tube formation by human umbilical vein endothelial cells (HUVEC) on Matrigel. One of the best inhibitors of this process was MDL 27032 (4-propyl-5-(4-pyridinyl)-2(3H)-oxazolane) which has been previously shown to inhibit protein kinase C (PKC) and cAMP dependent protein kinase (PKA) in vein cytosolic extracts. Pretreatment of HUVEC for 18-20 hr with 100 μM MDL 27032 completely inhibited tube formation on Matrigel; 50 μM was the estimated IC_{50}. These concentrations of the compound did not inhibit [^{3}H]-thymidine incorporation into HUVEC DNA over a 24 hr period, and thus were not cytotoxic during the experiment. A 4-methyl analog of MDL 27032 (MDL 27044) which is ineffective as an inhibitor either for PKA or PKC was much less effective in preventing tube formation of HUVEC on Matrigel (IC_{50} > 100 μM). MDL 27032 (50 μM) inhibited neovascularization in yolk sac membranes of chick embryos, whereas MDL 27044 (50 μM) had only a slight inhibitory effect. These studies suggest that the PKA/PKC inhibitor, MDL 27032, has anti-angiogenic activity as it can disrupt both HUVEC differentiation into capillary-like structures on basement membrane preparations and normal neovascularization in ovo.

Late Abstracts

A NEW QUANTITATIVE METHOD FOR ASSESSING ANGIOGENESIS AND ANTI-ANGIOGENIC AGENTS USING RECONSTITUTED BASEMENT MEMBRANE, HEPARIN, AND FGF. Antonino Passaniti, Peter V. Long, Joseph A. Haney, Rebecca R. Pauly, and George R. Martin. National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore, MD 21224.
Angiogenesis is critical to normal development, growth, and wound healing and participates in many disease processes such as tumor growth and atherosclerosis. A number of useful models for studying angiogenesis have been developed by implanting various angiogenic agents or encapsulated tumor cells in the cornea of rabbits or the chorioallantoic membrane of the chick. However, these models are difficult to quantitate. For this reason, we have developed a new angiogenesis model which allows quantitative analysis of angiogenic and anti-angiogenic factors. A solution of basement membrane proteins (Matrigel) was mixed with FGF and heparin and injected subcutaneously into mice. Under these conditions, Matrigel forms a solid gel which slowly releases FGF. Invasion of blood vessels was apparent within12 hours and was maximal at 48 hours. Neovascularization persisted up to 7 days after injection. In this system, 1-10 ng/ml FGF and 16-64 U/ml heparin induced a massive ingrowth of blood vessels which could be assessed quantitatively using a sensitive hemoglobin assay. Attempts to culture infiltrating cells from these blood vessels resulted in the isolation of primarily macrophages and endothelial cells. Heparin and FGF are essential component of the Matrigel mixture since no angiogenesis was observed when FGF or heparin was excluded. The addition of primary cultures of smooth muscle cells to the matrix in the presence of FGF and heparin inhibited the development of angiogenesis. Alterations in the angiogenic response were observed when induced in old hosts and in response to various anti-angiogenic factors. This system offers a rapid quantitation of angiogenesis and will allow analysis of the role of aging in the vascular response to tumors, diabetes, and atherosclerosis.

FGF, Endothelial Cell Growth Factors and Angiogenesis

A NEW QUANTITATIVE METHOD FOR ASSESSING ANGIOGENESIS AND ANTI-ANGIOGENIC AGENTS USING RECONSTITUTED BASEMENT MEMBRANE, HEPARIN, AND FGF.

Antonino Passaniti, Peter V. Long, Joseph A. Haney, Rebecca R. Pauly, and George R. Martin. National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore, MD 21224.

Angiogenesis is critical to normal development, growth, and wound healing and participates in many disease processes such as tumor growth and atherosclerosis. A number of useful models for studying angiogenesis have been developed by implanting various angiogenic agents or encapsulated tumor cells in the cornea of rabbits or the chorioallantoic membrane of the chick. However, these models are difficult to quantitate. For this reason, we have developed a new angiogenesis model which allows quantitative analysis of angiogenic and anti-angiogenic factors. A solution of basement membrane proteins (Matrigel) was mixed with FGF and heparin and injected subcutaneously into mice. Under these conditions, Matrigel forms a solid gel which slowly releases FGF. Invasion of blood vessels was apparent within12 hours and was maximal at 48 hours. Neovascularization persisted up to 7 days after injection. In this system, 1-10 ng/ml FGF and 16-64 U/ml heparin induced a massive ingrowth of blood vessels which could be assessed quantitatively using a sensitive hemoglobin assay. Attempts to culture infiltrating cells from these blood vessels resulted in the isolation of primarily macrophages and endothelial cells. Heparin and FGF are essential component of the Matrigel mixture since no angiogenesis was observed when FGF or heparin was excluded. The addition of primary cultures of smooth muscle cells to the matrix in the presence of FGF and heparin inhibited the development of angiogenesis. Alterations in the angiogenic response were observed when induced in old hosts and in response to various anti-angiogenic factors. This system offers a rapid quantitation of angiogenesis and will allow analysis of the role of aging in the vascular response to tumors, diabetes, and atherosclerosis.

SMOOTH MUSCLE CELLS INVADE RECONSTITUTED BASEMENT MEMBRANE AND INHIBIT ANGIOGENESIS: IMPLICATIONS FOR THE DEVELOPMENT OF ATHEROSCLEROSIS.

Rebecca R. Pauly, Linda Cheng, Edward G. Lakatta, and Antonino Passaniti. National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore, MD 21224.

Atherosclerosis is a complex disease involving the migration and proliferation of smooth muscle cells (SMC). In addition to SMC, platelets, endothelial cells (EC), and monocytes-macrophages and their factors have been implicated in the development and progression of the lesion. Normally, SMC residing within the media contain abundant contractile proteins and are nonproliferating (differentiated). In response to injury, SMC migrate from the media to the intima where they intertact with extracellular matrix (ECM) and EC. We have investigated the relationship between SMC differentiation and the motility, invasiveness, and interaction of these cells with ECM. SMC from rat aorta readily proliferated on tissue culture plastic in response to serum. SMC showed a strong chemotactic response to PDGF in Boyden chamber assays and were also able to invade through a reconstituted basement membrane barrier (Matrigel). Their migration in both assays was inhibited by TGF-β. In contrast, SMC cultured on Matrigel ceased proliferating and migrated to form multi-cellular cord-like structures. The ability to migrate and form cords was inhibited by cycloheximide. The cells rapidly resumed proliferation when re-plated on plastic. The interaction of SMC and EC was studied using a newly developed *in vivo* quantitative angiogenesis assay. We found that SMC in the presence of FGF, heparin, and Matrigel induced a 10-fold decrease in the extent of angiogenesis compared to controls. We conclude that (1) ECM is a regulator of SMC differentiation and proliferation, (2) SMC have the ability to invade ECM, and (3) SMC alter the behavior and proliferation of EC.

A NOVEL PROTEIN KINASE INHIBITOR BLOCKS IN VITRO ANGIOGENESIS, Paul S. Wright, Doreen Cross-Doersen and Alan J. Bitonti, Merrell Dow Research Institute, Cincinnati, OH 45215

Tube formation on basement membrane preparations, Matrigel, has been used as an in vitro model of angiogenesis because the endothelial cells migrate through the matrix and differentiate into capillary-like structures. Inhibitors of cellular kinases prevented capillary-like tube formation by human umbilical vein endothelial cells (HUVEC) on Matrigel. One of the best inhibitors of this process was MDL 27032 (4-propyl-5-(4-pyridinyl)-2(3H)-oxazolane) which has been previously shown to inhibit protein kinase C (PKC) and cAMP dependent protein kinase (PKA) in vein cytosolic extracts. Pretreatment of HUVEC for 18-20 hr with 100 μM MDL 27032 completely inhibited tube formation on Matrigel; 50 μM was the estimated IC_{50}. These concentrations of the compound did not inhibit [^{3}H]-thymidine incorporation into HUVEC DNA over a 24 hr period, and thus were not cytotoxic during the experiment. A 4-methyl analog of MDL 27032 (MDL 27044) which is ineffective as an inhibitor either for PKA or PKC was much less effective in preventing tube formation of HUVEC on Matrigel (IC_{50} > 100 μM). MDL 27032 (50 μM) inhibited neovascularization in yolk sac membranes of chick embryos, whereas MDL 27044 (50 μM) had only a slight inhibitory effect. These studies suggest that the PKA/PKC inhibitor, MDL 27032, has anti-angiogenic activity as it can disrupt both HUVEC differentiation into capillary-like structures on basement membrane preparations and normal neovascularization in ovo.