GROWTH INHIBITORY AND CYTOTOXIC POLYPEPTIDES

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Plenary Sessions

Poster Sessions

Transforming Growth Factor-Beta and Related Factors - I

D 001 THE CONTROL OF PATTERN FORMATION BY DECAPENTAPLEGIC, A TGF-ß HOMOLOGUE IN *DROSOPHILA*, William M. Gelbart, Ronald K. Blackman, Richard W. Padgett, R. Daniel St. Johnston, Laurel A. Raftery, Holly A. Irick, Leila M. Posakony, M. Macy D. Koehler, Seth Findley and Robert Ray, Dept. of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138.

Decapentaplegic (*dpp*) is a >45 kb gene in the fruit fly, *Drosophila melanogaster*, which encodes a single polypeptide product controlling several morphogenetic events. As inferred from cDNA sequence analysis, this product is a member of the transforming growth factor-ß (TGF-ß) family of secreted factors. *The organization of the decapentaplegic gene.* The *dpp* gene is divided into three regions, which map in the order dpp^{shv} -- dpp^{Hin} -- dpp^{disk}. The entire polypeptide coding capacity of *dpp* is contained within the 6-7 kb of the dpp^{Hin} region; this region also contains sufficient transcriptional and regulatory information to drive all *dpp* expression necessary for survival through the embryonic period. On the 5′ side of this region is the dpp^{shv} interval; this region, which is >11 kb contains several alternative promoters and 5′ untranslated exons, all of which join with a common splice acceptor site to encode the identical polypeptide. The remainder of the dpp^{shv} region contains 5′ *cis*-regulatory sequences. All transcripts terminate within dpp^{Hin}. Immediately on the 3′ side of dpp^{Hin} is a 4 kb region containing two tyrosyl-tRNA genes. Their role, if any, in *dpp* expression is uncertain. Even further 3′ is the dpp^{disk} region, which contains >25 kb of 3′ tissue-specific *cis*-regulatory elements. Thus, *dpp* consists of a relatively small but complex transcription unit which is driven by *cis*-regulatory information spread over >45 kb of DNA. *The developmental roles of the decapentaplegic product.* These *cis*-regulatory elements modulate the expression of the putative secreted TGF-ß-like factor to control several events in pattern formation; most of these involve specification of epidermal patterns. These include determination of dorsal vs. ventral ectoderm in the early embryo, and proximal-distal patterning as well as other aspects of the morphogenesis of the adult appendages derived from the imaginal disks of the larva. Analyses of genetic mosaics and transcript localization suggest that the *dpp* product is required only in subsets of imaginal disk cells. In the best studied disk (the wing disk), this subset of cells appears to coincide with a lineage restriction called the anterior/posterior compartment boundary. *In situ* localization of transcripts suggests the possibility that proper *dpp* expression might also required for normal development of the digestive system. Recent observations on the structure of *dpp*, its regulation and its developmental role will be discussed.

D 002 TGF-BETA RECEPTORS AND ACTIONS, Joan Massagué, Sela Cheifetz, Ronald Ignotz, Frederick Boyd, and Anna Bassols. Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01655

TGF-ß is representative of a complex family of structurally-related growth and differentiation factors. Eight of the ten known TGF-ß related factors are the result of homo- and heterodimeric combination of six different mammalian gene products all present in humans. The TGF-ß subgroup in this family includes TGF-ß1, TGF-ß2 and the heterodimer , TGF-ß1.2. The activin/inhibin subgroup has four members, and the MIS subgroup has just one. The interaction of TGF-ßs with cells occurs via a set of structurally and functionally distinct cell surface receptors with K_d = 10-50 pM. Two of the three TGF-ß receptor types are glycoproteins of 53 K (type I) and 73 K (type II) that bind TGF-ß1 10-20 times better than TGF-ß2. The third TGF-ß receptor type is a large glycoprotein (∿300K) with a 100-120 K protein core, and a similar affinity for TGF-ß1 and TGF-ß2. Practically all cells tested exhibit at least one of these three TGF-ß receptors types.

Certain hematopoietic progenitor cells exhibit only type I TGF-ß receptors. TGF-ß1 inhibits potently the proliferation of these cells in response to colony stimulating factors (Multi-CSF, GM-CSF). TGF-ß2 which binds poorly to type I receptors has only ∿1% of the potency of TGF-ß1. Thus, occupancy of type I TGF-ß receptors correlates with inhibition of hematopoietic progenitor cell proliferation. Occupancy of type III TGF-ß receptors correlates with inhibition of epithelial cell proliferation, adipogenic and myogenic differentiation, and stimulation of chondrogenesis because TGF-ß1 and TGF-ß2 are equally potent at exerting these actions. The expression of cell adhesion proteins is also regulated by the TGF-ßs via type III receptors. The effect on cell adhesion receptors occurs at pre- and post-translational levels and results in enhanced adhesion of cells to extracellular fibronectin and collagen substrata. The effect of TGF-ß on chondroitin sulfate proteoglycans occurs also at two levels: enhanced expression of the proteoglycan core protein and increased size of the glycosaminoglycan chains. The effects of TGF-ß on cell adhesion proteins and receptors may play an important role in the regulation of expression of adipogenic, myogenic and "transformed" phenotypes.

None of the three TGF-ß receptor types appears to recognize activins or inhibins, but a fourth receptor type might exist which crossreacts with these factors and TGF-ß. It is also possible that the loss of growth inhibitory responsiveness to TGF-ß in certain human tumors might be due to loss of TGF-ß receptors. These possibilities are currently under study.

D 003 TRANSFORMING GROWTH FACTOR-BETA AND CONTROL OF EMBRYOGENESIS, TISSUE REPAIR, AND CARCINOGENESIS, Anita B. Roberts, Kathleen C. Flanders, Ursula I. Heine*, and Michael B. Sporn, Laboratories of Chemoprevention and *Comparative Carcinogenesis, National Cancer Institute, Bethesda, MD 20892.

Transforming growth factor-beta (TGF-beta) is often characterized as a growth inhibitor based on its ability to potently inhibit the growth of a variety of cultured cells including epithelial cells, fibroblasts, lymphocytes, endothelial cells, and hepatocytes. It also can block the action of many different mitogens acting on these cells, such as the actions of IL-1, EGF and PDGF on epithelial or fibroblastic cells, the action of IL-2 on lymphocytes, and the action of FGF on endothelial cells. Yet, under appropriate conditions, TGF-beta can synergize with these same mitogens to stimulate growth. Thus alterations in the culture conditions, in the age or state of differentiation of the cells, or in the particular set of growth factors acting together with TGF-beta, can result in a "switching" of the direction of the TGF-beta effect. Perhaps it is because of the ability of this peptide to swing the pendulum of growth and differentiation that the distribution of its receptors is nearly universal. It might be expected that a molecule with these properties might play a pivotal role in embryogenesis where programs of growth and differentiation must be turned on and off in an orchestrated fashion. Indeed, TGF-beta mRNA is expressed at high levels throughout embryogenesis in the mouse. Immunohistochemical studies of TGF-beta distribution in early mouse embryos demonstrate that the peptide is closely associated with tissues derived from mesenchyme, such as connective tissue, cartilage, and bone. TGF-beta is also conspicuous in tissues derived from neural crest mesenchyme, such as the palate, larynx, facial mesenchyme, nasal sinuses, meninges, and teeth. Staining in all of these tissues is greatest during periods of morphogenesis or when remodeling of mesoderm occurs, such as during the formation of digits from limb buds, formation of the palate, and formation of the heart valves. The ability of TGF-beta to regulate synthesis of extracellular matrix proteins such as proteoglycans, fibronectin, and types I, II, and V collagen and to control proteolysis of these proteins is probably central to many of its developmental effects. These same mechanisms are reiterated in repair and remodeling processes in adult tissues and aberrantly in pathological processes such as carcinogenesis.

1. Sporn, M. B., et al. (1987). J. Cell Biol. 105, 1039-1045.
2. Heine, U. I., et al. (1987). J. Cell Biol. 105, in press.

D 004 A LOCALIZED XENOPUS MATERNAL mRNA ENCODES A TGF-BETA-LIKE FACTOR D. Tannahill, D. L. Weeks and D. A. Melton, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138.

Our recent studies demonstrate that Xenopus eggs contain a small number of maternal mRNAs localized to either their animal or vegetal poles. One such RNA, called Vg1, that is localized to the vegetal pole of oocytes and eggs has been of special interest. Vg1 RNA is made early in oogenesis and then is translocated to the vegetal pole to form a tight crescent in mature oocytes. As a consequence of its vegetal location in oocytes, Vg1 RNA is inherited by the endodermal cells of a cleaving fertilized egg. Vg1 RNA is present in the cleaving embryo throughout the blastula stage after which time Vg1 RNA is destroyed. Sequence analysis shows that Vg1 encodes a protein of 360 amino acids (~41.8kd). Homology searches have revealed that the carboxyl terminal 120 amino acids show significant similarity to members of the TGF-B protein family, the greatest similarity (~48%) being with the sequence of the Decapentaplegic locus of Drosophila. Most striking are the presence of 7 conserved cysteine residues presumably required for dimer formation. The putative Vg1 protein also has a potential signal sequence for insertion into the ER and a potential cleavage site for release of the last 114 carboxyl terminal amino acids. Therefore we predict that the Vg1 protein may encode a small secreted growth factor similar to the TGF-B family of polypeptides. Mesoderm induction in Xenopus is thought to involve a signal being released from vegetal cells to which animal cells can respond such that they are able to change their fate. This together with the fact that heterologous growth factors can induce mesoderm in isolated animal caps suggests that Vg1 may play a role in the formation of mesoderm.

Transforming Growth Factor-Beta and Related Factors - II

D 005 MULLERIAN INHIBITING SUBSTANCE: A FETAL GROWTH INHIBITOR WITH HOMOLOGY TO TGF-β, Richard L. Cate, R. Blake Pepinsky, Lesley K. Sinclair, Elizabeth G. Ninfa, Sheryl L. Haletky, Kashi Ramesh, and Ray Rezaie, Biogen Research Corp., Cambridge, MA 02142.
Mullerian Inhibiting Substance (MIS) is a testicular glycoprotein expressed early in the sexual develoment of the male embryo. MIS causes regression of the Mullerian duct which, in the normal female embryo, develops into the uterus, vagina, and Fallopian tubes. MIS is synthesized and secreted as a 140 kd dimer composed of two 70 kd monomeric subunits. The bovine and human genes for MIS have now been isolated, and sequence analysis has revealed a highly conserved C-terminus that shows marked homology with TGF-β and other members of the TGF-β family. Unlike TGF-β, which is proteolytically cleaved and subsequently activated by the release of the 25 kd C-terminal domain, MIS apparently does not require processing to be active. Recombinant human MIS (140 kd dimer) produced by CHO cells containing the human MIS gene causes regression of the rat Mullerian duct in vitro. However, MIS may be processed and activated in the environment of the Mullerian duct. We are investigating this possibility by generating fragments of MIS with various proteases. Various constructs encoding for different domains are also being generated and expressed in CHO cells. The results of these experiments and other aspects of MIS synthesis and structure will be discussed.

D 006 MULLERIAN INHIBITING SUBSTANCE: A TYROSINE KINASE INHIBITOR WITH ANTICANCER EFFECTS.
Patricia K. Donahoe, Pediatric Surgical Research Laboratory, Massachusetts General Hospital, Boston, MA 02114
Mullerian Inhibiting Substance, a fetal regressor originally purified from mammalian testicular Sertoli cells and more recently found in Granulosa cells of the ovary, has a profound effect on the developing Mullerian duct where it causes a series of Mesenchymal-epithelial morphologic changes resulting in complete desolution of the Mullerian duct. This substance was purified to homogeneity from bovine sources and tryptic digests sequenced and used to synthesize oligonucleotide probes which successfully screened a cDNA library made from bovine mRNA to detect a bovine MIS cDNA. This then was used to screen a human genomic library from which was selected a MIS genomic clone for human MIS. A construct inserted and amplified in CHO cells directs the production of human recombinant MIS which has been tested in a number of assays, where it has been shown to be identical to bovine MIS. The MIS producing CHO cells have been adapted to serum free conditions and human recombinant MIS purified to homogeneity. Human recombinant MIS caused regression of embryonic Mullerian duct in organ culture, inhibits phosphorylation of the EGF receptor in A-431 cells, inhibits meiosis in rat oocytes, and inhibits the growth of a human endometrial cell line implanted in the subrenal capsule of immunosuppreseed mice. MIS appears to initiate its effects via a receptor mediated event which results in minimal serine phosphorylation and complete inhibition of tyrosine phosphorylation on the EGF receptor, as indicated by whole cell assays using P^{32} in which the labeled EGF receptor is immunoprecipitated with EGF receptor antibody or antibody to phosphotyrosine, followed by acid hydrolysis and thin layer chromatography or high pressure liquid chromatography. Purification of the MIS receptor has proven difficult because of the inability to ^{125}I label MIS.

Preliminary studies using labeled anti-idiotypic antibody to MIS, however, indicate that a moiety of approximately 200,000 molecular weight may be part of the presumptive MIS receptor. This can now be confirmed with metabolically labeled MIS of sufficiently high specific activity. Human endometrial cells (HTB-111), after trypsinization into a single cell suspension, were compacted into a Fibrin-throbin clot, and 1 mm fragments implanted beneath the renal capsule of immunosuppressed mice. Human recombinant MIS injected in the tail vein or intraperitoneally after ingrowth of blood vessels had been shown to extend into the implanted tumor, caused inhibition of tumor growth. Use of this modified subrenal capsule assay will be extended to further demonstrate in vivo efficacy of MIS as an anticancer agent as the human recombinant MIS is amplified and sufficient material of adequate purity becomes available.

D 007 AUTOCRINE GROWTH INHIBITION OF HUMAN BREAST CANCER CELLS. Marc E. Lippman, Cornelius Knabbe, Gerhard Zugmaier, Lalage Wakefield, Kathleen Flanders, Anita Roberts, and Robert B. Dickson. Medical Breast Cancer Section, Medicine Branch and the Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.
The mechanisms by which growth inhibitory substances such as antiestrogens slow the growth of human breast cancer cells are of great interest. In previous work from our laboratory we demonstrated that trophic substances such as estrogens increased secretion of a group of stimulatory growth factors including insulin-like growth factor I, insulin-like growth II, transforming growth factorα, platelet derived growth factor, and an epithelial cell stimulating activity. Secretion of these activities was inhibited by antiestrogens. We wonder whether antiestrogens could induce the release of growth inhibitory substances. We developed a serum-free assay system in which conditioned media from hormone-dependent cells treated with antiestrogen was assayed on hormone independent, estrogen receptor negative, human breast cancer cells which are intrinsically unresponsive to antiestrogens. Growth inhibitory acitivity was found and after extensive purification much of the activity was found to be due to transforming growth factorβ. Synthesis and secretion of transforming growth factorβ by human breast cancer cells was shown by radioreceptor analysis, immunoprecipitation, bioassay, and specific messenger RNA production. Positive regulation of transforming growth factor activity was shown by growth inhibitory stimuli such as serum starvation, antiestrogen treatment, and glucocorticoids whereas growth stimulation by estrogens and growth factors lowered transforming growth factorβ secretion. Inhibition by exogenously added transforming growth factorβ or autocrine inhibition was reversed by specific antibodies directed against transforming growth factorβ. Thus, transforming growth factorβ appears to be an appropriately regulated autocrine growth inhibitor of human breast cancer cells <u>in vitro</u>. The role of transforming growth factorβ as an <u>in vivo</u> regulator of neoplastic progression is under investigation.

Tumor Necrosis Factor

D 008 STRUCTURE AND FUNCTION OF THE TNF RECEPTOR, Abla A. Creasey, Ralph Yamamoto*, and Charles R. Vitt, Departments of Cell Biology and *Protein Chemistry, Cetus Corporation, Emeryville, CA 94608.
Tumor Necrosis Factor (TNF), a 17 kDa polypeptide hormone from macrophages and other cell types, is a pleiotropic cytokine with cytotoxic, growth proliferative and immunomodulatory activities. Human and mouse recombinant TNF have been labeled with ^{125}I to characterize their interaction with cellular receptors on cells that respond to the various biological activities of this factor. Receptor number did not correlate with cytotoxicity on TNF sensitive and resistant tumor cells, growth proliferation activity on fibroblasts and on activated granulocytes. However, a threshold receptor number was necessary for a response. Using ^{125}I human recombinant TNF (^{125}IHurTNF) and a bifunctional crosslinking agent 1,5, difluoro-2,4-dinitrobenzine, we found that ^{125}I HurTNF was preferentially crosslinked to four cellular polypeptides of molecular weight 54 kDa, 75kDa, 95kDa and 138 kDa in two breast carcinoma cell lines (MCF-7 and BT-20). In contrast, ^{125}I HurTNF was preferentially crosslinked to only three (54,75, and 95 kDa) of the four polypeptides on TNF resistant tumor cell lines, (MCF-7R, SKBR3) and normal fibroblasts (Hs27F and GM2504). ^{125}I mouse recombinant TNF was also preferentially crosslinked to three polypeptides 54, 75 and 95 kDa on mouse L929S and human MCF-7 cells. The migration of crosslinked polypeptides from human cells on SDS gels did not change appreciably under reducing and nonreducing conditions however those crosslinked on mouse cells migrated faster under reducing conditions. These results suggest that the TNF receptor is complex, may be composed of multiple subunits, three of these subunits are somewhat conserved between human and mouse cells and that one (138 kDa) may be associated with TNF cytotoxicity in human cells.

D 009 PLEIOTROPIC ACTIVITIES OF TUMOR NECROSIS FACTOR (TNF). Jan Vilček, Jian-Xin Lin, Vito Palombella, Rena Feinman, Yihong Zhang, Luiz F.L. Reis and Junming Le, Department of Microbiology, New York University Medical Center, New York, NY 10016.
TNF is cytostatic/cytotoxic for many tumor cell lines and this activity is important for the monocyte-mediated killing of tumor cells (1). However, the actions of TNF on cell growth vary, depending on the target cell, TNF dose and culture conditions. TNF was found to be mitogenic in the diploid human FS-4 cells (2), in BALB/c 3T3 cells, rat NRK cells, murine plasmacytoma cells and in a cloned line of human A673 rhabdomyosarcoma cells. Addition of TNF to quiescent FS-4 cells produced a rapid and transient stimulation of c-fos and c-myc mRNA levels (3). The kinetics of c-myc mRNA stimulation were different from those seen with serum, EGF or activators of protein kinase C, but they were similar to those seen with the adenylate cyclase activator, forskolin. Combined treatment of growth-arrested FS-4 cells with TNF and EGF produced a strong synergistic stimulation of DNA synthesis, suggesting that TNF substantially increased the number of cells entering S phase when added in combination with EGF. This synergism between TNF and EGF may be related to the earlier demonstrated stimulation of EGF receptor synthesis and expression by TNF (4). A similar degree of synergism was seen between TNF and PDGF. Mitogenic action was also seen with TNF in quiescent BALB/c 3T3 cells and this effect was synergistic with either EGF or PDGF. However, in 3T3 cultures TNF was also cytotoxic; this cytotoxic action was dose-dependent and more pronounced in subconfluent, cycling cells than in quiescent cells. TNF was also shown to protect quiescent FS-4 cells from infection with EMC virus (5). Recent evidence suggests that this antiviral action of TNF is mediated by "classical" interferon(IFN)-beta and not by the so-called IFN-beta$_2$ (a.k.a. BSF-2, 26 K protein or IL-6). Since a preparation of recombinant human IFN-beta$_2$/BSF-2 showed no stimulatory or inhibitory effect on DNA synthesis in FS-4 fibroblasts, it is likely that classical IFN-beta, and not "IFN-beta$_2$", acts as an autocrine negative regulator of growth in this cell system.

(1) Urban et al. (1986) PNAS 83, 5233; Philip and Epstein (1986) Nature 323, 86; Feinman et al. (1986) J. Immunol. 138, 635.
(2) Vilček et al. (1986) J. Exp. Med. 163, 632.
(3) Lin and Vilček (1987) J. Biol. Chem. 262, 11908.
(4) Palombella et al. (1987) J. Biol. Chem. 262, 1950.
(5) Kohase et al. (1986) Cell 45, 659.

Retinoblastoma and Wilm's Tumor Genes

D 010 MOLECULAR GENETICS OF CANCER PREDISPOSITION, Marc F. Hansen and Webster K. Cavenee, Ludwig Institute for Cancer Research, (Montreal Branch), 687 Pine Ave., West, Montreal Quebec H3A 1A1 Canada.
A strong case for a genetic diathesis in human cancer can be made through the molecular analysis of familial aggregations of mixed tumor types. This is particularly true for childhood cancers which have a very low incidence when compared to that of adult cancer and for which common environmental factors appear to play little etiological role. In studies designed to apply molecular genetic analysis toward defining the lesions which predispose to human cancer, we found that the clinically associated tumors retinoblastomas and osteosarcoma share a pathogenetic mechanism entailing aberrant chromosomal segregation events during mitosis which lead to tumor cells homozygous for recessive mutant alleles at the RB1 locus on human chromosome 13 band q14. Whether the RB1 locus is composed of a single gene or several closely related genes is examined using Field Inversion Gel Electrophoresis technology. This combination of analyses suggest that a rational explanation for the sequential occurrence in these children of two different tumor types is the initial inheritance of a predisposing recessive mutation.

D 011 CHARACTERIZATION OF THE HUMAN RETINOBLASTOMA SUSCEPTIBILITY GENE AND ITS ENCODED PROTEIN: Wen-Hwa Lee, Jin-Yuh Shew, Frank Hong, Robert Bookstein, Lih-Jiuan Young, and Eva Y-H. P. Lee, Experimental Pathology Program and Center for Molecular Genetics, Department of Pathology, M-012, University of California, San Diego, La Jolla, CA 92093.

Recent evidence indicates the existence of a genetic locus in chromosome 13q14 that confers susceptibility to retinoblastoma, a cancer of the eye in children. A gene encoding a messenger RNA (mRNA) of 4.6 kilobases (kb), located in the proximity of esterase D, was identified as the retinoblastoma susceptibility (RB) gene on the basis of chromosomal location, homozygous deletion, and tumor-specific alterations in expression. Transcription of this gene was abnormal in six of six retinoblastomas examined: in two tumors, RB mRNA was not detectable, while four others expressed variable quantities of RB mRNA with decreased molecular size of about 4.0 kb. In contrast, full-length RB mRNA was present in human fetal retina and placenta, and in other tumors such as neuroblastoma and medulloblastoma. DNA from retinoblastoma cells had a homozygous gene deletion in one case and hemizygous deletion in another case, while the remainder were not grossly different from normal human control DNA. The gene contains at least 18 exons distributed in a region of over 200 kb. Sequence analysis of complementary DNA clones yielded a single long open reading frame that could encode a hypothetical protein of 928 amino acids. To further substantiate and identify the RB protein, we have prepared rabbit antisera against a trypE-RB fusion protein. A purified anti-RB IgG immunoprecipitated a protein doublet with apparent molecular weight of 110-114 kD. The specific protein(s) were present in all cell lines expressing normal RB mRNA, but were not detected in all retinoblastoma cell lines examined. Antigenically related proteins were observed in different species such as quail, mouse, rat and monkey. Biochemical fractionation and immunofluorescence studies indicated that the majority of the protein was located within the nucleus. Furthermore, the RB protein can be metabolically labeled with 32P-phosphoric acid, indicating that it is a phosphoprotein. This protein was retained by and eluted from DNA cellulose columns, suggesting that it was associated with DNA binding activity. A hypothesis on mechanims of RB gene involvement in human oncogenesis was proposed.

Growth Factors: Modulation of Gene Expression

D 012 FIBROBLAST GROWTH FACTOR REGULATION OF SKELETAL MUSCLE GROWTH AND DIFFERENTIATION. Steve Hauschka, Jean Buskin, Jeff Chamberlain, Chris Clegg, Cyndy Gartside, Jim Jaynes, Jane Johnson, Pat Noel, Brad Olwin, and Jennifer Seed. Dept of Biochemistry, Univ. of Washington, Seattle, WA 98195.

Clonal analysis of the myogenic cell lineage during chick and human limb development has disclosed a variety of muscle colony-forming (MCF) cells. All MCF cells are FGF responsive although some will grow in the absence of FGF; others exhibit absolute dependence on FGF for either their clonal survival or their subsequent differentiation. In all cases FGF initially represses the onset of terminal differentiation. However, even in the continuous presence of FGF, differentiation eventually begins. This suggests that cell density or extracellular matrix components may trigger differentiation independently of FGF removal. In a model cell culture system using a permanent line of mouse skeletal muscle myoblasts (MM14) purified bovine acidic and basic FGF both stimulate proliferation and repress terminal differentiation. bFGF is about 30 times more potent than aFGF--with half-maximal activity in clonal assays of 1 and 30pM respectively. Addition of low or high molecular weight heparin in the 100ng-1mg/ml range inhibits bFGF-mediated effects whereas heparin stimulates aFGF-mediated effects in the 1ng-1ug/ml range, and then becomes inhibitory in the 3ug-1mg/ml range. Such differential heparin-FGF effects on myoblasts could serve as an alternative in vivo mechanism for controlling muscle growth and differentiation. When FGF is removed from the medium of proliferating MM14 myoblasts, G1-phase cells withdraw from the cell cycle and undergo an irreversible commitment to a post-mitotic phenotype and initiate transcription of muscle-specific genes. Within one cell cycle (12.5h) *the entire cell population becomes post-mitotic. One aspect of the post-mitotic* mechanism appears to be the irreversible loss of specific growth factor receptors. FGFR and EGFR both disappear from the cell surface within 24h of FGF removal. While this would explain how the myocyte post-mitotic state is maintained, a current paradox is that cell surface FGFR and EGFR actually increase 2-3 fold during the first 12h of FGF deprivation. Thus at a time when the entire cell population is already committed to a post-mitotic phenotype, cell surface growth factor receptors are still present. Further studies of this problem will be described. At the level of gene regulation, FGF represses muscle-specific gene expression independently of cellular proliferation. *Analysis of muscle-creatine kinase expression indicates that the gene is regulated via a tissue-*specific enhancer element that activates transcription in the absence of FGF. The intracellular signal pathway between cell surface FGFR and muscle nuclear proteins that bind specifically to the enhancer element is under investigation.

D 013 THE GENOMIC RESPONSE TO GROWTH FACTORS, K. Ryder, S. Hartzell, and D. Nathans, Howard Hughes Medical Institute Laboratory and Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Lau and Nathans (Proc. Natl. Acad. Sci. 84:1182-1186, 1987, reported the identification by cDNA cloning of 10 genes that are activated rapidly and transiently following exposure of quiescent BALB/c 3T3 mouse cells to serum, platelet-derived growth factor, or fibroblast growth factor. Inhibitors of protein synthesis superinduce these "immediate early" genes by causing prolonged gene transcription and stabilization of the corresponding mRNAs. In general, this set of genes is regulated coordinately with c-fos or c-myc.

cDNAs derived from the mRNAs of three immediate early genes have been sequenced. One cDNA encodes a protein that is rich in serine and proline. It does not have detectable homology to any protein whose sequence is available for comparison. A second cDNA encodes a protein whose amino acid sequence suggests that it is a transmembrane protein, i.e, it has a hydrophobic N-terminal segment typical of a signal peptide, a segment with four potential N-glycosylation sites, a highly hydrophobic stretch, and a short C-terminal tail. Its sequence is similar to that recently described for human tissue factor. (Spicer et al Proc. Natl. Acad. Sci. 84:5148-5152, 1987).

The third cDNA encodes a protein homologous to that encoded by the avian sarcoma virus 17 oncogene v-jun. (We refer to this immediate early gene as jun-B.) Homology between the jun-B and v-jun protein is in two regions: one near the N-terminus, and the other at the C-terminus. The latter was shown by Vogt et al. (Proc. Natl. Acad. Sci. 84:3316-3319, 1987) to have regions of sequence similarity to the DNA-binding domain of the yeast transcriptional regulatory protein GCN4 and to the oncogenic protein fos. Southern blots of human, mouse and chicken DNA indicate that jun-B and c-jun are different genes, and that there may be other vertebrate genes related to jun-B and c-jun. These findings suggest that there is a jun family of genes encoding related transcriptional regulatory proteins. The jun-B protein, and perhaps other members of the jun family, may play a role in regulating the genomic response to growth factors.

D 014 REGULATION OF GENE EXPRESSION BY PLATELET-DERIVED GROWTH FACTOR, Barrett J. Rollins, Patricia Oquendo and Charles D. Stiles, Harvard Medical School and the Dana-Farber Cancer Institute, Boston, MA 02115

Much attention has focused upon induction of the c-myc and c-fos proto-oncogenes by platelet-derived growth factor (PDGF); however PDGF stimulates expression of between 10-30 other genes in appropriate target cells. The structure of these other PDGF-inducible genes (which we have termed "competence" genes) and their function in the cellular response to PDGF are largely unknown. We have examined the structure and regulation of the original PDGF-inducible competence genes isolated from Balb/c-3T3 cells. Our data may provide insights into function of these genes. The PDGF-inducible "JE" and "KC" genes encode secretory proteins with cytokine-like properties. In addition, PDGF induces other cytokine-like proteins including MCSF, EPA/TIMP and interleukin-6 (also known as type 2 beta interferon and BSF 2). The data highlight another parallel between the response of T-lymphocytes to antigens and the response of fibroblasts to PDGF. Just as a T cells secrete a panel of interleukins as a component of activation, it appears that fibroblasts secrete a panel of cytokines in response to PDGF.

Interferons - I

D 015 INTERFERONS AS GROWTH INHIBITORS: TOWARD A MOLECULAR MECHANISM. Judith Chebath, Philippe Benech, Daniel Wayman, David Peretz, Batya Cohen and Michel Revel, Dept of Virology, Weizmann Institute of Science, Israel. The (2'-5') oligo A synthetase E is strongly induced by all interferon (IFN) species and is increased in differentiating cells undergoing terminal growth arrest as a result of autocrine IFN secretion. We identified the functional sequences from the promoters of the human and of two murine genes for this enzyme, by fusion to a CAT gene and transient expression in transfected human cells. IFN-∝, β, ɣ and autocrine IFN-β2 activated expression. A 72 bp segment of the human gene (E-IRS), immediately upstream of the multiple RNA starts was found to contain two required elements: "A" near the 5' end of the E-IRS acts as a constitutive enhancer for the Herpes TK promoter, while B in 3' functions as an IFN-activated enhancer. The sequence GGAAAN-GAAAC conserved in the human and mouse element B appears to be the target of IFN action. A synthetic element B oligonucleotide of 16bp binds specifically to a nuclear protein, the binding increases 2 hours after IFN and is altered at 12 hours. Promoter competition experiments indicate an additional repressor binding site downstream of the E-IRS. The (2'-5') oligo A synthetase cDNA encoding the 40 Kd form was fused to the SV40 early promoter and cotransfected with DHFRcDNA in CHO cells. After amplification, clones expressing the human enzyme constitutively at levels 500 fold higher than CHO control cells were isolated. Replication of the picornavirus Mengo was found to be decreased three logs in these CHO-E cells. The reduction was proportional to the enzyme level. VSV was not affected. The ability of these CHO-E cells to form colonies when plated at low densities was reduced. Plating in agar showed that while the CHO control cells produced anchorage independent colonies of large size, the CHO-E cells produced only few very small colonies. Thus, high expression of this IFN-induced enzyme appears to profoundly affect the growth properties of these transformed cells.

D 016 REGULATION OF EXPRESSION AND FUNCTIONS OF INTERFERON-INDUCIBLE GENES, Ganes C. Sen, Raj K. Tiwari, Rakesh Kumar and Jyotirmoy Kusari, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.
In contrast to other cell lines, in HeLaM cells induction of transcription of four mRNAs, 561, 2-5(A) synthetase, 1-8 and 6-16, by interferon-α needed ongoing protein synthesis. The process of induction could be operationally divided into two steps: only the first of which needed ongoing protein synthesis. The characteristics of the two IFN-generated signals, signal 1 and signal 2 which mediate these steps, were studied in depth for induction of mRNA 561. IFN-α could generate both signals but IFN-γ could generate only signal 1. As a result, this mRNA was not induced by IFN-γ alone or by IFN-α plus cycloheximide, an inhibitor of protein synthesis. Efficient induction was however observed in HeLaM cells which had first been treated with IFN-γ and then with IFN-α plus cycloheximide. In addition to IFN-α and IFN-γ , epidermal growth factor and platelet derived growth factors could also produce signals which were functionally equivalent to signal 1. On the other hand functional equivalents of signal 2 could be produced by double-stranded RNA. Several lines of evidence indicated that induction of mRNA 561 by dsRNA was not mediated through induction of IFN. The induced transcription of mRNA 561 is a transient process. It is diminished to an uninduced level after a few hours of IFN-treatment even if IFN-α is constantly present in the culture medium. In such a desensitized cell, the transcription of the gene can however be induced efficiently by dsRNA suggesting that this inducer can bypass the IFN-α -induced desensitization process. In Daudi cells, this desensitization process is inoperative causing a continuous synthesis of IFN- α-induced gene products. Some of these products may be toxic to the cells and their accumulation may be responsible for the profound anticellular effects of IFN-α on Daudi cells.
For correlating different IFN-induced cellular phenotypes with the products of different IFN-inducible genes we have been studying the expression of these genes in cell lines which are partially responsive to IFNs. In human RD-114 cells both IFN-α and IFN-γ inhibit retrovirus production but they do not inhibit replication of VSV or EMCV. In these cells mRNAs 561, 1-8 and 6-16 were induced normally by IFN-α suggesting that the products of these genes are not sufficient for inhibiting VSV or EMCV. Recently we have isolated two clonal derivatives of mouse JLSV-11 cells in one of which, but not in the other, EMCV replication is inhibited by IFN whereas VSV replication is inhibited by IFN in both lines. We are currently comparing the expression of the genes in the 2-5(A) synthetase/RNase L pathway in these two sister lines. Information obtained from these studies will be useful in assessing the role of this pathway in anti-EMCV action.

Growth Factors and Growth Inhibitors in Neoplasia

D 017 THE HUMAN trk LOCUS: A NOVEL TRANSMEMBRANE RECEPTOR, M. Barbacid, D. Martin-Zanca, R. Oskam, F. Coulier, and G. Mitra, BRI-Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701

trk is a new member of the tyrosine protein kinase family of hormone receptors. Nucleotide sequence analysis of trk cDNA clones revealed the three classical domains of growth factor receptors. The extracellular domain contains a putative signal peptide, 13 consensus N-glycosylation sites and 11 unclustered cysteines. The transmembrane region consists of a stretch of 26 hydrophobic amino acids. Finally, the 266-amino acid long kinase catalytic domain is followed by a short carboxy-terminal tail of 15 amino acids which includes a single tyrosine residue. The trk locus was first identified as part of an oncogene activated in a human colon carcinoma biopsy by a somatic rearrangement that fused a non-muscle tropomyosin gene with the transmembrane and catalytic domains of the trk proto-oncogene.

The primary translational product of the trk proto-oncogene is a glycoprotein of 110 kd with a polypeptide backbone of 79 kd. gp110^{trk} is further glycosylated to yield the mature product, gp140^{trk} which becomes anchored across the plasma membrane. Both gp140^{trk} and gp110^{trk} have in vitro autophosphorylating activity specific for tyrosine residues. The trk oncogene codes for a 70 kd molecule which also has an associated tyrosine kinase activity. p70^{trk}, unlike gp140^{trk}, does not appear to be associated with cellular membranes. Thus, suggesting that its transforming properties might be exerted by a mechanism involving phosphorylation of non-physiological substrates. To examine the role of tropomyosin sequences in the malignant activation of the trk oncogene. We have replaced them by other cytoskeletal (β-actin) or unrelated (β-globin) genes. The resulting chimeras completely failed to transform NIH3T3 cells in spite of directing the synthesis of proteins that retained tyrosine protein kinase activity. These results suggest that tropomyosin may contribute to the transforming properties of the trk oncogene by allowing p70^{trk} to interact with a specific subset of cytoplasmic substrates.

Finally, we have observed that trk proto-oncogene sequences can efficiently recombine in vitro to yield novel transforming genes. We have identified thirteen new trk oncogenes by transfecting NIH3T3 cells with nontransforming plasmids carrying either the tyrosine kinase domain or the entire coding sequences of the trk proto-oncogene. Each of these oncogenes expressed a novel trk-related protein of aberrant size with tyrosine protein kinase activity. Biochemical characterization of these proteins indicates that whereas some are located in the cytoplasm others are transmembrane proteins. These results raise the possibility that the trk proto-oncogene may serve as a marker to identify multiple substrates whose unscheduled phosphorylation may contribute to malignant transformation.

D 018 GROWTH, DIFFERENTIATION, AND NEOPLASTIC TRANSFORMATION OF HUMAN BRONCHIAL EPITHELIAL CELLS. Curtis C. Harris, Brenda Gerwin, Ke Yang, George Mark, Tohru Masui, Andrea Pfeifer, Roger Reddel, and John F. Lechner, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892

We are investigating the molecular and cellular mechanisms controlling growth and differentiation of human bronchial epithelial cells and the dysregulation of these controls during the multistage process of carcinogenesis. Our results and those of others are consistent with the hypothesis that the preneoplastic and neoplastic bronchial epithelial cells have a selective clonal expansion advantage because of diminished responsiveness to negative growth factors and/or autocrine production of positive growth factors. Aberrations in regulation of the multistage pathway leading to terminal squamous differentiation of the bronchial epithelial cells are also observed. The role of activated proto-oncogenes in controlling proliferation and squamous differentiation is being studied by transfection of expression vectors containing ras, myc and raf in the sense or antisense orientation singly or in combination into normal, SV-40 immortalized or neoplastic bronchial epithelial cells. Activated ras and myc proto-oncogenes cause abnormalities in growth and squamous differentiation pathways of human bronchial epithelial cells.

D 019 REGULATION OF CELL PROLIFERATION BY TRANSFORMING GROWTH FACTORS, Harold L. Moses, Vanderbilt University School of Medicine, Nashville, TN 37232.

Two types of transforming growth factors (TGF) have been purified and well characterized, TGFα and TGFß. TGFα is a 5.6 kD single chain EGF-related molecule that binds to the EGF receptor and has biological effects very similar to those of EGF; it is mitogenic for most cell types including normal epithelial cells. TGFß is a 25 kD homodimer of 12 kD subunits that has its own specific cell surface receptors. While growth stimulatory for selected mesenchymal cells, TGFß inhibits proliferation of most cell types including normal epithelial cells. Using cultured skin keratinocytes as a model system for normal epithelial cells, the production of and response to TGFα and TGFß has been examined along with potential mechanisms of growth inhibition by TGFß. The keratinocytes are stimulated to proliferate by EGF and TGFα. TGFα is produced by adult and neonatal skin keratinocytes, and this production is autoregulated. TGFß, on the other hand, is a potent inhibitor of keratinocyte proliferation. The mechanism of growth inhibition by TGFß appears to involve selective inhibition of expression of growth factor inducible genes necessary for cell proliferation. The keratinocytes also synthesize and release TGFß, but in a latent form; the major regulatory step in TGFß action may be at the level of activation of the latent form. Normal autocrine stimulation by TGFα and autocrine inhibition by TGFß is implied and changes in this autocrine regulation may be important in neoplastic transformation of epithelial cells. Both increased autocrine stimulation by endogenous TGFα or decreased inhibition by TGFß could lead to an increased proliferative potential and thereby contribute to the neoplastic phenotype.

D 020 CSF-1 AND ITS RECEPTOR (THE c-fms PROTO-ONCOGENE PRODUCT) IN CELL TRANSFORMATION, Charles J. Sherr, Martine F. Roussel, Carl W. Rettenmier, Esther F. Wheeler, and Jean Michel Heard, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105.

The macrophage colony stimulating factor, CSF-1 or M-CSF, is a homodimeric glycoprotein that stimulates the proliferation, differentiation, and survival of cells of the mononuclear phagocyte lineage. A human 1.6 kb CSF-1 cDNA specifies a polypeptide that includes an N-terminal signal peptide (residues -32 to -1), a biologically active moiety (residues 1 - ca. 165), a transmembrane spanning segment (residues 166-188) and a short C-terminal tail (residues 189-224). The precursor is dimerized through interchain disulfide bonds, and externalized on the plasma membrane as a 68 kd homodimer which is proteolyzed to yield a soluble 44 kd growth factor. Truncation of the precursor after residue 158 results in the production of a soluble, biologically active growth factor that is rapidly secreted. A 4 kb CSF-1 cDNA encodes a 554 amino acid polypeptide containing 298 additional amino acids interposed aminoterminal to the transmembrane segment. Intracellular proteolysis of the larger precursor within its unique domain results in the rapid secretion of a 86 kd homodimer. Cotransfection of the human c-fms gene together with these CSF-1 cDNAs into mouse NIH-3T3 cells induces morphologic transformation and tumorigenicity in nude mice. By contrast, introduction of the 1.6 kb CSF-1 cDNA into a CSF-1 dependent mouse macrophage cell line abrogates its factor dependence but does not induce tumorigenicity, suggesting that mature macrophages are relatively refractory to persistent autocrine signals which can transform fibroblasts.

The v-fms oncogene product differs from the normal CSF-1 receptor by scattered point mutations and by a truncation of its distal carboxylterminus that removes a presumed negative regulatory site of tyrosine phosphorylation. The altered receptor functions constitutively as a CSF-1 independent tyrosine kinase that is refractory to down modulation by CSF-1 and phorbol esters. Introduction of the v-fms gene into CSF-1 dependent macrophage or IL-3 dependent myeloid cell lines renders them factor independent and tumorigenic without affecting the synthesis, affinity, or turnover of normal CSF-1 or IL-3 receptors. To determine if the v-fms gene can directly initiate hematopoietic malignancies in animals, murine bone marrow cells infected with a v-fms-containing retroviral vector were used to reconstitute lethally irradiated mice. The majority of recipients contained dominant provirus-positive clones in their spleens which repopulated secondary lethally irradiated animals, giving rise to both erythroleukemias and B cell lymphomas. Thus, the v-fms gene can transform cells of several hematopoietic lineages both in vitro and in vivo, including those that do not normally express CSF-1 receptors.

Interferons - II

D 021 MECHANISMS OF THE ANTITUMOR EFFECTS OF INTERFERON IN MICE

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DBA/2 mice were injected intraperitoneally (i.p.), subcutaneously (s.c.), or intravenously (i.v.) with lines of Friend erythroleukemia cells (FLC) either sensitive (745) or resistant (3CI8) to various biologic effects of mouse interferon (IFN)α/β. Evidence is presented that 3CI8 cells were not only resistant to IFNα/β in vitro,but also resistant in vivo to the antiviral effects of IFN and to the enhancing effects of IFN on the expression of cell surface H-2 antigens and the level of 2-5A synthetase activity. IFN treatment proved highly effective in inhibiting the primary growth of 745 and 3CI8 FLC injected i.p. and s.c. ; in inhibiting the development of tumor metastases in the liver and spleen after s.c. or i.v. inoculation of FLC ; and in increasing the survival time and overall survival of tumor inoculated mice. The use of the interferon resistant 3CI8 FLC line suggests that the antitumor effects of IFN in these experimental systems were not due to a direct effect of IFN on the tumor cells, but were host-mediated. The host-mediated component may be different, however, depending on the site of tumor growth. For example, IFN induced a marked ischemic necrosis of established s.c. FLC tumors which was preceded by IFN induced damage to the endothelium of small tumor vessels. The marked inhibition of the development of visceral tumor metastases by IFN even when treatment was initiated after tumor cells were present in target organs may result from the interaction of IFN treated hepatocytes with tumor cells. We have not found evidence for an important role for macrophages or cells of the immune system in the antitumor effects of IFN in any of these experimental systems. We conclude that IFN can exert a very potent antitumor effect in mice, but that various mechanisms may be operative.

D 022 INTERFERON-β_2/B-CELL DIFFERENTIATION FACTOR BSF-2/HEPATOCYTE STIMULATING FACTOR,

Pravinkumar B. Sehgal[1], Lester T. May, Uma Santhanam, Stephen B. Tatter, Anuradha Ray and John Ghrayeb[1], The Rockefeller University, New York, NY 10021 and [1]Centocor, Malvern PA 19355

The elemental property of proteins of molecular weight in the range from 23 to 30 kDa derived from the human "β_2-interferon/B-cell differentiation factor BSF-2/hepatocyte stimulating factor" ("IFN-β_2") gene appears to be hormone-like communication between peripheral damaged tissues and the hepatocyte. IFN-β_2 proteins, derived from a highly polymorphic gene located at 7p15-21 in the human genome, have emerged as major mediators of the hepatic "acute phase response." When added to cultures of human hepatoma cell line Hep3B2, rIFN-β_2, as well as natural IFN-β_2, enhances secretion of positive acute phase reactants (e.g. fibrinogen, α_1-anti chymotrypsin, complement C3, α_1-acid glycoprotein, haptoglobin, etc.) and inhibits secretion of negative acute phase reactants (e.g. albumin). A rabbit antiserum to E. coli-derived rIFN-β_2 inhibits the antiviral, hepatocyte stimulating and B-cell differentiation activities of appropriate IFN-β_2 preparations. This antiserum immunoprecipitates a complex series of at least six forms of IFN-β_2 secreted by induced human fibroblasts and monocytes (p23, p25, p25.5, gp28, gp29, and gp30). The chemical structures and biological properties of each of the individual forms are under investigation. The regulation of IFN-β_2 gene expression is particularly adapted to the role of communication between damaged tissues and the hepatocyte. IFN-β_2 gene expression is enhanced in fibroblasts, epithelial cells, cells of the monocytic lineage and in lymphocytes by stimuli generated during infection, inflammation or other tissue damage (e.g. thrombosis). The regulation of IFN-β_2 gene expression has been investigated in different tissues using several different techniques: nuclear runon transcription assays, mRNA blot-hybridization assays and protein secretion assays. In human fibroblasts, IFN-β_2 gene expression is enhanced by several cytokines that affect cell growth (TNF, IL-1, PDGF and IFN-β_1), by bacterial LPS and by virus infections. In human monocytes, IFN-β_2 is enhanced by LPS, TNF, IL-1 or PHA; its expression is also enhanced in human tonsillar T-cells by phorbol esters (TPA). In fibroblasts, protein kinase C-activating or [Ca^{2+}]-elevating agents rapidly increase transcription of the IFN-β_2 gene. The nucleotide sequence of the 5'-flanking region of the IFN-β_2 shares distinctive similarities with the serum-responsive enhancer element in the human c-fos gene. Experiments involving genetic manipulations of the IFN-β_2 promoter in heterologous expression systems are in progress in an attempt to delineate the particular sequence elements involved in the regulation of its expression in different tissues.

D 023 MOLECULAR BIOLOGY OF THE MX SYSTEM. Charles Weissmann, Markus Aebi, Hubert Hug, Maria Costas, Peter Staeheli*, Mathieu Noteborn, Heinz Arnheiter+, Otto Haller* and Charles Samuel#. Institute of Molecular Biology I and *Institute of Virology, University of Zürich, Zürich, Switzerland, +Laboratory of Molecular Biology, NINCDS, Bethesda, MD, # present address: Dept. Biol. Science, University of California, Santa Barbara, CA.

Specific resistance of mice to influenza virus is due to an interferon (IFN)-induced protein designated Mx. Mx cDNA has been cloned and sequenced (Staeheli et al., Cell 44:147, 1986). Cells of different species, when transformed with Mx cDNA under the control of a constitutive promoter, acquire specific resistance to influenza virus in the absence of IFN treatment (Staeheli et al., Cell 44:147,1986; Noteborn et al., J.Interferon Res., in press). The murine Mx gene consists of 14 exons, which are distributed over at least 60 kb of DNA. The Mx promoter and the first exon, comprising a non-coding sequence of 27 bp, are separated by an intron of >30 kb from the second exon, which contains only the first codon of the Mx coding sequence.

The Mx promoter was linked to a ß-globin transcription unit. Surprisingly, it was induced as efficiently by virus as by IFN. The CAP site is preceded by a TATA box and an Sp1 binding site; the 5′ boundary of the promoter region required for maximal induction by both IFN and virus was located about 140 bp upstream of the CAP site; at least two distinct regions are involved in the response to the two inducers. So far, the region responsive to IFN could not be distinguished from that responding to virus. The responsive regions contain elements found in the 3′ moiety of the Friedman-Stark consensus sequence, however they are dispersed; a typical Friedman-Stark consensus region is found neither in Mx nor in several other IFN-inducible genes.

A 33-nucleotide segment of the Mx promoter, when linked to a minimal ß-globin promoter (containing only the TATA box and the CAP site) mediated IFN and virus response, provided that it was preceded by an SV40 enhancer. A tetrameric repeat of the sequence GAAACT which occurs in the Mx promoter was also capable of mediating response to both inducers. This suggests that IFN and virus induction are mediated by a factor or factors that recognize related sequences on the promoter.

IFN-induced human cells produce 2 Mx-related mRNAs, the cDNAs of which have been cloned. Their sequences are related but distinct, showing that there are two distinct Mx-related genes in man.

Tumor Suppressor Genes

D 024 GENES WITH POTENTIAL FOR SUPPRESSING MALIGNANT PHENOTYPES OF KIRSTEN SARCOMA VIRUS-TRANSFORMED NIH/3T3 CELLS. Makoto Noda, Hitoshi Kitayama, Yoko Aida, Yoshikazu Sugimoto, Tomoko Matsuzaki and Yoji Ikawa, Tsukuba Life Science Ctr., The Institute of Physical and Chemical Research, Yatabe, Tsukuba, Ibaragi 305, Japan.

In investigating the molecular mechanisms of cell transformation induced by oncogenes, phenotypic revertants in which the oncogenes are intact and yet fail to fully express their transforming ability would be useful. In initial attempts by chemical mutagenesis to isolate such revertants from Kirsten murine sarcoma virus (Ki-MSV)-transformed NIH/3T3 cells (designated DT), we could obtain some "flat" revertants which behave dominantly in the cell fusion experiments with normal or Ki-MSV-transformed partners (1,2). Recently, we have identified a genetic alteration in the vicinity of the promotor region of fibronectin gene in one of the revertants, which probably explains the deregulated overexpression of the fibronectin mRNA and its product found in this particular revertant (3). These observations suggested potential usefulness of this system for detecting genes which dominantly suppress transformation when transcriptionally activated.

In a new series of experiments, we employed transfection of cDNA expression library constructed with mRNA from normal human fibroblas, instead of chemical mutagenesis, to facilitate the identification of the genes responsible for reversion. After enrichment of flat populations among the transfected DT cells by several different protocols, we have isolated at least 10 independent clones of revertants which exhibit i) stably flat morphology, ii) persistent expression of v-ras protein, and iii) resistance to super-infection with Ki-MSV. Southern blot analysis indicated that those revertants harbor between one and ten copies of cDNAs most of which seem to be directly rescuable into E. coli, by virtue of closely linked bacterial ori and drug resistance markers, by transfection of the revertant DNA which had been fragmented and, then, circularized. Identification and characterization of biologically active cDNAs thus recovered are in progress.

1) Proc. Natl. Acad. Sci. USA. 80, 5602-5605 (1983).
2) Adv. Viral Oncol. 6, 103-127 (1987).
3) Oncogenes and Cancer (eds. S. A. Aaronson et al., Japan Sci. Soc. Press, Tokyo, 1987) pp.261-267.

D 025 GROWTH AND INHIBITION OF HUMAN MAMMARY EPITHELIAL CELLS OF NORMAL AND TUMOR ORIGIN, Ruth Sager, Vimla Band, Anthony Anisowicz, and Deborah Zajchowski, Dana-Farber Cancer Institute, Boston, MA 02115

A primary goal of research in this laboratory is the identification and cloning of genes whose encoded products inhibit or kill tumor cells while leaving normal cells unaffected. One tumor type we have chosen to use for these studies is the human mammary carcinoma. Normal human breast cells of lumenal epithelial origin grow well in serum-free supplemented MCDB 170 (Hammond et al., PNAS, 1985) whereas tumor cell lines are grown in α-MEM plus 10% FCS. Both classes of cells will grow, however, in a novel medium that we have developed, making it possible to compare properties of cells grown under similar conditions and to carry out transfection-selection experiments in which transfectants resembling normal cells can be recovered. The natural cytotoxic agent TNF and the dye Rhodamine-123 are both much more toxic to cells from some mammary tumors than are normal human mammary epithelial cells as we have reported at this meeting (Poster), and their synergistic cytotoxicity produces a differential toxicity of more than 10^6 as determined by plating efficiencies. We are also comparing the expression of proto-oncogenes and growth factors in these cells. One of the noteworthy differences is in the expression of the gro gene, whose transcription is tightly regulated in normal fibroblasts (Poster; Anisowicz et al., PNAS, (1987). The quantity of this mRNA in normal mammary epithelial cells is significantly higher than that present in tumor-derived cells. Studies investigating the control mechanisms for this differential expression will be presented.

D 026 NEGATIVE GROWTH CONTROL IN CELLULAR SENESCENCE, O.M. Pereira-Smith, Don A. Kleinsek, Andrea L. Spiering, and J.R. Smith, Department of Virology and Epidemiology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

Fusion of normal human fibroblasts with any number of immortal human cell lines yielded hybrids that had limited division potential in culture. This result indicated that the phenotype of cellular senescence was dominant, and that cellular immortality resulted from recessive changes in the cell. This idea was further tested by fusing different immortal cell lines with each other, and led to the identification of 4 complementation groups for cellular immortality. When cell lines within a complementation group were fused with each other, immortal hybrids were obtained and when fusions were performed between groups, hybrids with finite lifespan were obtained. Therefore, there are at least 4 pathways to cellular immortality. In a separate series of experiments, senescent cells have been found to produce a protein inhibitor(s) of initiation of DNA synthesis, and to contain a high abundance of mRNA that inhibit DNA synthesis. The mRNA(s) appears to act by inhibiting the initiation of DNA synthesis, as assayed by micro-injection into your HDF cells. We have constructed a λGT11 cDNA library from senescent HDF cells. cDNA synthesis was equally efficient with senescent mRNA templates or mRNA obtained from young HDF cells (PDR of 35). The size of the cDNA inserts was analyzed by release of the cDNA from the lambda vector by EcoR1 digestion followed by a Southern analysis using nick-translated human genomic DNA as probe. The cDNA inserts range from 0.4 to ≥4.0 kilobases (Kb) in length, with an average size of 2 Kb. Representation of several mRNAs in the library that are constitutively expressed in HDF cells has been confirmed. This cDNA expression library contains 1.8×10^7 recombinants. The estimated high abundance of message (0.1-1.0%) for the inhibitor(s) should permit the cloning of its sequences using either nucleic acid or antibody probes. Our current hypothesis is that cellular senescence is the result of a genetic program, the end point of which is production of an inhibitor of DNA synthesis; and that cellular immortality results from recessive changes in this program.
(Supported by USPHS grants AG05333 and AG04749 and T32-CA09197.)

TGF-Beta and Related Molecules

D 100 POST-TRANSCRIPTIONAL REGULATION OF EGF-INDUCIBLE GENES IN MOUSE KERATINOCYTES BY TGFß REQUIRES PROTEIN SYNTHESIS. Charles C. Bascom, Robert J. Coffey, Nancy J. Sipes, and Harold L. Moses. Vanderbilt Medical School, Nashville, TN 37232.
Balb/c mouse keratinocytes (BALB/MK), a highly EGF-dependent cell line, are reversibly growth arrested by pM concentrations of transforming growth factor type ß (TGFß) at a point distal to EGF:EGF receptor interactions. The effects of TGFß are also not due to a general inhibition of macromolecular synthesis because neither total RNA nor protein synthesis are significantly altered by TGFß. These data suggest that TGFß's inhibitory effects on BALB/MK cells may be due to selective changes in mRNA expression and protein production. As an attempt to understand TGFß's mechanism(s) of action, we initially examined the expression of EGF-inducible genes in both rapidly growing and quiescent, EGF-stimulated BALB/MK cells following treatment with TGFß. In either growth state, expression of c-myc and KC were inhibited by TGFß while c-fos induction by EGF in quiescent cells was unaltered by TGFß. In contrast, TGFß actually increased expression of ß-actin in the BALB/MK cells. Nuclear run-on experiments indicated that TGFß's inhibitory effects on c-myc are post-transcriptional. Furthermore, pre-treatment with cycloheximide abrogated the inhibition of both c-myc and KC by TGFß. The data suggest that the mechanism of TGFß reduction of c-myc expression is different from that observed with tumor necrosis factor.

D 101 **ONCOSTATIN M DIFFERENTIALLY REGULATES THE ACTION OF TYPE 1 TRANSFORMING GROWTH FACTOR-β (TGF-β1)**, T.J. Brown, M. N. Lioubin, and H. Marquardt, Oncogen, Seattle, Washington 98121
The proliferative and biosynthetic effects of oncostatin M and TGF-β1 on nontransformed cells were investigated in vitro. Normal diploid fibroblasts of mesenchymal origin isolated from the human embryonic palate (HEPM) were found to be highly responsive to mitogenic stimulation induced by physiologic concentrations of TGF-β1. Conversely, oncostatin M demonstrated antiproliferative activity at physiologic doses. TGF-β1 stimulated 2-3 fold increases in total protein synthesis and secretion of extracellular collagen while oncostatin M induced moderate decreases in this regard. The mitogenic effect of 50 pM TGF-β1 was not abrogated in the presence of 50 pM oncostatin M although collagen biosynthesis remained at control levels. Therefore, at these doses, oncostatin M did not interfere with the mitogenic signal induced by TGF-β1 but did regulate the specific pattern of protein synthesis induced by TGF-β1. Scatchard analysis of [^{125}I]-TGF-β1 binding studies revealed two classes of cell surface receptors on HEPM cells that were quantitatively decreased following pretreatment with 100 pM TGF-β1 (↓50%). Oncostatin M did not interfere with TGF-β1 receptor binding or receptor down-regulation. Therefore, oncostatin M differentially regulates TGF-β1 action at site/s distal to the receptor. Normal palatogenesis involves mesenchymal cell growth and synthesis of extracellular matrix components necessary for the elevation and fusion of apposing palatal shelves. These data suggest a regulatory role for oncostatin M and TGF-β1 in specific processes of embryogenesis and tissue remodeling.

D 102 **RECOMBINANT TGF-β1 PRECURSOR PRODUCED BY CHO CELLS IS GLYCOSYLATED**, Amy Brunner, Mario N. Lioubin, Larry Gentry, Tony Purchio, Oncogen, 3005 First Avenue, Seattle, Washington 98121

Transforming growth factor-β1 (TGF-β1) consists of two identical disulfide linked subunits having molecular weights of approximately 12,000. Analysis of cDNA clones coding for human (Derynck et al., 1985, Nature 316:701), mouse (Derynck et al., 1986, J. Biol. Chem. 261: 4377) and simian (Sharples et al., 1987, DNA 6:239) TGF-β1 indicates that it is synthesized as a large precursor polypeptide, the carboxy terminus of which is cleaved to yield the mature TGF-β1 monomer. Recombinant simian TGF-β1 has been expressed to high levels in Chinese hamster ovary cells (Gentry et al., 1987, Mol. Cell. Biol., in press). Using site-specific antibodies, it was shown that these cells secreted mature as well as precursor forms of TGF-β1. Three potential glycosylation sites exist in the precursor region of TGF-β1 (Asn-82, -136, and -177). The large molecular weight forms of TGF-β1 (90-110 Kd band) bound to Con A Sepharose and were specifically eluted by α-methyl mannoside. Labeling with [^{3}H]-glucosamine indicated that the precursor region, but not mature TGF-β1, was glycosylated. Digestion with neuraminidase and subsequent polyacrylamide gel analysis indicated that sialic acid residues were present. Digestion with N-glycanase caused the precursor proteins migrating as diffuse 44 Kd to 56 Kd and 30 Kd to 42 Kd bands under reducing conditions to migrate as two sharp 40 Kd and 28 Kd bands suggesting most, if not all, of the sugar residues were removed. Translation of TGF-β1 RNA transcripts produced by SP6 polymerase resulted in the synthesis of a 40 Kd polypeptide suggesting that the species represents the unmodified amino acid backbone of TGF-β1.

D 103 INDUCTION OF TGFβ GENE TRANSCRIPTION IN THE REGENERATING AND CARCINOGEN-ALTERED RAT LIVER, Brian I. Carr, Ting H. Huang, Keiichi Itakura, and Normand Marceau, City of Hope National Medical Center, Duarte, CA 91010 and Universite Laval Quebec.

We and others previously found that TGFβ is a potent, non-toxic inhibitor of mitogen-induced DNA synthesis in primary cultures of adult rat hepatocytes. Using a cDNA clone (Genentech) we investigated TGFβ gene expression in quiescent, regenerating after partial hepatectomy (PH) and neoplastic liver and hepatoma lines. We found that PH liver had increased TGFβ gene transcripts beginning at about 8 hrs after PH, with a peak at 36 hrs and return to normal after 9 days. Separation of the liver into its constituent cell types followed by mRNA extraction and re-probing revealed that increased TGFβ gene transcripts were confined to the Kuppfer and endothelial cell population and were not in hepatocytes. Increased expression was also found in the liver of rats 2 days after birth and in fetal liver at 14 days post-insemination. Elevated levels were also found in continuous cultures of oval and bile duct cells. Transcripts were undetectable in normal human liver, but were abundant in the human hepatoma lines HEP-G2, HEP-3B, PLC/PRF/5 and SK-HEP-1. Elevated levels were also found in rat liver-derived lines BRL-3A and clone 9 and the H4IIE and nTC rat hepatomas, but not the slowly-growing MH_1C_1 and MH7777 hepatomas. The hepatocarcinogen diethylnitrosamine (DEN) induced high transcript levels after single injections in a dose-dependent manner. These results suggest that the liver is a paracrine organ with respect to TGFβ gene expression, which may be induced by carcinogens and by growth stimulation.

D 104 MULLERIAN INHIBITING SUBSTANCE INHIBITS EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE, F.G.Cigarroa, J.P.Coughlin, *M.White, P.K.Donahoe, and D.T.MacLaughlin, Massachusetts General Hospital, *Joslin Diabetic Center, Boston, MA 02114

Mullerian Inhibiting Substance (MIS) is a 140kd glycoprotein produced by Sertoli cells from both the fetal and neonatal testis. In the embryo, it causes active regression of the Mullerian duct contributing to the normal male phenotype. In addition, both bovine and human recombinant MIS have been shown to inhibit growth of primary tumors and transformed cell lines of Mullerian origin, as well as A-431 cells rich in Epidermal Growth Factor receptors.

Experiments have linked MIS activity with Epidermal Growth Factor (EGF). In an organ culture assay, EGF inhibits MIS activity in a dose dependent manner. Based on this observation, and on the ability of MIS to inhibit A-431 colony growth in soft agar, A-431 cells were used to study the mechanism of MIS action. A-431 membranes were co-incubated with 10nM MIS, 26nM EGF, 0.136uM $AT^{32}P$, and the required EGF cofactor Mn^{++}(1mM). MIS inhibited EGF stimulated autophosphorylation of the EGF receptor (EGFr), while controls lacking MIS, stimulated such phosphorylation. This inhibition was not affected by a 2000, 80, or 4 fold increase in the concentrations of EGF, Mn^{++}, or $AT^{32}P$, respectively, making competition for EGF binding sites or sequestration of these substrates by MIS unlikely. In intact A-431 cells, MIS also inhibited EGFr autophosphorylation. Using anti-EGFr and anti-phosphotyrosine antibodies, we have shown that MIS inhibits EGF induced phosphorylation of tyrosine residues on the EGFr. These results suggest that MIS is an inhibitor of EGFr tyrosine kinase, making MIS the first natural inhibtor of this enzyme.

D 105 EFFECT OF TRANSFORMING GROWTH FACTOR-BETA (TGF-β) ON COLLAGEN GEL REGULATION OF FIBROBLAST MATRIX PROTEIN PRODUCTION. Richard A.F. Clark, Larry D. Nielsen, and John M. McPherson, National Jewish Center, Denver, CO 80206, Collagen Corp., Palo Alto, CA 94303.

Neonatal human dermal fibroblasts (NHDF) synthesize large amounts of collagen and fibronectin (FN) when grown in vitro on tissue culture (TC) plastic. Production of these matrix proteins is suppressed when NHDF are grown in collagen gels. Since TGF-β is known to stimulate the production of matrix proteins in many cell types, we have studied the effects of TGF-β on NHDF in collagen gels. Equal numbers of cells were plated near confluence on TC plastic or were suspended in hydrated fibrillar collagen gels and maintained in medium containing 10% serum. The cells were then incubated for 12 hrs in serum-free medium containing various concentrations of TGF-β 1 and finally labeled for 10 hrs with ^{14}C proline. Collagen production was determined from the reduction in non-dialyzable label after collagenase digestion of the newly synthesized protein. FN was estimated from densitometry scans of collagenase resistant proteins on SDS-PAGE autoradiography. When fibroblasts were plated on TC plastic, maximum TGF-β stimulation of non-matrix protein production was 2.0 fold, while maximum stimulation of collagen and FN production was 5.4 fold and 4.2 fold respectively, indicating a selective enhancement of matrix protein production. In the absence of TGF-β, cells growing in collagen gels produced only about 50% as much collagen and FN as cells growing on TC plastic. TGF-β, however, stimulated cells in collagen gels to produce FN at the same level as cells on TC plastic, but did not override the collagen gel suppression of collagen production. The differential effect of TGF-β on fibronectin versus collagen production when NHDF are grown in collagen gels indicates that the biological effects of TGF-β can be modulated by the extracellular matrix.

D 106 AUTOCRINE INHIBITION OF MINK LUNG EPITHELIAL CELL GROWTH BY TGF-BETA 2, David Danielpour, Linda L. Dart, Kathy C. Flanders, Anita B. Roberts, and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Transforming Growth Factor Beta (TGF-beta), a potent modulator of cell growth, differentiation, and the expression of extracellular matrix components in a variety of cell types, is now recognized to exist as two distinct homodimers, sharing 71% sequence homology (TGF-beta 1 and TGF-beta 2). TGF-beta 1 has been previously suggested to function as an autocrine inhibitor of the growth of various cell types. Here we present evidence that TGF-beta 2 may function as an autocrine inhibitor for mink lung epithelial CCL-64 cells. Both TGF-beta 1 and TGF-beta 2 are known to be highly potent inhibitors of the growth of these cells. We have developed antisera against native TGF-beta 1 and TGF-beta 2 in turkeys, each of which specifically blocks both the receptor binding and the growth inhibitory activities of each of these peptides. Unlike the anti-TGF-beta 1 and control IgG fractions, the purified anti-TGF-beta 2 IgG fraction was able to stimulate DNA synthesis by over 500% above controls, strongly suggesting a role for TGF-beta 2, rather than TGF-beta 1 as an autocrine inhibitor of CCL-64 cell growth. Further experiments on the regulation of this experimental system will be presented.

D 107 TRANSFORMING GROWTH FACTOR-B1 (TGF-B1) RECEPTORS ON DEVELOPING AND MATURE T CELLS, Larry R. Ellingsworth, Patricia Segarini, James Dasch and Debra Nakayama, Collagen Corporation, Palo Alto, CA 94303.

TGF-B1 is a potent growth inhibitor of developing and mature T cells. The biological action of TGF-B1 is mediated through a complex of high (280 KD) and low (95-85 KD and 65 KD) molecular weight cell surface glycoproteins. In this study, we have evaluated the effects of T cell mitogens (interleukin-1 (IL-1) and lectins) upon the expression of the TGF-B1 receptor complex on murine thymocytes, murine splenic T cells and human peripheral blood T cells. The high and low molecular weight receptors were identified by affinity crosslinking 125-I-TGF-B1 with disuccinimidyl suberate. These results show that unactivated (G_0) thymocytes (murine) and mature T cells (murine and human) appear to constitutively express only the low molecular weight (95-85 KD and 65 KD) forms of the TGF-B1 receptor; the high molecular weight (280 KD) form was not detected on G_0 T cells. Upon mitogenic stimulation for 24 hrs, however, there was a great enhancement in the expression of the low molecular weight receptors (95-85 KD and 65 KD) and the appearance of the high molecular weight (280 KD) form of the TGF-B1 receptor. Antibodies which neutralize the anti-proliferative action of TGF-B1 did specifically block the binding of 125-I-TGF-B1 to both the high and low molecular weight forms of the receptor, suggesting that these are the biologically relevent receptors. These results suggest that a regulatory relationship may exist between the mitogenic action of IL-1 and the growth-inhibiting action of TGF-B1.

D 108 EPITOPE-DEPENDENT IMMUNOHISTOCHEMICAL LOCALIZATION OF TRANSFORMING GROWTH FACTOR-BETA, Kathleen C. Flanders, Nancy L. Thompson, Larry R. Ellingsworth*, Mary E. Kass+, Michael B. Sporn, and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892, *Connective Tissue Research Laboratory, Collagen Corporation, Palo Alto, CA 94303, and +Department of Pathology, Washington Hospital Center, Washington, D.C. 20010.

Transforming growth factor-beta-1 (TGF-beta) is a multifunctional regulator of cell growth and differentiation. TGF-beta bioactivity has been found in a number of cultured cell lines, as well as a variety of tissues. We have generated polyclonal antibodies to peptides corresponding to several regions of the TGF-beta molecule and have used these antibodies for immunohistochemical localization of TGF-beta. Two different staining patterns have been observed depending on the antibody used: in one pattern, staining is largely extracellular and associated with extracellular matrix, while in the second pattern, staining is intracellular and localized in a variety of cell types. This differential staining pattern has been observed in a number of systems, including cultured cell pellets, mouse embryonic, neonatal and adult tissues, bovine papilloma wart tissue and human colon tumors. Furthermore, the differential staining patterns are observed with antibodies raised not only to different peptides, but also to different synthetic batches of a single peptide corresponding to the amino-terminal 30 amino acids of TGF-beta. Presumably, the antisera have been raised against different epitopes of the peptide. We are currently investigating the molecular mechanism for the basis of the different staining pattern.

D 109 MODULATION OF CELLULAR PROLIFERATION, HYPERTROPHY, BEHAVIORAL ORGANIZATION, AND GENE EXPRESSION BY TGF-β IN CULTURES OF NEONATAL AND ADULT AORTIC SMOOTH MUSCLE CELLS, Linda V. Goodman, Gloria J. Badiner, Mark W. Majesky*, and Richard A. Majack, The Upjohn Company, Kalamazoo MI 49001, and *University of Washington, Seattle, WA 98195.

Vascular smooth muscle cells (SMC) express and respond to TGF-β in a complex fashion determined in part by cell density and developmental age. TGF-β is the primary determinant of the "hill-and-valley" pattern of organization diagnostic for adult SMC in culture. The growth response of adult cells to TGF-β depended on cell density: sparse cells were markedly inhibited by the peptide whereas confluent cells showed growth promotion. Confluent SMC rendered quiescent by serum deprivation (0.5% FBS for 72 h) expressed growth-related genes within 4 h of TGF-β treatment. Levels of Type I collagen message were markedly induced by TGF-β independent of cell density, growth inhibition, and growth promotion. Neonatal (12 day) aortic SMC, which grow in culture in a monolayer pattern of organization, were growth-inhibited by TGF-β when treated at subconfluent densities; their growth was unaffected at higher cell densities. Confluent (monolayered) cultures formed multilayers within 24 h of exposure to TGF-β. Cell number assays showed that formation of the hill-and-valley morphology was not associated with an increase in cell number. Changes in Coulter volume distributions were observed in these cells following TGF-β treatment, suggesting that cellular hypertrophy may be an additional, primary response of these cells. In contrast to adult cells, neonatal SMC responded to TGF-β with an enhanced expression of fibronectin.

D 110 EXPRESSION OF FIBRONECTIN RECEPTORS ELEVATED BY TRANSFORMING GROWTH FACTOR-β. Ronald A. Ignotz and Joan Massague, University of Massachusetts Medical School, Worcester, MA 01655.

The fibronectin receptor is a member of larger family of receptors, the cytoadhesions, that are responsible for attachment of cells to the extracellular matrix and homing of cells to specific sites or tissues. As a model system, we examined the fibronectin receptor for modulation by TGF-β. We find that TGF-β enhances the ability of mouse thymocytes to attach to a fibronectin substratum; this attachment being inhibited by a peptide that mimics the cell binding region of fibronectin. To characterize the molecular basis of this phenomenon, we utilized mouse 3T3-L1 cells in immunoprecipitation experiments. TGF-β increases the biosynthesis and cell surface expression of fibronectin receptors. This occurs through two complementary mechanisms; elevated mRNA levels for receptor β-subunit and faster kinetics of receptor β-subunit precursor to product conversion. Pulse-chase experiments show that in the absence of TGF-β, the maturation of β-subunit is slow, requiring at least 5 hr for 50% conversion whereas in the presence of TGF-β 50% conversion takes about 2 hr. The increased expression of the fibronectin receptor appears to be specific for TGF-β since neither EGF nor IGF-II increase fibronectin receptor biosynthesis. Thus, modulation of fibronectin receptors by TGF-β may be an important mechanism in regulation of cell migrations during wound healing, embryonic development and differentiation, and the homing of cells to tissues.

D 111 TRANSFORMING GROWTH FACTOR ß INHIBITS BOTH EPIDERMAL GROWTH FACTOR AND ONCOGENE-INDUCED TRANSIN. L.D. Kerr*, C. Machida, L. L. Muldoon, K.D. Rodland, B.E. Magun, and L.M. Matrisian*. *Dept. Cell Biology, Vanderbilt Univ., Nashville TN 37232 and Dept. Cell Biology and Anatomy, Oregon Health Sciences University, Portland OR 97201

Transin is a secreted metalloproteinase transcriptionally induced by epidermal growth factor (EGF) and by a number of oncogenes, including v-src and H-ras. We have previously shown that TGFß inhibits EGF induction of rat transin RNA (Mol. Cell Biol. 6, 1679, 1986). We have extended these studies and demonstrated a similar effect of TGFß on EGF-induced transin RNA and protein in mouse (Balb\c 3T3) and human (foreskin fibroblast) cells. EGF binding studies in Rat-1 cells shows no significant effect of TGFß on EGF receptor number or affinity. TGFß alters EGF-induced transin RNA levels by decreasing transcription as determined by nuclear run-on assays. We have also examined the effects of TGFß on oncogene-induced transin using Rat-1 cells transformed by temperature-sensitive mutants of src (LA-24) and H-ras (Rtsll.3) oncogenes. A three hour pre-incubation with 5ng/ml TGFß followed by a shift to the permissive temperature eliminates the induction of transin RNA and protein seen in control cells following the temperature shift. These experiments suggest that the inhibitory effects of TGFß occur at the level of transin gene expression and provide a system to examine transcriptional inhibition by TGFß.

D 112 INDUCTION OF MEMBRANE-ASSOCIATED INTERLEUKIN-1 IN CULTURED HUMAN MACROPHAGES BY TRANSFORMING GROWTH FACTOR-β. Jorma Keski-Oja[1] and Mikko Hurme[2], Departments of Virology[1] and Immunology and Bacteriology[2], University of Helsinki, SF-00290 Helsinki, Finland.

An important function of both transforming growth factor-β (TGFβ) and interleukin-1 (IL-1) is to affect pericellular proteolytic activity by inducing the production of the type-1 plasminogen activator inhibitor. Therefore we performed a study on TGFβ regulation of the expression of IL-1 in cultured human macrophages and human fibroblasts. The cells were incubated with TGFβ (0.1-20 ng/ml) for 24 h. The production of IL-1 was assayed by augmentation of the thymocyte proliferative response to phytohemagglutinin as the indicator. The membrane-associated IL-1 activity was quantitated by adding paraformaldehyde-fixed macrophages to the thymocyte assay. It was found that like lipopolysaccharide, TGFβ increased the expression of IL-1 activity in the cells in a dose-dependent manner. Analysis of the secreted and membrane-bound forms of IL-1 showed that the majority of this activity was of the membrane-bound form. Assays of preparations from human dermal and lung fibroblasts showed no secreted IL-1 in their medium. The induction by TGFβ of membrane-bound IL-1 in macrophages offers an important regulatory mechanism for targeted regulation of proteolysis and connective tissue formation in healing and inflammation processes. In addition, the data suggest that TGFβ could have a regulatory function in the activation of T lymphocytes.

D 113 EXPRESSION OF THE TGF-β GENE IN THE OVARY AND ITS ROLE DURING MEIOSIS OF RAT OOCYTES, Michael Knecht, Pei Feng, Kevin Catt, and Edward Gelmann, NICHD and NCI, NIH, Bethesda, MD 20892

Although growth factors have major roles in initiating the mitotic division of many cells, their effects on meiosis are largely uncharacterized. In cumulus-oocyte complexes obtained from immature rats, TGF-β as well as such growth factors as EGF, IGF-I, and IGF-II accelerated the resumption of meiosis, as indicated by the breakdown of the nuclear germinal vesicle in oocytes arrested in the prophase to metaphase stage of the first meiotic division. The stimulatory effects of TGF-β on oocyte maturation were concentration-dependent with a 2-fold rise in germinal vesicle breakdown during a 60 min incubation. TGF-β action was reversed by inhibitors of meiosis such as cAMP and hypoxanthine, and did not involve the expansion of the granulosa-cumulus cell layers from the oocyte. The importance of the granulosa-cumulus cells was further emphasized by the lack of effect of the growth factor in denuded oocytes. The levels of the 2.5 kb species of TGF-β mRNA, as detected by hybridization with a labeled cRNA probe and washing with RNase A to reduce background labeling, were elevated during culture of granulosa cells for several hours. However, the amounts of TGF-β transcripts were not markedly altered during stimulation of granulosa cell maturation by the gonadotropin, follicle-stimulating hormone. These results indicate that TGF-β may have an autocrine role during ovarian development, and that the stimulatory signals produced by the growth factor during oocyte maturation are transferred from the somatic cells to the germ cells.

D 114 MODULATION OF PROTEOLYTIC ACTIVITY AND ANCHORAGE-INDEPENDENT GROWTH OF CULTURED CELLS BY SARCOMA CELL-DERIVED FACTORS: RELATIONSHIPS TO TRANSFORMING GROWTH FACTOR-ß, Marikki Laiho, Dept. of Virology, University of Helsinki.

We have previously described factor(s) produced by 8387 fibrosarcoma cells which can affect plasminogen activator (PA) activity of cultured cells. Since then transforming growth factor-ß has been established as a major growth inhibitor that regulates both the expression and activity of PAs and their endothelial-type inhibitor (PAI-1). The present study was undertaken for further characterization of the 8387 fibrosarcoma cell-derived factor(s) as modulators of PA activity and cell growth and to investigate the relationships with TGFß. The fibrosarcoma cell-derived proteins were partially purified from serum-free conditioned culture medium using Bio-Gel P-10 chromatography. Two separate fractions of apparent Mr 16.000 and 12.000 contained factors that simultaneously decreased the secretion of PA activity in human lung fibroblasts and inhibited the colony formation of A549 lung adenocarcinoma cells in soft agar assays. When the effects of these factors were studied in detail, it was found that both factors had similar effects on the production of *urokinase-type PA and PAI-1 in various cell lines and on the anchorage-independent growth of murine* AKR-2B fibroblasts in a fashion similar to that of TGFß. The immunological relationships between the Mr 16.000 and 12.000 factors and TGFß were therefore studied using neutralizing polyclonal anti-TGFß-antibodies (1). The TGFß-antibodies efficiently inhibited the effects of the Mr 16.000 factor but not that of the Mr 12.000 factor in biological assays. The results suggest that 8387 fibrosarcoma cells produce two major, distinct growth inhibitors, one of which is closely related to TGFß.

1. J. Keski-Oja, R. M. Lyons & H.L. Moses, Cancer Res. 47 (1987) in press.

D 115 COMPARISON OF LATENT TGFß IN SERUM AND CELL CONDITIONED MEDIUM, Russette M. Lyons, Jorma Keski-Oja and Harold L. Moses, Vanderbilt, Nashville, TN 37232.

Although TGFß stimulates proliferation of some fibroblastic cells, it is the most potent growth inhibitor thus far described for many cell types. TGFß is a highly ubiquitous molecule and is produced by normal as well as neoplastically transformed cells. Platelets are a rich source of TGFß and have been used extensively for its purification. Both platelets and cells in culture release TGFß in a latent form which is irreversibly activated by acid treatment. An analysis of pH dependency for TGFß activation in previous studies suggests that at least two pools of latent TGFß may be present in cell conditioned medium. One pool can be activated by mild acid or base, while a second pool requires strong acid or base for activation. In contrast, the majority of latent TGFß present in human serum appears to be in a form which requires strong acid or base treatment for activation. In addition, previous studies indicate that plasmin, a broad spectrum protease, activates the same pool of latent TGFß in cell conditioned medium as that activated by mild acid or base. Similar and higher concentrations of plasmin as that used for activation of latent TGFß in cell conditioned medium had no effect on latent TGFß in serum. These results suggest that the majority of latent TGFß in serum is not accessible for activation by plasmin or mild pH treatment. In addition, molecular sieve chromatography studies indicate that the latent TGFß in serum has an apparent molecular weight which is much greater than that found in cell conditioned medium. Thus, cells in culture may secrete TGFß in a form different from that released by platelets.

D 116 **cDNA CLONING AND SEQUENCE ANALYSIS OF TRANSFORMING GROWTH FACTOR-BETA 2,** Linda Madisen, Nancy R. Webb, Timothy M. Rose, Hans Marquardt, Tatsuhiko Ikeda, Daniel Twardzik, Saeid Seyedin, and A.F. Purchio

A cDNA clone coding for human TGF-Beta 2 was isolated from a tamoxifen treated human prostatic adenocarcinoma cell line (PC-3) using oligonucleotide probes. The cDNA sequence predicts that TGF-Beta 2 is synthesized as a 442 amino acid polypeptide precursor from which the mature 112 amino acid TGF-Beta 2 subunit is derived by proteolytic cleavage. A comparison of the protein sequences encoded by human TGF-Beta 1 and TGF-Beta 2 cDNAs indicates that the proteins share an overall homology of 41%. The mature and amino-terminal precursor regions show 71% and 31% homology respectively. Northern blot analysis identified TGF-Beta 2 transcripts of 4.1 kb, 5.1 kb, and 6.5 kb using mRNA from several different sources. Analysis of polyadenylated RNA from tamoxifen treated PC-3 cells showed that these cells contain higher amounts of TGF-Beta 1 transcripts than TGF-Beta 2 although they produce more TGF-Beta 2 protein than TGF-Beta 1. This suggests that there is a post-transcriptional level of regulation for the production of these proteins.

D 117 A FACTOR PRODUCED BY NORMAL PROSTATIC STROMA STIMULATES PROLIFERATION OF METASTATIC PROSTATE CELLS, Robert N. McEwan, Donald B. Carter, Ed Sherwood*, James M. Kozlowski*, The Upjohn Company, Kalamazoo, MI 49007 and *Northwestern University Medical School, Department of Urology, Chicago, IL 60611.

In the normal tissue environment epithelia and stroma are separated by a basement membrane which acts as an important anchor and inducer for epithelial cell function as well as being a semipermeable filter for signals transduced between the epithelial cells and stroma. We have separated these two populations and grown them *in vitro* separated by a costar Transwell culture chamber. Stroma or stromal-conditioned supernatants stimulate metastatic prostate cells (PC3) to proliferate. When stroma are grown opposite primary epithelia there is no stimulation of proliferation. If the Transwell membrane is coated with collagen I and III there is proliferation in the absence of stroma. Experiments are underway to characterize the nature of this stromal-derived factor.

D 118 PURIFICATION AND CHARACTERIZATION OF A LATENT HIGH MOLECULAR WEIGHT FORM OF TRANSFORMING GROWTH FACTOR-BETA 1. Kohei Miyazono, Christer Wernstedt, Ulf Hellman, and Carl-Henrik Heldin. Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-751 23 Uppsala, Sweden.
Human transforming growth factor-β1 (TGF-β1) was purified from human platelets in a latent high molecular weight complex using a six-step procedure. It is composed of three different proteins with apparent M_rs of 13,000, 40,000, and 125,000-160,000 on SDS-gels under reducing conditions. The M_r = 13,000 subunit was identified as TGF-β1 by amino acid sequencing; the active molecule is M_r = 25,000 dimer stabilized by disulfide bridges. There was no evidence for the presence of TGF-β2 in human platelets in the purified material. Analysis by SDS-gel electrophoresis revealed that the M_r = 40,000 and the M_r = 125,000 - 160,000 subunits are linked by disulfide bonds, forming a complex of M_r = 210,000. The M_r = 40,000 subunit was identified as N-terminal part of the TGF-β1 precursor by N-terminal amino acid sequencing; the sequence start at a leucine residue, 30 amino acid from the initiation site, indicating that the hydrophobic signal peptide is cleaved at this point. Partial amino acid sequencing of the M_r = 125,000 - 160,000 protein revealed no homology to known protein sequences. TGF-β1 binds non-covalently to the M_r = 210,000 complex, and was separated and activated after incubation at extreme pH, in 0.02 % SDS, or in 8 M urea.

D 119 TGFß INDUCTION OF INTERFERONS IN FIBROBLASTIC AND EPITHELIAL CELLS, Jennifer A. Pietenpol, Jana R. Wolfshohl, Harold L. Moses, Dept. Cell Biology, Vanderbilt School of Medicine, Nashville, TN 37232.
Transforming growth factor type beta (TGFß) is a polypeptide with cellular modulating activities. Under certain conditions some fibroblastic cell lines are stimulated by TGFß, however, most cell types are inhibited by this growth factor. TGFß induces interferon-ß2 (IFNß2) in both mouse (AKR-2B) and human (HEL-299) fibroblastic lines. JE, a gene cloned by Cochran et al. (Cell 33:939, 1983) was also found to be induced by TGFß in AKR-2B and HEL-299 cells. Recent studies (Rollins, Morrison, and Stiles, submitted) have indicated that JE has homology to both human IFNß2 and colony stimulating factor-I. Preliminary data suggest that TGFß also induces interferon-ß1 in human fibroblasts (HEL-299). While the mechanism of growth control by TGFß in fibroblasts is unclear, it can be postulated that this growth modulator acts through the induction of interferon. IFNα/ß inhibits EGF and insulin stimulated DNA synthesis in AKR-2B cells, whereas, it has no inhibitory effects in mouse keratinocytes. However, TBFß is a potent growth inhibitor in keratinocytes. It appears that TGFß regulates interferon-related genes differently in epithelial cells.

D 120 A NUCLEAR FACTOR 1 BINDING SITE MEDIATES THE TRANSCRIPTIONAL ACTIVATION OF A TYPE I COLLAGEN PROMOTER BY TRANSFORMING GROWTH FACTOR BETA, P. Rossi[1], G. Karsenty[1], A. B. Roberts[2], N. S. Roche[2], M. B. Sporn[2] and B. de Crombrugghe[1], [1]The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX and [2]Laboratory of Chemoprevention, NCI, NIH, Bethesda, MD.
Transforming growth factor-beta (TGF-β) causes an increase in the synthesis of several extracellular matrix proteins in fibroblasts and an increase in the steady-state levels of their RNAs. In DNA transfection experiments, TGF-β stimulates the activity of the mouse α2 (I) collagen promoter 5 to 10 fold in mouse NIH 3T3 fibroblasts and rat osteosarcoma cells. TGF-β also stimulates the α1 (III) collagen promoter but neither the SV40 early promoter nor the tyrosine amino-transferase promoter. A deletion analysis indicates that a segment of the α2 (I) collagen promoter between -350 and -300 is needed for the stimulatory effect of TGF-β. DNA binding experiments show that nuclear extracts of NIH 3T3 fibroblasts contain a factor, identified as Nuclear Factor 1 (NF1), which binds between -315 and -295 in the mouse α2 (I) collagen promoter. Insertion of the α2 (I) collagen promoter sequence which is protected by NF1, 5' to the SV40 early promoter, confers TGF-β inducibility to this promoter. A 3 bp substitution mutation in the NF1 binding site of the α2 (I) collagen promoter, which is unable to bind NF1, abolishes the response of the promoter to TGF-β and is unable to confer TGF-β inducibility to the SV40 promoter. The NF1 binding site, present at the origin of replication of adenovirus 2 and 5, inserted 5' to the SV40 promoter, also confers TGF-β inducibility to this promoter. These results indicate that a NF1 binding site mediates the transcriptional activation of the mouse a2 (I) collagen promoter by TGF-β.

D 121 PROPERTIES OF A GROWTH INHIBITORY FACTOR PRODUCED BY FETAL UROGENITAL SINUS IN ORGAN CULTURE. David R. Rowley and Donald J. Tindall, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

In order to address the role of paracrine factors in stromal-epithelial interactions, we reported previously a growth-inhibitory factor which was produced by fetal rat urogenital sinus in organ culture (UGS factor). UGS factor inhibited growth, stimulated protein secretion and altered morphology of NBT-II cells (bladder epithelial cells) in culture. In order to assess the homology of UGS factor with other known growth inhibitors, we further characterized UGS factor production and its physicochemical properties. Factor secretion by UGS explants could be maintained chronically (3-6 months) in serum-containing medium. Defined media preparations were useful for only acute periods (2-10 days). Factor production was unaffected by the use of collagen gels as explant substrate. The biological activity was a linear function of UGS factor concentration and was saturable. A maximum of 40-50% growth inhibition was achieved. NBT-II cells and MCF-7 cells were growth inhibited by both UGS factor and transforming growth factor-beta (TGF-β). Moreover, UGS factor was stable when exposed to acid (pH 3.5, 1hr) or warming (80°C, 10 min), properties shared with TGF-β. Dot-blot analysis suggested a strong immunoreactivity of UGS factor with anti-TGF-β polyclonal antisera. In addition, another anti-TGF-β antisera was neutralizing to UGS factor biological activity. Accordingly, anti-TGF-β antibodies were used in experiments to determine the molecular weight of UGS factor. Gel electrophoresis and Western analysis suggested molecular weights of 55-60 and 110-120 kilodaltons under reducing conditions. These molecular weights do not correlate with known forms of TGF-β. It is not yet clear whether UGS factor represents a novel polypeptide or a form of TGF-β which has not been previously reported.

D 122 TRANSFORMING GROWTH FACTOR β : RECEPTOR DIFFERENCES ON MYELOID LEUKEMIC CELL LINES. K. T. Sill [1], G. K. Sing [2], J. R. Keller [3], L. Ellingsworth [4] and F. W. Ruscetti [5], [1,3] P.R.I. Inc., [2,5] L.M.I., NCI-FCRF, Frederick, MD. and [4] Collagen Corp., Palo Alto, CA. Recent experiments in our laboratory have shown that transforming growth factor β (TGF-β) is a potent inhibitor of normal human hematopoiesis. Therefore, the effects of TGF-β1, were studied on human myeloid leukemic cell lines. TGF-β inhibits the proliferation of KG-1 cells (myeloblasts) as measured by ^{3}H-thymidine incorporation with an ED_{50} of 0.2 ng/ml while having no effect on the proliferation of HL-60 cells (promyelocytes). The differential response of the myeloid leukemic cell lines to TGF-β suggests that there may be differences at the receptor level. Therefore, receptor binding and crosslinking studies were employed to identify the nature of the TGF-β receptor on these cell lines. Three structurally distinct cell surface glycoproteins have been described as high affinity TGF-β receptors, a 280 Kd receptor (a component of a larger, 600 Kd receptor complex) a 85 Kd and a 65 Kd receptor. Receptor binding studies with ^{125}I TGF-β indicate specific binding to KG-1 with no detectable binding to HL-60. Crosslinking studies with ^{125}I TGF-β to KG-1 and HL-60 demonstrate that KG-1 expresses the 280 Kd component and the 85 Kd and 65 Kd receptor, while no crosslinking was observed with HL-60. The loss of TGF-β receptor expression may play a role in the development of some myeloid leukemias.

D 123 THE COOPERATION BETWEEN TRANSFORMING GROWTH FACTOR-β AND TUMOUR NECROSIS FACTOR IN THE GROWTH REGULATION OF NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS IN HUMANS. Garwin Sing, Jonathan Keller, Larry Ellingsworth and Francis Ruscetti, Lab. of Mol. Immunoregulation and Program Resources Inc., BRMP, NCI-FCRF, Frederick, and Collagen Corp., Palo Alto, CA. Transforming growth factor-β (TGF-β) is a 25 kDa polypeptide which inhibits the proliferation of immune cells, and has recently been shown in this laboratory to regulate the proliferation of hematopoietic progenitor cells in humans and mice. Since tumor necrosis factor (TNF) also inhibits hematopoietic cell growth in vitro, this present study was undertaken to evaluate the influence of combinations of TNF-α and TGF-β on normal human hematopoiesis as well as on the proliferation of leukemic myeloid and virus-infected lymphoid cell lines. The 50% effective dose (ED_{50}) required to inhibit GM-CSF driven proliferation of normal human bone marrow cells was 10x lower with a combination of TNF and 0.1 ng/ml TGF-β than if TNF was used alone, while colony formation after 14 days was almost completely inhibited in the presence of TNF+ 0.1 ng/ml TGF-β. This synergistic effect could also be demonstrated on the proliferation of the promonocytic cell line U937. However, TNF alone or in combination with TGF-β showed no effect on the proliferation of transformed T and B cell lines. These results suggest that a combination of these two soluble mediators might have a potential role to play in the treatment of various myeloid, but not lymphoid leukemias.

D 124 CHARACTERIZATION OF TGFß-RESISTANT TRANSFORMED KERATINOCYTES, N.J. Sipes, R.M.Lyons,*B.E. Weissman and H.L. Moses, Vanderbilt, Nashville, TN 37232 and *Children's Hospital of Los Angeles, Los Angeles, CA 90027.
Transforming growth factor type ß (TGFß) is a potent growth inhibitor for normal epithelial cells. As some carcinoma cells have been shown to be nonresponsive to TGFß, it is proposed that loss of TGFß sensitivity could play a role in carcinogenesis. We utilize the mouse keratinocyte cell line, BALB/MK to study growth control of normal epithelial cells. We also have a Kirsten ras-transformed derivative of these cells, KC cells (Weissman and Aaronson, Mol.Cell.Biol.5:3386,1985). Both the MK and KC cells are growth inhibited by TGFß. We have isolated variants of the KC cell line that are not growth inhibited by concentrations of TGFß that arrest the nonselected parental cells. Evaluation of TGFß binding to KC and KCR cells demonstrated that ligand-receptor interactions are operative in the resistant cells. TGFß causes specific reductions in EGF-inducible gene expression [c-myc and KC (a PDGF-inducible gene)] in the sensitive MK and KC cells; however, these genes were similarly suppressed by TGFß treatment of KCR cells despite their resistance. These results suggest that the mechanism(s) of TGFß resistance is distal to receptor binding and early signal transduction pathways. Also, changes in genetic regulation of p21 or other proteins could be a result of random integration of v-ras. Integration sites of v-ras and p21 expression levels in sensitive versus resistant cells are currently under investigation. The understanding of mechanisms by which neoplastic cells avoid growth inhibition by TGFß would provide insight into potential ways to restore TGFß responsiveness and thus growth control.

D 125 CELL TYPE SPECIFIC EXPRESSION OF TRANSFORMING GROWTH FACTOR-BETA IN NEONATAL AND ADULT MOUSE TISSUE. Nancy L. Thompson, Kathleen C. Flanders, Larry R. Ellingsworth*, Anita B. Roberts and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892 and Connective Tissue Research Laboratory, Collagen Corporation*, Palo Alto, CA 94303.
Transforming growth factor beta (TGF-beta) expression has been assayed at the whole organ level in a variety of normal and neoplastic tissues using protein bioassay or northern blot analysis. However, such analyses cannot reveal cell type specific expression in histologically complex tissues and few studies have addressed whether RNA differences are reflected at the protein level. Recently, immunohistochemically detectable TGF-beta has been localized to sites of active morphogenesis during mouse embryonic development (Heine, U. et. al., J. Cell Biol., in press). We have extended these immunohistochemical studies to include several neonatal and adult mouse tissues and now report cell type specific localization of TGF-beta using polyclonal antibodies to synthetic peptides corresponding to the N-terminal 30 amino acids of TGF-beta 1. Cell types stained with these antibodies using the avidin-biotin-peroxidase detection system include costal chondrocytes, renal tubular cells, cardiac myocytes and retinal pigment epithelial cells. Staining is greatly reduced when antibody peparations are preadsorbed on a TGF-beta-Sepharose affinity column, thus demonstrating specificity. These tissue localization studies further implicate TGF-beta as an endogenous regulator of normal cell growth and differentiation.

D 126 A NEW METHOD FOR THE PURIFICATION OF TYPE BETA TRANSFORMING GROWTH FACTOR FROM PLATELETS UTILIZING EXCLUSIVELY VOLATILE ACIDIC SOLVENTS AND RESULTING IN A HIGH PROTEIN RECOVERY. A.J.M. van den Eijnden-van Raaij, I. Koorneef, S.W. de Laat and E.J.J. van Zoelen, Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands.
A new method was developed for the purification of type beta transforming growth factor from human platelets. This method implies a combination of gel filtration, weak cation exchange HPLC and reverse phase HPLC, separating proteins according to size, charge and hydrophobicity, respectively. All steps are carried out at low pH using exclusively volatile acidic buffer solutions. The sterile conditions and easy removal of salt by lyophilization facilitate the quantification of the growth factor by biological assays. Homogenous preparations of the acid-stable, highly basic transforming growth factor beta are recognized by TGF ß1-specific antibodies raised to a synthetic oligopeptide identical to the N-terminal residues 1-29 of human platelet TGF ß1. Using the present method pure platelet TGF ß is obtained in very high yield. 40 units of outdated platelets yield 400 ug pure TGF ß, which is about a 10-20-fold higher yield than reported for other purification procedures.

D 127 INHIBITION OF ANDROGEN-INDEPENDENT HUMAN PROSTATE CANCER CELLS BY TGFβ. G. Wilding, G. Zugmeier C. Knabbe K. Flanders, and E Gelmann Medicine Branch, NCI, Bethesda MD. Men with metastatic prostate cancer inevitably develop androgen-independent tumors which are resistant to control by existing treatment modalities. Addition of an inhibitory factor, such as TGFβ, may inhibit growth. To better understand the growth control mechanisms of androgen-independent prostate cancer, we examined the effects of TGFβ on the growth of human prostate cancer cells in vitro. Specifically, the growth control of two androgen--independent lines, DU145 and PC3, and one androgen-responsive line, LNCaP, was explored. Growth in monolayer was inhibited in a dose responsive fashion by TGFβ in 2 of the lines. ^{3}H-thymidine uptake was decreased to 50% control in DU145 cells by TGFβ(5ng/ml). When anchorage-independent growth in soft agar was examined both PC3 and DU145 formed fewer colonies (55% and 16% of control, respectively) in the presence of TGFβ. LNCaP cell growth in monolayer and soft agar was unaffected by TGFβ. Scatchard analysis showed 1500 and 2900 TGF binding sites/cell on DU145 and PC3 cells, respectively (Kd = 10^{-12}M) while receptors on LNCaP cells could not be identified. Analysis of conditioned media confirmed secretion of TGFβ into the media by DU145 and PC3 cells but not LNCaP cells. Using a 1kb TGFβ cDNA probe, Northern blot analysis confirmed the presence of TGFβ mRNA in PC3 and DU145 cells but not LNCaP cells. To evaluate the inhibitory effects of TGFβ on DU145 cell growth in vivo, studies are underway using TGFβ infusions in a nude mouse model. These data indicate that TGFβ might serve as an important role as an autocrine inhibitory factor in prostate cancer.

D 128 AUTO-INDUCTION OF TGFß1 mRNA IN A FIBROBLASTIC AND AN EPITHELIAL CELL LINE. Jana F. Wolfshohl, Charles C. Bascom, Robert J. Coffey, and Harold L. Moses. Vanderbilt Medical School, Nashville, TN 37232.
The recent demonstrations that TGFα and PDGF can amplify expression of their own genes suggest that normal growth regulatory mechanisms might involve autocrine signals to carefully modulate the growth response. We have further examined for auto-induction by TGFß1 in both murine AKR-2B fibroblasts and Balb/c mouse keratinocytes (BALB/MK). In AKR-2B fibroblasts, in which TGFß1 induces latent stimulation of DNA synthesis by enhancing c-sis expression, induction of TGFß1 mRNA is observed as early as 1 hr after TGFß1 treatment with maximal induction after 3-6 hrs. In BALB/MK cells, which are reversibly growth inhibited by TGFß1, induction of TGFß1 mRNA is also observed but only after 12-24 hrs following the addition of TGFß1. The auto-induction of TGFß1 expression in the BALB/MK cells is blocked by cycloheximide, suggesting that it is dependent upon protein synthesis. Furthermore, nuclear run-on experiments are underway to determine whether this accumulation of TGFß1 mRNA is a transcriptional or a post-transcriptional event. Because TGFß1 is secreted by these cells in a latent form, it is not clear whether increased TGFß1 mRNA levels would result in an increase in active material.

Tumor Necrosis Factor

D 200 EVIDENCE FOR TUMOUR NECROSIS FACTOR PRODUCTION IN CANCER, Frances Balkwill, *Richard Osborne, Frances Burke, Stuart Naylor, *Denis Talbot, Helga Durbin, +Walter Fiers, Imperial Cancer Research Fund, London, WC2A 3PX, U.K. *I.C.R.F. Dept of Medical Oncology St. Bartholomews and Homerton Hospitals, London, U.K. +University of Ghent, Belgium.
Using an ELISA assay we have detected highly labile tumour necrosis factor-α, TNF-α activity in 50% of 226 freshly obtained serum samples from cancer patients wich active disease. In contrast, only 3% normal individuals and 18% of cancer patients in durable complete remission showed low levels of this factor. Serum samples from patients with ovarian and oat cell carcinoma were more frequently positive (69% and 63%) than those from patients with lymphoma (26% positive). When RNA preparations from peripheral blood mononuclear cells PBMC and solid tumours were probed with TNF-α cDNA, we found TNF-α message in 8 of 11 cancer patient PBMC but only 1 of 8 normal PBMC. In addition, TNF-α message was detected in 2 of 6 colorectal tumours. TNF-α production may be involved in host response cancer but could also contribute to the symptoms and evolution of the disease.

D 201 SYNERGISTIC CYTOTOXIC EFFECTS OF RECOMBINANT TUMOR NECROSIS FACTOR AND RHODAMINE-123 ON HUMAN MAMMARY TUMOR EPITHELIAL CELL LINES, Vimla Band, Paul Yaswen, Martha Stampfer, Andrew Wasserman, and Ruth Sager, Dana-Farber Cancer Institute, Boston, MA 02115. Cytotoxic effects of recombinant human tumor necrosis factor- (TNF) and Rhodamine-123 (R-123) on normal human breast epithelial and tumor cell lines were investigated. TNF was cytotoxic to tumor cell lines whereas experimentally-induced tumor cells, immortalized cells, and normal mammary epithelial cells were several fold more resistant. Tumor lines showed elevated uptake and retention of R-123 and elevated cytotoxicity as compared to normal and immortalized non-tumorigenic mammary epithelial cell lines. When cells were treated with TNF plus R-123, synergistic cytotoxicity was observed with the clinical tumor lines, whereas experimentally-induced tumor cells, immortalized cells and normal cells were affected much less. Thus, the synergistic cytotoxicity appears to require initial sensitivity to both TNF and R-123. In the content of chemotherapy, the synergistic cytotoxicity of TNF plus R-123 may permit considerable dose-reduction in circumstances in which the tumor cells are TNF-sensitive.

D 202 γ-IFN ACCELERATES DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA IN VITRO, R.Black, Z-Q. Zou, J. Ridge, J.B. Harford, and E. Chang, USUHS, FDA, NICHD, Bethesda, Md. 20814 The antiproliferative activity of interferon(IFN) has been evaluated as a potential therapeutic agent in a variety of model systems. In this report, the effect of γ-IFN on human squamous cell carcinomas(HSCCA) has been characterized. Upon treatment with 200U/ml γ-IFN, the HSCCA cell line, A431, appears to be stimulated to undergo terminal differentiation. In monolayer culture, this process is associated with nearly total cell death within 6 days of initiation of treatment and is accompanied by a dramatic enhancement of epidermal growth factor receptor(EGFR) 10kb mRNA expression at 24-48 hrs post treatment. This antiproliferative effect has been examined further in a panel of 5 HSCCA cell lines. Northern analysis has confirmed an association between the inhibition of growth by γ-IFN and the increase in EGFR mRNA expression. To establish conditions in which growth is most effectively inhibited, the HSCCA cell lines were treated with γ-IFN and/or tumor necrosis factor(TNF). Although γ-IFN alone is a potent inhibitor of cell growth of 4 of the 5 cell lines, the two mediators appear to act synergistically. In a 3-dimensional agar disc culture system, γ-IFN induced A431 differentiation is characterized by expression of a 67kd keratin species, specific for differentiated squamous cells, and extensive cell death. In order to evaluate the therapeutic potential of γ-IFN/TNF treatment in a system more closely approximating the in vivo situation, the 3-dimensional culture system has been used to maintain 1° explants of HSCCA tumors during treatment. 50-60% of the tumors have responded to this treatment regimen. Experiments are in progress to determine whether elevated EGFR may be used as a marker for sensitivity of tumors to γ-IFN/TNF treatment.

D 203 GROWTH-INHIBITION OF B-LYMPHOID CELL LINES: CELL CYCLE CONTROL AND C-MYC RNA REGULATION, Heidi K. Blomhoff[1], Jon Lømo[1], Rune Blomhoff[2], Harald Holte[1], Trond Stokke[1], Erlend B. Smeland[1], Steinar Funderud[1] and Tore Godal[1], [1]Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; [2]University of Oslo, Norway. By comparing the effects of five different growth-inhibitors on the B-lymphoid cell lines Reh and Raji, we found that down-regulation of c-myc RNA was not a prerequisite for reduced cell-proliferation, but was associated with G_1 arrest. The adenylate cyclase activator forskolin and the phorbol-ester TPA both reduced the c-myc RNA levels within 3 hours and increased the proportion of cells in middle to late G1 within 3 days of treatment. On the other hand, interferon-γ, tumor necrosis factor-α and the anti-class II monoclonal antibody 33-1 did neither reduce the c-myc RNA level nor block the cells in G_1. Growth-inhibition was not associated with differentiation.
The effect of forskolin on the cell-cycle distribution was studied in more detail, and it became evident that prior to the G_1 arrest, the Reh cells were transiently delayed in G_2 for 10-15 hours. This effect was however dependent on where in the cell cycle forskolin was added. Thus, cAMP-mediated growth-inhibition might involve perturbation of the cell-cycle in both G_2 and G_1.

D 204 COMPARATIVE GROWTH MODULATORY EFFECTS OF A LIVER DERIVED GROWTH INHIBITOR (LDGI), TRANSFORMING GROWTH FACTOR β, (TGF-β) AND RECOMBINANT TUMOR NECROSIS FACTOR (TNF-α) IN RAT AND HUMAN CELL CULTURE SYSTEMS, Mrunal S. Chapekar, Anthony C. Huggett, and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD. 20892.

Growth modulatory effects of LDGI, TGF-β, and rTNF-α were examined in three human and five rat cell culture systems using a rapid semiautomated system developed to measure DNA DNA content and synthesis in adherent cells (Richards et al Exp.Cell.Res.159:235-246,1985). Normal rat liver epithelial (RLE) cells were highly sensitive to LDGI (ID_{50}= 0.2ng/ml) and TGF-β (ID_{50}= 0.3ng/ml), and exhibited a moderate sensitivity to rTNF-α after two days of treatment (ID_{50}> 5000 units/ml). Aflatoxin transformed RLE, on the contrary, were resistant to the antiproliferative effects of TGF-β and rTNF-α, but showed some sensitivity to LDGI (ID_{50}= 1.5ng/ml). Human breast carcinoma (MCF-7) and rat hepatoma (Rueber) cells were extremely sensitive to rTNF-α (ID_{50}= 10 and 20 units/ml respectively); exhibited sensitivity at relatively higher concentrations of LDGI (ID_{50}= 1 ng/ml), but were resistant to the antiproliferative effects of TGF-β. Rat hepatoma UVM 7777 cells, however, were resistant to the growth inhibitory effects of all the three modulators. The rate of DNA synthesis in rat kidney fibroblasts (NRK) and human foreskin fibroblasts was significantly stimulated in response to all the modulators. These data demonstrate that LDGI, TGF-β and rTNF-α exert positive and negative modulations in different cell systems, and indicate that the effects of LDGI are different from those of TGF-β and rTNF-α in some cell systems.

D 205 REGULATION OF TNF α AND TNF β mRNA BY HEMATOPOIETIC GROWTH FACTORS, François DAUTRY and Dominique WEIL, Laboratoire d'Oncologie Moléculaire, Institut Gustave Roussy, 94805 Villejuif, FRANCE.

During our investigation of gene regulation by IL2 and IL3 in murine hematopoietic cell lines, we have found that TNF genes are part of the primary response (i.e. independent of de novo protein synthesis) to these growth factors. In two lymphocytic cell lines (CTLL-2 and B6.1) both TNF α and TNF β genes are induced by IL2. This expression does not correlate with the cytotoxic activity, in agreement with the absence of data implicating TNF in lymphocyte cytotoxicity. Furthermore actively growing cells, and not cells growth arrested by IL2 deprivation, express substantial levels of both messages indicating that its expression is part of the normal cell cycle. Similarly, the IL3 - dependent myeloid cell line FDCP-2 expresses the TNFα message in response to IL3 stimulation. Since TNF is a growth regulator of both T lymphocytes and monocytic precursors, these results suggest the participation of TNF in the control of the proliferative response to IL2 and IL3. We are currently investigating this possible autocrine loop with antibodies to TNF and recombinant TNF.

D 206 TUMOR NECROSIS FACTOR MODULATES EGF RECEPTOR KINASE ACTIVITY IN SENSITIVE BUT NOT RESISTANT HUMAN TUMOR CELLS, Nicholas J. Donato[1], Gary L. Gallick[2], Jordan U. Gutterman[1] and Michael G. Rosenblum[1]. Departments of [1]Clinical Immunology and [2]Tumor Biology, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Several human tumor cell lines are sensitive to the cytotoxic and cytostatic effects of recombinant tumor necrosis factor (TNF). The molecular basis for sensitivity and resistance is unknown but does not appear to be due to differences in TNF binding. We have examined the effect of TNF on EGF receptor kinase activity in resistant and sensitive cell lines with equivalent TNF binding sites per cell. In cell lines sensitive to TNF (ME-180, BT-20), the effects of TNF on EGF-R kinase were similar to those observed with EGF. In cells resistant to TNF action, TNF had no effect on EGF-R kinase. In metabolicly labelled ME-180 cells, TNF induced an increase in orthophosphate-labelled EGF receptor, whereas there was no change in the amount of ^{35}S-methionine incorporated. Addition of TNF to immune complexes from sensitive cells resulted in modulation of EGF-R kinase activity identical to what was observed in intact cells. These results suggest that TNF interacts directly with EGF receptor, or in a complex between EGF-R and other proteins to modulate the kinase activity of this receptor.

D 207 SPECIFIC REDUCTION IN SRC KINASE ACTIVITY IN HT-29 HUMAN COLORECTAL CARCINOMA CELLS CORRELATES WITH GROWTH INHIBITION BY INTERFERONδ AND TUMOR NECROSIS FACTOR, Gary E. Gallick[1], Rachelle B. Crosbie[1], Nicholas Donato[2], Michael Rosenblum[2] and Catherine L. Novotny[1] Departments of [1]Tumor Biology and [2]Clinical Immunology, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.
In human colorectal tumors and cell lines a specific 30-60 fold increase in src kinase activity is observed with respect to normal colonic mucosa. To study the mechansim of src "activation" in colon tumor cell lines, we examined the effects on src kinase activity after growth inhibition by recombinant Tumor Necrosis Factor (TNF) and interferon δ (IFNδ). 50% growth inhibition was observed after 72 hours growth using 50,000 IU/ml δTNF or 500IU/ml IFNδ . At these concentrations, the agents were cytostatic rather than cytotoxic. In immune complex kinase assays from these cultures, the autophosphorylation of $pp60^{src}$ was reduced 15-20 fold in cells growth-inhibited by either agent as compared to control cultures. A similar reduction was observed in phosphorylation of the exogenous substrate, enolase. However, no substantial changes were observed in the level of $pp60^{src}$ between growth-inhibited and control cells as determined by immunoblotting. A ten fold reduction in autophosphorylation and substrate phosphorylation was observed after 1hr; however, no effect was observed by direct addition of the agents to the immune complexes. In HT-29 cells resistant to growth inhibition by IFNδ growth in 2000 u/ml of this agent induced no changes in src kinase activity. These results suggest that regulation of src kinase activity may play an important role in growth control of human colon cells.

D 208 A RELATIONSHIP WITH DIFFERENTIATION INDUCING FACTOR TO LYMPHOTOXIN. U.Gullberg, M.Lantz, C.Peetre, I.Olsson. Dept of Hematology, University of Lund, Sweden.
Differentiation Inducing Factor (DIF) is released from the HTLV I-infected T-lymphocyteline HUT-102. DIF has been partly characterized and has maturation and growth inhibitory effects on hematopoietic cells. DIF is compared to Tumor Necrosis Factor (TNF) an Lymphotoxin (LT) on the basis of functional, biochemical and antigenic similarities.
DIF, TNF and LT showed similar growth supression of myeloid cell lines. However, HL-60 wild type was resistant to this growth inhibition but with higher concentrations all three factors induced HL-60 to monocyte like cells.
The mw of DIF was 45kD judged from assay of biological activity after SDS-PAGE. Isoelectric focusing revealed a heterogeneity with pI of 5.4-5.8. This is distinct from TNF with a mw of 17kD in SDS-PAGE and a pI of 6.0. LT resolves as 20 or 25kD species on SDS-PAGE.
An antigenic relationship was found between DIF and LT since a neutralizing antiserum to LT neutralized the DIF effect. In addition, a monoclonal antibody to LT coupled to Sepharose did bind DIF in a reversible way.
Competition experiments indicated that TNF and LT recognize the same receptor. This is in consistence with results from other cell systems and may explain the similar effects on hematopoietic cells. An activity, that cochromatographed with DIF through several steps of purification, competed with binding of both TNF and LT. Activation of proteinkinas C with PMA or diacylglycerol rapidly decreased the number of binding sites for TNF and LT.
Detailed biochemical and structural data will be required to establish the exact identity and relationship of cytokines released from T-lymphocytes.

D 209 TUMOR NECROSIS FACTOR STIMULATES PROLIFERATION OF HUMAN OSTEOSARCOMA CELLS AND TRANSCRIPTION OF MYC MESSENGER RNA, Martina Kirstein* and Corrado Baglioni, *The Rockefeller University, New York, NY 10021, State University of New York at Albany, Albany, NY 12222. The objective of this study was to establish whether human recombinant tumor necrosis factor (TNF) can significantly stimulate the proliferation of some tumor cells. Treatment with TNF had little or no effect on the growth of human and murine cells cultured in medium with high serum concentration. Two tumor cell lines, SK-MEL-109 melanoma and HOS osteosarcoma cells, were adapted to grow in medium supplemented with 0.5% serum. The growth of these SK-MEL-109-LS cells was inhibited by TNF, but that of HOS-LS cells was greatly stimulated by TNF in a dose-dependent way. Treatment with 10 ng/ml of TNF resulted in a two-fold increase of the rate of cell division. This effect of TNF was also shown by measuring DNA and protein synthesis. The continuous presence of TNF however was not required for its mitogenic activity on HOS-LS cells. Interferons abolished the mitogenic activity of TNF on HOS-LS cells. Addition of polypetide growth factor such as insulin or epithelial growth factor did not enhance the growth-stimulatory activity of TNF. The level of *myc* mRNA was increased after 30 min of treatment with TNF. Interferons did not interfere with the induction of *myc* mRNA. This shows that for HOS-LS cells TNF is a growth factor, which presumably induces transcription of competence genes.

D 210 Differentiation of HL-60 Cells is Associated with Increases in the 35 kDa Substrate of the EGF Receptor/Protein Kinase, Felicia William*, Stanley Cohen# and Andrew S. Kraft*, Division of Hematology/Oncology*, University of Alabama, Birmingham, Al 35294, and the Department of Biochemistry#, Vanderbilt University, Nashville, TN 37232.
The addition of epidermal growth factor (EGF) to A431 human epidermoid carcinoma cells stimulates the phosphorylation of a 35 kDa protein. This 35 kDa protein has been identified as lipocortin 1, a protein which may inhibit phorpholipase A_2 in situ. Because lipocortin 1 is found both in undifferentiated HL-60 cells and mature monocytes, we have examined changes in lipocortin 1 during differentiation of HL-60 cells. To accomplish these studies, we have raised a polyclonal antibody in rabbits to an amino terminal peptide of the 35 kDa protein. This antibody recognizes a single 35 kDa protein in HL-60 and A-431 both by immunoprecipitation and western blotting. Using this antibody, we have demonstrated that differentiation of HL-60 cells towards macrophages with PMA (.1 uM) induces increases over 72 hours in the 35 kDa protein. Also, vitamin D_3 which induces differentiation towards macrophage phenotype and dibutyrl C-AMP which stimulates granulocyte changes stimulates increases in 35 kDa protein content. In contrast, bryostatin 1 which activates protein kinase C, but does not cause differentiation,does not effect changes in 35 kDa levels. Using our antipeptide antisera we have established an RIA to quantitate changes in the levels of the 35 kDa protein. The 35 kDa protein partially purified from differentiated HL-60 cells will bind to membranes in a calcium-dependent fashion and can be removed from the membrane by treatment with PLA_2 or PLC. These experiments demonstrate that the cellular content of a known growth factor substrate, the 35 kDa protein,can be modified during differentiation.

D 211 INDEPENDENT REGULATION OF TUMOR NECROSIS FACTOR AND LYMPHOTOXIN GENE EXPRESSION IN HUMAN TUMOR CELLS, Martin Krönke, Gabriele Hensel, and Klaus Pfizenmaier, Clinical Research Group "BRWTI" of the Max-Planck-Society, 3400 Göttingen, FR Germany.
Tumor necrosis factor (TNF) and lymphotoxin (LT) are two partially homologous cytokines produced by macrophages and cell types of hematopoietic origin. It appears that these agents may play both protective and pathological roles during parasite infections, autoimmune disorders, and neoplastic diseases. TNF and LT bind to the same cellular receptor and have very similar biological activities. We here present evidence that certain leukemic cell lines constitutively express both TNF and LT mRNA. However, only LT, but not TNF, is secreted into the culture supernatant. Immunoprecipitation analysis revealed a cell-associated 26 kD TNF protein, which presumably is integrated in the cell membrane. Following stimulation with PMA, a soluble 17 kD TNF molecule can be detected, suggesting an additional regulatory level for TNF secretion. 11 of the 17 tumor cell lines investigated lacked TNF or LT mRNA expression. Following stimulation with PMA, 9 of these 11 tumor cell lines accumulated TNF mRNA. In contrast, only 3 cell lines were induced to synthesize LT mRNA, indicating differential requirements for TNF and LT gene activation, respectively. Various cytokines of recombinant source can also induce TNF mRNA synthesis. For example, interferon (IFN)-alpha, IFN-gamma, LT, and TNF itself, all are able to elicit TNF mRNA accumulation in HL-60 cells. The kinetics of TNF mRNA induction depends on the cell type investigated, suggesting that regulation of TNF gene expression is different in different tissues. TNF and LT production by tumor cells may contribute to pathological syndroms of neoplastic diseases like cachexia, lytic bone lesions etc. and, thus, questions the value of TNF treatment in certain tumor entities.

D 212 EFFECTS OF DEXAMETHASONE AND INTERFERON-GAMMA ON CACHECTIN/TNF BIOSYNTHESIS, Christina Luedke and Anthony Cerami, Rockefeller University, New York, NY 10021.
Cachectin/Tumor Necrosis Factor has been implicated as an early mediator of both endotoxic shock and the cachexia of chronic disease. Since exogenous cachectin can reproduce these lethal effects, we hypothesize that the synthesis of endogenous cachectin in response to natural stimuli must be tightly controlled. We are interested in defining the normal controls on cachectin biosynthesis and the pharmacologic treatments which might best limit its production. Initial studies in our lab by Beutler et al. (Science 232:977, 1986) showed that dexamethasone significantly decreases the amount of cachectin secreted by endotoxin-stimulated murine peritoneal macrophages, and that it acts at both transcriptional and post-transcriptional levels. Our recent work focuses on the mechanisms of dexamethasone suppression through studies of transcription rates, mRNA stability, and in vitro translation of macrophage mRNA. These studies include the effects of interferon-gamma, which strongly induces cachectin mRNA in the presence of endotoxin and is able to increase significantly the amount of cachectin secreted by dexamethasone-treated macrophages. Preliminary results indicate that dexamethasone causes an incomplete post-transcriptional block to cachectin synthesis which may be broken through by high levels of cachectin mRNA and that the message produced by dexamethasone-treated cells is competent for translation.

D 213 THE MITOGENIC ACTION OF TNF IN HUMAN FIBROBLASTS: INTERACTIONS WITH EGF AND PDGF.
Vito J. Palombella, John Mendelsohn, and Jan Vilcek, New York University Medical Center, New York, NY 10016 and Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
We have previously shown that tumor necrosis factor (TNF) can increase the number of epidermal growth factor (EGF) receptors on human FS-4 fibroblasts and that this increase may be related to the mitogenic action of TNF in these cells. Here we showed that TNF stimulated the growth of FS-4 fibroblasts in a chemically-defined, serum-free medium in the absence of EGF. Anti-EGF receptor antibody, which blocked the mitogenic effects of EGF in FS-4 cells, did not inhibit the mitogenic action of TNF in serum-free or serum-containing medium, indicating that EGF or an EGF-like molecule was not responsible for the mitogenic effects of TNF. However, the simultaneous addition of TNF and EGF to cells grown in serum-free medium resulted in a synergistic stimulation of DNA synthesis and cell growth. The actions of TNF and EGF were also examined in growth-arrested FS-4 cells and were compared to the action of platelet-derived growth factor (PDGF). In the absence of other growth factors, TNF was a relatively weak mitogen in growth-arrested cells, compared to EGF or PDGF. Nevertheless, TNF synergized with EGF or high doses of PDGF in stimulating DNA synthesis. Furthermore, antibodies specific for TNF or the EGF receptor were used to selectively inhibit the actions of these two factors, after specific incubation periods, in growth-arrested cells treated concurrently with EGF and TNF. To produce an optimal stimulation of DNA synthesis, EGF had to be present for a longer period of time than TNF. We conclude that in their synergistic action on growth-arrested FS-4 cells, EGF was responsible for driving the majority of the cells into S phase, while TNF appeared to make the cells more responsive to the mitogenic action of EGF. The findings indicate that TNF can cooperate with, and enhance the actions of, EGF in promoting DNA synthesis and cell division.

D 214 ALTERATIONS IN CYTOKINE BINDING INDUCED BY RECOMBINANT HUMAN TUMOR NECROSIS FACTOR ALPHA AND GAMMA INTERFERON ON HUMAN PANCREATIC CARCINOMA CELLS. Arthur B. Raitano, Murray Korc, and Philip Scuderi, Arizona Cancer Center, University of Arizona, Tucson, AZ, 85724. We have previously described two human pancreatic carcinoma cell lines, Colo 357 and ASPC-1, that exhibit enhanced sensitivity to the combined cytotoxic effects of rhTNF-α and rhIFN-γ. The present study was undertaken to examine the role of receptor modulation as a possible mechanism for these effects. Binding studies were performed using I^{125} labelled rhTNF-α and rhIFN-γ. Cytotoxic effects were measured using the MTT cytotoxicity assay. Both cell lines specifically bound relatively high levels of IFN-γ and low levels of TNF-α. Preincubation of ASPC-1 cells with TNF-α resulted in a 2-fold increase in IFN-γ binding at 4^{o} and 37^{o}C. Upregulation occured within 4 hours of pretreatment and required RNA and protein synthesis. Preincubation of Colo 357 cells with IFN-γ resulted in a moderate increase in TNF-α binding. In a short term cytotoxicity assay, 24 hour pretreatment of cells with one cytokine followed by a 48 hour incubation with the other, was not as effective as a 48 hour simultaneous incubation. Extending the incubation period to 7 days increased the cytotoxic effects on both cell lines. In a 7 day incubation period, IFN-γ no longer required the presence of TNF-α to be maximally effective. In contrast, 24 hour pretreatment of cells with IFN-γ increased the long term cytotoxic action of TNF-α. Conclusions: 1. Both Colo 357 and ASPC-1 cells behave similarly with respect to the cytotoxic effects induced by TNF-α and IFN-γ. 2. These cell lines behave differently with respect to their ability to bind TNF-α and IFN-γ after pretreatment with the reciprocal cytokine. 3. The additive/synergistic cytotoxic effect of TNF-α and IFN-γ may involve cytokine receptor modulation and as yet undefined post-receptor mechanisms.

D 215 γ-INTERFERON AND TUMOR NECROSIS FACTOR MODULATE THROMBOSPONDIN PRODUCTION BY HUMAN BLOOD MONOCYTES. Bruce Riser, James Varani, Brian Nickoloff, and Vishva Dixit. University of Michigan, Ann Arbor, MI. 49109
Human periferal blood monocytes (HPBM) synthesize, secrete and bind the extracellular matrix molecule thrombospondin (TSP). Exposure of HPBM cultures to the cytokines γ-interferon (γ-IFN) or tumor necrosis factor (TNF) resulted in a marked increase in TSP production. This may relate to the ability of HPBM to recognize, bind, and effect lysis of human squamous carcinoma cells (HSCC) in vitro. These tumor cells which also synthesize and bind TSP via specific receptors (utilizing TSP as an adhesion molecule), were sensitive to in vitro lysis by HPBM. Further, the HSCC line UM-SCC-11B which exhibits high levels of TSP production and specific receptor binding was highly sensitive to in vitro HPBM-mediated cytolysis. In contrast, the HSCC cell line UM-SCC-22B which exhibits low TSP production and specific receptor binding was largely resistant to killing. In addition, rabbit polyclonal antibodies to TSP were able to interfere with HPBM-mediated cytolysis of HSCC. These results suggest that TSP may play an important role in leukocyte-HSCC interactions serving as an intracellular bridge and that the cytokines γ-IFN and TNF may act to regulate this anti-tumor activity by modulation of HPBM surface TSP or TSP receptors.

D 216 DIFFERENTIAL REGULATION OF ONCOGENE EXPRESSION IN TRANSFORMED MOUSE FIBROBLASTS BY IFN-GAMMA AND TNF-ALPHA, Barbara Seliger and Klaus Pfizenmaier, Clinical Research Group "BRWTI" of the Max-Planck-Society, 3400 Göttingen, FR Germany.
Both tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) exhibit antineoplastic activity on a braod range of animal and human tumor cell lines. In order to define the potential mechanisms of both TNF-alpha- and IFN-gamma-action on tumor cells, we investigated the effect of murine recombinant TNF-alpha and IFN-gamma on mouse fibroblasts transformed by distinct retroviral vectors carrying the v-mos, c-myc and v-Ha-ras oncogene, respectively. The data obtained indicate that treatment with both cytokines induces phenotypical reversion of v-mos and c-myc, but not Ha-ras-transformed cells, as revealed from a strongly reduced proliferative capacity in monolayer culture, inhibition of soft agar colony formation and changes in morphology. Phenotypical reversion of mos- and myc-transformants was preceded by a selective reduction of oncogene-specific steady RNA levels. Removal of TNF-alpha and IFN-gamma, respectively, from these revertants resulted in complete retransformation. In v-Ha-ras-transformants, no inhibition of neoplastic phenotype was observed despite a downregulation of oncogene-specific RNA levels by IFN-gamma treatment. Our data further indicate that IFN-gamma action of LTR-controlled oncogene expression occurred at the transcriptional level, apparently due to inhibition of LTR-activity. In contrast, TNF primarily affected LTR-controlled oncogene expression at the post-transcriptional level. However, in both instances, de novo protein synthesis was not required for this cytokine action.

D 217 PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC FOR MURINE TUMOR NECROSIS FACTOR. Kathleen F. Sheehan and Robert D. Schreiber, Dept. Pathology, Washington Univ. Sch. Med. St. Louis, MO 63110.
Monoclonal antibodies (MoAb) to Murine Tumor Necrosis Factor (rMuTNFα) were produced following immunization of Armenian hamsters with purified, E. coli-derived recombinant TNFα (rMuTNFα). Antibody-producing cultures were identified using an ELISA that employed solid-phase rTNFα. Of 576 wells plated, 318 were growth positive and 48 produced antibody. Supernatants from 36 cultures neutralized the lytic activity of rMuTNFα. Purified MoAb from 2 different cloned cell lines were tested for their ability to inhibit natural TNFα and TNFß (Lymphotoxin). TN3-19.12 and TN3-72.16 inhibited 100% of the lytic activity in culture wells containing 20 units rMuTNFα (50% inhibition at inputs of 19.12 and 72.16 of 28 and 200 ng respectively, 100% inhibition at inputs of 300 ng of either MoAb). Both MoAb also inhibited all the activity contained in supernatants of a stimulated T cell clone that produced both TNFα and TNFß (as judged by Northern blot analysis). Moreover, all the lytic activity was removed from the T cell supernatant following absorption on a TN3-19.12 Sepharose column but not on a control column containing irrelevant hamster IgG. The epitopes for either MoAb survived SDS-PAGE and transfer to nitrocellulose and thus permitted detection of TNFα by Western blot analysis. Either MoAb has been used in conjunction with rabbit polyvalent anti-rMuTNFα to establish a TNF-specific ELISA. Attempts to identify antibodies that react only with TNFα and not TNFß are ongoing. These unique reagents should be useful in assessing the role of TNF in immune regulation.

D 218 RECOMBINANT INTERLEUKIN-1 (IL-1β) REGULATES THE DIRECT AND MONOCYTE-MEDIATED TUMORICIDAL ACTIVITY OF TUMOR NECROSIS FACTOR (TNF), Diana M. Smith, Gregory A. Lackides and Lois B. Epstein, University of California, San Francisco, CA 94143.
Studies in our laboratory indicate that IL-1β is an important regulatory monokine which suppresses the direct and monocyte-mediated tumoricidal activity of TNF. The direct cytotoxicity of TNF and IL-1β alone, and in combination for murine fibrosarcoma WEHI 164 clone 13 cells was determined in an 18 hr tetrazolium microcytotoxicity assay in the presence of actinomycin D. The data show that the tumor targets were highly sensitive to the cytotoxic effects of TNF alone and relatively resistant to lysis by IL-1β. When cells were treated with TNF and IL-1β in combination, higher concentrations of TNF were required to reduce cell viability by 50% than with TNF alone. Pretreatment of human monocytes with either TNF or IL-1β also enhanced monocyte-mediated cytotoxicity for WEHI 164 tumor targets in a short term ^{51}Cr-release assay. When monocytes were pretreated for 24 hr with TNF and IL-1β in combination, the cytotoxicity observed was less than the expected additive effects of TNF and IL-1β and in many cases was less than that observed with TNF or IL-1β alone. The antagonistic effect of IL-1β and TNF was more pronounced when the cytotoxic phase of the assay was extended from 8 hr to 18 hr. These results show that IL-1β regulates the direct and monocyte-mediated tumoricidal activity of TNF and indicate the importance of exploring the cellular interactions of various biologic response modifiers prior to their use in clinical trials.
Supported by NIH grant CA 44446.

D 219 INHIBITORY EFFECT OF RECOMBINANT HUMAN TUMOR NECROSIS FACTOR ON THE IN VITRO GROWTH OF NORMAL HUMAN MEGAKARYOCYTIC PROGENITOR CELLS, B.Völkers,A.Ganser,J.Greher,C.Carlo Stella+,D.Hoelzer,Dept.of Hematology, Univ.of Frankfurt,D-6 Frankfurt ,F.R.G.,+Dept.of Int.Med.&Med.Therapy,Pavia Human tumor necrosis factor a cytokine with an in vitro cytotoxic effect to different malignant human cell lineages has recently been shown to inhibit in vitro growth of normal hematopoietic progenitor cells,however little is known about its effect on normal human megakaryopoiesis.In this study the effect of recombinant human tumor necrosis factor(rhTNF-∝/Knoll,F.R.G.)on the growth of normal human bone-marrow derived megakaryocytic progenitors (CFU-Mk)was tested in a clonal culture system containing 30% human plasma in IMDM,5% PHA-leucocyte conditioned medium,0.9% methylcellulose,$5x10^{-5}$M 2-ME and 1 U/ml rh-erythropoietin.Addition of rhTNF-∝(1 U-300 U/ml)to unseparated bone-marrow cells resulted in a dose dependent inhibition of CFU-Mk(50% inhibition at 10 U/ml;complete inhibition of CFU-Mk colony formation at 300 U/ml in all experiments).Removal of adherent cells and T-lymphocytes from the bone-marrow target cells only slightly influenced the inhibitory activity of rhTNF-∝.(50%ID-30 U/ml,100%ID-300 U/ml).It is concluded that rhTNF-∝ markedly inhibits the growth of CFU-Mk and the inhibitory effect does not appear to be mediated by autologous monocytes and T-lymphocytes in the bone-marrow samples.
Supported by the Deutsche Forschungsgemeinschaft(Grant No. GA 333/1-1)

D 220 HIERARCHY OF MOLECULAR MECHANISMS CONTROLLING TUMOR CELL SENSITIVITY TO THE CYTOTOXIC ACTION OF LYMPHOTOXIN/TNF, Carl F. Ware, Frederick Coffman, Lora M. Green and William H. Fletcher, University of California, Riverside, CA 92521 and the Jerry Pettis VA Hospital, Loma Linda, CA 92357. Several levels of control appear to be in operation that determine whether a tumor cell will undergo a cytotoxic response to lymphotoxin (LT) or tumor necrosis factor (TNF). Expression of specific high affinity receptors is a prerequisite but not sufficient for lysis to occur. We have recently established that the sensitivity to the cytotoxic action of LT/TNF is strongly correlated with target cells that are gap junction imcompetent (Fletcher et al., J. Immunol. 139:956, 1987). Within tumor cell lines, we have found pre-existing subclones which differ markedly in both dose response and kinetic behavior upon addition of LT/TNF. Two subclones of the human cervical carcinoma ME-180 cell line express the same number of TNF receptors with identical TNF binding constants, are both gap junction deficient as measured by dye transfer, yet differ more than 40 fold in their sensitivity to TNF. At saturating levels of TNF (30 nM), cell death in the sensitive clone begins earlier (8 hr vs 20 hr) and proceeds faster than in the resistant clone (6% lysis/hr vs 1%/hr). Addition of the specific DNA topoisomerase II inhibitor VM-26 to the resistant subclone abolishes these differences and the resulting time course of cell death is indistinguishable from the sensitive subclone. These results indicate that a hierarchy of processes are operative in determining the response of tumor cells to these toxins.
Supported by NCI CA35638, HD13704 and HD21318.

D 221 ANTIPROLIFERATIVE ACTIVITY OF INTERFERONS AND TUMOR NECROSIS FACTOR ON HUMAN COLON CARCINOMA CELLS, V.L.Y. Wong, M.A. Anzano, D.J. Rieman, L. Aronson, B. Dalton* and R. Greig, Dept. of Cell Biology and *Dept. of Immunology and Anti-infectives, Smith Kline & French Laboratories, King of Prussia, PA 19406

Recombinant human interferon beta (IFN-ß) inhibited in a time- and dose-dependent manner the proliferation of 18/18 human colon carcinoma cell lines in monolayer cultures and 8/9 lines in a soft agar assay. Four human fibroblast lines were unaffected. Inhibitory activity was neutralized by polyclonal antibodies against natural IFN-ß. Repetitive treatment with IFN-ß (50 units/ml) at 2 day intervals for 8 days caused > 90% inhibition. In addition, natural human interferon-alpha (IFN-α), recombinant human interferon-gamma (IFN-γ) and recombinant tumor necrosis factor (TNF) each inhibited to varying degrees (30-80%) the proliferation of several colon carcinoma cell lines. However, when employed in combination, IFN-ß, IFN-γ and TNF induced a synergistic blockade of colon carcinoma cell growth. The potent inhibitory effect of these lymphokines provides an informative in vitro system for examining the pharmacology of colon epithelial cell proliferation.

Growth Inhibitory and Cytotoxic Polypeptides

Interferons, Recessive and Suppressor Genes

D 300 FUNCTIONAL ANALYSIS OF THE RETINOBLASTOMA GENE. Rene Bernards, Stephen Friend, Robert Weinberg, Whitehead Institute, Cambridge, MA 02142. Several lines of evidence suggest that retinoblastoma (Rb) arises because of the loss of the activity of a gene that is localized on chromosome 13, band q14. We have recently isolated a cDNA clone from this region and have provided strong circumstantial evidence to suggest that this cDNA encodes the retinoblastoma gene. The ultimate proof of this however can only be provided by demonstrating that the isolated gene can modulate the oncogenic phenotype of retinal cells. We have therefore constructed retroviral vectors that direct the synthesis of RNA that is complementary to the mRNA specified by the putative retinoblastoma gene. These retroviruses were used to infect primary cultures of human embryonic retinal cells. RNA analysis shows that infected cells produce large amounts anti sense Rb mRNA. The properties of the anti sense-producing retinal cells will be discussed

D 301 RELATIONSHIP OF CELLULAR ONCOGENE EXPRESSION TO DIFFERENTIATION OF DAUDI CELLS INDUCED BY INTERFERONS OR TPA, Michael J. Clemens, Nigel A. Sharp, Vivienne J. Tilleray and Dirk R. Gewert, Cancer Research Campaign Group, Dept. of Biochemistry, St George's Hospital Medical School, London SW17 ORE and Imperial Cancer Research Fund, London WC2A 3PX. Treatment of the Daudi line of Burkitt lymphoma cells with human interferons inhibits proliferation and induces differentiation to a plasma cell phenotype. These responses also occur if the cells are treated with the phorbol ester, TPA. Both interferons and TPA down regulate expression of the c-myc oncogene in this system. In addition expression of the Epstein Barr virus-activated cellular oncogene c-fgr is inhibited by interferon treatment. Although TPA can mimic the effect of interferon on cell differentiation it does not induce 2'5'oligo(A) synthetase or the interferon-sensitive mRNAs 6-16 or 9-27. Thus activation of protein kinase C is not sufficient to explain all the effects of interferon treatment on gene expression. We have previously reported that inhibition of ADP-ribosyl transferase activity by nicotinamide analogues such as 3-methoxybenzamide inhibits interferon- or TPA-induced differentiation of Daudi cells (Exley et al., PNAS, Sept. 1987). We now show that 3-methoxybenzamide superinduces c-myc mRNA in this system; although this effect is partially counteracted by interferon or TPA treatment, the elevated level of c-myc gene expression may be sufficient to prevent differentiation and allow cell proliferation to continue.

D 302 **ALTERED GENE EXPRESSION IN ONCOGENE-TRANSFORMED MOUSE CELLS PERSISTENTLY REVERTED BY PROLONGED INTERFERON TREATMENT**, Sara Contente, Kaylene Kenyon, Dvorit Samid and Robert M. Friedman, Dept. of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Prolonged treatment of RS 485 (NIH 3T3 transformed by LTR-activated c-Ha-ras) with IFN-β resulted in phenotypic reversion accompanied by reduction in ras-encoded mRNA and p21. This reversion persists in more than 99% of the cells long after IFN is removed. These persistent revertants(PRs) are non-tumorigenic in nude mice and do not grow in soft agar, yet they re-express high levels of ras mRNA and p21. These PRs resist retransformation by the oncogenes EJras, v-Ha-ras, v-Ki-ras, and v-abl. In contrast, they were transformed following transient treatment with 5-aza-2'-deoxycytidine (5AzadC), a cytidine analogue that is known to modulate gene expression previously controlled by DNA methylation. It is therefore possible that prolonged treatment with IFN has induced altered patterns of gene expression, via an effect on DNA methylation, that allow the cells to express high levels of ras mRNA and p21 yet remain non-transformed. One of the approaches that we are taking to investigate the molecular mechanisms involved in this anti-oncogenic effect of interferon is identifying those cell messages whose expression has been altered. We have constructed cDNA libraries from RS 485 and a PR (PR4), and screened for differential expression using cDNA probes from each cell line. Several messages that are overexpressed in each line have been isolated. These messages are being subcloned in mammalian expression vectors and will be transfected into each cell line to evaluate their potential biological activity in two ways. First, messages that are overproduced in one line will be artificially overexpressed in the other to determine if such expression leads to reversion or re-transformation. Second, messages cloned in the anti-sense orientation will be transfected into the cell lines to determine if normal expression of these messages is required for the maintenance of either the transformed or reverted state.

D 303 SUPPRESSION OF THE TUMORIGENIC PHENOTYPE OF A WILMS' TUMOR CELL LINE BY INTRODUCTION OF A NORMAL CHROMOSOME 11

Steven F. Dowdy, Bernard E. Weissman, and Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717

Wilms', a pediatric nephroblastoma, has been associated with deletions of the p13 band of chromosome 11. The function of the deleted genetic material in the etiology of Wilms' is unknown, but has been postulated to contain a gene(s) that suppresses the malignant phenotype. The role of this genetic information was investigated by introducing a normal chromosome 11, t(X/11)(Xqter:Xq26::11q23:11pter), via the Microcell Mediated Chromosome Transfer (MMCT) technique into a karyotypically normal Wilms' tumor cell line, G401. The G401 microcell hybrids contain 47 chromsomes, with the t(X/11) retained by HAT selection, and express similar in vitro characteristics as the parental cell line. However, the ability of these hybrids to form tumors in nude mice is completely suppressed compared to the G401 parent. MMCT of additional chromosomes, X and 13, into G401 showed no effect on tumorigenicity. Further, a t(X/11p) (Xqter:Xq22::11q13:11pter) was introduced via MMCT into G401 to regionalize the tumor suppressing gene(s). These t(X/11p) microcell hybrids demonstrated complete suppression of the tumorigenic phenotype. The ability of genetic information in the pter to q13 region of chromosome 11 to control the malignant expression of Wilms' tumor cells and additional G401 microcell hybrids will be discussed.

D 304 ANALYSIS OF EXON/INTRON RELATIONSHIPS OF THE RETINOBLASTOMA GENE,

Huei-Jen S. Huang, Lih-Jiuan Young, Tony Oro, Hoang To, Robert Bookstein, Eva Y.H.P Lee and Wen-Hwa Lee, Experimental Pathology Program, Department of Pathology M-012, University of California, San Diego, La Jolla, CA 92093. A restriction map of the retinoblastoma (RB) gene was constructed by aligning overlapping genomic clones isolated from both a normal human genomic library and a library made from DNA of retinoblastoma cell line Y79. Exons were initially identified as minimal-length *Hin*dIII or *Eco*RI restriction fragments containing sequences hybridizing to RB cDNA clones. To further define exon/intron junctions, oligonucleotides were synthesized according to the complete cDNA sequences and used as primers in sequencing genomic clones. Preliminary data indicates the presence of at least 19 exons; of these, 15 are completely defined. Restriction mapping and sequencing showed that the terminal exon was about 1900 bp in length and included the translation stop codon. Eight other exons were ~120 bp; five exons were ~60 bp; and one was 32 bp. Large genes with numerous small exons may be especially susceptible to mutation. Establishing exon/intron relationships will provide a basic framework to understand secondary structure of the RB gene product, $pp110^{RB}$.

D 305 HUMAN RECOMBINANT INTERFERON GAMMA (rhIFN-g) INHIBITS THE GROWTH OF A CHILD T-CELL LYMPHOMA IN NUDE MICE. C. Jemma, M. Giovarelli, T. Musso, R. Arione, G. Benetton, G. Forni, Inst. Microbiol., University of Turin, Turin Italy.

T-lymphoma cells from a lymph node biopsy of a boy currently in remission did not grow in vitro whereas they formed a tumor in splenectomized and sublethally irradiated (S-I) nude mice and became serially transplantable. After the fourth transplant, the cells (ST4) display a membrane phenotype and karyotype similar to that of the biopsy. When cultured in vitro in the presence of increasing amounts of rhIFN-g they show a dose-related enhancement of proliferation. However when ST4 cells cultured in the presence of 10 or 100 IU/ml of rhIFN-g were injected into S-I nude mice, they formed tumors in 80% only, and the latency time was significantly extended as compared to ST4 cells cultured in medium only. Moreover, no tumor growth was found when S-I nude mice challenged with ST4 cells received daily injections of 100 IU of rhIFN-g around the challenge area. By contrast, 100% tumor takes were found in the control mice daily injected with Hanks' only. Histologic pictures show that rhIFN-g mediated tumor inhibition is not associated with infiltrations by leukocytes from S-I nude mice. Moreover, functional data show that rhIFN-g is unable to activate lymphocyte, macrophage or granulocyte reactivity of S-I nude mice. These data suggest that rhIFN-g directly inhibits the oncogenic capacity of ST4 cells in vivo without impairing their in vitro growth capacity or enhancing the immunoreactivity of S-I nude mice.

D 306 LIGAND BINDING INDUCES A CONFORMATION CHANGE IN THE PLATELET-DERIVED GROWTH FACTOR RECEPTOR, Mark T. Keating and Lewis T. Williams, University of California, Department of Medicine, Cardiovascular Research Institute, and Howard Hughes Medical Institute, San Francisco, CA., 94143

The pleotrophic effects of platelet-derived growth factor (PDGF) are mediated by its cell surface receptor. We have examined the effect of ligand binding on PDGF receptor conformation using antisera directed against specific receptor domains. Antisera directed against the carboxy terminus of the cytoplasmic domain specifically recognized the PDGF-stimulated form of the receptor. In soluble extracts of cells stimulated by PDGF, this antibody precipitated 5 - 7 fold more receptor protein than in comparable extracts from unstimulated cells. This effect was elicited by three different forms of PDGF (AA and BB homodimers and AB heterodimers) and was reversed by suramin, a compound that inhibits PDGF binding. Control antisera directed against the extracellular region and other cytoplasmic sequences precipitated unactivated and activated forms of the receptor equally well. After denaturation, activated and unactivated forms of the receptor were recognized equally well by all antisera. Ligand binding, therefore, elicits a change in the conformation of the PDGF receptor, exposing a carboxy terminal domain that is cryptic in unstimulated cells.

D 307 KINETIC COMPLEXITIES IN THE HUMAN INTERFERON RECEPTOR INTERACTION, David Leavesley and Joyce Taylor-Papadimitriou, Imperial Cancer Research Fund, London, WC2A 3PX, U.K.

Intrinsically labelled Hu.IFNα_2 has been used at low, biological concentrations (10pM) to characterize the early events of the IFN-receptor interaction in human and bovine epithelial cells. Clear differences are observed, manifest in association, dissociation, internalization and processing kinetics. The interaction in bovine(MDBK) cells is classical, indicative of a single receptor-ligand binding event, whereas the interaction in human(BT20) cells is complex and non-classical. Evidence for multiple human IFN-receptor interactions comes from competition and dissociation analysis, and Scatchard analysis complicating interpretation. The complexity is not due merely to putative multiple receptor species but could also be attributed to post-binding events. Could these complexities in the human cell relate to IFNs' growth regulating action?

Post-binding interactions of the IFN-receptor complex have been analysed using *in situ* cell fractionation with weak acid and mild detergent treatments after binding of ^{125}I-labelled Hu.IFNα_2. We have identified a series of successive stages in the receptor binding and post-binding processing of the ligand-receptor complex. This processing in bovine cells is dissimilar to processing in human cells suggesting alternative mechanisms. Degradation at near physiological concentrations (25pM) is apparently a first order event and very slow, possibly reflecting the time required to establish some of IFNs activities. There is evidence to indicate cytoskeletal involvement and the effects of colchicine treatment implicate possible microtubule involvement.

D 308 THE EXPRESSION OF THE NUCLEAR ANTIGEN KI-67 IN INTERFERON-TREATED CELLS, Erik Lundgren, Dan Lundblad, Göran Landberg och Göran Roos, Inst. Cell Mol. Biol., and Dept. Cytol., University of Umeå, S-901 87 Umeå, Sweden.

Ki-67 was expressed as measured by immunofluorescense in nuclei and nucleoli in the interferon (IFN) sensitive cell line Daudi and the glioma line 251 MG. IFN reduced the frequency of positive Daudi cells concommitant with their accumulation in G0. The glioma cells on the other hand were arrested with a DNA content representative for S-phase cells. No reduction of Ki-67 expression was noted. In control experiments it was shown that drugs blocking Daudi cells in G2 did not inhibit Ki-67 expression, while removal of growth factors from the glioma line reduced the expression to very low levels. Thus, we conclude that IFN regulates Ki-67 in an indirect way as a consequence of its ability to block cells in G0, the effect being the same as when growth factors are removed from factor dependent cells. The function of Ki-67 is not known, but it obviously serves as a marker for non-resting cells.

D 309 LACK OF EXPRESSION OF AMINOACYLASE-1 IN SMALL CELL LUNG CANCER: EVIDENCE FOR INACTIVATION OF GENES ENCODED BY CHROMOSOME 3p., Y.E. Miller, B. Kao and A. Gazdar, Eleanor Roosevelt Inst. for Cancer Res., V.A. Med. Ctr., Univ. Co. Health Sci. Ctr., Denver, CO and NCI and Naval Hosp., Bethesda, MD.
A characteristic deletion of chr. 3p(14-23) has been described in small cell lung cancer (SCLC). Because aminoacylase-1 (ACY-1) has been assigned to chr. 3p21, we examined its expression in SCLC, using both an electrophoretic assay and a monoclonal antibody-based ELISA. 29 SCLC cell lines were tested; six had undetectable activity and antigen. 34 other human tumor cell lines were tested; all are positive for ACY-1. A variety of human and rat tissues, including lung, brain, pancreas, liver and kidney, are positive for ACY-1. Erythrocytes are the only ACY-1 negative normal cell found to date. Eight SCLC tumors have been examined; one has undetectable ACY-1, six have 10% or less compared to sixteen other human tumors. All ACY-1 negative samples are positive for a control enzyme, LDH, arguing against non-specific degradation of enzymes in these samples. Because the assays do not distinguish ACY-1 isozymes, loss of a single allele is not detectable in these experiments. The data suggest that complete inactivation of ACY-1, perhaps due to loss of all alleles, is specific for SCLC and occurs more frequently than does complete loss of esterase D activity in retinoblastoma. Identification of molecular probes for ACY-1 and closely linked genes may be useful for understanding the abnormality of chromosome 3p in SCLC.

D 310 POLYMORPHIC DNA MARKERS REVEAL A LOSS OF CHROMOSOME 3P ALLELES IN THE MAJORITY OF SMALL CELL LUNG CANCER, S.L. Naylor[1], A. Marshall[1], C.H. Hensel[1], B.E. Johnson[2], A.Gazdar[2], J.D. Minna[2], and A.Y. Sakaguchi[1], [1]The University of Texas Health Science Center, San Antonio, TX 78284 and [2]NCI-Naval Oncology Branch, Bethesda, MD.
Karyotypic studies of small cell lung cancer (SCLC) by Whang-Peng et al have identified a deletion in chromosome 3 at band p14-p23. Our studies are to determine if DNA is lost from chromosome 3p in the tumor and to characterize the gene(s) that may be deleted in SCLC. Four polymorphic DNA markers were mapped to the p14-p23 region of chromosome 3: pMS1-37 detects and MspI polymorphism at the D3S3 locus at 3p14.2, p12-32 as MspI polymorphism at the D3S2 locus at 3p14.2-p21, and pH3H2 and pH3E4 subclones of λH3 detect HindIII polymorphisms at a locus at 3p21. DNA was isolated from normal and tumor tissue from 26 individuals with SCLC, ten of which had established tumor cell lines. Three individuals had only tumor lines and normal lymphoid lines available. Twenty-eight patients were informative, i.e. heterozygous in their normal tissue, and allele loss was detected in 25 of 28. Of the individuals exceptional for allele loss, one patient who did not show allele loss in either of two informative polymorphisms in tumor tissue or in a tumor cell line, had an unusual case history. One individual, SCLC10, lost an allele of pH3H2, but remained heterozygous for p12-32 and pMS1-37, indicating the critical region of deletion is closer to pH3H2. The loss of DNA from chromosome 3p suggests that this region might play a role in the etiology of small cell lung cancer. Transcribed sequences being developed for this region should allow us to determine whether the deletion leads to loss of a recessive cancer suppressor gene or uncovers a recessive oncogene.

D 311 ALTERED SV40 EARLY REGION INHIBITS CELL PROLIFERATION IN A TRANSGENIC MOUSE FAMILY, Paul A. Overbeek, Michael J. Kovac, and William Shawlot, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.
Expression of the early region of SV40 in tissues of transgenic mice usually predisposes the expressing cells to tumorigenic transformation. However in one of our families of transgenic mice an altered SV40 early region dominantly inhibits cell proliferation. The transgenic mice carry a murine α-crystallin promoter linked to a truncated version of the early region of SV40. Heterozygous mice show lens cataracts and microphthalmia. Homozygous mice have lenses that are less than 5% of normal size. Lens epithelial cell proliferation and differentiation are altered. Lens fiber cells are absent. In addition, homozygous mice have altered hair follicle development due to insertional inactivation of the downless gene. Genomic clones of the transgenic insert have been isolated. The clones will be used to define the extent of the SV40 early region that integrated, and to begin studies of the mechanism by which the altered early region protein(s) inhibit lens cell proliferation. In addition, the genomic sequences flanking the site of integration will be used to begin the molecular characterization of the downless gene and its role in controlling local patterns of morphogenesis.

D 312 AUGMENTATION OF CELL GROWTH BY ANTISENSE BETA-1 INTERFERON mRNA, Paula M. Pitha and N. Babu K. Raj, The Johns Hopkins University Oncology Center, Baltimore, MD 21205

To examine the role of antisense beta-1 interferon mRNA in regulation of cell growth, we transfected plasmids, in which the expression of human anti beta-1 interferon mRNA was directed by the beta-1 promoter, into human fibroblast cells and the stably transfected colonies were selected by resistance to GM418. From the 15 colonies slected and grown into cultures, two cell lines (D, L) show a constitutive expression of anti beta-1 mRNA. In the L line which shows high levels of anti beta-1 mRNA, the inducible expression of beta-1 interferon gene was altered and induced synthesis of interferon markedly inhibited. Furthermore, the D line shows altered morphology and increased growth rate when compared to the parental line and the other transfected clones. The morphological changes could not be reverted by incubation of the L cells with exogenous beta-1 interferon. These results show that autocrine synthesis of beta-1 interferon may have a growth regulatory function.

D 313 TYPE I AND TYPE II INTERFERON TRANSCRIPTIONAL RESPONSE ELEMENTS OF THE I-8 GENE FAMILY, Laurence E. Reid, Adrian Brasnett, Trevor Dale, A.M. Ali Imam, George R. Stark and Ian M. Kerr, Imperial Cancer Research Fund, PO Box 123, 44 Lincoln's Inn Fields, London WC2A 3PX, UK. Expression of the human 1-8 gene family is increased more than tenfold in response to Type I or Type II IFNs. Polyclonal antisera against a common peptide recognize at least 3 IFN-inducible 15kDa proteins on Western blots. Four distinct cDNAs have been cloned and sequenced, including one of full length for one specific member (code 9-27). A genomic cosmid carrying the 9-27 and two additional 1-8 genes has been isolated. All three genes have been subcloned and shown to respond to both Type I and Type II IFNs upon transfection into rodent cells. The 9-27 5' upstream region has been shown to contain a sequence (GGAAATAGAAACT) which is highly homologous with a sequence motif known to be involved in the response of the 6-16 gene to Type I IFNs (A.C.G. Porter, Y. Chernajovsky, G.R. Stark, I.M. Kerr, submitted). An oligonucleotide corresponding to the homologous region competes with the Type I response element of the 6-16 gene, for IFN-specific bands, in gel retardation assays. Deletion, construct, and band shift analyses are in progress to define the Type II response element and factors interacting with it.

D 314 BIOCHEMICAL CHARACTERIZATION OF INTERFERON-INDUCED REVERTANTS OF RAS-TRANSFORMED CELLS, Donata Rimoldi, Robert M. Friedman and Dvorit Samid, USUHS, Bethesda MD 20814 Cloned persistent revertants (PR's) of Ha-ras-transformed NIH3T3 cells obtained after prolonged treatment with interferon (IFN) α/β have been described (Samid et al., Mol. Cell. Biol. 1987, 7: 2196-2200). PR cells maintain a stable non-tumorigenic phenotype long after IFN withdrawal although they express high levels of ras mRNA and p21. In order to elucidate the molecular mechanism of reversion we compared the expression of growth-related genes in the original transformed cells and PR's. No significant difference was detected in the steady-state level of mRNA of genes such as myc and fos in growing cells. In contrast, Northern blot analysis of RNA isolated from PR cells showed, when compared to the original ras-transformed cells, an increased level in the 1.7 Kb message for (2'-5') oligoadenylate synthetase (2-5 A synthetase), a gene induced by IFN and associated with down-regulation of cell proliferation. This message, although inducible upon treatment with exogenous IFN, was not detectable in untreated NIH3T3 cells. Furthermore, the level of 2-5 A synthetase mRNA detected after induction with equal amounts of IFN α/β was considerably higher in PR cells compared to both NIH3T3 and parental transformed cells. Expression of the major histocompatibility complex class I genes (H-2 class I genes), another set of genes characteristically induced by IFN, was also found to be elevated in PR's. Although these results suggest a possible production of autocrine IFN by PR cells, preliminary experiments with neutralizing antibodies raised against mouse IFN α/β indicate that the known α and β forms of IFN are unlikely to be involved in the induction of these genes.

D 315 TWO MOLECULAR FORMS OF THE HUMAN INTERFERON-γ RECEPTOR: LIGAND BINDING, INTERNALIZATION AND DOWN REGULATION, Dina G. Fischer, Daniela Novick, Patricia Orchansky and Menachem Rubinstein, Dept. of Virology, Weizmann Institute of Science, Rehovot 76100, Israel

The receptors for human interferon-γ (IFN-γ) on peripheral blood monocytes and various cells of non-hematopoietic origin were thoroughly characterized and compared. The receptors of all cell types exhibited a similar affinity for IFN-γ (Kd ~ $1x10^{-10}$M) and in all cases receptor mediated endocytosis and ligand degradation were demonstrated. However the receptors differed in their molecular weights (95,000 in HeLa cells and 140,000 in monocytes, assuming a 1:1 ligand to receptor ratio) as concluded from experiments of cross-linking to ^{125}I-IFN-γ. Lower molecular weight species were obtained as well, particularly in monocytes. Such species could represent either degradation products or subunit structures. The monocyte and HeLa receptor responded differently to an excess of ligand. A significant receptor down-regulation was observed when monocytes were incubated with an excess of ^{125}I-IFN-γ whereas no such down-regulation was observed in HeLa cells or in normal fibroblasts. This differential response was observed both in the presence or in the absence of a protein synthesis inhibitor. The receptor on monocytes was found to be acid-labile while that on HeLa cells was resistant to acid treatment. These and additional experiments indicate that the monocyte receptor is inactivated following internalization while the HeLa receptor retains its structure and recycles back to the cell surface. The difference in the properties and fate of these two receptor subtypes is probably related to the differential functions of IFN-γ in various cell types.

D 316 INTERFERON RECEPTOR INTERACTION: A STUDY USING MONOCLONAL ANTIBODIES TO HuIFNα, Moira Shearer and Joyce Taylor-Papadimitriou, Imperial Cancer Research Fund, London, WC2A 3PX.

Monoclonal antibodies to HuIFNα species have been isolated as tools to study IFN-receptor interaction (M. Shearer, J. Taylor-Papadimitriou, D. Griffen and F. Balkwill; 1984, J. Immunology 133; 3096). The antigenic determinants of four of these have been identified using hybrid and analogue IFNs. Amino acids in the 107-113 region of HuIFNα$_2$ are implicated in the epitopes recognized by three of the antibodies while the fourth antibody recognises IFNs with arginine at position 121 (J. Taylor-Papadimitriou, M Shearer and D. Griffen, 1987, J. Immunology 139; *in press*). Binding of IFN to its receptor on human and bovine cells in the presence of excess concentrations of these four antibodies is inhibited and as expected the biological activity of IFN is neutralized. However binding of IFN in the presence of equimolar concentrations of the antibodies has shown that the 107-121 region of IFN containing the antigenic determinants is exposed and able to bind antibody when IFN is bound to its receptor on human but not when bound to its receptor on bovine cells. At equimolar concentrations the antibodies partially inhibit internalisation of IFN in human cells and also partially inhibit biological activity. Studies using radiolabelled antibody to determine receptor levels have shown that, contrary to the published data, the IFN receptor is only marginally down regulated in human cells and is fact blocked by bound ligand.

These antibodies are being used to follow the fate of the IFN-receptor complex.

D 317 SUPPRESSION OF RSV-INDUCED TUMORIGENICITY IN AVIAN EMBRYOS.
A.W. Stoker, A.R. Howlett, and M.J. Bissell. Cell Biology Lab, Lawrence Berkeley Laboratory, Berkeley, CA .

We have shown previously (Nature, 309:552, 1984) that the sarcomagenic action of Rous sarcoma virus (RSV) is suppressed in the early chicken embryo. Injection of RSV into day 3-4 embryo limb buds leads to viral expression in the entire muscle tissue with later expression in connective tissue (Oncogene Res. 1:255, 1987). Using monoclonal antibodies we show the presence of $pp60^{v-src}$ (the transforming tyrosine kinase) and elevated phosphotyrosine in histologically normal muscle and other tissue 7-10 days post infection. Since the embryos die of viremia by day 12-14, we have developed an ASLV vector packaging cell system, to supply replication-defective (rd) v-src expressing viruses. The use of rd viruses has permitted the embryos to survive beyond day 14, and viral protein expression is detected in tissues of a proportion of these older embryos. We are examining infected and uninfected embryo tissues histologically to ascertain whether there is a stage or differentiation-specific switch to tumor permissiveness during later development. We are also examining the expression of TGF-β and PDGF in these tissues, to determine if a relationship exists between expression of these factors and the supression phenotype.

D 318 INACTIVATION OF THE HUMAN RETINOBLASTOMA GENE IN RETINOBLASTOMA AND OTHER TUMORS Anne T'Ang, Shikha Chakraborty, Jin Qian, A. Linn Murphree, Paula Ladne, Yuen Kai Fung, Divisions of Ophthalmology, Hematology and Oncology, Department of Pediatrics & Microbiology, USC School of Medicine, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

The human retinoblastoma gene belongs to a class of recessive genes implicated in a number of embryonic tumors that have a genetic predisposition. We have previously demonstrated that the development of retinoblastoma in hereditary, as well as sporadic cases, correlates with the inactivation of the human retinoblastoma gene at the DNA and RNA level. We showed that, in about 40% of retinoblastomas, structural defects can be readily detected using the cloned cDNA of the human retinoblastoma gene. The structural defects involved homozygous deletion at the 3' end or internal region of the Rb gene locus as well as homozygous total deletion and hemizygous deletion. It is interesting to observe that clinically, patients with the hereditary form of retinoblastoma often develop other types of tumors, including the most frequent ones, fibrosarcoma and osteosarcoma. We demonstrated that, in clinically related osteosarcoma, homozygous internal deletion of the retinoblastoma gene is observed. Similarly, we showed that homozygous internal deletion and 3' deletion of the retinoblastoma gene was detected in a fibrosarcoma cell line. Reintroduction of chromosome 13 into this cell line leads to suppression of its tumorigenicity and reduction in its saturation density in vitro. We have recently extended these observations to other types of human tumors clinically not related to retinoblastoma. We report here the detection of structural changes of the retinoblastoma gene locus in human breast tumor and human melanoma. These changes include homozygous internal deletion, homozygous total deletion, hemizygous deletion, and homozygous 3' deletion of the Rb genomic locus. Moreover, structural changes in the form of amplification of part of an exon are also observed.

D 319 STRUCTURAL FEATURE AND EXPRESSION OF THE HUMAN RETINOBLASTOMA cDNA CLONE Anne T'Ang, Koichiro Mihara, Hong-Ji Xu, Donna Muzny, A. Linn Murphree, Thomas Caskey, Lily Shi, Yuen Kai Fung, Division of Ophthalmology, Hematology and Oncology, Department of Pediatrics & Microbiology, USC School of Medicine, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

We have previously reported the isolation of the cDNA clone of the human retinoblastoma gene. Nucleotide sequence analysis have shown the following features: (1) The longest reading frame starts from base 321 to base 2769 including a polypeptide of 816 amino acids. The overall amino acid composition is not particularly biased. There is a stretch of proline rich region in the carboxy-terminal domain. However, no transmembrane domain can be observed. A stretch of extremely GC-rich region at the 5' untranslated region is observed. Almost half of the messenger RNA is composed of a long stretch of 3' untranslated region with many stop codons. The most striking feature of the nucleotide sequence is the presence of 15 stretches of different nucleotide sequences, each of which is repeated once elsewhere in the genome. These sequences are found to be distributed all over the entire cDNA clone, both in the coding and non-coding region. One third of these sequences are found to be concentrated in the middle of the coding region. Interestingly, this is the region that is most frequently deleted in the tumor samples. We propose that the existence of such structures may predispose the locus to deletion. A comparison of our cDNA sequence with computer data bank through Bionet shows no sequence homology with any known gene. However, since the gene is highly conserved, we have used the human cDNA clone as a probe and isolated an Rb homologue of mouse as well as Drosophila. We are currently expressing the cDNA clone in a number of the bacterial systems to generate antigens for the production of antibodies.

D 320 ISOLATION AND CHARACTERIZATION OF THE GENOMIC CLONE OF THE RETINOBLASTOMA GENE Rei Takahashi, Tomoko Hashimoto, Jin Qian, Anne T'ang, Hua Zheng, Linn Murphree and Yuen Kai Fung. Division of Ophthalmology, Hematology/ Oncology, Department of Pediatrics & Microbiology, USC School of Medicine, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

Retinoblastoma is a childhood desease which occurs in hereditary and non-hereditary forms. The genetic locus Rb-1 indicated in the development of the disease has been located to the human chromosome at 13q14. It has been suggested that inactivation of both alleles of the Rb-1 locus is responsible for tumorigenesis. Recently a cDNA clone which has the property of the retinoblastoma gene was isolated in our laboratory. We demonstrated that 40 % of retinoblastoma showed structural changes by Southern blot analysis (*Science* vol. 236, 1657, (1987)). In order to understand the structural changes in the remaining 60% of retinoblastoma, we attempt to define the genomic locus of the human retinoblastoma gene. Using cDNA as a probe, a number of overlapping clones corresponding to the human Rb gene were isolated. These clones extend over a region of more than 200 kb of genomic DNA and include at least 14 exons. Estimation of the total length and microscopic change of the locus is being done by pulsed field gradient gel electrophoresis. Work is in progress to characterize and extend the cloned region to fully define the entire Rb gene.

D 321 HIPPOCRATIN (INTERFERON-ß$_2$/B-CELL DIFFERENTIATION FACTOR/HEPATOCYTE STIMULATING FACTOR): MEDIATOR OF THE HEPATIC ACUTE PHASE RESPONSE.
S.B. Tatter, U. Santhanam, L.T. May, and P.B. Sehgal, The Rockefeller University, New York, NY 10021.
The human "hippocratin" gene encodes proteins previously called interferon-ß$_2$, B-cell differentiation factor (BSF-2), and hepatocyte stimulating factor. We have expressed the hippocratin c-DNA in *E. coli* and raised a specific polyclonal antibody to the expressed protein. This antibody decorates several polypeptides secreted by human fibroblasts and monocytes on immuno-blots. Tunicamycin treatment of human fibroblasts inhibits synthesis of the triplet centered at 29 kDa but not of the triplet centered at 25 kDa. We confirm that various recombinant and natural hippocratin polypeptides stimulate secretion of acute phase reactants by Hep-3B2 human hepatoma cells. These reactants include fibrinogen, complement C3, α_2-macroglobulin, α_1-antichymotrypsin, α_1-acid glycoprotein, and α-thiol proteinase inhibitor as assayed by immunoprecipitaion and/or rocket immuno-electrophoresis. This stimulation is abolished by preincubation with the anti-hippocratin antiserum. Secretion of albumin, a negative acute phase reactant, is diminished by treatment with hippocratin preparations. α-thiol proteinase inhibitor has not been previously described as a human acute phase reactant. Preliminary results indicate that its plasma level is elevated (approximately 50%; similar to the increase in fibrinogen) in hospitalized patients manifesting an acute phase response as determined by increased erythrocyte sedimentation rate and increased plasma C-reactive protein. In view of the ancient descriptions and the historical importance of what is now called the acute phase response we propose that the polypeptides derived from the IFN-ß$_2$/BSF-2/HSF gene be named hippocratins and that they be further designated by their apparent molecular weights in SDS polyacrylamide gels. Furthermore, the gene encoding these polypeptides should be designated the hippocratin gene.

D 322 PROPERTIES OF (TRANSFORMED) CELL LINES WHICH CONSTITUTIVELY PRODUCE INTERFERON. M. van Heuvel, M. Govaert-Siemerink, J. Bosveld, E. Zwarthoff and J. Trapman. Erasmus University, Rotterdam, The Netherlands.
Two types of cell lines were constructed which constitutively synthesize various Mu IFN-α subspecies. 1. In CHO (hamster) cell lines we investigated a possible relationship between constitutive IFN production, expression of IFN-induced genes and biological properties of the cells. The producer cell lines were protected against viral infection by the Mu IFN, but did not respond to its growth inhibitory action. Three mRNA's which are normally induced in CHO cells by IFN treatment (1-8, 15k and 2,5A) were constitutively present in comparable amounts in IFN producer cell lines. One mRNA (54k) was diminished in the producer lines as compared to IFN-treated cells. Our data exclude a role of 1-8, 15k and 2,5A in cell growth inhibition in this system. 2. In a series of H-ras transformed mouse 3T3 cell lines we studied the effect of constitutive IFN production on their oncogenic properties. No obvious differences were found in cell morphology, absence of contact inhibition, anchorage independency and H-ras mRNA levels in IFN producers as compared to control cells. However, when injected into nude mice, IFN producers gave rise to small tumors or did not grow at all, whereas the control nonproducers formed large tumors within two weeks.

D 323 EFFECT OF γ-INTERFERON ON KERATINOCYTE BIOSYNTHESIS AND EXPRESSION OF THROMBOSPONDIN, James Varani, Bruce Riser and Brian Nickoloff, Univ. of Michigan Ann Arbor, MI. 49109
Undifferentiated human keratinocytes biosynthesize thrombospondin (TSP), secrete TSP into the culture medium and bind it to high affinity cell surface receptors. TSP is a potent cell - substrate adhesion factor for keratinocytes. Basal adhesion of keratinocytes to plastic culture dishes in the presence of bovine serum albumin or to type IV collagen-coated culture dishes can be suppressed by pretreatment of the cells with polyclonal or monoclonal antibodies to TSP. This suggests that TSP serves as an endogenous adhesion factor for keratinocytes and that it may play an important role in regulating the interaction of keratinocytes with the extracellular matrix. Treatment of keratinocytes with γ-interferon (IFN-γ) (150 - 600 U/ml) suppresses TSP biosynthesis. There is a concomitant inhibition of TSP secretion and cell surface expression. The same treatment also inhibits keratinocyte attachment and spreading on plastic culture dishes and type IV collagen-coated culture dishes. Treatment with two other cytokines; i.e., ß-interferon and tumor necrosis factor, do not have these effects, although tumor necrosis factor can act syngergistically with IFN-γ to further suppress TSP production and utilization. Since TSP is localized in vivo at the dermal-epidermal junction, these results suggest that TSP may be an important adhesion molecule for keratinocytes. They also provide the first evidence that inflammatory mediators such as IFN-γ can modulate the synthesis, secretion and utilization of this extracellular matrix molecule by these cells.

D 324 INTERFERON INDUCTION OF 2-5A SYNTHETASE AND CONTROL OF CELL GROWTH.
B.R.G. Williams, M.N. Rutherford, G.E. Hannigan, G. Duckworth-Rysiecki and D.R. Gewert.* Division of Infectious Diseases, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Ont. Canada and * ICRF Labs. Lincolns Inn Fields, London, England.
Interferons (IFNS) induce a rapid rise in transcription of a number of genes including that coding for 2-5A synthetase. We have investigated IFN and growth factor responsive elements in the 5' flanking region of the human 2-5A synthetase gene. Using transient expression assays we have detected an IFN responsive element which confers IFN inducibility on heterologous recorder genes. The 5' boundary of this element is 60 bp upstream of the most 5', IFN-induced transcriptional start site. The 3' portion of this element includes a region which shows homology to the core consensus sequence occurring in other IFN responsive genes. Within this element we have detected a 40 bp sequence which is capable of specifically binding a protein present in nuclear extracts from IFN-treated cells. This DNA binding protein activity is induced with kinetics similar to those observed for IFN-induced transcription of the 2-5A synthetase gene.
We have established cell lines expressing high constitutive levels of 2-5A synthetase under control of the adenovirus major late promoter. These cells are resistant to virus infection and show altered growth characteristics providing further evidence for 2-5A synthetase as an antiviral protein and suggesting a direct role for this enzyme in controlling cell growth.

D 325 ALTERATIONS IN GENE EXPRESSION AT VARIOUS STAGES OF NEOPLASTIC TRANSFORMATION OF SYRIAN HAMSTER EMBRYO (SHE) CELLS. Roger W. Wiseman, Michael E. Lambert*, Patricia W. Lamb, James I. Garrels*, and J. Carl Barrett. NIEHS, Research Triangle Park, NC, 27709 and *CSH Laboratory, Cold Spring Harbor, NY, 11724.
Neoplastic transformation of normal diploid SHE cells is a multi-step process. We have examined [^{35}S]methionine-labeled proteins in total cell lysates from 27 SHE cell derivatives at various stages of transformation for alterations in gene expression using the QUEST system of quantitative two-dimensional gel analysis. A SHE cell protein database has been established and utilized to compare the levels of more than 1000 proteins in each cell lysate after a 4hr labeling period. Derivatives of 2 chemically induced immortal SHE cell lines have been studied in detail. Spontaneously occuring, anchorage-independent varients of these preneoplastic lines have been examined, as well as tumor-derived SHE cell lines induced by chemical carcinogens or transfection with polyoma virus, v-src, or v-H-ras and v-myc DNA. Several lines of evidence indicate that the loss of a tumor suppressor function represents an additional stage in SHE cell transformation and clonal derivatives of each preneoplastic line which either express (sup$^+$) or have lost (sup$^-$) a tumor suppressor function have also been compared. A dramatic reduction of several tropomyosin isoforms (TM-1, TM-2 and TM-3) is observed in each of the sup$^-$ varients relative to sup$^+$ clones or normal SHE cells; these tropomyosins are also reduced in all anchorage-independent and tumor-derived SHE cell lines examined except those induced by transfection with polyoma virus DNA. Additional proteins which are specifically induced or repressed at each stage of transformation will also be discussed.

D 326 EFFECT OF INTERFERON ALPHA ON GROWTH AND PROTO-ONCOGENE EXPRESSION IN NORMAL HUMAN FIBROBLAST. M. Yaar, M. Peacocke, A.V. Palleroni and B.A. Gilchrest, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111 and Dept. of Experimental Oncology and Virology, Hoffmann-La Roche, Inc., Nutley, New Jersey.
Interferons (IFN's) are a family of glycoproteins with documented anti-proliferative activities in a variety of normal and transformed cell types. The mechanism of this growth inhibition is not understood but is characterized by growth arrest as opposed to cytotoxicity and is frequently reversible when IFN is neutralized or removed from the culture medium. We have examined the effect of recombinant IFN alpha and beta on the growth characteristics of human dermal fibroblasts. Cells grown in 10% fetal bovine serum (FBS) for 7 days in culture demonstrate a 50% reduction in cell counts in the presence of IFN alpha 5000 U/ml as compared to 16% growth inhibition when IFN beta was used. This effect was entirely reversible when IFN alpha was removed from the medium. Northern blot analysis of the same fibroblasts, grown to 70% confluence, serum starved for 3 days and then stimulated with 20% FBS in the presence or absence of IFN alpha, showed no change in c-fos, c-myc, c-Ha-ras in the first 20 hours after stimulation despite a 50% reduction in growth rate of the IFN treated cells. This suggests that IFN induced growth arrest is not mediated in these normal cells by suppression of the cell cycle specifc proto-oncogenes.

D 327 **ANTIFIBROGENIC EFFECTS OF GAMMA-INTERFERON IN MURINE SCHISTOSOMIASIS.** MA Zern, FR Weiner, M-A Giambrone, S. Takahashi, L Biempica and MJ Czaja, Liver Research Center, Albert Einstein College of Medicine, Bronx, NY,

We have previously determined that γ-interferon dramatically inhibits total collagen synthesis and decreases types I and III procollagen mRNA levels in cultured fibroblasts. Although the interferons have been used in the treatment of chronic hepatitis B virus infections because of their antiviral properties, they have not been examined as anti-fibrogenic agents in liver disease. To evaluate the effects of γ-interferon on an animal model of hepatic fibrogenesis, mice were infected with 50 cercaria of S. mansoni. Half of the mice were then given daily IM injections of 100,000 anti-viral units of recombinant mouse γ-interferon (a gift of Genentech, Inc., So. San Francisco, CA) for four weeks beginning four weeks postinfection. The mice were sacrificed and their liver samples were analyzed histologically, for total collagen content, and by molecular hybridization techniques. Liver histology revealed a marked decrease in the amount of fibrosis in infected animals treated with γ-interferon. Likewise HPLC analysis demonstrated that infected mice treated with interferon had a 65% decrease in total collagen content as compared to untreated, infected mice. Northern blot hybridizations of total RNA extracted from the livers of interferon-treated, infected mice revealed a 70% decrease in steady-state levels of type I procollagen mRNA and an 85% decrease for type III procollagen mRNA from the levels in nontreated, infected animals. In contrast, γ-interferon treatment of the infected mice increased the mRNA content of the constitutive protein β-actin several-fold. These findings demonstrate that γ-interferon therapy profoundly inhibits fibrogenesis in vivo in the murine schistosomiasis model. Analysis of the effect of this therapeutic agent on a broad spectrum of fibrotic liver disease is indicated.

D 328 SUPPRESSION OF THE TRANSFORMED PHENOTYPE IN HUMAN-RAT CELL HYBRIDS EXPRESSING ROUS SARCOMA VIRUS $pp60^{src}$, Arturo Zychlinsky and Allan R. Goldberg, The Rockefeller University, New York, NY 10021

We isolated cell hybrids from the fusion of normal human fibroblasts with Rous Sarcoma Virus transformed rat cells that showed normal morphology, that did not grow in soft agar and were markedly less tumorigenic in nude mice than the transformed parent.

We demonstrated that the provirus containing the *src* oncogene was present in the hybrids by Sendai Virus mediated fusion of those cells with permissive chicken fibroblasts. The virus rescued by cell fusion still encoded a fully transforming *src* gene product. Southern hybridization of the parent cell lines' genomic DNA to the clones' genomic DNA proved that the cells were true hybrids.

The amount of $pp60^{src}$ determined by immunoprecipitation and the level of tyrosine kinase activity were shown to be similar in the fusants and the RSV transformed parent cell line. Nonetheless, the hybrids presented a normal phenotype. The mechanism of suppression is currently under investigation.

Steroid Effects; Other Growth Inhibitors

D 400 c-FOS INDUCTION ACCOMPANIES CELL CYCLE BLOCK BY ACTH, Eico Kimura and Hugo A. Armelin, Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20.780, São Paulo, Brasil.

Among the long known effects of adrenocorticotropin (ACTH) on the mouse malignant adrenocortical Y-1 cell line, are: a) steroidogenesis stimulation; b) cell shape change; c) cell cycle arrest at G_1. Proto-oncogene c-Ki-ras amplification and over expression in Y-1 cells has recently been reported by others and confirmed by us. We have also shown that these cells secrete a competence growth factor and that normal revertants isolated from the Y-1 line have lost both c-Ki-ras amplified sequences and growth factor secretion. Here we report that ACTH causes both transient induction of the c-fos proto-oncogene expression and reduction of the c-myc proto-oncogene expression. Upon ACTH treatment of exponentially growing cells, DNA synthesis begins to decrease by 4-5h, reaching negligible levels by 15h, whereas c-fos mRNA peaks by 30 min. and is no longer detectable by 2h. On the other hand, c-Ki-ras mRNA levels and growth factor secretion are not affected by the hormone. mRNA levels were determined by Northern hybridization using specific coding sequences as probes. Secreted growth factor's activity was determined by DNA synthesis stimulation in growth arrested Balb-3T3 monolayers.

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D 401 **ISOLATION AND PARTIAL STRUCTURAL CHARACTERIZATION OF A NOVEL HEPARIN-BINDING, NON-FGF-LIKE ENDOTHELIAL CELL GROWTH FACTOR.** Peter Böhlen, Peter Gautschi-Sova, Urs Albrecht, Stephanie Lehmann, and Daniel Huber. Department of Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland.

A protein with mitogenic activity for bovine aortic endothelial cells (BAEC) was purified to homogeneity from bovine brain using the standard procedure for the isolation of fibroblast growth factors (FGF), involving tissue extraction at pH 4.5, ammonium sulfate precipitation, Heparin-Sepharose affinity chromatography, and cation exchange and reverse-phase HPLC. The protein has a molecular weight of 18 kDa, and is clearly distinguished from FGFs by (a) lesser affinity to heparin (elution from Heparin-Sepharose at 1 M NaCl), (b) retention behavior on cation exchange and reverse-phase HPLC, (c) amino acid composition, and (d) an amino-terminal sequence not related to that of any known protein. The mitogenic activity for BAECs is similar to that of acidic FGF. The growth factor was also isolated from rat and chick brain and possesses a completely conserved amino-terminal sequence suggesting an important biological function.

D 402 A POLYPEPTIDE GROWTH INHIBITOR FROM LACTATING MAMMARY GLAND (MDGI). F.-D. Böhmer and R. Grosse, Central Institute of Molecular Biology, Department of Cellular Biochemistry, Academy of Sciences of the GDR, 1115 Berlin-Buch.

The present state of knowledge on a new polypeptide growth inhibitor (MDGI, mammary-derived growth inhibitor) of mammary epithelial cells, purified from lactating bovine mammary gland, is summarized. Identification of the about 13 kDa inhibitor was performed by establishing co-purification of inhibitory activity with the respective polypeptide using different methods, by immunoneutralization experiments and chemically by determination of the entire amino acid sequence. An almost identical polypeptide was identified in milk fat globule membranes. Anti-MDGI antibodies precipitated only an about 13 kDa protein from the mixture of in vitro translation products of poly-A-mRNA from lactating bovine mammary gland. Cell biological and sequence data clearly distinguish MDGI from hitherto established growth inhibitors as interferons and TGF beta. However, extensive homology of MDGI to retinoic acid- and fatty acid - binding proteins as well as to myelin P2 and to a differentiation associated protein (p422) has been found. Specific anti MDGI-IgG reacted with the homologeous proteins and also with a fibroblast growth inhibitor (FGR-s) purified by Wang and associates

In addition to polyclonal anti-MDGI antibodies, peptide-specific antibodies directed against MDGI sequences 69-78 and 121-131 were raised. With both types of antibodies immunoreactive antigens in mammary tissue were characterized by immunoprecipitation experiments and immunocytochemistry at the electronmicroscopical level. Of particular interest is an antigen closely associated with the euchromatin of differentiated mammary epithelial cells.

The data raise the possibility that MDGI defines a new family of growth regulatory molecules.

D 403 OSTEOGENESIS INHIBITORY PROTEIN INHIBITS GLYCOSAMINOGLYCAN AND COLLAGEN SYNTHESIS IN VITRO. Anna G. Brownell, Nancy Gerth and G.A.M. Finerman. Chapman College, Orange, CA 92666 and UCLA, Los Angeles, 90024.

We isolated a noncollagenous bone matrix protein which is biologically active in inhibiting de novo bone formation in vivo and cartilage formation is organ culture. The present report outlines the biological activity of partially purified protein in vitro. Organ cultures of neonatal mouse triceps muscle were stimulated by bone morphogenetic protein to undergo cartilage differentiation as the first stage of endochondral bone formation. Collagen synthesis was measured using uptake of ^{14}C-proline and synthesis of ^{14}C-hydroxyproline; glycosaminoglycan synthesis was followed by uptake of $^{35}SO_4$ into pronase - resistant, trichloracetic acid precipitated macromolecules. Both collagen synthesis and glycosaminoglycan synthesis were inhibited in the presence of osteogenesis inhibitory protein at concentrations between 0.01 and 0.1 ug/ml. The protein is effective at inhibiting chondrogenic gene expressions only if added to the culture system at a critical stage of cell differentiation -- once the cells have initiated collagen synthesis, the protein is not inhibitory. However, once collagen synthesis has been inhibited, removal of the inhibitory protein does not restore the biosynthetic process. General protein synthesis was not affected. Synthesis of glycosaminoglycans is not as tightly controlled; once the inhibitory protein is removed, the synthesis of these molecules is restored to control levels. These data demonstrate that the protein is not cytotoxic but acts as an inhibitor of cellular differentiation. Supported by a research grant to AGB from the National Institutes of Health (AR37440).

D 404 THE MOLECULAR BASIS OF THE ANTIPROLIFERATIVE ACTION OF RETINOIDS: TRANSCRIPTIONAL CONTROL OF THE TRANSGLUTAMINASE GENE, E. Antonio Chiocca, Peter J.A. Davies, and Joseph P. Stein, University of Texas Medical School, Houston, Texas 77225
Vitamin A and its metabolites (retinoids) are potent and ubiquitous antiproliferative agents in both transformed and non-transformed cells. The molecular events responsible for this activity are poorly understood. We have shown that retinoic acid causes a rapid increase in the levels of the intracellular enzyme tissue transglutaminase (TGase) in mouse peritoneal macrophages and in human promyelocytic leukemia (HL60) cells. To evaluate the possibility that the gene for this enzyme may thus constitute a molecular marker for retinoid action, we cloned a TGase cDNA. Northern blot analysis revealed that the TGase mRNA levels in macrophages or in DMSO-primed HL60 cells increased within 15 minutes of retinoic acid treatment. The retinoid effect was not inhibited by cycloheximide, indicating that it was directly related to the expression of the TGase gene. The half-lives of the TGase mRNA from cells treated with retinoic acid or from control cells were similar. This implied that retinoic acid did not induce alterations in the stability of the TGase mRNA and that it was regulating the TGase gene at the level of transcription. It thus appears that the tissue TGase gene is the first direct biochemical index for retinoid action. Determination of the molecular mechanisms involved in the retinoid regulation of the expression of this gene will provide us with an insight into the events critical to the retinoid control of cellular proliferation.

D 405 ROLE OF SHIGA-TOXIN BINDING GLYCOLIPIDS IN B-LYMPHOCYTE DIFFERENTIATION, Amos Cohen[1], Vicente Madrid-Marina[1], Zeev Estrov[2], Melvin H. Freedman[2], Clifford A. Lingwood[3] and Hans-Michael Dosch[1], Divisions of Immunology/Rheumatology[1], Hematology/Oncology[2] and Bacteriology[3], Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.
The bacterial products Shiga-like-toxins (SLT) bind to the cell surface glycolipid globotriosylceramide (Gbose$_3$cer). SLT cytotoxicity is highly selective towards limited types of human cells. Human Burkitt B-lymphoma cells (Daudi) were found sensitive to the cytotoxic action of SLT. The toxin was specifically bound by the glycolipids Gbose$_3$cer and galabiosylceramide found on the surface of sensitive Burkitt lymphoma cells. Mutant Daudi cells (SLT^R_{20}) selected for resistance to SLT cytotoxicity were deficient in SLT-binding glycolipids and failed to bind SLT to their surface suggesting a role for these glycolipids in the mediation of SLT cytotoxicity. Of a number of normal and transformed lymphoid and myeloid cells screened for SLT sensitivity, only B-lymphoid cells were found susceptible to SLT action. Correspondingly, B-lymphoid cells were the only cells expressing the SLT-binding glycolipids. Limiting dilution analysis, of SLT-sensitive B-cell subsets, revealed that IgG and IgA committed cells are sensitive to SLT cytotoxicity, whereas IgM producing cells are relatively resistant. These observations may explain the exclusive production of anti-SLT antibodies of the IgM class previously found in Shigella infected humans. In addition, the restricted cell specificity of SLT may be of a potential use in the therapy of B-cell malignancies.

D 406 ISOLATION AND CHARACTERIZATION OF HUMAN DEFENSIN CDNA, Kathleen A. Daher, Robert I. Lehrer, and Mitchell Kronenberg. University of California, Los Angeles, CA 90024.
Defensins are a family of cysteine-rich microbicidal peptides found in the phagocytic cells of many mammals. There are three defensin peptides in human neutrophils, designated human neutrophil peptide (HNP)-1 HNP-2 and HNP-3, which make up about 30% of the neutrophil's granule protein. These peptides exhibit considerable anti-microbial and cytotoxic activity *in vitro* and are believed to contribute significantly to the neutrophil's arsenal of killing mechanisms. The HNPs are 29-30 amino acids in length and are identical in sequence except at their N-terminal amino acid. Using an oligonucleotide probe which was synthesized based on the C-terminal amino acid sequence of the HNPs, four defensin clones were isolated from a cDNA library prepared from HL-60 cells, a human promyelocytic leukemia cell line. Nucleotide sequence analysis indicates that the defensin coding unit has a 94 amino acid open reading frame, including a hydrophobic signal sequence. Following signal peptide removal, the 74 amino acid pro-defensin must be further cleaved to yield the mature peptides. Sequence data indicate that HNP-1 and HNP-3 differ by few nucleotide substitutions and therefore must have diverged recently. 0.75 kb defensin mRNA was detected in human bone marrow cells and in some myelogenous leukemia leukocytes, but not in normal peripheral blood leukocytes. Thus it is likely that defensin synthesis is completed in bone marrow neutrophil progenitor cells before their terminal differentiation and release to the blood stream.

D 407 EVALUATION OF THE ANTI-PROLIFERATIVE AND DIFFERENTIATION-INDUCING ACTIVITY OF RETINOIDS IN HUMAN PRO-MYELOCYTIC LEUKEMIA (HL-60) CELLS, Peter J.A. Davies, E. Antonio Chiocca, Susmita Poddar, William McKinney and Joseph P. Stein, University of Texas School of Medicine, Houston, Texas 77225.
Retinoids are a class of polyisoprenoid lipid molecules that can induce striking changes in the growth and differentiation of a number of transformed cells. Human promyelocytic leukemia (HL-60) cells respond to retinoic acid with decreased proliferative activity and granulocytic differentiation. Although these changes in phenotype are dramatic, their appearance is characterized by a marked delay (24-48h) suggesting that a complicated set of changes in cellular function precede their expression. To investigate the early components of retinoid action we have searched for gene products acutely regulated by retinoic acid. Using DMSO-primed HL-60 cells, we have found that retinoic acid acts as an acute inducer of a limited number of gene products, the most prominent of which is tissue TGase. Retinoic acid also causes the rapid suppression of expression of another set of gene products, one of which is c-myc. We have used the induction of tissue Tgase and the suppression of c-myc as indices to probe the mechanism and regulation of retinoid-regulated gene expression in HL-60 cells. Our studies suggest that there may be two pathways of retinoid action in HL-60 cells. One is a negative regulatory pathway, exemplified by the suppression of c-myc expression that may be linked to the inhibition of proliferative activity. The second is a positive pathway, operating at the level of transcriptional activation, that is linked to the induction of the differentiated phenotype.

D 408 DIFFERENTIATION OF CYTOTROPHOBLASTS IN VITRO, CHANGES IN GENE EXPRESSION. Nathan de Groot, Ofer Giladi, Abraham A. Hochberg, Hebrew University, Jerusalem, Israel.
Cytotrophoblasts were isolated from human term placenta and incubated in a M-199 medium supplemented with 20% newborn calf serum. The cells adhere to the culture dish, aggregrate and fuse to form multinuclear syncytiotrophoblasts. RNA was prepared from freshly isolated cytotrophoblasts or from cultured cells following various periods in culture. The expression of specific sequences was examined using Northern blotting of cellular RNA and hybridization with specific cDNA probes. Freshly isolated cytotrophoblasts contain a high level of c-fos transcripts before being exposed to serum-containing medium. After plating, there is some increase in the amount of c-fos transcripts, which then declines rapidly to undetectable levels after 3 hours. C-sis transcripts reach a peak after 6 hours in culture and decline thereafter to a constant, nonzero level. C-myc transcripts are first detected after 4 hours in culture, reach a peak at 6-7.5 hours and then decline. RNA sequences coding for the 2'-5' oligoadenylate synthetase (OAS) first appear after 6 hours in culture, and reaches a 30-fold higher level at 24 hours; and then declines to low levels. Hybridization to a hCG-β cDNA probe was detectable only at 120 hours of culture, in agreement with the level of the protein. hPL transcripts increase considerably in amount with time in culture.

D 409 GROWTH INHIBITORY ACTION UPON FIBROBLASTS OF A NOVEL FACTOR RELEASED BY A HUMAN PROMYELOCYTIC LEUKEMIC CELL LINE (HL-60), Klaus Dittmann and Petro E. Petrides, Munich University School, 8000 Munich 70, West Germany.
A central issue in tumor biology is the understanding of the interactions between tumor cells and their microenvironment. Cocultivation of the human promyelocytic cell line HL-60 with normal human skin fibroblasts results in a reversible loss of attachment of fibroblasts to tissue culture plastic dishes or their extracellular matrix (ECM) resp. and the concomitant cessation of proliferation of these cells. At the same time terminal differentiation of HL-60 cells is induced. This effect can also be obtained by the addition of medium conditioned by the HL-60 cells to fibroblasts in culture. When HL-60 cells are induced with phorbolesters to differentiate into macrophages, the production of the growth inhibitory factor is discontinued. Upon contact with conditioned medium fibroblasts loose their spindle like appearance, round up and cease to divide. Replacement of HL-60 conditioned medium with unconditioned medium leads to a recovery of attachment to ECM and mitogenic activity. Preincubation of conditioned medium with sodium-benzoyl-L-arginine amid, a protease inhibitor, abolishes the growth inhibitory activity indicating that a proteolytic enzyme is involved the mechanism of action of this factor. Fractionation of the HL-60 conditioned medium by gel fitration experiments reveals a molecular weight for the activity in the range of 1000 -2000 d. Our results indicate that the concerted action of a proteolytic enzyme (either produced by HL-60 cells or fibroblasts) and a low molecular substance (presumably a polypeptide) mediates the growth inhibitory effect of the leukemic cells upon the fibroblasts. Supported by a grant from DFG (SFB 324)

D 410 A LOW MOLECULAR WEIGHT INHIBITOR OF HEMOPOIETIC PLURIPOTENT STEM CELL PROLIFERATION. Martine Guigon*[1], Maryse Lenfant*, Joanna Wdzieczak-Bakala*, Dominique Sotty*, Hiroko Izumi*[1], Jean-Yves Mary+ and Emilia Frindel*. *INSERM U.250, Institut Gustave-Roussy, Villejuif - *ICSN/CNRS, Gif s/Yvette - +INSERM U.263, Paris - France.

We have previously reported that a dialysable factor extracted from fetal calf bone marrow is able to inhibit pluripotent stem cell (CFU-S) entry into DNA synthesis after irradiation or Cytosine Arabinoside (Ara-C) treatment. This factor has been shown to increase the proportion of mice surviving administration of quasi-lethal doses of the drug. Partial purification has been achieved by ultrafiltration and gel chromatography on BioGel P-2. The protective effect of the CFU-S inhibitor has been confirmed using this partially purified material. When administered to mice which received 4 injections of high doses of Ara-C, a single injection of 4µg/mouse of the inhibitor 4 hours before the last Ara-C injection increased the percentage of survivors from 25 to 50 % (p 0.02) Mice alive at day 10 were still alive at day 200. The survivors recovered normal hemopoiesis about one month after treatment. When applied to EMT6 tumor-bearing mice, the CFU-S inhibitor increased similarly the number of Ara-C treated surviving mice, without altering the response of the tumor of the drug. The increase in animal survival induced by the inhibitor is similar to the protective effect of bone marrow grafting. In view of clinical applications in bone marrow protection, studies in vitro are now in progress on human normal and malignant cells (M.G.) with the pure molecule (tetrapeptide : E.F., M.L., D.S., J.W-B.).

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D 411 EXPRESSION OF ONCOSTATIN M AND PROPERTIES OF THE RECOMBINANT PROTEIN, Nancy Gunderson, Scott Austin, Vincent Ochs, Timothy Rose, Najma Malik, Joyce M. Zarling, Peter S. Linsley, and Cha-Mer Wei, ONCOGEN, 3005 First Ave., Seattle, WA 98121

Oncostatin M (Onco M) is a single chain glycoprotein of M_r~ 25,000 which is released by TPA-induced U937 cells (1) and activated human T cells (2), and which inhibits growth of certain human tumor cell lines *in vitro*. This novel growth regulator may also act synergistically with other polypeptide factors (2). In order to produce sufficient quantities of Onco M for further study of its biological activities, including *in vivo* anti-tumor effects, we have expressed DNA sequences encoding this polypeptide in a variety of systems. The polypeptide has been overexpressed in *E. coli*, using lambda P_L and *tac* promoter systems, to levels of approximately 20% of total cellular protein. Preliminary experiments indicate that following renaturation and refolding, recombinant Onco M is capable of inhibiting growth of A375 melanoma cells. Although the native polypeptide contains both N- and O-linked oligosaccharides, these do not appear essential for biological activity. Various recombinant proteins are being purified; studies of structural features and biological activities of these are in progress.

1) Zarling, JM, et al. (1986) PNAS *83*:9739-9743.

2) Brown, TJ, et al. (1987) J. Immunol., in press.

D 412 ASSAYING THE QUALITY OF cDNA LIBRARIES, Frederick S. Hagen, Charles L. Gray, and Joseph L. Kuijper, ZymoGenetics, Inc., Seattle, WA 98103. Once a cDNA library has been constructed, it is very useful to be able to easily assess the quality of the library. Because actin is a ubiquitous sequence, this assessment has been accomplished by probing filter lifts and Southern blots of libraries with an actin cDNA probe. These methods provide information about the percent of actin positive clones and the degree of completeness of cDNA clones in a library. This approach is used routinely to gain a greater degree of confidence that newly constructed cDNA libraries are useful for screening for clones of interest. Many cDNA clones for low abundant mRNA, such as factor V, factor VII, GM-CSF, GPIB, and PDGF receptor, have been obtained from libraries which exhibit percent actin positives of 0.1 to 1.0 %. Unsuccessful attempts have been made at obtaining clones from libraries with 10 to 100 times lower values for actin positive clones and thus now time is not wasted in screening such libraries, rather new libraries are constructed. Southern analysis of cDNA libraries with an actin probe provides information about whether a particular library may contain partial or full length clones of interest. Full length cDNA clones of 3 to 4 Kb have been obtained from libraries that exhibit full length actin bands by Southern analysis.

D 413 INHIBITORY DIFFUSIBLE FACTOR (IDF_{45}) AFFECTS DIFFERENTLY THE STIMULATION OF RNA AND DNA SYNTHESIS INDUCED IN CHICK EMBRYO FIBROBLASTS BY VARIOUS MITOGENS. L. HAREL*, J. DELBE*, J. VILLAUDY*, C. BLAT*, A. GOLDE*.*Institut de Recherches Scientifiques sur le Cancer Villejuif BP. 8 France.*Institut Curie, Pavillon Regaud Paris 75005 France.
Inhibitory Diffusible Factor (IDF_{45}) purified from conditioned medium of 3T3 dense cultures (1) was able to inhibit, in chick embryo fibroblasts (CEF) DNA synthesis and cell growth (2) stimulated by serum. By contrast the stimulation of DNA synthesis by v-src gene expression (in Ny 68 infected CEF transfered to 37°C) was poorly inhibited by IDF_{45} (2). The inhibitory efficiency of IDF_{45} has been tested on CEF stimulated by different mitogens : PDGF, TPA, IGF_1, and insulin which increased, RNA synthesis in the first two hours after their addition. This stimulation was differently inhibited by IDF45 (from 0 to 100%) following the mitogens. The stimulation of DNA synthesis was, in the same manner, differently inhibited by IDF_{45} following the growth factor.
(1) BLAT, C., CHATELAIN, G., DESAUTY, G., and HAREL, L. FEBS LETTERS. 203, N°2 175-180. (1986).
(2) C. BLAT, J. VILLAUDY, D. ROUILLARD, A. GOLDE, and L. HAREL. J. of Cell Physiol. 130 416-419 (1987).

D 414 POSSIBLE ROLE FOR ALU INTERDISPERSED REPETITIVE SEQUENCES IN NEGATIVE REGULATION OF CELL GROWTH, C. Michael Fordis, Kazuichi Sakamoto, Christopher D. Corsico, Wolfgang Holter, R. Bruce Helmly, Tazuko H. Howard, Abdelfattah M. Attallah, and Bruce H. Howard, National Cancer Institute, Bethesda, MD 20892.
To study mechanisms by which mammalian cell growth can be suppressed, we have performed DNA-mediated gene transfer experiments in which candidate growth inhibitory genes are cotransfected with a cell surface marker plasmid. Cells that transiently express the plasmid-encoded surface marker (the Tac subunit of the human IL-2 receptor) are identified by FACS or affinity cell sorting and analyzed with respect to DNA replication (BrdUrd labeling index or ^{3}H-thymidine incorporation). Our results suggest that transfection with interspersed repetitive sequence (IRS) elements of the 7SL RNA/Alu and THE families can transiently suppress the growth of recipient HeLa or CV-1 cells. Our current hypotheses concerning IRS-mediated growth suppression mechanisms will be discussed, and evidence will be presented that such transfection-related growth inhibition may be relevant to physiological growth regulation.

D 415 PURIFICATION AND CHARACTERIZATION OF A LIVER DERIVED GROWTH INHIBITOR, Anthony C. Huggett, Mrunal S. Chapekar, Peter J. Wirth, Henry C. Krutzsch, James B. McMahon and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892. The purification of a growth inhibitory protein for liver cells isolated from rat liver has been previously reported (McMahon et al., P.N.A.S. USA, 1982, 79:456-460). SDS-PAGE analysis of this product with sensitive silver staining failed to demonstrate a pure protein. A new purification scheme for this inhibitor was developed which yielded a product that has an inhibitory activity 1000-fold greater (ID_{50} = 50 pg/ml for rat liver epithelial cells, 250 pg/ml for primary hepatocytes) than that previously reported although the final preparation was still not pure. The growth inhibitor which has a molecular weight in the range 17-25 Kd and a pI of about 5.5, was labile to heat, low pH and organic solvents, resistant to reducing agents and sensitive to treatment with trypsin under conditions favoring protein unfolding. The inhibitors, which produces a reversible inhibition of liver epithelial cell proliferation, was shown to be different to other well characterized growth inhibitors including TGF- . In an attempt to obtain a pure preparation, a new approach to the purification of this growth inhibitory protein using reverse phase HPLC has recently been devised. Initial results have indicated that more than one protein may be responsible for the growth inhibitory activity.

D 416 CURRENT STATUS OF FLY AND MOSQUITO OOSTATIC HORMONE, Thomas J. Kelly, Edward P. Masler, Albert B. DeMilo, Robert A. Bell and Stephen B. Haught, U.S. Department Agriculture, Agricultural Research Service, Insect Reproduction Laboratory, Beltsville, MD 20705. *Oostatic activity* has been demonstrated in saline extracts of mature ovaries from the house fly, Musca domestica, fruit fly, Drosophila melanogaster and mosquito, Aedes atropalpus. Extracts inhibit ovarian maturation at a similar previtellogenic stage in all species. This stage of ovarian maturation in A. atropalpus responds to the cerebral neuropeptide, egg development neurosecretory hormone (EDNH), by producing insect steroid hormones, or ecdysteroids. Although ecdysteroid production is blocked in females injected with oostatic extracts, co-injection of the major physiological ecdysteroid, 20-hydroxyecdysone, does not restore ovarian maturation. Thus a more general mechanism of oostatic hormone action is suggested. *Ovarian maturation inhibitory activity* reaches a maximum in post-vitellogenic ovaries and correlates with ecdysteroid synthesis inhibitory activity in in vitro ovarian cultures. The major portion of inhibitory activity from extracts of house fly ovaries elutes in highly polar region during RP-HPLC and has an apparent molecular weight of <2500 Daltons as estimated using HP-SEC.

D 417 HEMOREGULATORY PEPTIDE MONOMER AND DIMER: INHIBITORY AND STIMULATORY EFFECTS ON MYELOPOIESIS IN VITRO AND IN VIVO, Ole D. Laerum, Olav Sletvold, Rune Smaaland and Walter R. Paukovits, The Gade Institute, Dept of Pathology, University of Bergen, Haukeland Hospital, N-5021 Bergen, Norway and Institute of Cancer Research, University of Vienna, A-1090 Vienna, Austria.

The hemoregulatory peptide, a synthetic analogue of a pentapeptide associated with human granulocytes, has been tested for biological effects in vitro and in vivo. In vitro it has a dose dependent inhibitory effect on myelopoietic colony formation (CFU-GM) both on human and mouse cells in concentrations from 10^{-6} to 10^{-13}M. Above and below these doses, the inhibitory effect disappears. Similar effects are found in vivo by single injections and infusions to mice, with main effect on CFU-GM. Myelopoietic cell proliferation and the numbers of peripheral blood granulocytes, as well as to some extent the multipotent stem cells (CFU-S) are also reduced. By oxidation, the peptide forms a homo-dimer which stimulates both human and mouse myelopoietic cells. The dimer is no growth factor, but enhances greatly the colony formation by CSF. Within 3 days after a single injection of the dimer to mice, the CFU-GM numbers are increased by 3 times, and increased levels persist for 11 days. However, exceeding single doses of 120 ng per mouse does not lead to further effect. Thus, both substances seem to have a dose dependent selective effect on myelopoietic cells. The monomer-dimer system may constitute a new type of regulation of myelopoiesis, possibly connected to a feedback mechanism excerted by granulocytes.

D 418 IMMUNOSUPPRESSION BY THE CYCLIC PEPTIDE DIDEMNIN B: EFFECTS ON INTERLEUKIN 2. S.J. LeGrue, T.L. Sheu and S. Sanduja. The University of Texas M. D. Anderson Hospital, Houston, TX, 77030. We investigated the mechanism by which the cyclic depsipeptide didemnin B (DB) inhibits lymphocyte proliferation. DB is a novel cyclic octapeptide obtained from a Carribean tunicate, and contains several unique structural attributes including hydroxy-isovalerylproprionate, statine, and lactylproline substituted onto an N-methylleucine. DB *inhibited the proliferation of both Con-A- stimulated murine splenocytes and the interleukin 2* (IL 2)-dependent T cell line CTLL-2 at equimolar concentrations (IC_{50} = 3 to 4 nM). Inhibition of CTLL-2 growth was partially reversible at DB concentrations up to 10 nM. Concentrations of DB that completely blocked mitogen-driven spleen cell blastogenesis (10 nM) only partially inhibited the synthesis and secretion of IL 2. Although DB blocked the growth of CTLL-2 cells in response to recombinant human IL 2, the suppression was not due to an uncoupling of the lymphokinetic signal, but was closely correlated with an inhibition of protein and RNA synthesis. In addition, DB significantly inhibited the incorporation of [^{3}H]-thymidine by CTLL-2 cells, even when added 24 hr after initial IL 2 stimulation. The influence of DB on protein synthesis was not attributable to an affect on the translational machinery, since addition of DB to an *in vitro* cell-free translation system did not inhibit protein synthsis. Thus, we conclude that DB functions as a cytostatic or antiproliferative compound, with no effect on the synthesis of, or signaling by, IL 2. (The U.T. Cancer Foundation).

D 419 IDENTIFICATION OF A MEMBRANE RECEPTOR FOR THE GROWTH REGULATOR, ONCOSTATIN M, Peter S. Linsley, Marcia Bolton-Hanson, Mohammed Shoyab, and Joyce M. Zarling, ONCOGEN, 3005 First Ave., Seattle, WA 98121

Oncostatin M (Onco M) is a polypetide growth regulator produced by U937 cells which have been induced to differentiate by treatment with phorbol esters. This factor inhibits the replication of certain human tumor cell lines, and shows synergistic effects with other growth regulators. Although there is obvious interest in the use of Onco M for treatment of human malignancies, little is known about the molecular mechanisms by which this factor exerts its cytostatic effects. As an initial step, we have identified and characterized a membrane receptor for this factor on human tumor cell lines. Onco M was purified to homogeneity from TPA-treated U937 cells and radiolabeled with ^{125}I. The iodinated protein retained approximately 25% of its original biological activity. Binding of ^{125}I-Onco M was specific and saturable, and was not inhibited by other known polypeptide growth regulators. Scatchard analysis of binding data obtained with different cell lines revealed that ^{125}I-Onco M bound to 1-2 x 10^4 binding sites per cell with a K_d of approximately 1 nM. To identify the Onco M receptor, we chemically cross linked ^{125}I-Onco M to its receptor using disuccinimidyl suberate. When the reaction products were examined by SDS PAGE, a single cross-linked complex of $M_r \sim 180,000$ was observed. This complex was observed with several cell lines which differed greatly in their sensitivity to Onco M. Neither receptor number, affinity nor receptor size correlated with sensitivity of different cell lines to the growth inhibitory activity of Onco M.

D 420 CLONING AND SEQUENCE ANALYSIS OF cDNA CLONE ENCODING A GROWTH REGULATORY PROTEIN ONCOSTATIN M, Malik, N., Austin, S.D., Zarling, J.M., Shoyab, M., Marquardt, H., and Wei, C.M. Oncogen, 3005 First Avenue, Seattle, Washington 98121

A growth inhibitory protein termed oncostatin M has been purified to homogeneity from the serum-free supernatants of U-937 histiocytic lymphoma cells which were treated with phorbol ester, TPA. Oncostatin M has been shown to inhibit the replication of A375, melanoma and other human tumor cell lines. However, the protein has no inhibitory effect on normal human fibroblasts (Zarling et al., PNAS 83, 1986). A 2.1 kb clone encoding oncostatin M has been isolated from a cDNA library constructed using poly (A^+) RNA obtained from TPA treated U937 cells. The nucleotide sequence of the clone was determined; the deduced amino acid sequence suggests that oncostatin M is a novel protein. The polypeptide encoded for by the cDNA clone is 26 Kd and is composed of 228 amino acids. The protein molecule is very hydrophilic and has two potential N-glycosylation sites. Northern blots of RNA isolated from various cell lines treated with TPA which were probed with the nick translated oncostatin M clone will be shown.

D 421 IDENTIFICATION, MOLECULAR ANALYSIS AND EXPRESSION OF A CELL PRODUCED GROWTH ARREST FACTOR, L. Mallucci[1], V. Wells[1], M.D. Waterfield[2], N.F. Totty[2], G.T. Scrace[2], T. Harris[3] and R. Boyle[3], Guy's Medical School, London, U.K.[1], Ludwig Institute, London, U.K.[2], Celltech, Slough, U.K.[3]

We have isolated in a pure state a cell produced protein which has cell growth inhibitory activity at nanogram concentrations. The protein which we term Growth Arrest Factor (GAF) has a M.W. of approximately 12,500 daltons and consists of about 129 amino acids. Partial peptide sequence analysis corresponding to about one fourth the size of the molecule has shown no homology with growth factors, TGF-beta, or with interferon molecules. Using synthetic probes from two amino acid sequences, clones have been obtained from a cDNA library. Northern blot analysis has shown greater expression of GAF mRNA in G_0 than in mixed populations. Under natural growth conditions GAF appears to have a cell growth regulatory role. Cell cycle linked levels (S phase) of 2-5A synthetase, an enzyme which is involved in RNA cleavage, are lowered by GAF. The anti-proliferative effect of GAF also extends to tumour cells.

D 422 CELLULAR LOCALIZATION OF GLUCOCORTICOID RECEPTOR mRNAs IN RAT BRAIN AND LIVER BY IN SITU HYBRIDIZATION, Martha F. Matocha, Guang Yang and Stanley I. Rapoport, Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.
In situ hybridization is a powerful technique to precisely localize transcripts of genes sensitive to changes in the cellular milieu. For this reason, we developed an in situ hybridization method to detect glucocorticoid receptor (GR) mRNAs in various tissues of rat. Brain or liver tissues fixed by perfusion or by post-fixation in 4% formaldehyde were cryostat-cut into 10 micron sections. Hybridization was carried out using radiolabeled antisense RNA probes transcribed from cDNAs complementary to different regions of the rat liver GR gene. The specificity of hybridization was determined in several ways: 1) in situ hybrids formed using different probes colocalized to the same cells; 2) agreement between the experimental and theoretical Tm values; and 3) decreased grain density on sections pretreated with RNase A. Direct autoradiography showed dense clustering of grains corresponding to antisense-cellular RNA hybrids over the cytosolic area of heptocytes. In brain temporal cortex, silver grains covered both the glia and neurons. Also, grain intensity was extremely high in the hippocampal region and localized primarily over the pyramidal cells of CA2. These results are consistent with the high levels of GR in liver and the regional distribution of GR in brain. They demonstrate the applicability of in situ hybridization to study the transcriptional control of GR gene expression.

D 423 HEPARIN-BINDING GROWTH FACTOR TYPE 1 IS AN AUTOCRINE REGULATOR OF GROWTH AND SECRETORY FUNCTION IN HUMAN LIVER CELLS Wallace L. McKeehan, Mikio Kan, Jinzhao Hou, Hiroyoshi Hoshi, and Per-Erik Mansson, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY 12946. HBGF-1 (acidic FGF) at 1 to 100 ng/ml reversibly inhibited growth of a well-differentiated, non-tumorigenic human hepatoblastoma-derived cell line (HepG2). The same concentrations of HBGF-1 specifically stimulated secretion of antigens related to the hepatocyte-derived proteinase inhibitor, inter-alpha trypsin inhibitor (IαTI). No stimulation of IαTI-related antigens by EGF, insulin, glucocorticoid or TGF-β could be detected. At concentrations below 1 ng/ml, HBGF-1 stimulated the growth of HepG2 cells. The inhibition of growth and stimulation of IαTI-related antigens correlated with the presence of an abundant, low-affinity (Kd = 1 nM) receptor on the HepG2 cells with apparent molecular weight of 130 kD. A less abundant, high-affinity (Kd = 10 pM) class of receptors correlated with growth stimulation by HBGF-1. Other malignant hepatoma cell lines that displayed only the high-affinity receptor were neither inhibited by HBGF-1 nor did HBGF-1 stimulate IαTI-related antigens. Expression of HBGF-1 mRNA in HepG2 cells was 5 to 10 times that of other cell types. No HBGF-2 (basic FGF) mRNA could be detected. These results implicate HBGF-1 as a potential autocrine regulator of liver cell growth and secretory function. Direction of effect of the hormone is determined by affinity of the HBGF receptor and amount of HBGF in the local cell environment.

D 424 DOES THE LACK OF ANDROGEN-REPRESSED CELL DEATH ASSOCIATED mRNA EXPRESSION RENDER A RAT VENTRAL PROSTATE EPITHELIAL CELL LINE ANDROGEN INDEPENDENT?, Michael L Montpetit and Martin Tenniswood, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5
In-vivo, castration results in the death of the epithelial cells at the distal end of the ductal network while the proximal epithelial cells and global fibroblast cell populations survive. Androgen ablation results in the expression of androgen-repressed mRNA sequences in the distal epithelial cells that have been closely associated with the active process of epithelial cell death in the prostate. Normal RVP epithelial cells in short term culture are androgen-dependent for secretory functions and cell survival. A series of rapidly-dividing epithelial (RDE) cell lines have been isolated from primary cultures of rat ventral prostate (RVP) epithelial cells. Unlike androgen-dependent secretory epithelial cells, the RDE cells in culture do not express androgen-dependent secretory proteins nor do they express the androgen-repressed cell death sequences found in the epithelial cells during prostatic regression. Data will be presented indicating that the loss of androgen-dependence for cell survival in RDE cells results at least in part from the inability of the cells to express the cell death sequences.

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D 425 SV40-TRANSFORMED HAMSTER CELLS SECRETE A NEGATIVE GROWTH FACTOR THAT MAY PROMOTE THEIR ONCOGENICITY. C.T. Patch, K. Murai, A. Roy and A.S. Levine, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

Hamster cells transformed by either adenovirus 2 (Ad2) or SV40 are oncogenic in immunoincompetent hamsters; however, only SV40-transformed cells induce tumors in immunocompetent animals. Differences in the expression of Class I MHC antigens or TSTA do not correlate with oncogenicity in this system. While there is a good correlation between oncogenic potential and resistance to in vitro lysis by NK cells and macrophages, other factors may be involved in the ability of SV40-transformed cells to cross the allogeneic barrier. We therefore sought to learn if these differences in oncogenicity could be influenced by growth factors. We have investigated the influence of media conditioned by these two transformed cell types on thymidine uptake in untransformed cells. We found that medium conditioned by Ad2-transformed cells acts synergistically with serum mitogens in stimulating DNA synthesis, while medium conditioned by SV40-transformed cells is inhibitory. We have partially purified the mitogenic inhibitor (MI) from SV40-conditioned media. MI has a MW of 30-40 kd as estimated by elution from gel-filtration HPLC; its activity is reduced by heat and low Ph, but is largely resistant to trypsin and disulfide reducing agents. MI does not compete with TGFβ (for binding to A549 cells) and its inhibitory action is completely reversible. NRK cells, blocked by MI, do progress through most of the G1 phase when serum mitogens are present; after removal of MI, the cells rapidly enter S phase. MI is much more inhibitory for untransformed cells than it is for the SV40-transformed cells that secrete it. Moreover, when SV40-transformed cells are at high density, MI stimulates their DNA synthesis. However, we have also found that MI is strongly inhibitory for hamster spleen lymphocytes. This result suggests that the MI secreted by SV40-transformed cells could influence their oncogenicity by inhibiting the mobilization of immune effector cells at the site of tumor growth.

D 426 HEMOPOIETIC STEM CELL INHIBITION BY HEMOREGULATORY PEPTIDES: A NEW APPROACH FOR REDUCING MYELOTOXICITY OF CYTOSTATIC DRUGS:

Paukovits W.R.[1], Laerum O.D.[2], Guigon M.[3], Paukovits J.B.[1], Binder K.A.[1]

From extracts of normal human leukocytes we have isolated a low molecular weight HEMOREGULATORY PEPTIDE (HP) which is a potent inhibitor of myeloid but not erythroid colony formation in vitro (dose: 10^{-13} - 10^{-7} M/l, max. 75-90% inhibition). In vivo it causes a decrease of the number of CFU-GM and CFU-S per femur. Sequence determination yielded pGlu-(Asp or Glu)$_2$-Cys-Lys. Based on this sequence synthetic analogues were prepared. The pentapeptide pGlu-Glu-Asp-Cys-Lys-OH (HP5b) is a very potent inhibitor of hemopoietic proliferation in vivo and in vitro. 80 to 90 % of CFU-GM are inhibited in vitro in doses between 10^{-7} and 10^{-13} Moles/l. In vivo CFU-S and CFU-GM are inhibited by 60 resp. 75 % with cumulative doses of 0.01 - 1 µg/mouse applied over 6 days by continuous infusion. HP5b, at a single dose of 0.6 µg per mouse, prevents the activation of hemopoietic stem cells CFU-S after ara-C application. This effect was utilized in further experiments in which we could significantly reduce the lethal effect of multiple high dose ara-C injections (4x900 mg/kg). HP5b (1 x 0.6 µg/mouse) saved the mice to about the same degree (survival improvement factor over ara-C group: 2.1) as a bone marrow graft, which was included in the protocol to show that the ara-C lethality in these experiments was related to hemopoietic damage.

Application of cytostatic drugs triggers the majority of the (normally) quiescent hemopoietic stem cells into active proliferation thus rendering them vulnerable to subsequent application(s) of the drug. The hematological side effects associated with this phenomenon frequently determine the maximum effectiveness of tumor chemotherapy. The prevention of stem cell activation by synthetic peptides like HP5b and the resulting reduction of hemopoietic damage may represent a novel approach to decrease hematotoxic side effects during certain forms of cancer chemotherapy.

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D 427 NRP - A NEGATIVE REGULATOR OF ERYTHROPOIESIS, F.G. Pluthero, D.F. Del Rizzo, D. Eskinazi and A.A. Axelrad, Dept of Anatomy, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Negative Regulatory Protein (NRP) is a growth factor produced in the bone marrow of C57BL/6 (B6) mice that specifically inhibits DNA synthesis f the early erythropoietic progenitor BFU-E, and our evidence indicates that it is involved in the physiological regulation of erythropoiesis. The effect of NRP is rapid (occurring in minutes) and reversible, as determined by a cell-suicide assay of BFU-E proliferation. We have examined the effects of other growth factors on BFU-E, and found that Transforming Growth Factor-B (TGF-B) also inhibits DNA synthesis, and its action. Interleukin-3 was found to oppose the action of NRP and TGF-B. We have purified NRP activity from the medium of a continuous cell line derived from B6 marrow, obtaining material active in nanogram amounts. Results of ion-exchange and lectin affinity chromatography indicate that NRP is a wheat-germ lectin-binding glycoprotein, neutral at physiological pH. Recent improvements in the purification of NRP have yielded enough protein for a preliminary molecular analysis, the results of which will be presented.

D 428 Anti-proliferative activities of soluble factors derived from a macrophage-like cell line (U937), Patricia Roth, Ken Taverner and John Wilkins, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Treatment of cells of the macrophage-like line, U937, with phorbol ester (TPA) results in the release of soluble factor(s) which inhibit the proliferation of several tumor cell lines, including cells of the U937 line. The factor(s) is cytostatic, as it does not affect the viability of target cells. In addition, the anti-proliferative effect on U937 cells and on cells of the myeloid leukemia line, K562, is long-lived, lasting several weeks after removal of supernatant. Proliferating U937 cells which have recovered from the inhibitory effect can be stimulated with TPA to produce antiproliferative activity. The antiproliferative activity can be isolated repeatedly from the same culture 48 hr, 72 hr and 96 hr after treatment with PMA. However, supernatants which are allowed to remain in culture for 72 hr or 96 hr without media replacement contain no more activity than 48 hr supernatants, indicating that once a maximum concentration of activity is reached, no additional activity is produced. These results suggest that factors in U937 supernatants may play a feedback regulatory role as well as having an anti-proliferative effect. Tumor necrosis factor (TNF) activity is also detected in U937 supernatants. However, the antiproliferative activity cannot be blocked by antibody to TNF. In addition, no synergy between TNF and factors contained in the supernatant can be demonstrated. The antiproliferative activity is heat and acid-stable and its molecular weight has been estimated at 50-80Kd by gel filtration. The physicochemical properties and mode of action of the factor(s) are currently being investigated.

D 429 ONCOGENE MODULATION BY A REVERSIBLE GROWTH INHIBITOR OF TRANSFORMED CELLS. Gail Seigel and Raphael Mannino, Albany Medical College, Albany, NY 12208

Defining mechanisms regulating cellular proliferation is vital to our understanding of such life processes as neoplasia, ageing, and embryogenesis. Succinylated Concanavalin A, (SCA), a non-toxic derivative of the lectin Concanavalin A, reversibly inhibits the growth of a variety of normal and transformed cell lines, simulating density-dependent growth inhibition. In such SCA-inhibited SV40 transformed 3T3 cells, levels of both SV40 large T antigen and p53 cellular oncogene product are greatly reduced as detected by flow cytometric analysis. This decrease is consistent throughout the cell cycle. Furthermore, reduction is dependent upon concentration and duration of SCA treatment, such that higher concentrations of SCA, as well as longer times of SCA exposure, lead to more pronounced decreases in detected levels of p53 oncogene product and SV40 large T antigen.

Upon reversal of SCA growth inhibition by alpha-methyl mannoside, cells once again enter a proliferative mode, with a corresponding increase to pre-SCA treatment levels of SV40 large T antigen seen within one hour after reversal, then higher levels at 4 hours post-reversal, returning to pre-SCA treatment levels by 8 hours post-reversal. Currently, studies are underway to determine whether control of p53 and SV40 large T antigen expression resides at transcription or translation.

D 430 ISOLATION AND CHARACTERIZATION OF MITOTIC INHIBITOR SECRETED BY TISSUE CULTURE CELLS, Chandrashekhar N.Shenoy, Shirin M.Marfatia and Kamlakar A.Chaubal, Biophysics Unit, Cancer Research Institute, Parel, Bombay 400012, INDIA

The growth of cells, passing through different stages of cell cycle, is a well regulated phenomenon. It depends on several factors which play important roles in controlling the overall growth of normal cells. However, no such mechanism of growth control is evident in malignant cells. Though the precise mechanism of growth regulation is not known, several substances affecting growth have been isolated, few theories have been postulated and evidence is accumulating for the definite role played by endogenous inhibitors.

It has been our observation that when the used up medium of Human Amnion cells (HA) in tissue culture was fractionated, using sephadex gel filtration, a fraction having absorption at 260 mu consistently occurred. The addition of this fraction to HA cells caused inhibition of mitosis without any toxicity and the action was reversible. However, the fraction was found ineffective on other cell types. Hence, the inhibitory action was specific.

Further investigations using microplanimetry, showed that the mitotic inhibitory fraction caused enlargement of cells. The effect of the fraction on macromolecular synthesis is being studied using autoradiography and cytophotometry. The chemical analysis of the fraction showed that it consists of protein and DNA. The molecular weight of the fraction was determined by sephadex gel filtration using molecular weight markers.

D 431 INHIBITION OF GLIAL CELL PROLIFERATION BY ISOLATED NEUROBLASTOMA CELL MEMBRANES AND GLYCOPEPTIDES, Peter A. Steck, Cynthia Swanson-Pfeiffer, and W.K. Alfred Yung, Department of Neuro-Oncology, M.D. Anderson Hospital and Tumor Insititute, Houston, TX 77030. Cell surface membrane preparations has been shown to inhibit cellular proliferation in several tissue culture systems. To investigate the possible growth and differentiation effects of neuronal cells on astroglial cells, we have utilized isolated membrane fractions from neuroblastoma cell to inhibit the growth and DNA synthesis of normal glial cells. The inhibitory effects of the membranes was shown to be dose dependent and reversible. Also, equal quantities of membranes from the glial cell proliferation. In addition, conditioned medium from the neuroblastoma cell did not inhibt the glial cell proliferation. Differentiation of glial cells by neuroblastoma cells was examined by their capability to induce morphological processes and glial filament protein. Current efforts are directed at chaacerizing the growth inhibitory factor(s) associated with the membrane fractions and preliminary evidence suggest the inhibitors are glycoproteins. These results implicate the role of cell-cell contact in the growth regulation of astroglial cells. This work is supported by grants from the J.S. Dunn Research Foundation, the Preuss Foundation, and NIH RR-5511-23.

D 432 THE MOLECULAR BASIS OF THE ANTIPROLIFERATIVE ACTION OF RETINOIDS: TRANSCRIPTIONAL CONTROL OF THE TRANSGLUTAMINASE GENE, E. Antonio Chiocca, Peter J.A. Davies, and Joseph P. Stein, University of Texas Medical School, Houston, Texas 77225
Vitamin A and its metabolites (retinoids) are potent and ubiquitous antiproliferative agents in both transformed and non-transformed cells. The molecular events responsible for this activity are poorly understood. We have shown that retinoic acid causes a rapid increase in the levels of the intracellular enzyme tissue transglutaminase (TGase) in mouse peritoneal macrophages and in human promyelocytic leukemia (HL60) cells. To evaluate the possibility that the gene for this enzyme may thus constitute a molecular marker for retinoid action, we cloned a TGase cDNA. Northern blot analysis revealed that the TGase mRNA levels in macrophages or in DMSO-primed HL60 cells increased within 15 minutes of retinoic acid treatment. The retinoid effect was not inhibited by cycloheximide, indicating that it was directly related to the expression of the TGase gene. The half-lives of the TGase mRNA from cells treated with retinoic acid or from control cells were similar. This implied that retinoic acid did not induce alterations in the stability of the TGase mRNA and that it was regulating the TGase gene at the level of transcription. It thus appears that the tissue TGase gene is the first direct biochemical index for retinoid action. Determination of the molecular mechanisms involved in the retinoid regulation of the expression of this gene will provide us with an insight into the events critical to the retinoid control of cellular proliferation.

D 433 EXPRESSION OF ANDROGEN REPRESSED MESSAGES IN THE RAT VENTRAL PROSTATE DURING REGRESSION. M. Tenniswood & J. Léger, University of Ottawa, Ottawa, Ont. Canada.
Growth and differentiation of the rat prostate is under the control of androgens. During castration-induced regression of the prostate the vast majority of epithelial cells within the gland die. This programmed cell death is an active process that requires protein synthesis and is antagonized by androgens. After castration a novel mRNA (TRPM-2) is expressed in the regressing prostate. This sequence is 2000 nucleotides in size and codes for a protein of about 46 kDa, with a pI of 5.9-6.3. We have isolated cDNA clones specific for TRPM-2, which have been used as probes to study the expression of the gene during regression. The expression of TRPM-2 peaks between days 4 & 6 following castration, as determined by Northern analysis and dot blot hybridization. We have also shown with the same type of analysis that two anti-androgens, namely flutamide and cyproterone acetate, also induce TRPM-2 expression, although these two anti-androgens do not completely suppress the expression of the prostate steroid-binding protein genes. In situ hybridization has established that TRPM-2 is expressed in the epithelial cells of the prostate after castration, and is expressed in other tissues including the involuting mammary gland and in the uterus of ovariectomized rats. These results suggest that TRPM-2 represents an endogenous, cytotoxic protein which is expressed in a clearly defined temporal manner by the epithelial cells of the prostate during castration or anti-androgen induced involution of the gland. We believe that TRPM-2 codes for a protein which is actively involved in the process of cell death.
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D 434 RETINYL ESTER TAKEN UP VIA THE LOW DENSITY LIPOPROTEIN RECEPTOR PATHWAY CAUSES DIFFERENTIATION OF HL-60 CELLS, Karl-Olaf Wathne, Kaare R. Norum and Rune Blomhoff, Institute for Nutrition Research School of Medicine, University of Oslo, Norway. Newly absorbed retinol is transported in association with chylomicrons and their remnants. In addition, following intake of high doses of retinol, significant amounts are also found in low density lipoprotein (LDL). As both chylomicron remnants and LDL may be taken up by cells via the LDL receptor, and retinoids inhibit proliferation of some leukemic cells, we have studied the uptake of retinol in leukemic cells via the LDL receptor pathway. The human promyelocytic cell line HL-60 contains saturable binding sites for LDL. HL-60 cells were incubated with LDL or chylomicron remnants labeled with (^{3}H) retinyl palmitate. Uptake of retinyl ester associated with both LDL and chylomicron remnants was observed. Futhermore, the presence of excess LDL reduced the uptake with 75-100%, indicating that the uptake of retinyl ester occurred via the LDL receptor in HL-60 cells. When HL-60 cells are exposed to lipoproteins containing retinyl esters, cell proliferation is inhibited and cell differentiation is induced in a time and concentration dependent way.

D 435 LOSS OF TGF-B-INDUCED INHIBITION OF GROWTH AFTER IMMORTALIZATION OF RODENT CELLS. V. Sorrentino and E. Fleissner, Kettering Institute for Cancer Research, New York. Evidence is accumulating that TGF-beta (TGF-B) is an important regulator of growth and differentiation. It stimulates the proliferation of various cells in vitro and in vivo. TGF-B also inhibits growth of epithelial cells, B and T lymphocytes, and hepatocytes. The TGF-B stimulatory or inhibitory effects on established fibroblast cell lines appear to depend on the simultaneous presence of other growth factors (e.g. EGF or PDGF). We have observed that TGF-B is strictly inhibitory on anchorage dependent growth of primary mouse and rat embryo cells, under the same doses and conditions that do not affect the proliferation of most of the established cell lines analyzed. To further examine the differences in cellular response to TGF-B in primary fibroblast cells and established lines, we analyzed the sensitivity to TGF-B in cells derived after prolonged passages of primary cell cultures. Alterations in the inhibitory effect of TGF-B are detected after immortal clones emerge from senescent crisis and become established. In clones from 2 out of 3 rat and 3 out of 4 mouse embryo cells we detected an increasing refractoriness to this inhibitory effect of TGF-B. This was a function of culture passage number. These data indicate that TGF-B plays a role in regulating the proliferation of normal fibroblasts. After immortalization clones emerging from senescent crisis have lost their sensitivity to the inhibitory effect of TGF-B. Loss of sensitivity to inhibitory control mechanism, i.e. alteration in some TGF-B mediated pathway, may leave cells without one physiologic mechanism controlling growth and in turn favor uncontrolled proliferation.