MECHANISMS OF ACTION AND THERAPEUTIC APPLICATIONS OF BIOLOGICALS IN CANCER AND IMMUNE DEFICIENCY

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Mechanisms of Action and Therapeutic Application of Biologicals in Cancer and Immune Deficiency Disorders

Hematopoietic Growth Factors

U 001 COMBINATION BIOTHERAPY IN VIVO AND IN VITRO WITH IL-1, IL-3, IL-5, G-CSF, AND GM-CSF, Malcolm A.S. Moore, and David Warren, Laboratory for Developmental Hematopoiesis, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

Recognition that primitive murine stem cells required IL-1 to upregulate receptors for various hematopoietic growth factors in order to detect their proliferative response in vitro led us to develop a human assay equivalent to the murine high-proliferative potential assay of 5-Fluorouracil-treated marrow. Selection of primitive, pluripotential human stems cells was undertaken by a combination of brief in vitro exposure to 4-hydroperoxy-cyclophosphamide and positive selection by panning with monoclonal antibodies that recognize the CD34 antigen present on clonogenic, and pre-clonogenic hematopoietic cells. Positive selection , followed by 7 day incubation with various CSF and Interleukin species, revealed an amplification of both IL-1-dependent and CSF-dependent progenitors, which in the case of IL-1 and IL-3 combinations was between 100-1,000 fold. Combinations of growth factors revealed additive and synergistic interactions, however, in certain systems, sequential addition of factors (e.g. IL-1,IL-3, IL-5, or IL-1, IL-3 and IL-4) was necessary to achieve optimal expansion of differentiated eosinophils, mast cell/basophils or neutrophils. In both murine and human systems, IL-6 (Interferon beta-2) was ineffective in synergising with CSFs in the various clonogenic assays used to demonstrate IL-1 activity. Complex interactions between IL-1 and other hematopoietic growth factors were observed in short-term cultures of bone marrow from patients with acute and chronic myeloid leukemia, myelodysplastic disorders and acquired or iatrogenic neutropenias.

In vivo studies have been undertaken in mice, dogs and cynmologous monkeys that confirm the biological significance of combination biotherapy using interleukins and CSFs under conditions of irradiation, chemotherapy and bone marrow transplantation.

U 002 GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF), Richard Weisbart, Department of Medicine, Division of Rheumatology, Veterans Administration Medical Center, UCLA/San Fernando Valley Program, Sepulveda, CA 91343.

GM-CSF is a hemopoietin capable of stimulating the proliferation and maturation of bone marrow progenitor cells to neutrophils, macrophages, and eosinophils. Its effects on hematopoiesis are now demonstrated both in vitro and in vivo. In addition to its effect on hematopoiesis, GM-CSF primes mature neutrophils to enhance responses to physiological stimuli and thereby potentiate host defense mechanisms including chemotaxis, IgA dependent phagocytosis, and oxidative metabolism. Clinical trials of GM-CSF are currently in progress, and will be useful in identifying which of the in vitro biological phenomena have physiological relevance in maintaining health and defining those which may participate in the pathophysiology of disease.

Interleukins

U 003 EXPRESSION OF FUNCTIONAL INTERLEUKIN-2 RECEPTORS IN HUMAN L-CHAIN/TAC TRANSGENIC MICE, Miyuki Nishi, Yasumasa Ishida, Shigetoshi Suzuki, Paschalis Sideras, Masazumi Takahashi, Masashi Kawaichi and Tasuku Honjo, Department of Medical Chemistry, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan.

The growth of mature T lymphocytes is regulated by interaction between interleukin-2 (IL-2) and its receptor. Three distinct binding sites of IL-2, namely low (Kd = 10 nM), intermediate (Kd = 100 pM) and high (Kd = 10 pM) affinity sites, were demonstrated on human and primate T lymphocytes. Chemical crosslinkage of labeled IL-2 to human T cells demonstrated two polypeptide chains p55 (L chain) and p75 (H chain) which bind IL-2 with low and intermediate affinities, respectively. The high-affinity binding was shown to be due to ternary complex formation of IL-2, L and H chains. Construction of various mutants of the L chain cDNA indicated that the L chain is not directly involved in growth signal transduction (1). Nonetheless, expression of the IL-2 receptor L chain is tightly regulated by antigen or mitogen stimulation. To understand the biological function of the L chain we initiated construction of transgenic mice using human L chain cDNA of the IL-2 receptor under the control of a constitutive promoter. Studies on the L-chain transgenic mice showed that functionally active IL-2 receptors with the high affinity were expressed on unstimulated spleen and thymus cells (2). The results indicate that the H chain of the IL-2 receptor is constitutively expressed in T cells.

(1) Kondo, S., Kinoshita, M., Shimizu, A., Saito, Y., Konishi, M., Sabe, H. and Honjo, T. Nature 327, 64-67 (1987)
(2) Nishi, M., Ishida, Y. and Honjo, T. Nature in press

U 004 MECHANISM OF TOXICITY OF RECOMBINANT INTERLEUKIN-2, James W. Mier, Gloria Vachino, Charles A. Dinarello, New England Medical Center, Boston, MA, 02111

Although highly effective in the treatment of patients with melanoma, renal cell carcinoma, or lymphoma, the administration of IL-2 alone or in conjunction with LAK cells results in a myriad of potentially lethal side effects including fever, chills, hypotension requiring pressors, renal and hepatic failure, myocardial infarction, and a bizarre capillary leak syndrome resulting in weight gain and the development of ascites and peripheral and pulmonary edema. The injection of IL-2 also induces a marked increase in the plasma levels of stress-associated hypothalamic-pituitary hormones such as ACTH and an increase in hepatic acute phase protein levels. To further characterize the mechanism of IL-2 toxicity, a series of experiments were carried out in New Zealand rabbits. These animals develop delayed fever in response to an infusion of IL-2 (100,000 units/kg/hr). The febrile response is not attenuated by pre-incubating the IL-2 with polymyxin B, an antibiotic that inactivates bacterial endotoxins. Furthermore, the various excipient detergents are non-pyrogenic rabbits, indicating that the fever resulting from IL-2 is due to neither the various additives included in the IL-2 formulation or to contaminant endotoxin. To determine if IL-2 is intrinsically pyrogenic, human fibroblasts and rabbit hypothalamic cells were cultured in IL-2-containing medium, which was subsequently assayed for prostaglandin E_2 (PGE_2). Although these cells responded to IL-1 with a 50-fold increase in PGE_2 production, IL-2 had no effect. Serial plasma samples were obtained from cancer patients undergoing treatment with IL-2 to determine if secondary pyrogenic cytokines could be detected in the circulation prior to the onset of fever. Using a sensitive and highly specific RIA, we were able to detect high levels (approximately 750 pg/ml) of TNF-alpha in the plasma two hours after a single injection of 100,000 units/kg of IL-2. These results suggest that the toxicity of IL-2 is secondary to the release into the circulation of TNF-alpha and other pyrogenic cytokines.

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U 005 CHARACTERIZATION OF THE BIOLOGIC PROPERTIES OF HUMAN INTERLEUKIN-3, Yu-Chung Yang, Robert G. Donahue, Allan R. Mufson, Makio Ogawa*, and Steven C. Clark, Genetics Institute Inc., Cambridge MA 92140, and *Medical University of South Carolina and VA Medical Center, Charleston, SC 29403.

The molecular cloning of the human gene encoding interleukin-3 (IL-3) has enabled us to produce substantial quantities of this growth factor for analysis of its effects both in culture and in primate models. Because of its ability to support colony formation by many types of hematopoietic cells, IL-3 is also known as multi-lineage colony-stimulating factor. The IL-3-responsive target cells found in normal bone marrow overlap considerably with those responsive to another multi-lineage hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF). A direct comparison of the ability of these factors to support the proliferation of primitive blast cell colonies has revealed that IL-3 is more effective than is GM-CSF in generating human blast cell colonies. Furthermore, the resulting blast cell colonies from the IL-3-supported culture can be replated with higher efficiency than those from the GM-CSF cultures. These studies indicate that although the pool of target cells for the two factors overlap considerably, the IL-3-responsive cells include a population of more primitive stem cells with a higher proliferative potential than those found in the pool of GM-CSF-responsive progenitors. The effects of IL-3 on stimulating hematopoiesis in normal monkeys are consistent with the results of the clonogenic culture systems. Continuous intravenous infusion of recombinant IL-3 for 7 days resulted in a modest elevation in circulating white cell count, typically reaching a maximum of 2-3 fold 3 days after stopping the administration. The elevation was due to increased numbers of neutrophils, eosinophils, monocytes and lymphocytes. In comparison, GM-CSF has been found to elicit a rapid leukocytosis in the same model, typically reaching 5 fold within 2-3 days after the start of a 7 day infusion. To test the possibility that the IL-3 effects may be more restricted to earlier progenitors, we pretreated the animals for 6 days with IL-3 to increase the progenitor pool size then administered a suboptimal dose of GM-CSF to support the maturation of the IL-3-generated immmature cells. As expected, the elevation in circulating white cell count of the IL-3-pretreated animals to the minimal dose of GM-CSF was significantly greater to that in the those animals not receiving IL-3. These studies suggest that IL-3 may not be sufficient to support the complete developmental program of many of the hemopoietic progenitors and that this insufficiency is likely to be due to the acquisition of dependency of the developing cells to other growth factors such as GM-CSF.

Cytotoxic Lymphokines and Monokines

U 006 BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF LEUKOREGULIN ACTION. Charles H. Evans, Susan C. Barnett, Balázs A. Gelléri, Paulette Furbert-Harris, Paul A. Sheehy*, Jeffrey L. Barker*, Patricia D. Baker, Anna C. Wilson, Esme K. Farley and Francesco D'Alessandro. Laboratory of Biology, National Cancer Institute and Laboratory of Neurophysiology*, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20892.

Tumor cell sensitivity to natural lymphocyte cytotoxicity can be up-regulated or down-regulated by exposure to lymphokines from activated lymphocytes. An increase in cytolysis by natural killer (NK) lymphocytes, for example, follows target cell exposure to leukoregulin. In contrast, target cell treatment with gamma-interferon renders the target cells more resistant to destruction by NK lymphocytes. We have recently ascertained that leukoregulin also up-regulates target cell sensitivity to lymphokine activated killer (LAK) as well as to NK lymphocytes. The molecular events underlying the 2-4 fold increase in tumor cell sensitivity to NK and LAK cytotoxicity induced by leukoregulin, a 50,000 dalton pI 5.1 glycoprotein lymphokine, were examined in human K562 erythroleukemia cells. Leukoregulin was purified from PHA stimulated human peripheral blood lymphocytes or monocytes or from TPA stimulated RPMI-1788 lymphoblastoid cells by sequential 10,000 MW exclusion ultrafiltration, 100 mM Tris-HCl, pH 7.4, DEAE ion exchange chromatography, pH 4-6 ampholine or immobiline IEF, and silica size exclusion HPLC. Treatment of K562 cells with 1-10 units leukoregulin/ml causes a sharp transient elevation in intracellular ionic calcium within one minute as detected by increased fluorescence of the calcium chelator indo-1. During the next several minutes a transient burst of rapidly opening and closing cation plasma membrane channel activity occurs in patch clamped cells. Cation ion channel activity is accompanied at 5-10 minutes by increasing plasma membrane permeability measurable by influx of fluorescent propidium iodide and efflux of intracellular fluorescein. Increased membrane permeability reaches its maximum within 1-2 hours and like the accompanying inhibition of cell proliferation is reversible. Membrane protein kinase C activity increases concomitantly with increased membrane permeability and enhanced sensitivity to NK and LAK cytotoxicity. Associated with the increase in target cell membrane permeability is an augmentation of cellular uptake of doxorubicin and other anti-metabolites as revealed by flow cytometric analysis. FITC-dextran uptake indicates that the leukoregulin membrane channel facilitates intracellular uptake of molecules as large as 10,000-20,000 daltons. This may permit entry of cytolytic molecules such as cytolysin, lymphotoxin or tumor necrosis factor leading to the target cell destruction characteristic of the final stage of the NK and LAK cytotoxic reactions.

U 007 LEUKOREGULIN AND LYMPHOTOXIN PRODUCED BY PHYTOHEMAGGLUTININ AND 12-O-TETRADECANOYL-PHORBOL-13-ACETATE OR CARBOXYMETHYL-PACHMARAN ACTIVATED HUMAN SPLEEN CELLS, Jian Ni, T.X. Ye and S.K. Yang, Department of Microbiology and Immunology, Second Military Medical College, Shanghai 200433, China. Previous studies showed that tumor-promoting phorbol 12-O-tetradecanoyl-phorbol -13-acetate (TPA) stimulated lymphocytes to produce immune interferon and interleukin 2. This study showed that TPA treatment alone induced only low levels of leukoregulin (LR) or lymphotoxin (LT), but TPA and phytohemagglutinin (PHA) treatment in combination of human spleen cells caused significant enhance -ment of LR or LT yields. The optimal conditions for the induction of LR using orthogonal experimental design were 1 X 10^7 cells/ml for human spleen cells, 3.1μg/ml for PHA, 12.5ng/ml for TPA, 70 hr for incubation time, 15% for bovine serum. This study also showed that Carboxymethyl-Pacharan (CMP), a derivant of pachyman, previously have been shown in animal models to stimulate the immunological function and enhance protective immunity to transplanted tumors, has no direct cytocidal effect on tumor cells can induce low levels of LR or LT, and costimulation with CMP and PHA produced some further enhancement of LR or LT production. Effects of different CMP concentrations and incubation times on LR or LT production were shown, CMP appears to be a low stimulator for LR or LT induction and best stimulating concentration of CMP was 25μg/ml and incubation time was 48-72 hr. Kinetics of LR or LT production by human spleen cells stimulated by different concentrations of CMP and PHA were also studied using orthogonal experimental design. Maximal production of LR or LT occurred at 48-72 hr, CMP concentration of 25μg/ml and PHA concentration of 5-10μg/ml. The inducing and enhancing effect of CMP have proved helpful in producing LR or LT for clinical use as an anticancer agent and other studies on the mechanisms of LR or CMP anticancer action. LR was also readily produced following esculentoside (Es) stimulation of fresh human spleen cells, the amount of LR produced, however, is lower compared to PHA. A tomato lectin extracted and purified from edible common tomato (Lycopersicon esculentum) was found to have no mitogenic activity on human spleen cells and displayed a dose-dependent inhibition of the mitogenic effects of PHA or concanavalin A and induction of LR or LT by PHA or TPA alone or in combination. The detection, purification and characterization of LR or LT were also studied.

U 008 CHARACTERIZATION OF ANTI-NKCF MONOCLONAL ANTIBODY: CROSS-REACTIVITY WITH RAT LGL DERIVED CYTOLYSIN. J.R. Ortaldo, R.T. Winkler-Pickett*, D. Reichardt, C.W. Reynolds, S. Giardina* and R. Kantor*, Laboratory of Experimental Immunology, BRMP, DCT, NCI, and *Program Resources, Inc., NCI-FCRF, Frederick, MD 21701-1013.

We have previously reported the initial characterization of a series of murine monoclonal antibodies (MoAbs) that neutralized rat natural killer cytotoxic factor (NKCF) activity, but had no effect on rat tumor necrosis factor (TNF) (Fed. Proc. 46:1227,1987). In addition, these MoAbs were capable of reducing the natural killer (NK) activity of rat large granular lymphocytes (LGL). The present studies have been performed to examine the antigenic relationship between NKCF and LGL cytolysin. The addition of purified anti-NKCF MoAbs was capable of blocking, in a dose dependent fashion, the cytolysin-mediated lysis of SRBC and YAC-1 target cells, with 50% inhibition being seen at <10 ug/ml of MoAb. In addition, these MoAbs were shown to inhibit the NKCF derived from human LGL. Biochemical analyses of supernatants containing NKCF from human and rat LGL have demonstrated a unique protein, with a reduced molecular weight of ~12,000 kD which reacts with the anti-NKCF MoAbs. Further studies are being performed using Western-blot analysis to examine the anti-NKCF MoAb cross-reactivity with rat granule proteins. These results suggest an antigenic cross-reaction between the rat LGL granule cytolysin and NKCF secreted from these same effector cells. In addition, a close antigenic relationship appears to exist between human and rat NKCF. Results from these biochemical characterizations and the implication of this cross-reactivity between cytolysin and NKCF will be discussed.

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Pathways of Cytokine Action

U 009 INTERACTIONS BETWEEN INTERFERON AND GROWTH FACTORS IN MODULATING CELL PROLIFERATION. Roy A. Levine[1], Tara Seshadri[1], Stephen R. Hann[2] and Judith Campisi[1]; [1]Department of Biochemistry, Boston University Medical School, Boston, MA 02118; [2]Cell Biology Department, School of Medicine, Vanderbilt University, Nashville, TN 37232.

Cell proliferation in higher eukaryotes is regulated by factors that provide both positive and negative growth regulatory signals. Tumor cells are often more sensitive to growth stimulatory signals or less sensitive to growth inhibitory signals.

Polypeptide growth factors provide growth stimularory signals. Many growth factors induce the expression of genes whose products are believed necessary for proliferation, including the c-myc and c-fos protooncogenes. Interferons (IFN), on the other hand, inhibit the proliferation of many cell types and, in lymphoid cells, IFN has been shown to reduce the levels of c-myc mRNA. It is possible, therefore, that positive and negative growth regulatory signals might act on an overlapping set of genes.

We are studying the actions of growth factors and antiproliferative interferons in cultured fibroblasts. In quiescent fibroblasts stimulated by growth factors, alpha/beta-IFN retards progress through GO/G1 and delays the onset of DNA synthesis. However, IFN had little or no effect on the induction of several growth factor-inducible mRNAs, including c-myc and c-fos mRNA. Moreover, for three mRNAs, IFN actually delayed the decline that normally occurs several hours after the initial induction. These mRNAs were ornithine decarboxylase (odc), fibronectin (fbn) and c-myc mRNA. c-myc and FBN mRNA accumulated on light and heavy polyribosomes and, in the case of c-myc, IFN caused a marked stabilization of the otherwise labile mRNA. It is well-established that cycloheximide, an inhibitor of protein synthesis, stabilizes c-myc mRNA and causes mRNAs to accumulate on polyribosomes. Indeed, IFN inhibited the induced rate of fbn protein synthesis and inhibited the accumulation of odc enzyme activity. c-myc protein synthesis is currently under investigation. The effects of IFN on mRNA levels and translation were apparent only several hours after cells had been stimulated by growth factors. Two chemically transformed fibroblast cell lines that fail to modulate c-myc mRNA levels in response to growth factors were resistant to the antiproliferative effects of IFN.

Our results suggest that, in fibroblasts, IFN inhibits the translation of selected mRNAs late in G1, by a growth factor-dependent mechanism, and that the c-myc mRNA is particularly sensitive.

U 010 INTRACELLULAR PATHWAYS OF CYTOKINE ACTION, Stanley Cohen, Department of Pathology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

Most studies of the mechanism of cell proliferation involve investigation of cell membrane associated or early transduction events such as binding of growth factors to their receptors, phosphorylation of membrane proteins, activation of protein kinases, and mobilization of calcium. We have chosen to study later biochemical events that are involved directly triggering the cell nucleus for DNA replication. We found that ctyoplasmic extracts of mitogen-activated, but not resting lymphocytes, contained a soluble factor that could induce DNA replication in isolated quiescent nuclei. This was found to be a heat labile protein with a molecular weight of approximately 100,000 daltons, which we called ADR. Although ADR is not a DNA polymerase, the reaction between nucleus and ADR is polymerasedependent.

We found that Interleukin 2 (IL-2) activation of T lymphocytes led to induction of intracytoplasmic ADR activity, and that ADR played a role in the sequence of intracellular events leading to activation for IL-2 mediated proliferation. Because of the nature of the defining assay, the locus of ADR action appears to be near the terminal end of the transduction pathway. Preparations rich in ADR activity have proteolytic activity as well. In addition, aprotinin, as well as a variety of other protease inhibitors, suppress the ADR-induced DNA synthesis in a dose-dependent fashion. ADR activity can be removed from active extracts by absorption with aprotininconjugated agarose beads, and can be removed from the beads by elution at pH 5.0. This latter suggests that ADR itself is a protease.

We have also detected an inhibitor of ADR activity in the cytoplasm of resting lymphocytes. This is a heat stable protein of approximately 60,000 daltons. In addition to suppressing the interaction of ADR with quiescent nuclei, this inhibitor could suppress DNA synthetic activity in replicative nuclei isolated from mitogenactivated lymphocytes. Interestingly, these preparations had little or no activity on replicative nuclei derived from several neoplastic cell lines. The resistance of tumor cell nuclei to spontaneously occurring the cytoplasmic inhibitory factors such as these may prove one explanation for the loss of growth in neoplastic cells.

U 011 THE RECEPTOR FOR THE MONONUCLEAR PHAGOCYTE COLONY STIMULATING FACTOR, CSF-1.

Charles J. Sherr[1], Martine F. Roussel[1], James R. Downing[1,2], Carl W. Rettenmier[1], A. Thomas Look[1,3], and Richard A. Ashmun[1,3], Departments of Tumor Cell Biology,[1] Pathology,[2] and Hematology-Oncology,[3] St Jude Children's Research Hospital, Memphis, TN 38105

The receptor for the macrophage colony stimulating factor, CSF-1 (M-CSF), is a transmembrane glycoprotein oriented with its ligand binding domain outside the cell and its tyrosine kinase domain at the inner surface of the plasma membrane. This receptor is encoded by the c-fms proto-oncogene, suggesting that genetic alterations in coding sequences and/or aberrant receptor expression might predispose to malignancy. Transduction of the human c-fms gene into fibroblasts renders them responsive to recombinant human CSF-1, demonstrating that physiologic substrates for the receptor kinase are expressed in these cells. Binding of CSF-1 to its receptor induces receptor autophosphorylation on tyrosine and phosphorylation of heterologous cell substrates, followed by rapid receptor degradation. Receptor degradation is also accelerated after exposure of macrophages to the phorbol ester, TPA. In cells in which protein kinase C is downmodulated, CSF-1 receptors reexpressed on the plasma membrane are still degraded in response to CSF-1, indicating that ligand- and phorbol ester-induced downmodulation are mediated through different pathways. CSF-1 treatment leads to the rapid induction of other genes (eg. c-fos and c-myc), suggesting that the receptor initiates a mitogenic response by ultimately affecting gene transcription. No physiologic substrates of the receptor kinase other than the receptor itself have been identified.

The v-fms oncogene transforms cultured fibroblasts and myeloid cells in vitro and induces malignancies of diverse hematopoietic lineages in vivo after retroviral-mediated gene transfer into murine bone marrow cells. Unlike the normal CSF-1 receptor, the v-fms gene product functions as a constitutive kinase and is not downmodulated in response to CSF-1 or TPA. Two genetic alterations are required to fully activate c-fms as an oncogene: (i) elimination of a C-terminal tyrosine residue (tyr^{969}) which is presumed to serve as a negative regulatory site of tyrosine phosphorylation, and (ii) a mutation which renders the receptor CSF-1 independent and constitutive as an enzyme. Studies with chimeric c-fms/v-fms constructs indicate that the v-fms-coded tyrosine kinase can be appropriately regulated by a portion of the c-fms ligand binding domain.

Monoclonal antibodies to the human CSF-1 receptor have been used to survey for receptor expression on human acute myelogenous leukemia (AML) cells. CSF-1 receptors were found to be expressed not only on cells with monocytic characteristics but also on a subset of AMLs lacking evidence of monocyte differentiation. Thus, c-fms may be inappropriately expressed in certain cases of AML and may contribute to human leukemia.

U 012 SYNERGY AMONG CYTOKINES IN THE TUMOR-DORMANT STATE, E. Frederick Wheelock, Lieping Chen, Yasuhiro Suzuki, Cheng-Ming Liu and Takayuki Morita, Dept of Pathology, Hahnemann University, Philadelphia PA 19102.

L5178Y lymphoma cells can be maintained in a tumor-dormant state in the peritoneal cavity of DBA/2 mice by immunologic mechanisms, and are restrained from progressive growth in many peritoneal cell (PC) cultures ('in vitro tumor-regressor' PC cultures) that are prepared from such mice. Restraint on tumor cell growth is dependent on the production and action of interferon-gamma (MuIFN-gamma) which requires murine tumor necrosis factor (MuTNF) to induce anti-tumor cytotoxicity in peritoneal macrophages and lymphocytes. In those PC cultures from tumor-dormant mice in which tumor cells proliferate progressively ('in vitro tumor-progressor' PC cultures), small concentrations of exogenous MuIFN-gamma can synergize with MuTNF and with HuIL-1 to induce anti-tumor cytotoxic activity. These experiments indicate the importance of combinations of small concentrations of cytokines in immunoregulation and in anti-tumor cell-mediated immune responses.

Treatment of 'in vitro tumor-regressor' PC cultures with antibody to MuIFN-gamma stimulated both PGE2 production and tumor cell growth. When PGE2 production was inhibited by indomethacin, antibody to MuIFN-gamma could not stimulate tumor cell growth, and the restraint on tumor cell growth was macrophage-mediated. This indicates that activated macrophages in 'in vitro tumor-regressor' PC cultures can remain cytotoxic in the absence of MuIFN-gamma as long as PGE2 production is inhibited. The increased PGE2 production and tumor cell growth in anti-MuIFN-gamma- treated 'in vitro tumor-regressor' PC cultures was not due to the action of MuIFN-gamma :anti-MuIFN-gamma immune complexes. MuIFN-gamma could not inhibit PGE2 production, but could block the tumor cell growth- enhancing effects of PGE2.

We propose that macrophages in tumor-dormant mice develop anti-tumor cytotoxic activity following exposure to endogenous MuIFN-gamma, and maintain this activity as long as MuIFN-gamma is produced. MuIFN-gamma does not inhibit production of PGE2, but does block the down-regulatory effects of PGE2. When MuIFN-gamma is not produced, macrophages lose cytotoxic activity and produce PGE2, which further down-regulates them, permitting the 'dormant' L5178Y cells to proliferate and terminate the tumor-dormant state.

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Monoclonal Antibodies

U 013 PRODUCTION OF MURINE IgG3 TO TUMOR ASSOCIATED ANTIGENS: AUGMENTATION OF THEIR ADCC CAPABILITY. Alton C. Morgan Jr.*, Scott Graves*, Wendy Sullivan*, Alan Mufson+, Malcolm Mitchell, Clive Woodhouse*. *NeoRx Corporation, Seattle, WA 98119; +Genetics Institute, Cambridge, MA 02140; Univ. of Southern California.

Murine IgG3 to tumor associated antigens have mediated anti-tumor effects in patients with malignant melanoma and neuroblastoma (1,2). Successful application of such antibodies to major neoplastic diseases require efficient immunization methods to elicit the antibodies, an understanding of the receptors and effector cell types with which the antibodies interact, and appropriate lymphokines for augmentation of cytolytic and inflammatory properties of the different effector cell types.

We have generated murine IgG3 to tumor associated antigens of colon cancer (3). These Mab are directed to glycolipids distributed homogenously on virtually all colon adenocarcinoma and on isolated cells of normal tissues including colon and pancreas. Unlike most of the IgG3 elicited to melanoma cells, the colon IgG3 typically produce 10-40ug/ml/10^6 cells in stationary culture and are therefore economically produced by fermentation methodology.

We and others have demonstrated that murine IgG3 are the most effective subclass at mediating mononuclear cell ADCC in short term assays (4,5). Large granular lymphocytes, the primary effector cell in such assays, can be augmented for ADCC by short exposure to low concentrations of IL-2 and even more effectively by a combination of IL-2 and GM-CSF. Mononuclear cell preparations from patients receiving prolonged IL-2 treatment with cytoxan pretreatment have demonstrated augmented ADCC.

Other effector cell types can also mediate cytolysis of tumor targets with murine IgG3. Human monocytes cultured in serum with M-CSF but not with IL-3 or GM-CSF, demonstrated enhanced ADCC with antigen positive tumor targets in a long term ^{3}H-thymidine release assay. Polymorphonuclear leukocytes can mediate ADCC but only in the presence of human serum (C3b receptor) or after short exposure to GM-CSF.

Clinical trials are currently underway in colon cancer with the murine IgG3, NR-CO-04 antibody alone. Trials with the antibody in combination with lymphokines, particularly IL-2, will be conducted in the near future.

1) A.N. Houghton et.al., Proc, Nat'l Acad Sci, USA, 82:1242, 1985
2) N-K.V. Cheung, et.al., J. Clin. Oncol. 5:1430, 1987
3) A.C. Morgan et.al., Hybridoma 3:233, 1984
4) J.R. Ortaldo et.al., J. Immunol. 138:3566, 1987
5) C. Anasetti et.al., J. Immunol. 138:2979, 1987

Differentiation and Immunomodulation

U 014 **SIGNALING MECHANISMS ASSOCIATED WITH INDUCTION OF HUMAN MONOCYTIC DIFFERENTIATION**

Donald Kufe, Junko Horiguchi, Eric Sariban, Matthew Sherman, David Spriggs, Richard Stone, and Barbara Weber, Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Boston, MA 02115

HL-60 promyelocytic leukemia cells differentiate along the monocytic lineage when exposed to phorbol esters, such as 12-0-tetradecanoyl-phorbol-13-acetate (TPA). The differentiated phenotype is characterized by growth inhibition, adherence, decrease of cell surface transferrin receptors, increase in monocyte surface markers and induction of alpha-naphthyl acetate esterase staining. In addition to down-regulation of c-myc expression, TPA-induced monocytic differentiation is also associated with induction of c-fos, c-fms, CSF-1, PDGF-1, PDGF-2 and tumor necrosis factor (TNF) transcripts. TPA activates the calcium and phospholipid-dependent protein kinase C (PKC). Monocytic differentiation is similarly induced by other agents, such as teleocidin, bryostatin 1 and phospholipase C, which also result in PKC activation. TNF is another inducer of monocytic differentiation that also increases levels of TNF, c-fms and CSF-1 mRNA. Indeed, one of the earliest events induced by TNF is transcriptional activation of the TNF gene. TNF has no detectable effect on hydrolysis of phosphatidylinositol phosphates and intracellular calcium levels in HL-60 cells. In contrast, the autoinduction of TNF gene expression appears to be mediated by an increase in arachidonic acid release. Moreover, intermediary metabolites of the 5-lipoxygenase pathway induce TNF expression, while the cyclooxygenase metabolite PGE_2 inhibits TNF-induced signaling. The monocytic phenotype induced by TPA is also mediated in part by increased arachidonic acid metabolism. Thus, inhibitors of phospholipase A_2 and arachidonic acid release also block TPA-induced TNF expression during monocytic differentiation. Similar findings have been obtained with other human myeloid leukemia cell lines and normal peripheral blood monocytes. In summary, these findings indicate that TPA and TNF induce changes associated with monocytic differentiation at least in part through the 5-lipoxygenase pathway of arachidonic acid metabolism.

U 015 REGULATION OF IL-2 RECEPTOR (TAC) AND HUMAN IMMUNODEFICIENCY VIRUS TYPE I (HIV-1) GENE EXPRESSION, John W. Lowenthal, Ernst Bohnlein, Dean W. Ballard, Miriam Siekevitz, B. Robert Franza and Warner C. Greene. Howard Hughes Med. Inst., Duke Univ., Durham NC 27710 and CSH Lab., Cold Spring Harbor, NY 11724.
Transfection studies with a series of deleted forms of the IL-2 receptor (Tac) promoter linked to the chloramphenicol acetyltransferase (CAT) gene identified a 46 bp region between -317 and -271 that was required for mitogen induced promoter activation in mature Jurkat T cells. In contrast, immature YT-1 T cells or Jurkat cells that produce the transactivator (tat-1) protein of HTLV-1 only required sequences downstream of -271. Oligonucleotides spanning different regions between -293 and -243 were tested in gel retardation assays for their ability to specifically bind inducible nuclear proteins. Incubation of PMA induced but not noninduced Jurkat nuclear extracts with radiolabeled oligo III (-291 to -245) resulted in the formation of two specific protein-DNA complexes whereas oligo II (-267 to -243) produced only one of these complexes. Incubation of PMA induced YT-1 nuclear extracts with oligo III also resulted in the formation of two specific complexes, but in contrast to Jurkat cells, oligo II produced two complexes, both of which were competed for by unlabeled oligo III. The upstream region of oligo III (oligo I, -293 to -270) failed to directly bind or compete for the binding of proteins in either cell type. The close correlation between the transfection results and the protein binding data was further strengthened by the finding that oligo III, when linked to an HSV thymidine kinase-CAT construct, imparted to the mitogen-unresponsive TK promoter a level of mitogen inducibility comparable to that achieved with the full length Tac promoter. The finding that the oligo III region functioned in either orientation and that a tandem sequence produced an additive effect suggests that this element may correspond to an enhancer sequence. Using mutated forms of oligo II, we mapped the binding site of one of the nuclear proteins to a 12 bp segment located between -267 and -256. This site was confirmed by in situ DNA footprinting and methylation interference. This binding site shares 9/11 nucleotides with the enhancer of the human immunodeficiency virus (HIV-1), a viral element that regulates mitogen inducibility of HIV-1 gene expression and binds the inducible nuclear proteins, NF-KB and HIVEN86A. Competition studies revealed that the HIV-1 enhancer and oligo III cross-competed with each other for the formation of the two complexes. Using a microscale DNA-affinity precipitation assay we demonstrated the direct binding of HIVEN86A to both oligo III and the HIV-1 enhancer. Our findings indicate that the HIV-1 virus utilizes the same nuclear protein(s) that is normally used in the regulation of IL-2 receptor gene expression and may offer an insight into the role of T cell activation in the control of latent versus lytic HIV-1 infection of T cells.

U 016 INDUCED DIFFERENTIATION OF ERYTHROLEUKEMIA CELLS (MELC) BY HEXAMETHYLENE BISACETAMIDE (HMBA): A MODEL FOR CONSIDERING CYTODIFFERENTIATION THERAPY, Paul A. Marks and Richard A. Rifkind, DeWitt Wallace Research Laboratories, Memorial Sloan-Kettering Cancer Center; Sloan-Kettering Division of the Graduate School of Medical Sciences, Cornell Univ., NY 10021.
Considerable evidence has accumulated to indicate that transformation does not necessarily destroy the differentiation potential of tumor cells. One striking example of this is the ability of polar compounds, such as HMBA, to induce MELC to express the differentiated erythroid phenotype including loss of proliferative capacity. While the mechanism of action of HMBA has not been defined, it is clear that inducer-mediated differentiation is a multi-step phenomenon. Among the early changes caused by the inducer are a decrease in diacylglycerol concentration; an increase in membrane bound PKC activity; the appearance of Ca^{2+} and phospholipid independent PKC activity in the cytosol; alterations in ion transport; and modulation in expression of a number of genes including down regulation of c-myb, c-myc and p53 and an increase in expression of c-fos. During this early period there is no detectable commitment of MELC to terminal cell division or induced expression of differentiated genes, such as, α^1- and β^{maj}-globin genes. HMBA commitment to irreversible terminal differentiation is first detectable by about 12 hr and proceeds in a stochastic fashion over the ensuing period of culture until about >95% of the population becomes recruited to commitment to terminal differentiation including terminal cell division. Commitment is associated with a persistent suppression of c-myb gene expression and elevated levels of c-fos mRNA, while the level of c-myc mRNA returns to that of uninduced cells. By 36 to 48 hr, transcription of α^1- and β^{maj}-globin genes increases 10 to 30 fold and that of the rRNA genes is suppressed. The in vitro studies with HMBA induction of MEL cells provide a basis for considering polar agents, such as HMBA, in clinical therapy of human cancers. Based on these in vitro studies it is likely that to be effective, HMBA must be administered in doses that can achieve optimal concentrations in the serum, probably in the neighborhood of 3 to 5 mM, that these concentrations be maintained over a prolonged period of time and that the biological effect of the agent be monitored by indicies of expression of differentiated phenotype and decreased proliferative rate of the tumor cells.

U 017 ISOLATION AND IDENTIFICATION OF THREE CLASSES OF BONE MARROW PROGENITORS: THE HEMATOPOIETIC STEM CELL, MULTI-POTENTIAL NON-STEM CELLS, AND AN EARLY B LINEAGE COMMITTED PRECURSOR, Gerald J. Spangrude, Shelly Heimfeld, George Tidmarsh, Jan Klein, Christa Muller-Sieburg, Jeff Friedman, and Irving Weissman, Laboratory for Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

We have identified the mouse hematopoietic stem cell. Phenotypically, it is Thy-1^{lo}, Sca-1^{+}, Lin^{-} (Lin^{-} means lacking markers for any of the known hematolymphoid lineages such as B220, Gr-1, Mac-1, L3T4, Lyt-2, etc.). Upon i.v. injection into lethally irradiated animals it gives rise mainly to one 12 day spleen colony for every 10-12 cells injected, compared to 1/6,700 unfractionated bone marrow cells. (The seeding efficiency of i.v. CFU-S is 1/6 to 1/10 cells.) The limiting number for thymic colonies following intrathymic injection is 1 per 5 cells injected, compared to 1/8000 bone marrow cells. The dose of these stem cells required to restore fully lethally irradiated animals is 20-50 cells to rescue half of the injected animals, as opposed to $>10^{4}$ whole bone marrow cells; all hematolymphoid lineages (B, T, erythroid, myeloid, etc.) are reconstituted.

A second multi-potent hematopoietic progenitor is Thy-1^{lo} $B220^{+}$ Mac-1^{+} (Gr-1^{+}). It gives rise almost exclusively to 7 day spleen colonies when injected i.v. into lethally irradiated animals. Approximately 1 spleen colony is found for every 250 cells injected. Injection of these cells into lethally irradiated Ly5 congenic animals along with host stem cells leads to (at least) short-term repopulation of donor-derived cells of all hematolymphoid lineages bearing the Ly5 marker (T, B, macrophage, granulocyte). Animals which were lethally irradiated and injected with over 1500 of these cells die at approximately 20 days following irradiation, presumably because this population lacks self-renewal capacity. We are currently testing the hypothesis that this multi-potent poorly self-renewing hematopoietic progenitor is a necessary intermediate between the hematopoietic stem cell and committed lineage precursors; and whether it is each cell (vs the population) which is multipotent.

The committed early B lineage precursor is Thy-1^{lo} $B220^{+}$ Mac-1^{-} Gr-1^{-}. These cells are poorly self-renewing in vitro. In vivo, however, as few as 2-7,000 cells are as potent as 500,000 whole bone marrow cells in giving rise to B$\mu\delta$ cells by 6 weeks following irradiation. The Thy-1^{lo} $B220^{+}$ committed B lineage precursor does not give rise to T cells or myelomonocytic cells, and represents the most highly enriched target cell population yet defined for Abelson leukemia virus in vitro leukemogenesis (1400 per 10^{6} plated compared to 60 per 10^{6} whole bone marrow cells).

Combination Therapy (joint)

U 018 COMBINATIONS OF CHEMOTHERAPY AND BIOMODULATION IN THE TREATMENT OF CANCER, Malcolm S. Mitchell, Departments of Medicine and Microbiology, U.S.C. Cancer Center, Los Angeles, CA 90033.

Biological response modifiers ("biomodulators") have emerged as an important new class of agents for treating cancers. While they have usually been tested alone, several are now suitable for use in combination with older modalities such as chemotherapy. Chemotherapy is usually but not always immunosuppressive. Several drugs cause far less suppression than others, and many can be immunostimulatory. Such drugs as doxorubicin, bleomycin, DTIC and cis-platin are in this category, but even "classical" immunosuppressive compounds such as cyclophosphamide (CY) can cause immunostimulation at low doses by inhibiting cellular and humoral suppressor influences. Chemotherapy should generally precede immunotherapy rather than being given concomitantly, to permit recovery of the immune response or to eradicate suppressor influences. The cytoreductive effects of chemotherapy also allow the immune response to act more effectively on the remaining tumor cells. Under special circumstances, biomodulation has been useful before chemotherapy, perhaps altering vascular permeability of the tumor, but this sequence may make the immune response more vulnerable to suppression. Several clinical trials with therapy to restore T cells have potentiated chemotherapy, such as in small cell lung cancer and melanoma. Low-dose CY has potentiated delayed hypersensitivity to autologous melanoma cells, although clinical responses have been somewhat uncommon. Most recently the combination of low-dose CY preceding IL-2 has caused complete or partial remissions in nearly 30% of patients with melanoma. DTIC in therapeutic doses before IL-2 has caused responses of the same order of magnitude early in the study. Doxorubicin and interferon also appear to be synergistic, and are being exploited for that effect in current clinical trials. The use of biomodulators that increase the uptake of chemotherapy into cancer cells, particularly immunoconjugates, that decrease its toxicity (by stimulating bone marrow stem cells or antagonizing immunosuppression), cause tumor maturation or inhibit metastases are all on the horizon, and promise to potentiate the efficacy of chemotherapy considerably. Thus, the interaction of biomodulators and chemotherapy is mutual, with the potential of improving the activity of each.

U 019 PERSPECTIVES FOR COMBINED BIOTHERAPY OF CANCER: BUILDING UPON REGIMENS USING TOLERABLE DOSES OF INTERLEUKIN-2, Paul M. Sondel, Jacquelyn A. Hank, Jeff A. Sosman, Peter C. Kohler, Depts. of Pediatrics, Human Oncology and Genetics, University of Wisconsin, Madison, WI 53792.

Although interleukin-2 can activate immune cells with anti-tumor activity when given in very high doses, it can also turn on immune reactions which cause significant life-threatening toxicity. We have evaluated sustained treatments with lower doses of interleukin-2. This regimen does have side effects but these are not severe and are well tolerated by cancer patients admitted to a general hospital ward without requiring intensive care unit monitoring or support. This regimen, utilizing interleukin-2 alone, can activate within the patient a 100-fold increase in the level of circulating lymphokine activated killer (LAK) activity in their white blood cells. At least some patients receiving this treatment have shown greater than 50% shrinkage of all measurable renal cell carcinoma. Thus, interleukin-2 can be given safely, with acceptable toxicity, utilizing a regimen that induces dramatic LAK activity, as well as measurable anti-tumor effects.

IL-2 remains a relatively new treatment with much room and need for further development. Yet, in less than three years of clinical testing, it is clear that this strictly immunological approach can be utilized in a number of regimens to activate a patient's own immune system, and that this activated immune response can cause a shrinkage of sizeable cancers in some patients. IL-2 is not a "magic bullet", nor a panacea, nor is the LAK activity that is induced by IL-2. In fact, the majority of patients receiving IL-2 show some side effects without any measurable tumor shrinkage. Nevertheless, the induction of reproducible clinical anti-tumor effects by a well characterized molecule which acts only through the immune system, provides some hope that further development of this approach may someday enable better clinical results. This will most certainly require combination of this approach with other modalities. Numerous clinical and laboratory teams are working rapidly toward this goal.

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Immunodeficiency Disorders

U 020 SINGLE AGENT AND COMBINATION CHEMOTHERAPY OF HIV INFECTIONS, Samuel Broder, M.D., National Cancer Institute, National Institutes of Health, Bldg. 10, Room 12N214, Bethesda, MD 20892.

We have found that with the ribose moiety of the molecule in a 2',3'-dideoxy configuration, almost every purine or pyrimidine suppresses HIV replication in vitro. The key determinant of anti-retroviral effect is the capacity of the target cell to anabolically phosphorylate the nucleoside analogue; a lack of effective anabolic phosphorylation will make a retrovirus appear to be drug resistant. Clinical trials with AZT (the azido analogue of dideoxythymidine) have shown good oral bioavailability and penetration across the blood/brain barrier in patients with AIDS. Recent studies have shown that AZT confers a significant survival advantage to patients with AIDS compared to placebo. AZT can provide significant clinical benefits to certain adults and children with AIDS-related neurologic disease. In the near future, we plan to combine AZT with GM-CSF therapy. Another dideoxy-analogue (dideoxycytidine) is now in phase I testing and preliminary results suggest that it has the capacity to inhibit replication in vivo (reduction of p24 antigenemia). A primary toxicity of dideoxycytidine is peripheral neuropathy. A pilot study testing the feasibility of administering AZT and dideoxycytidine in an alternating weekly regimen has been initiated. The preliminary data suggest that the toxicity of each agent can be reduced with preservation of clinical activity. However, these observations should be confirmed in a larger study before clinical conclusions are drawn. We are also now testing a broad range of compounds which have different mechanisms of anti-viral effects and toxicity. One class of novel anti-retroviral agents is the phosphorothioate family of oligodeoxynucleotides. Such compounds have several complex mechanisms by which they exert an anti-retroviral effect in vitro. Under some conditions they are competitive inhibitors of template-primer in the process of reverse transcription. However, there are preliminary data to suggest that ordered-sequences of such compounds set in an anti-sense configuration can block the expression of viral genes in chronically infected cells.

Late Additions

U 021 TUMOR NECROSIS FACTORS: STRUCTURE, EXPRESSION AND PROPERTIES, David V. Goeddel, Molecular Biology Department, Genentech, Inc., South San Francisco, CA 94080.

Tumor necrosis Factor (TNF-α) and Lymphotoxin (TNF-β) are cytokines that were named for their in vitro and in vivo anti-tumor properties, but are now known to have a wide range of shared biological activities. TNF-α and TNF-β are synthesized transiently and tissue-specifically following a variety of mitogenic stimuli. The genes for TNF-α and TNF-β are closely linked on Chromosome 6 in humans, and code for mature proteins having 30% amino acid identity. Purified recombinant TNF-α and TNF-β have been examined in anti-tumor and anti-viral model systems. Many properties of the TNFs are synergistically enhanced by Interferon-γ.

U 022 FUTURE DIRECTIONS IN BONE MARROW TRANSPLANTATION, Robert Peter Gale, M.D., Ph.D. University of California, Los Angeles, 90024.

Bone marrow transplantation is increasingly used to treat human disease; over 3,000 are performed annually worldwide. Most transplants are in persons with cancer, usually leukemia. Other diseases treated by bone marrow transplantation include aplastic anemia, solid tumors, immune deficiency disorders, and genetic and metabolic diseases such as thalassemia, osteopetrosis and the mucopolysaccharidoses. About one-half of transplants are from HLA-identical siblings; the other half are autotransplants. Recently an increasing number of transplants have been from HLA-partially matched related donors or from HLA-partially or fully matched unrelated persons. The overall success rate in transplants is about 50%. In some diseases, such as aplastic anemia and thalassemia, success rates exceed 70%. In some instances, transplantation is the preferred therapy; in other instances it remains investigational. The major problems in transplantation include graft-rejection, graft-vs.-host disease, disease recurrence, tumor cell contamination of autologous bone marrow, viral infection (particularly CMV). Innovative approaches to these problems include in vitro removal of T-cells or tumor cells using monoclonal antibodies or drugs, development of high-dose chemotherapy or radiotherapy schedules, antiviral drugs, and others. Development of HLA-typed donor pools, has permitted the expansion of transplants to individuals without conventional donors. Future directions of bone marrow transplantation will depend on reducing the treatment related morbidity and mortality. One direction is the likely use of autotransplants as a vector of genetic engineering - initial candidates may be thalassemias and adenine deaminase deficiency. It may also be possible to use bone marrow transplantation to achieve specific immunologic tolerance as a prelude to other organ grafts such as heart or kidney. Other directions include the use of transplants in autoimmune disorders and possibly in aging.

U 023 RETROVIRAL MEDIATED TRANSFER AND EXPRESSION OF GENES IN MOUSE HEMATOPOIETIC CELLS, Arthur W. Nienhuis, David Bodine, Stefan Karlsson, Cynthia Dunbar, Peter Wong, Siu-Wah Chung and Timothy Browder, Clinical Hematology Branch, National Heart, Lung and Blood Institute, Bethesda MD 20892.

Retroviral vectors provide an efficient means to introduce genes into hematopoietic stem and progenitor cells. We have developed producer clones that generate high titres of retroviral particles containing the human beta globin gene. Fifty to 100% of mouse primative multipotential progenitors (CFU-S) are infected, depending on the conditions of bone marrow culture. Hematopoietic growth factors such as IL-3 and IL-1 may enhance infection frequency. Human globin gene expression has been documented in nearly 90% (31 of 35) spleen foci derived from CFU-S containing an integrated proviral genome although the level of expression is only 1-5% of that of the mouse beta globin gene. Circulating red cells containing human beta chains are present in long-term recipient animals between 3 and 8 weeks post-transplantation but disappear thereafter. Under the conditions of infection used to date, gene transfer into repopulating stem cells is much less efficient than into CFU-S.

In a second series of experiments retroviral vectors containing the mouse IL-3 coding sequences have been used to introduce and express that gene in hematopoietic stem and progenitor cells. A myeloproliferative process results in which mice have markedly elevated WBC, bone marrow hyperplasia, and organ enlargement due to myeloid cell infiltration. This proliferative process appears to occur by an autocrine mechanism and has been observed in secondary recipients of bone marrow from animals exhibiting the syndrome. These experiments provide an animal model for chronic myelocytic leukemia and can be used, for example, to study evolution to acute leukemia.

Hematopoietic Growth Factors; Interferons, Interleukins, Cytotoxic Lymphokines and Monokines

U 100 TUMOR CELL LINES RESISTANT TO CHEMOTHERAPEUTIC DRUGS ARE EFFICIENTLY KILLED BY LAK CELLS AND ACTIVATED MONOCYTES. P.Allavena,M.D'Incalci G.Damia and A.Mantovani "Mario Negri" Institute, Milano, ITALY.

We have studied the in vitro susceptibility to the cell-mediated cytotoxicity of human cell lines:the colon carcinoma Lovo, the breast carcinoma MCF7 and the transformed intestinal I-407 and their variants (Lovo/Dx,I-407/Dx and MCF7a) resistant to Doxorubicin and also to VP16, Vincristine and m-AMSA. LAK cells and activated monocytes expressed cytotoxicity levels on multidrug resistant tumor cells comparable to those of the parental cells. Moreover a murine reticolosarcoma of the ovary (M5) and its Cis-platinum and a Cyclofosfamide resistant variants were studied because these types of resistance have different characteristics as compared to the pleiotropic resistance. Also in this case LAK cells efficiently killed both drug-resistant and drug-sensitive lines. These findings provide a rational for immunological approaches designed to eliminate drug resistant tumors, indipendently of the resistance mechanism.

U 101 IMMUNOTHERAPEUTIC EFFECTS OF INTERLEUKIN-2 AND CYCLOPHOSPHAMIDE ON PROGRESSION OF MURINE HEPATIC METASTASES, Yoav Barnavon, Hiroyuki Iwaki, Evelyn Darnell, Jane Merrill, Jerry Bash and Marc K. Wallack. Mount Sinai Medical Center, Miami Beach, FL 33140.

Effective adjuvant treatment for colon cancer patients with hepatic metastases is sorely needed. Cyclophosphamide (Cy) therapy can result in both direct antitumor and indirect immuno-modulatory effects. By combining Cy with low-dose interluekin-2 (IL-2) therapy we may potentiate a synergistic antitumor effect and avoid the toxic side effects associated with high-dose IL-2 therapy. We therefore tested this hypothesis in a murine model.

C-C36 cultured colon adenocarcinomas were injected intrasplenically into syngeneic BALB/c mice in order to produce experimental hepatic metastases. Mice were assigned to four groups: Cy treatment, IL-2 treatment, combined treatment and controls. Cy was administered at a dose of 5 mg/kg I.P. on day 3 following tumor challenge. IL-2 (Hoffmann-La Roche) was administered at a dose of 25,000 units I.P., BID, on days 9, 10 and 11 following challenge. Mice were sacrificed 4 weeks following tumor challenge and total liver weight was used as a measure of hepatic tumor burden.

The average liver weight and SEM were calculated for each group: 2.03 ± 0.36 for controls 1.59 ± 0.21 for IL-2 treatment, 1.21 ± 0.08 for Cy treatment and 1.08 ± 0.03 for combined treatment. The combined treatment group showed signifiant tumor burden reduction when compared to the other groups: $p = 0.031$ vs controls, $p = 0.026$ vs IL-2 treatment alone, $p = 0.098$ vs Cy treatment alone. We are presently performing survival studies to confirm these results and in vitro immune function assays to illucidate possible immune mechanisms involved.

U 102 INTERLEUKIN-1 AND -2 EFFECTS ON HYPOTHALAMIC CORTICOTROPIN RELEASING HORMONE (CRH) SECRETION, Renato Bernardini, Samuel J. Listwak*, Aldo E. Calogero, Philip W. Gold* and George P. Chrousos, DEB/NICHD and *BPB/NIMH, Bethesda, MD 20892.

During an inflammatory response, the hypothalamic-pituitary-adrenal (HPA) axis is activated, probably as a result of hypothalamic CRH and/or other ACTH secretagogue action. Interleukin-1 (IL-1) and possibly IL-2 may be among the products of the immune system that activate the HPA axis during an immune reaction. We have shown that IL-1 and IL-2 stimulate secretion of immunoreactive CRH by rat hypothalami in organ culture in a dose-dependent fashion.

In this study we examined the role of arachidonic acid metabolites (AAM) on IL-1 and IL-2 stimulated CRH secretion. Explanted rat hypothalami were exposed to 10^{-8}M IL-1 or IL-2 alone or in presence of inhibitors of AAM biosynthesis. Indomethacin (INDO), nordihydroguaiaretic acid (NDGA) or eicosatetraynoic acid (ETYA), which respectively block cyclooxygenase, 5-lipoxygenase, or both enzymes, were employed. In another experiment prostaglandins (PG)E_2 or $F_{2\alpha}$, thromboxane (TX) B_2, the TXA_2 receptor agonist U-49,661 or leukotriene D_4 (LTD_4) were tested for possible CRH secretagogue activity. Concentrations examined ranged from 10^{-15} to 10^{-5}M. Both INDO (1 uM) and ETYA (10 uM), but not NDGA (up to 100 uM), prevented IL-1-induced CRH increase ($p < 0.05$). None of these enzyme inhibitors employed affected IL-2-induced CRH increase. Cyclooxygenase metabolites $PGF_{2\alpha}$ and TXB_2, as well as the TXA_2 receptor agonist U-49,661, stimulated hypothalamic CRH secretion ($p < 0.05$). We conclude that IL-1, but not IL-2, induces hypothalamic CRH secretion mediated by cyclooxygenase metabolites.

The mechanism of the response to IL-1 is thus similar to that shown for the fever response to this cytokine.

U 103 RECOMBINANT CYTOKINES ENHANCE THERAPEUTIC ACTIVITY IN COMBINATION CHEMOIMUNOTHERAPY OF METASTATIC DISEASE, Paul L. Black, D.L. Longo, M.P. Castelli, B.F. Lenz, and J.E. Talmadge, Preclin. Eval. Lab., PRI and BRMP, NCI-Frederick Cancer Research Facility, Frederick, MD 21701 and Smith Kline & French Labs, King of Prussia, PA 19406.
rM GM-CSF, rH G-CSF, rH IL-1α, rM IFN-g, and rH IL-2 all accelerated recovery of bone marrow stem cell function when administered after the chemotherapeutic agent cyclophosphamide (CY). rM GM-CSF, rH G-CSF, and especially rH IL-1α also protected bone marrow from subsequently administered CY. However, the sequence and timing of administration of the biological response modifiers (BRMs) relative to the CY were critical, or else increased myelodepression resulted. The identification of the optimal therapeutic protocol for combination chemoimmunotherapy of experimental pulmonary metastases was similarly complex. The combination of a single administration of CY and chronic rH IL-2 (i.p., qd, beginning 48 hr after CY) proved effective in treating animals even with heavy tumor burdens (CY was not administered until 2 wk after i.v. inoculation of tumor cells). In similar circumstances, rH IL-1α and rH G-CSF also had additional therapeutic activity compared with either CY or BRM alone. A combination protocol involving administration of rH IL-1 at 20 hr before CY and chronic administration (beginning 48 hr after CY) of rM IFN-g or rH IL-1α after CY also had good therapeutic activity. Therapeutic activity in chemoimmunotherapy studies correlated with rapid restoration of stem cell function (CFU-C frequency and number) after CY, but not with immunomodulatory activity. Combination chemoimmunotherapy thus provides greater therapeutic efficacy compared with chemotherapy or BRM therapy alone.
Research supported by DHHS contract No. N01-23910 with Program Resources, Inc.

U 104 IN VIVO EFFECTS OF RECOMBINANT INTERLEUKIN-2 ON ANTI-TUMOR AND ANTI-VIRAL NATURAL IMMUNITY IN INDUCED OR NATURAL IMMUNODEFICIENCY STATES, L. D. Butler, C. P. Browne, N. K. Layman, P. Riedl and J. Tang, Lilly Research Labs., Indianapolis, IN 46285.
We have examined the ability of in vivo treatment of mice with recombinant Interleukin-2 (rIL2) to affect natural immunity measured against tumor (Yac-1) or virally infected (Herpes simplex type 1) target cells in natural or induced immunodeficiency states. The rIL2 effect is dose and time dependent and is strain related. The latter parameter correlating with the pretreatment natural immunity level of the strain. rIL2 therapy results in a noticeable increase in asialo-GM-1 positive spleen cells as well as IL2 receptor positive cells in most of the conditions examined. Notably, in mice bearing the scid (severe combined immunodeficiency) mutation, similar increases in anti-tumor and anti-viral immunity were noted even in the absence of reported T cell receptor rearrangements. In this strain, rIL2 therapy causes increases in asialo-GM-1 and Thy-1 positive cells but no increases in LYt-2 or L3T4 positive cells. The changes in IL2 receptor expression were not as obvious as in other conditions. In vivo rIL2 administration is differentially effective in enhancing natural immunity in various induced immunodeficiency states (immunotherapeutic antibodies, irradiation, immunosuppressive drugs or cytoreductive drugs). Notably, in these induced immunodeficiency states, although natural immunity is commonly enhanced, the number of spleen cells recovered is often only marginally affected. Thus, as expected, a limiting aspect in this use of rIL2 is the number of potentially responsive cells present in the immunodeficiency condition. In addition, correlations between the rIL2 effect, several of the immunodeficiency conditions and vascular leak syndrome are discussed.

U 105 THERAPEUTIC ACTIVITY OF RECOMBINANT HUMAN IL-1 (RH IL-1) IN COMBINATION CHEMO-IMMUNOTHERAPY OF METASTATIC DISEASE, M. Paola Castelli, Paul L. Black, Barbara F. Lenz, and James E. Talmadge, Preclin. Eval. Lab., PRI, NCI-Frederick Cancer Research Facility, Frederick, MD 21701 and Smith Kline & French Labs, King of Prussia, PA 19406.
rH IL-1 has myeloprotective and reconstitutive properties when administered before or after exposure to lethal irradiation or alkylating agents. These activities correlate with an increase in the frequency and total number of colony forming units-culture (CFU-C) as well as cellularity in the bone marrow and peripheral blood. rH IL-1 as a single agent has shown minimal therapeutic activity in the treatment of metastatic disease, but it has additive therapeutic activity when administered chronically in combination with cyclophosphamide (CY). However, the protocol (dose, sequence, and timing) of rH IL-1 and CY administration are critical to this therapeutic activity. The combination of rH IL-1 given 20 hr before a single injection of CY and rH IL-1 administered chronically (qd) beginning 48 hr after CY significantly prolongs survival in mice bearing B16 melanoma experimental metastases. The mechanism of this increased therapeutic activity was investigated by comparing therapeutic activity with hematopoietic and effector cell functions in several anatomical sites. Therapeutic activity correlates with increased stem cell functions (CFU-C frequency and number), but not with natural killer, cytolytic T lymphocyte, or macrophage tumoristatic activities at any site. In treatment of metastatic disease, rH IL-1 appears to have its greatest therapeutic activity as a myeloprotective/restorative agent in combined chemo-immunotherapy.
Research supported by DHHS contract No. N01-23910 with Program Resources, Inc.

U 106 CYTOTOXIC PROTEINS OF NATURAL KILLERS, O.Yu. Chertov, A.L. Krasnoselsky Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 117871 Moscow, USSR; L.P. Saschenko, N.V. Gnuchev, E.M. Lukanidin, Institute of Molecular biology, USSR Academy of Sciences, 117334 Moscow, USSR.

We found that the cytotoxic factor secreated by natural killers upon the contact with target cells consists mainly of the 67 K protein. This protein resembles the poreforming protein of cytotoxic T-lymphocytes - perforin in its ability to lyse target cells in the Ca^{++}-dependent manner.
We purified perforin of cytotoxic T-lymphocytes and the poreforming protein of NK cells and determined their N-terminal sequences, which appeared to be different.
We also found that cytotoxicity of NK cells is determined not only by the Ca^{++}-dependent poreforming protein but also by other Ca^{++}-dependent proteins.

U 107 INTERACTIVE EFFECTS OF IFNαA/D AND IL2 ON IN VITRO AND IN VIVO LAK ACTIVITY, N. Chikkala, I. Lewis, J. Ulchaker, J. Stanley, R. Tubbs, and J. Finke, Cleveland Clinic Foundation, Cleveland, OH 44106.

Previously we showed that the combination of IL2 (Cetus) and IFNαA/D (Hoffman-LaRoche) was more effective than either cytokine alone in inhibiting the lung metastases in mice bearing B16-BL6 melanoma. In an attempt to understand the immune mechanism by which the combination inhibited lung metastases, we assessed the effects of IL2 and IFNαA/D on LAK activity in vitro and in vivo. In vitro studies with splenocytes showed that IFNαA/D had a synergistic effect on IL2 augmented LAK activity against YAC-1 targets but had no significant effect on IL2 augmented LAK activity against B16-BL6 targets at most of the IFNαA/D doses tested. At the highest tested dose (10,000 U/ml), IFNαA/D inhibited the generation of LAK activity. Additional studies showed that IFNα A/D did not influence the frequency of LAK precursors under limiting dilution conditions. Treatment of B6 mice with the combination induced an increase in the lytic activity of splenocytes over that observed with either cytokine alone. Treatment of mice with these cytokines had minimal effects on LAK precursor frequency in the spleen. Even though IL2, IFNαA/D and the combination augmented kill against YAC-1 in the lungs, none of these treatments induced significant kill against B16-BL6. Limiting dilution studies with lung cells revealed that treatment with IL2 caused a significant increase in the frequency of cytotoxic cells against YAC-1 and B16-BL6 over that observed in control mice. Moreover, when injected with IL2, IFNαA/D did not inhibit the IL2 induced increase in LAK cells precursors directed against YAC-1 or B16-BL6. However, the frequency of LAK cells that infiltrate in response to cytokine treatment is relatively low when compared to the number of $Thy1^+$ infiltrating cells. It appears that many of these cells do not proliferate in vitro in response to IL2.

U 108 HUMAN rIL-3 PROMOTES THE GROWTH OF HUMAN BASOPHILS, Barbara Fagg*, K. Hirai, K. Nakajima and B. Stadler, *Biotechnology, SANDOZ LTD., CH-4002 Basle, and Clinical Immunology, University of Berne, CH-3010 Berne, Switzerland

Human basophil-like cell promoting activity (BaPA) has been partially purified from human lectin-stimulated spleen cell conditioned medium (Hirai et al, 1988). It stimulates human basophil development from bone marrow cells as determined by morphological criteria and increase in cell associated histamine content. The effect could not be reproduced by other lymphokines (IL-1, γINF, IL-2, G-CSF, GM-CSF) present in spleen cell conditioned medium. In the murine system, IL-3 and GM-CSF are distinguished by the unique ability of IL-3 to stimulate the growth of basophils or "mast" cells. We therefore asked whether human BaPA is in fact hIL-3 by (1) examining the effect of rhIL-3 on human basophil production. The BaPA could be entirely reproduced by rhIL-3. (2) purified BaPA was titrated in the CML assay alone and in the presence of neutralizing antibodies for hGM-CSF and hIL-3. BaPA stimulated CML proliferation to an equivalent level to IL-3, this activity was not neutralized by antibody to GM-CSF but was eliminated by anti-IL-3. Taken together the results indicate that hIL-3 in addition to its other haemopoietic activities also has basophil growth promoting activity.

U 109 SUBACUTE TOXICITY OF HUMAN RECOMBINANT INTERLEUKIN-2 IN CYNOMOLGUS MONKEYS, T.J. Hayes, T.D. Anderson, R.J. Tudor, and B. Rushton. Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ and Drug Safety Department, Roche Products Ltd., Welwyn Garden City, UK.

Dose-related toxicity has been limiting with some treatment regimens with human recombinant interleukin-2 (rIL-2) in humans. Reports on the toxicologic assessment of rIL-2 in animals are limited to findings in rodents. In this study groups of four monkeys each were treated by bolus IV injections once a day for two weeks with doses of 0 (vehicle control), 0.7, 2, and 7 million units (BRMP) of rIL-2/kg/day. Coughing and dypsnea occurred in high-dose monkeys. Moribundity and/or mortality occurred in three of four high-dose monkeys after one week of treatment. Dose-related anemia, lymphocytosis, eosinophilia, thrombocytopenia, and increased ESR were observed. Values for serum BUN, creatinine, bilirubin and triglycerides were increased; serum values for total protein, albumin, and sodium were decreased. Dose-related necropsy observations included pleural effusions, ascites, and increased spleen, lung, and kidney weights. The doses and duration of treatment in this study were greater than those used in clinical trials and were chosen to allow characterization of toxicity. The findings in cynomolgus monkeys are remarkably similar to those reported for rIL-2 toxicity in man and demonstrate that data of predictive toxicologic relevance to humans can be gained by testing a human recombinant cytokine in a laboratory animal.

U 110 ANTI-TUMOR AND HEMATOPOIETIC EFFECTS OF RECOMBINANT INTERLEUKIN-1-ALPHA (IL-1) IN NORMAL AND TUMOR BEARING MICE. Candace S. Johnson, Linda C. Stork, Paul G. Braunschweiger and Philip Furmanski, AMC Cancer Research Center, Denver, CO, 80214, and Children's Hospital, Denver, CO, 80218.

IL-1, a macrophage derived product, is a multifunctional cytokine that broadly potentiates hematopoiesis and induces the synthesis of hematopoietic colony stimulatory factors (CSF) in vitro and in vivo. To determine the potential for use of IL-1 in enhancing chemotherapeutic efficacy, normal mice and mice bearing the RIF-1 tumor (14 days after tumor implant) were treated with a single injection of 0.5μg of recombinant human IL-1 alpha (Hoffmann-LaRoche). By 6 hrs after treatment, IL-1 alone caused marked hemorrhagic necrosis of the RIF tumor in vivo and significantly reduced tumor blood flow. Clonogenic tumor cell viability and proliferation were also significantly reduced in IL-1 treated animals at 24 and 48 hrs post treatment. At 48 hrs, IL-1 significantly increased macrophage (CFU-C), granulocyte (CFU-G) and macrophage-granulocyte (CFU-GM) progenitor cell compartments in both normal and tumor-bearing mice. The numbers of immature erythroid progenitor cells (BFU-E) were significantly stimulated by IL-1, whereas mature erythroid progenitors (CFU-E) were significantly suppressed. In normal animals, IL-1 hastened myeloid recovery following cyclophosphamide (CP) treatment. In RIF-1 tumor bearing mice, IL-1 alone had no effect, but when IL-1 was given together with CP (150mg/kg), tumor regrowth delay was significantly increased over that observed with CP alone. These results demonstrate that IL-1 has profound hematopoietic and anti-tumor effects which could be utilized to enhance the therapeutic efficacy of cytotoxic drugs. Supported by NIH grant CA33188 and a gift to AMC from Richard L. Robinson.

U 111 IL-2 AND LYMPHOKINE ACTIVATED KILLER CELLS (IL-2/LAK) IN SOLID TUMORS. L.M. Jost, J. Gmur, O. Oelz, C. Sauter, P. Groscurth and R.A. Stahel. Division of Oncology and Department of Medicine, University Hospital, CH-8091 Zurich, and Institute of Anatomy, University of Zurich, CH-8057 Zurich, Switzerland.

The tolerance of an IL-2 analog (r-met Hu IL-2 [ala-125], Ortho Pharmaceutical) and effectiveness of IL-2/LAK was examined in 12 patients (pts) with metastatic solid tumor (6 hypernephroma, 3 melanoma, 3 extragonadal germ cell tumors refractory to chemotherapy). IL-2 was administered as bolus at 30'000 U/kg q8 hrs on days 1-5 and days 12-20. Leukapheresis was done days 8-12, lymphocytes were activated in vitro for 3 to 4 days and reinfused on days 12, 13 and 15. One pt was retreated upon relapse. The pts received 100% of planned IL-2 on days 1-5 and 75% (range 21-100%) on days 12-20. The mean number of reinfused cells was 7.2 $x10^{10}$ (5.0-9.5 $x10^{10}$). All pts had fever and malaise. Mean weight gain was 10% (6-15%). One pt developped a gram-negative sepsis due to a contaminated commercially supplied medium and required intubation. Eight pts required pressors because of hypotension and low urinary output. Grade III/IV toxicity was noted during the following number of courses (numbers in parenthesis attributed to gram negative sepsis): hematologic: 1/1(1), GI 1/0, renal 1(1)/0, skin 3/0, CNS 1(1)/0, pulmonary 5/1(1). No toxic death occurred. Partial response (PR) was seen in 2/6 renal cell carcinoma, 1/3 melanoma and 0/3 extragonadal germ cell tumors. Duration of PR was 1, 3+ and 4+ months. Mixed response (MR) was seen in 2/6 hypernephroma and 1/3 melanoma. One pt with melanoma underwent biopsies of skin lesions during treatment. Infiltration with characteristically altered lymphocytes and patterns of tumor cell destruction similar to those of NK- and T-cell mediated lysis were documented by light and electron microscopy. Follow up studies will examine ways to administer IL-2/LAK with less toxicity thus allowing repeated courses of treatment to be given for responders.

U 112 REGULATION OF M-CSF, G-CSF, AND GM-CSF mRNA LEVELS AND PROTEIN SECRETION IN HUMAN MONOCYTES, Mei-Ting Lee, Martha B. Ladner, Cetus Corporation, Emeryville, CA. 94608, Ken Kaushansky, Univ. of Washington, Seattle, WA. 98195

Macrophages and granulocytes are hematopoietic cells that have differentiated from bone marrow progenitor cells in response to specific colony stimulating factors. Recent studies have identified stimulated monocytes to be a source of the glycoproteins M-CSF, G-CSF, and GM-CSF. Monocytes also secrete the bioactive lipid prostaglandin E2, which often is a negative regulator of monocyte function. Thus the presence of a stimulating agent (lipopolysaccharide-LPS) and/or a naturally secreted inhibitor (prostaglandin E2-PGE-2), may have a significant effect on the production of CSFs. Using freshly isolated-LPS induced human blood monocytes, we investigated several parameters of M-CSF, G-CSF, and GM-CSF mRNA and protein production. The monocytes were cultured in polypropylene tubes (nonadherent) or adhered on plastic in 10% Human AB serum. Transcripts were identified by Northern analysis. Monocyte cultures that are not LPS stimulated produce low levels of CSF mRNA. Only G-CSF and GM-CSF mRNAs are detectable at 24h in nonadherent monocytes treated with 100 ng/ml LPS and 10^{-6}M indomethacin (an inhibitor of prostaglandin synthesis). Adherent monocytes, cultured under the same conditions, contain transcripts for G-CSF, GM-CSF, and M-CSF. If the monocytes are allowed to adhere for 24h in 10% HAB serum and then LPS treated for an additional 24h, only high levels of M-CSF transcripts are detectable. The addition of indomethacin increases the amount of M-CSF message at 48h (only M-CSF measured). Indomethacin also increases the amount of secreted protein for all three CSFs, as shown by RIA, proliferation assay,and morphology of bone marrow colony cells. When incubated in the presence of PGE-2 and LPS, monocytes contain G-CSF mRNA but no GM-CSF or M-CSF mRNA. We postulate that depending upon the activation state of the monocyte or macrophage, different CSFs are produced which differentially regulate the recruitment of various hematopoietic progenitors.

U 113 THE ROLE OF RECOMBINANT HUMAN IL-3 IN NORMAL HEMOPOIESIS
A.F. Lopez*, P. Dyson△, M. Elliott*, J. Russell△, L.B. To△, S. Milton*, J.R. Gamble*, Yu-Chung Yang§, G. Wong§, S. Clarke§, C. Juttner△ and M.A. Vadas* From the Divisions of *Human Immunology and △Haematology, The Institute of Medical and Veterinary Science, Frome Road, South Australia, and §Genetics Institute, Cambridge, MA.

Recombinant human IL-3 (rh IL-3) was tested for its ability to stimulate the proliferation and function of various types of human hemopoietic progenitor cells. rh IL-3 acted at early stages in hemopoiesis stimulating mixed colonies and megakaryocytes, as well as day 14 myeloid colonies containing granulocytes macrophages and eosinophils. However, myeloid cells at later stages of differentiation were substantially less responsive to rh IL-3. Thus, rh IL-3 stimulated relatively low numbers of day 7 colonies, and had a very small effect on a population of enriched promyelocytes and myelocytes. The loss of responsiveness to IL-3 with differentiation was most notable using mature cells. In this case rh IL-3 stimulated the function of eosinophils and macrophages but not that of neutrophils. In addition the concentration of rh IL-3 necessary for stimulation of mature cell function was higher than that necessary for day 14 colonies. Unlike rh IL-3, rh GM-CSF was a strong stimulus for mature cells and less concentrations of rh GM-CSF were necessary for a full effect on mature cells compared to progenitor cells.
These experiments show that rh IL-3 is primarily a stimulator of the early stages of hemopoiesis in contrast to rh GM-CSF.

U 114 IDENTIFICATION AND CHARACTERIZATION OF NOVEL IFNγ-INDUCED GENES. Andrew D. Luster, Richard Weinshank, and Jeffrey V. Ravetch. Sloan-Kettering Cancer Institute, New York, NY 10021. Gamma-interferon (IFNγ) is a potent activator of the cellular immune response. To characterize the molecular mechanism of the IFNγ-induced phenotype, two novel genes induced by IFNγ in human cells have been isolated. cDNA sequence analysis revealed that these genes encode previously undescribed proteins of 10kd and 30kd (referred to as IP-10 and IP-30, respectively). The deduced amino acid sequence of IP-10 reveals that it is a member of a newly emerging family of cytokines that include α-granule platelet proteins and proteins induced by transformation. The IP-10 protein is expressed during the development of a cutaneous delayed cellular immune response by keratinocytes, endothelial cells and infiltrating dermal mononuclear cells. Pronounced IP-10 expression is seen in the epidermis and dermis in the cutaneous lesions of tuberculoid leprosy and psoriasis. IP-10's homology to chemotactic and mitogenic proteins suggests that it may play a role in the directed migration, activation and proliferation of local and blood-borne cells that characterize an inflammatory response. In addition, the genomic organization of the IP-10 gene has been determined and an IFNγ-inducible DNase I hypersensitive site has been identified 250 bp upstream of the RNA initiation site defining a punitive cis-acting DNA sequence. The IP-30 gene encodes a 30kd protein that is present and inducible in cells of the hematopoietic lineage and absent but inducible in non-hematopoietic cells. The IP-30 protein has a processing-secretion pattern and an immunofluoresence pattern that suggest that it is a lysosomal protein. This is of particular interest since IFNγ is known to induce in cells a resistance to many intracellular pathogens.

U 115 EARLY LYMPHOMYELOID STEM CELLS CIRCULATE IN HAIRY CELL LEUKEMIA: THEIR PROLIFERATIVE AND DIFFERENTIATIVE RESPONSE TO RECOMBINANT HEMATOPOIETIC GROWTH FACTORS. Rita Michalevicz*, Eli Sahar** and Michel Revel***. *Inst. of Hematology, Ichilov Hospital, Tel-Aviv 64239, **Biotechnology, Tel-Aviv University and ***Virology, Weizman Inst. Rehovot, Israel.

Hematopoietic stem cells giving birth to lymphomyeloid-erythroid-mekagaryocytic (LGEM) colonies circulate in the peripheral blood of Hairy Cell Leukemia patients (1). These stem cells were isolated from HC, B and T cells using monoclonal antibodies and FACS. 2 to 16% of the HC peripheral blood cells were positive for My10 antibodies found previously useful for enrichment of multipotential progenitors in normal bone marrows but not normal peripheral blood. All LGEM, LGM and BFU-E grew in the My10 positive fraction with a 12 fold enrichment. The negative fraction yielded no colony growth. These stem cells grow in liquid cultures and require the presence of PHA-LCM. The aim of this work consisted in defining whether any cloned hematopoietic growth factor acts on proliferation and/or differentiation of LGEM colonies. Interleukin 3, granulocyte macrophage colony stimulating factor (rIL3 and rGM) promote growth of GM and BFU-E colonies but do not support formation of early LGEM colonies in weekly liquid cultures. Synergistic combinations using IL3 and IL1 or IL3 and IL6 (rIFN beta 2) resulted in increased numbers of LGEM. Results of cultures with My10 liquid cultures including factor combinations will be presented using a model of early stem cells with multipotential differentiative capacity and self-renewal ability. (1) Michalevicz R and Revel M: Proc.Natl.Acad.Sci.USA 84:2307,1987. rIL3 and GM-CSF are a gift from Sandoz Ltd. Basel.

U 116 INHIBITION OF B16 MELANOMA IN VIVO BY rHu-IL 1-THE ROLE OF PMNS-. Mary E. Neville, Kathleen M. Pezzella and James J. Huang. Medical Products Dept., E. I. du Pont de Nemours, Glenolden, PA 19036.

We have examined the ability of multiple injections of rIL-1B to inhibit the growth of B16 melanoma in syngeneic female C57BL/6 mice. When 0.5-1.0 mg/kg of rIL-1 was injected daily intratumorally into intradermal tumors for 3 to 7 days, a dose dependent inhibition of tumor growth was observed ranging from 36% to 93%. Other routes of injection of rIL-1 (i.m., i.d., i.p.) also inhibited tumor growth but to a lesser degree (27% to 50%). Inhibition of tumor growth was rapid, occurring within 3 days, suggesting that inhibition was probably not due to classical cellular or humoral immune responses. Furthermore histology of regressing tumors showed an influx of PMN's into necrotic areas of the tumors with little or no influx of mononuclear lymphoid cells. This influx of PMN's into regressing tumors was further documented by the quantitation of myeloperoxidase activity. Ten fold increase of myeloperoxidase was observed in regressing tumors as compared to sham-treated tumors. These results suggest that PMN's may have tumoricidical activity in vivo which may be augmented by IL-1.

U 117 MODULATION OF MCF-7 CELLS IN CULTURE BY THYMOSINS, Karen K. Oates, Gail Ginsburg and Marcia McGinty, George Mason University, Fairfax, VA 22030.

Several studies have identified the production of ectopic hormones by various cancers. One of the best models of growth factor synthesis by tumors has been the MCF-7 human breast cancer cell line. We have identified immunoreactive thymosin alpha one (TA1) in supernatants of MCF-7 cells in culture. To test the growth modulating effects of thymosin alpha one and the parent compound thymosin fraction five (TF5) two assays were performed, an anchorage dependent cloning (ADC) and a tritiated thymidine incorporation assay. The thymosins were treated in a dose dependent manner, with and without the hormone beta estradiol, which is a known stimulator of the MCF-7 cells.

The results indicated that TAI (1×10^{-9} mg/ml) with estradiol (1×10^{-9} M) increases the number of ADC by approximately 380% from MCF-7 cells treated with estradiol alone. The incorporation of tritiated thymidine by TAI (10^{-7}) with estradiol treated cells increased 193% from cells treated with estradiol alone. TF5, in combination with estradiol was inhibitory at all concentrations tested in the ADC assays (inhibition varied from 56 to 46%). Only one concentration of TF5 (6×10^{-4} mg/ml) in combination with estradiol had a marginal stimulatory response in the proliferation assay. Thymosin fraction five with estradiol was inhibitory (88 to 69%) in the proliferation assay when compared to cells treated with estradiol alone. Scanning electron micrographs indicate increased density of surface microville formation over that of thymosin alpha one treated cells (5 day exposure). This confirms our observation that the cells treated with thymosin alpha one have become activated. These results imply that thymic hormones may be used to stimulate (TAI) and inhibit (TF5) proliferation of the MCF-7 breast cancer cells in culture.

U 118 EFFECTS OF rGM-CSF ON HUMAN GRANULOCYTE (PMN) ADHERENCE AND CHEMOTAXIS

Reto Obrist, Regine Landmann and Paul Obrecht, Div. of Oncology, Dept. of Internal Medicine of the University, CH-4031 Basel, Switzerland.

In an under-agarose assay recombinant GM-CSF has been shown to inhibit migration of human PMN. This may be due to changes in granulocyte adherence. We tested human PMN after in vitro exposure to recombinant GM-CSF synthesized by chinese hamster ovary cells for adherence, absolute and relative migrating cell numbers in a modified Boyden chamber system. A 10' incubation does not enhance adhering PMN numbers, whereas after a 30' preincubation the number of adhering cells increased from 521+/-10.2 in medium to 744+/-8.3 at 10pg/ml CSF per high power field (hpf). Random migration increases from 32+/-1.9 in medium to 148+/-8.9 per hpf at 10pg/ml CSF. In contrast, directed chemotactic migration to 5% endotoxin activated human serum (AHS) and 10-8M fMLP is inhibited in a dose dependent fashion, decreasing from 529+/-10.9 in medium to 486+/-11.0 per hpf in 10pg/ml CSF against AHS and from 342+/-9.9 to 237+/-8.9 against fMLP. Similar trends, but less pronounced are found after a 30' CSF preincubation. Relative migration correcting for changing cell numbers reveals the decrease in fMLP-induced migration to be real, whereas the decrease with AHS is due to changing adherent cell numbers. The relative decrease is not dose dependent in the range of 1 to 1000 pg/ml. In conclusion rGM-CSF induces PMN adherence and inhibits fMLP - but not AHS - stimulated migration.

U 119 M-CSF AUGMENTS MURINE MACROPHAGE ANTIBODY-DEPENDENT KILLING (ADCC) OF TUMOR CELLS INDUCED BY INDIVIDUAL LYMPHOKINES, Peter Ralph, and Ilona Nakoinz, Cetus Corporation, Emeryville, California 94608.

Macrophage colony-stimulating factor (M-CSF) was investigated as a stimulator of ADCC to the murine R1.1 thymoma target by murine peritoneal exudate macrophages which were elicited by proteose peptone. Both an ^{125}IUdR-release and a viable cell count assay were used. The latter assay avoids radiation damage, and the fate of the targets can be determined over a long period. Pretreatment of macrophages in culture with lymphokine (LK) from Concanavalin A-induced mouse spleen cells or human blood mononuclear cells moderately stimulated ADCC. Preincubation of macrophages with conventional or recombinant human M-CSF or immunoaffinity-purified mouse M-CSF alone had little effect. However, M-CSF greatly enhanced ADCC to the tumor target when used as a costimulant with LK, IFNγ, IFNα, IFNß or IL-2. Incubation of macrophages with LK plus M-CSF for two days generated the highest level of ADCC, with a plateau at 1000 U/ml M-CSF. At 1 U/ml IFNγ or IL-2, or 5 U/ml IFNα or IFNß, M-CSF boosted ADCC activity to that using 10-fold of the LK alone. IL1, IL4 and TNF had little or no stimulating activity for ADCC alone or with M-CSF, and the other hemopoietic growth factors IL3 and GM-CSF did not promote this effector function alone or with IFNγ. We previously showed that M-CSF boosted macrophage antibody-independent killing of TU-5 sarcoma targets with or without LK (Cell. Immunol. 105:270, 1987). These studies thus show that M-CSF is a positive regulator of both macrophage nonspecific tumor lysis and ADCC.

U 120 TIME LAPSE VIDEO MICROSCOPY OF rHuIFN-g IN INDUCED CYTOLYSIS IN SQUAMOUS CARCINOMA TISSUE CULTURES

Wm. J. Richtsmeier, M.D., Ph.D., Johns Hopkins University, Balto., MD

We have observed the cytolytic effect of rHuIFN-g in sensitive squamous cell carcinoma (SCC) tissue cultures. Three to four hours after treatment with rHuIFN-g, this effect begins abruptly in a few cells which previously appeared morphologically similar to surrounding cells. Affected cells demonstrate multiple, rapid, short, pseudopodial extensions in a random pulsion-type appearance which spreads over the entire surface of the cell as it "rounds-up". This effect increases in the population over the next twelve hours. For affected cells it continues for variable period usually lasting several hours before cells cease moving and die either by fragmenting into small vesicles or decompressing internally into large vesicles. Cells which undergo this pseudopodial-pulsion morphologic change cease the typical translocation motility observed in control cultures. In contrast, many other rHuIFN-g treated cells remain unaffected and continue to translocate normally for days. Treatment of cells with EGF produces a similar pseudopodial pulsion, morphologic appearance but it occurs faster than after rHuIFN-g treatment, and cells eventually revert to normal. The enhanced cytolytic effect in SCC tissue cultures after treatment with both rHuIFN-g and EGF may point to a common hyperkenetic activity in these cells.

U 121 IDENTIFICATION OF IL-1 ANTAGONISTS IN CLINICALLY RELEVANT TARGET CELLS AND PHYSIOLOGIC MODELS. J.E. Shaw, B.L. Maloff, E.M. Bruin, T.M. DiMeo, D. Fox. E.I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898.

IL-1 is a polypeptide hormone, produced by mononuclear phagocytes, which may play a role in the pathogenesis of immunoinflammatory diseases. A primary screen, based on IL-1 stimulated PGE_2 release in MRC-5 fibroblasts, has been developed to detect potential antagonists of IL-1. Approximately 1% of compounds tested (taken from a pool of random, directed synthesis, and standard compounds) have some activity in this screen. Inhibitory compounds are evaluated for toxicity using a human neutrophil LDH assay. Human neutrophil chemotactic response to IL-1 is used to confirm the IL-1 antagonist properties of these compounds in a clinical target cell. Approximately 2/3 of the compounds detected by PGE_2 inhibition inhibit IL-1 stimulated chemotaxis. For example, compound #62574 has an IC_{50} of 1.4×10^{-6} M in the PGE_2 release assay, and inhibits IL-1-stimulated chemotaxis by 92% at 100 μM. A physiologic screen, involving inflammation mediated by cellular influx in response to IL-1 injection in mouse ears, has been developed to screen inhibitors in vivo. Standard anti-inflammatory compounds are ineffective in this model. Importantly, activity in vitro can be correlated with efficacy in vivo; about 1/3 of the IL-1 antagonists identified in the primary screen ameliorate the inflammatory effects of IL-1. Using this strategy, a number of IL-1 antagonist leads have been identified for evaluation in classic inflammatory models, such as delayed-type hypersensitivity.

U 122 MODULATION OF PHENOTYPIC AND FUNCTIONAL PROPERTIES OF HUMAN PERIPHERAL BLOOD MONOCYTES BY INTERLEUKIN-4 (IL-4). Anje A. te Velde, Jan P.G. Klomp, Benito A. Yard, Jan E. de Vries*, and Carl G. Figdor. The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; *Unicet, Dardilly, France.

Highly purified peripheral blood monocytes were cultured in the presence of recombinant IL-4. After day 5 of culturing the cells acquired a macrophage-like appearance, with increased cell size and extensive processes, suggesting that IL-4 may induce monocyte-macrophage differentiation. This notion is supported by the observed increased expression of MHC class II antigens, which is thought to be associated with monocyte differentiation. Exposure of monocytes to IL-4 resulted in a dose-dependent increase in the expression of MHC class II antigens, which became already apparent after 20 hours of incubation, while maximal expression was obtained after incubation for 6 days. Similarly, IL-4 increased the expression of CR3 and p150,95 antigens, two members of the LFA-1 family, while the expression of the third member, LFA-1, remained unchanged during culture. Furthermore it was shown that IL-2 inhibited the secretion of cytostatic and chemotactic compounds. Supernatants of monocytes cultured with IL-4 for 20 hours were, in contrast to control cultures, much less effective in inhibiting the growth of A375 melanoma cells. In addition these supernatants failed to direct the migration of freshly isolated monocytes in a chemotaxis assay. Further analysis revealed that these supernatants exhibited reduced IL-1 activity, as measured in a mouse thymocyte proliferation assay, which might explain the low cytostatic and chemotactic activity. Taken together these results show that IL-4 modulates monocyte phenotype and function and may induce monocyte-macrophage differentiation in vitro.

U 123 PRODUCTION OF INTERLEUKIN 1 α (IL1α) BY EPSTEIN-BARR VIRUS (EBV) CONTAINING CELLS FROM NASOPHARYNGEAL CARCINOMA (NPC), Thomas Tursz, Pierre Busson, Marc Lipinski and Hiro Wakasugi, Institut Gustave Roussy, 94805 Villejuif, France.

Nasopharyngeal carcinoma (NPC) is a human epithelial cancer constantly associated with the Epstein-Barr virus (EBV). Investigations on this tumor have been limited so far by the difficulty to grow NPC cells in vitro in the long term. We have obtained and characterized two transplantable NPC tumors, C15 and C17, which have been propagated in nude mice for 30 and 9 passages, respectively. Epithelial markers are detectable in both tumors. C15 contains 30 copies of the EBV genome and C17 only 2 copies. Karyotype examination and oncogene analysis have revealed substantial abnormalities. No CR2 (the C3d/EBV receptor on B cells) molecules can be found on any of the two tumors which, in contrast, constitutively express HLA Class II antigens. C15 and C17 cells spontaneously produce large amounts of Interleukin 1 (IL1). Supernatants from short-term cultures of fresh NPC biopsies also displayed IL1 activities. In most cases, IL1 from NPC cells exhibited biochemical, immunochemical and molecular characteristics of IL1α. Several EBV+ lymphoblastoid B cell lines have been reported to produce IL1 and to use it as an autocrine growth factor (PNAS 1987, 84: 804). A relationship might exist between EBV infection and constitutive production of IL1. IL1 produced by NPC cells in vivo could explain the marked T cell infiltrate observed in NPC tumors.

U 124 INDUCTION OF A NOVEL MYELOPOIETIC INHIBITORY MECHANISM IN BONE MARROW AND SPLEENS OF MICE INJECTED WITH PGE2 OR RECOMBINANT HUMAN INTERLEUKIN 1ß, Louis M. Pelus, Dept of Hematopoietic Regulation, Sloan Kettering Institute, New York, NY 10021.

We have previously demonstrated that single bolus i.v. injection of prostaglandin E2 (PGE2) in intact mice results in significant suppression of total CFU-GM per femur or spleen, and a selective decrease in the proportion of CFU-GM in S-phase of the cell cycle within 6 hrs post injection. Recent studies now indicate that coculture of bone marrow or spleen cells from mice injected with PGE2 with control marrow cells results in significant suppression of normal CFU-GM. As few as 5,000 marrow or spleen cells from mice sacrificed 6 hrs after a single injection of 0.01-10 ug PGE2 inhibit normal CFU-GM proliferation from 50,000 marrow cells by 45-60%, n=24. Kinetic analysis indicates that this suppressor mechanism is first observed within 3 hrs post injection, becomes maximal between 6 and 12 hrs and is no longer observed by 24 hrs post PGE2 injection. Negative selection by C'-mediated cytolysis with lineage specific monoclonal antibodies indicate a monocytic lineage [GMA1.2+, MAC1+, F4/80+]. Positive selection by immunoadherence with each of these antibodies confirmed these results and indicated that as few as 500-1000 positively selected cells are sufficient to maximally inhibit CFU-GM from 50,000 control normal marrow cells (range 42-60%). Given the ability of IL1 to stimulate PGE production, we investigated its ability to induce the equivalent short lived suppressive mechanism. Single i.v. administration of 200-3000 U/mouse (0.15-2.4 ug) recombinant human IL1ß and sacrifice after 6 hrs, resulted in induction of marrow and spleen cells capable of suppressing normal CFU-GM (42-49%, n=6), a 15-30% reduction in femoral and splenic CFU-GM and no change in femoral or splenic cellularity. Pretreatment of mice with indomethacin (100 ug/mouse, s.c. in 15% gelatin) 24 hrs prior to IL1 injection completely blocked the induction of suppressive marrow and spleen cells. Kinetic analysis following injection of 500 U/mouse IL1 indicated the presence of suppressive marrow and spleen cells at 6, 18 and 24 hrs post IL1 injection with loss of suppressor activity by 48 hrs post injection. However, when animals were pretreated with indomethacin, no suppressive mechanism could be detected at any time point, and significant enhancement of femoral and splenic CFU-GM was observed. Enhancement of total femoral CFU-GM in the range of 75-80% was observed at 48-72 hrs post IL1 injection, while total splenic CFU-GM was increased 275-500% in the 24-72 hrs post injection. These results indicate that in intact mice IL1 induces a myelopoietic suppressor mechanism as a consequence of its ability to initiate PGE biosynthesis, which can be blocked by pretreatment with indomethacin. These findings have considerable implication to preclinical and clinical trials of recombinant human IL1.

Mechanisms of Action and Therapeutic Application of Biologicals in Cancer and Immune Deficiency Disorders

Pathways of Cytokine Action, Monoclonal Antibodies, Differentiation and Immunomodulation

U 200 ANALYSIS OF MELANOMA ANTIGENS BY ANTIBODIES PRODUCED IN MELANOMA PATIENTS TREATED WITH VACCINIA MELANOMA ONCOLYSATES, Jerry A. Bash, Evelyn S. Darnell, Marc K. Wallack, Eleuthere Leftheriotis and Jacques Pourtokaillon, Mount Sinai Medical Center, Miami Beach, Florida 33140, Institut Merieux and Centre Leon Berard, Lyon, France.

Viral oncolysates have been shown to elicit immune responses to tumor cell membrane antigens which are poorly immunogenic in uninfected tumor cell lysates. Vaccinia melanoma oncolysates (VMO) prepared from vaccinia-infected allogeneic melanoma cell lines have been shown to elicit a wide variety of antibodies in melanoma patients. These include both IgG and IgM antibodies which are melanoma restricted in their reaction patterns as determined in both a Staphylococcus protein A (SpA) and enzyme-linked immunosorbant assay (ELISA). Recent evaluation of sera from melanoma patients treated with VMO in ELISA assays have produced evidence that IgG antibody to melanoma cell surface antigen(s) is of prognostic significance. Sera from a similar study in France have been shown to contain antibodies reactive with a ganglioside antigen, and the pattern of increases and decreases in IgG/IgM titer have shown to be correlated with disease-free survival. Current studies evaluating the antigenic content of VMO by SDS-PAGE (Western Blotting) have shown reactivity of patient sera with components of uninfected tumor lines. Comparison of antibodies produced by several patients in conjunction with clinical responses may improve the prognostic value of specific antibody monitoring and serve to identify relevant antigenic components in VMO which may be used as markers in production of more effective vaccines.

U 201 THE INDUCTION OF LYSIS OF RENAL CELL CARCINOMA AND OTHER TUMORS BY BISPECIFIC ANTIBODY REAGENTS USING NATURAL KILLER (NK) CELLS TCRαβ AND TCRγδ CTL AS EFFECTOR CELLS. Bolhuis, R.L.H., Segal, D., van Dijk J., Warnaar, S. and Braakman,E.. Dept. of Immunology,Rotterdam Cancer Institute,Rotterdam, the Netherlands

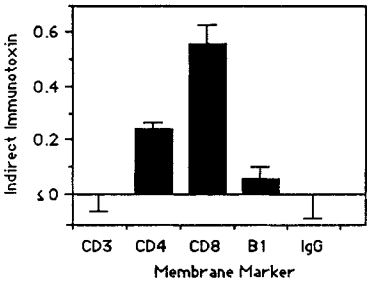
We have characterized T cell receptor (TCR^-)/$CD3^-$ NK cells; $TCR\alpha\beta/CD3^+$ cytotoxic T lymphocytes (CTL) and $TCR\gamma\delta/CD3^+$ CTL phenotypically and functionally. These populations of cytotoxic cells are clearly distinct on the basis of their TCR gene rearrangements. These differences are also reflected by major histocompatibility complex (MHC)-restricted regulatory and cytolytic functions. We have researched the regulation of lytic activity by the CD2,3,16 and framework TCRαβ and TCRγδ effector cell surface molecules and through which activation sites lytic activity can be induced in these cells. To this end we have also used monoclonal antibodies (MoAb's) which are specific for CD2,3,16 for assessment of the regulation processes and made use of bispecific antibody reagents recognizing activation sites on the effector cells on the one hand and (tumor) target associated antigens on the other. The results obtained show that efficient lysis of tumor cells can be obtained. Moreover, data suggest that multi-chain activation processes are involved and that additional target cell "specific" structures may play a role in target cell interactions with retargeted cloned effector cells. The distinct effector cell types show differential activation states depending on the "CD" specificity of the bispecific reagents. The differential activation obtained with various bispecific antibody preparations and distinct effector cell types will enable finetuning of activation mechanisms of the cellular immune system. The availability of distinct types of lymphocyte subpopulations with their own circulation and homing patterns as well as lymphokine producing capacities will be important for patient taylored therapy. This ultimately will allow the design of refined therapeutic strategies for the adoptive and/or active immunotherapy of cancer and infectious disease.

U 202 ENDOCYTOSIS OF A RICIN A CHAIN IMMUNOTOXIN REACTING WITH CD5 ANTIGEN ON LYMPHOCYTES AND LEUKEMIC CELLS, V. Byers[1,2], I. Pawluczyk[1], M. Garnett[1], E. Austin[1], P. Scannon[2], and R. Baldwin[1], 1. Cancer Research Laboratories, University of Nottingham, U.K., 2. XOMA Corporation, Berkeley, CA 94710.

The cytotoxicity of ricin A chain (RTA) immunotoxins (IT) is influenced by their capacity to be endocytosed following binding to cell surface antigens so allowing internalized RTA to reach the intracellular target organelle. The rate of endocytosis of monoclonal antibody H65 recognising the CD5 antigen on T lymphocyte and T leukemic target cells has been investigated using antibody linked to a conjugate of tetramethylrhodamine isothiocyanate (TRITC) with human serum albumin (H65-HSA-TRITC). These conjugates are constructed so that when bound to target cells the TRITC fluorescence is quenched. Following endocytosis and degradation the TRITC product is released so yielding intracellular fluorescence following UV excitation which can be measured by flow cytofluorimetry (Garnett and Baldwin, 1986). Endocytosis of H65-HSA-TRITC on human lymphocytes and MOLT-4 leukemic target cells initially occurs very rapidly (0-15 minutes) followed by a further continuous uptake of conjugate over 4 hours. This was further investigated using a procedure designed to quantitate endocytosis of ^{125}I-labeled H65 antibody in which cell bound non-endocytosed and endocytosed material is determined. The rate of endocytosis of H65 RTA IT was also determined using this technique and these findings are related to IT cytotoxicity for target cells.

U 203 EFFECT OF INTRAVENOUS GAMMAGLOBULIN ON THE OPSONOPHAGOCYTIC ACTIVITY OF PRETERM SERUM AGAINST COAGULASE-NEGATIVE STAPHYLOCOCCI, Amos Etzioni, Nora Obedeanu, David Merzbach and Avraham Benderly, Depts. of Pediatrics, Clinical Immunology and Microbiology, Rambam Medical Center and Faculty of Medicine, Technion, Haifa, Israel.
Recent reports have described cases of septicemia caused by coagulase-negative staphylococci (CNS) in pre-term neonates, mainly due to the use of artificial intravenous (i.v.(devices. It was interesting to examine if i.v. IgG therapy, known to be effective in group B streptococcal infections in similar patients, also had beneficial activity in CNS infections. Opsonophagocytosis of CNS by normal polymorphonuclear leukocytes (PMNL) in the presence of cord blood serum (CBS) supplemented by the commercial IgG preparation Sandoglobulin (SG) was investigated, using luminol-dependent chemiluminescence (CL). It was found that with two different CNS strains, SG had a concentration dependent enhancing effect on the CL response. This effect was demonstrated in the presence of native as well as inactivated CbS, and of sera from pre-term infants (28-33 weeks). It is concluded that i.v.-SG therapy is effective at clinically attainable concentrations in the treatment of pre-term infants with severe CNS infections.

U 204 ANTI-CD4 AND ANTI-CD8 ANTIBODIES MEDIATE POKEWEED ANTIVIRAL PROTEIN (PAP) INDIRECT IMMUNOTOXIN ACTIVITY. B.M. Goldstein, P.Pedroso, S-A. Johnson, A. Schultz and J.K. Weltman, Rhode Island Hospital-Brown University, Providence, R.I. 02903.
CD4 and CD8 differentiation antigens of T-cell subsets are shown, for the first time, to be potential targets for immunotoxins. Mouse antibodies (0.1-1 μM) against T and B cell membrane markers were incubated with MOLT4 cells ($CD3^-$,$CD4^+$,$CD8^+$) and screened for the ability to mediate activity of an Fab'-PAP disulfide-linked indirect immunotoxin conjugate (Cancer Res. 47, 5552, 1987). Indirect immunotoxin activity is given in the figure, as mean inhibition of protein synthesis (n=5). Monoclonal antibodies against CD4 and CD8 mediated indirect immunotoxin activity ($p<.0001$). These results suggest that immunotoxins against CD4 and CD8 can modulate T cell subset activity.



U 205 TREATMENT OF HUMAN B CELL LYMPHOMA WITH ANTI-CD19 ANTIBODY. A. Hekman, A. Honselaar, J. Sein, M. de Rie*, C. Melief, Ph. Rümke, The Netherlands Cancer Institute and *Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Four patients with B cell non-Hodgkin's lymphoma stage IV were treated with the mouse monoclonal antibody (moab) CLB-CD19 (isotype IgG2a), directed against the B-cell specific antigen CD19 that is not expressed on bone marrow stem cells or plasma cells. The main purpose of this phase I/II clinical trial was to determine pharmacokinetics and toxicity. The first 3 patients were treated with 225 mg moab i.v. in an escalating schedule over 4 consecutive days. Moab in the circulation reached peak levels of 30-70 μg/ml on day 4 and slowly decreased over 10-14 days. The fourth patient received 60 mg twice weekly during 3 weekly during 3 weeks. This resulted in continuous presence of the moab in the serum, lasting until 1 week after the last infusion. Infusion of the moab induced temporary reductions of circulating B cells, as well as antigenic modulation. In one patient circulating lymphocytes remained depressed for several weeks. The antibody penetrated to bone marrow, lymph nodes and lymphoma lesions in the skin, inducing modulation of the antigen. Plasma immunoglobulin levels did not change significantly within 2 months after treatment. There was no serious toxicity and none of the patients developed antibodies against mouse immunoglobulins. Two patients remained in progression, and two showed some tumor regression. On the basis of these results the next group of patients will receive higher doses of antibody over longer periods.

U 206 INTRAVENOUS INFUSIONS OF MOUSE MONOCLONAL ANTIBODIES (MAb 17-1A) USING DIFFERENT DOSAGE SCHEDULES. PLASMAPHARMACOKINETICS, SERUM IMMUNE REACTIVITY, ANTIBODY INDUCTION AND SIDE EFFECTS, C.H. Janson, J-E. Frödin, G. Masucci, H. Wigzell, H. Mellstedt, Karolinska Inst. and Karolinska Hosp., 104 01 Stockholm, Sweden.
In the treatment of solid tumors with naked MAb, a lot of factors have to be taken into consideration. One of these is the dosage schedule of MAb. Max. saturation on the tumor cells should be achieved to obtain optimal lysis. At the most 8% of a given dose reach the tumor, probably less. Thus, in pt with metastatic disease at least 5g, probably 10-15g, should be given. From a pharmacokinetic point of view a sustained plasma level of MAb for a longer time period should be pursued. To meet these requirements, increasing dose intensity schedules of MAb have been used. The half-life of a single infusion of MAb was approx. 22h and non-dose dependent. MAb was detected in serum for one week after 75% of the infusions while after 25% MAb was present for 2 weeks. To maintain a constant level, MAb had to be administered every other day. 400 mg of MAb at each infusion time has been given by this schedule. Total dose administered up to now is 7.6g. The immune reactivity of the pt serum i.e. the ability to bind to the target structures, had a similar half-life as mouse IgG indicating the presence of intact MAb molecules. All pts developed IgG and IgM antibodies. The titers increased by each infusion. IgG anti-mouse antibodies have been detected in serum for more than 1 year. By increasing anti-mouse titers there was a decrease in the max. conc. of MAb but no change in half-life of MAb. Immediately after infusions of MAb, there was a consumption of antibodies probably by immune complex formation. The side effects were usually mild. No signs or symptoms related to immune complex formation were noted. Allergic reactions were seen at 3/150 infusions. After 10-20% of the single infusions diarrhoea, fever, abdominal pains, vomiting/nausea were recorded. When giving the pts infusions every second day GI symptoms were noted after most infusions. In one pt we had to discontinue treatment due to non-tolerable abdominal pains. Intensive MAb therapy can be given on a safe basis. Very high doses of MAb alone on a continuous basis should probably be given with the aim to achieve an optimal therapeutic effect.

U 207 **FIBRONECTIN INCREASES LYMPHOCYTE PROLIFERATION BY MEDIATING ADHESION BETWEEN IMMUNOREACTIVE CELLS.** Hans-G. Klingemann, Shoukat Dedhar, Fred R. Kohn, Gordon L. Phillips. Terry Fox Laboratory, B.C. Cancer Research Center and Division of Hematology, University of British Columbia, Vancouver
Previous studies have shown that fibronectin (FN) is capable of enhancing the alloantigen-induced proliferation of lymphocytes from both normal individuals and patients immunocompromised by bone marrow transplantation*. We asked if this effect is mediated through the adhesive forces of FN which could strengthen the binding between accessory cells (monocytes) and lymphocytes. We looked at the distribution of the FN receptor on mononuclear cells using a polyclonal antibody against the FN receptor (provided by Dr. M. Pierschbacher). Indirect immunofluorescence, flow cytometric analysis and immunoprecipitation studies confirmed the presence of the FN receptor on monocytes and also on T-cells and B-cells. Adhesion studies using FN coated plastic plates demonstrated binding of monocytes and T-cells, but not B-cells to FN. This binding could be inhibited by a polyclonal antibody against FN as well as by Arg-Gly-Asp (RGD) containing peptides known to interfere with binding of FN to its receptor. Also, a polyclonal antibody against FN and the RGD peptide was able to inhibit the FN induced increase in the mixed lymphocyte reaction. Finally, we sought to determine if helper and/or suppressor lymphocytes are preferentially stimulated by FN. Using corresponding monoclonal antibodies and flow cytometric analysis, we found that both helper and suppressor cells proliferate to the same extent in a 6 day MLC. We suggest that FN plays a role in regulating alloantigen induced lymphocyte proliferation by increasing adhesion between immunoreactive cells.
*Klingemann et al. Transplantation 1986, 42:412

U 208 ***DOES TUMOR NECROSIS FACTOR HAVE A ROLE IN ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY?*** *Laura Timares Lebow, & Benjamin Bonavida*
Microbiology and Immunology UCLA School of Medicine, Los Angeles California 90024
Our laboratory has shown that human NK cells can mediate both natural killer (NK) and antibody-dependent cell-mediated cytotoxicity and that these cells secrete natural killer cytotoxic factors (NKCF) when co-cultured with NK sensitive tumor target cells or antibody-sensitized NK resistant tumor target cells. The recent availability of recombinant tumor necrosis factor (rTNF) has prompted us to examine the relationship of this monokine to NKCF and to its relevance in the lytic mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC). We have shown previously that NKCF posses a TNF antigenically related cytotoxic component. Experiments by others demonstrate that NK cells express TNF mRNA and protein. However, the involvement of TNF in the lysis of target cells during NK-CMC remains controversial and has not been examined for ADCC.

The questions addressed specifically in this study are the following: 1.) does the secreted TNF contribute to the cytotoxicity observed during ADCC, 2.) does antibody sensitization induce TNF receptors on NK insensitive ADCC target cells, and 3.) can NKCF or rTNF lyse Ab-sensitized NK resistant target cells? M Ab specific for rTNF were utilized in blocking studies on ADCC reactions. Binding studies were performed with radiolabled rTNF to examine the role of TNF receptors on NK tumor target cells and Ab-sensitized tumor target cells. Long term ^{51}Cr release assays were employed to examine susceptibility to NKCF and rTNF on NK and ADCC target cells. Findings demonstrate that mAb specific for rTNF can partially inhibit ADCC, as has been shown previously for the NK system. However, induction of TNF receptors could not be detected on Ab-sensitized target cells and this correlated with an inability to lyse Ab-sensitized NK-resistant target cells with rTNF or NKCF.

These experiments suggest that TNF or an antigenically related molecule may be involved, at least partially, in ADCC. And, if TNF contributes to the lytic mechanism during ADCC, it must do so in a TNF receptor independent manner.

LTL is a predoctoral trainee supported by USPHS National Institutional Research Service Award CA-09056)

U 209 MACROPHAGE ACTIVATION INDUCED BY A BIOLOGICAL RESPONSE MODIFIER DERIVED FROM SERRATIA MARCESCENS, *C. McCall, *L. Weimer, +P. Campbell, +D. Riches and *R. Urban, *Cell Technology, Inc., Boulder, Colorado, +The National Jewish Center for Immunology and Respiratory Medical Center, Denver, Colorado.

The recently developed biological response modifier ImuVert® consists of an optimal combination of membrane vesicles and ribosomes, both derived from the bacterium Serratia marcescens. When injected intraperitoneally in mice, ImuVert elicits large numbers of macrophages within 48 hours which demonstrate enhanced non-specific and specific phagocytic capacity, and increased ability to kill intracellular Listeria monocytogenes. A single i.p. injection of ImuVert results in a persistent elevation of the number of macrophages in the peritoneal cavity lasting 14-21 days, and cells harvested at 14 days show tumoricidal activity against P815 mastocytoma cells. ImuVert alone is able to activate bone marrow monocytes in-vitro from both LPS sensitive and LPS resistant mice. This fact, and the minimal toxicity of ImuVert observed in several species including man, indicate that endotoxin does not play a significant role in the mechanism of action of this BRM.

U 210 PRODUCTION OF CHIMERIC MOUSE/HUMAN MONOCLONAL ANTIBODIES WITH SPECIFICITY FOR HUMAN B-CELLS. Inger Sandlie, Grete Evensen and Steinar Funderud. The Norwegian Radium Hospital. Oslo. Norway

We have developed a hybridoma cell line (AB-1) producing a monoclonal antibody directed against the CD19 antigen on human B cells. The binding of specific antibody to CD19 leads to a strong inhibition of B cell activation and proliferation. Also, it is well established that murine monoclonal antibodies bound to target cells can mediate lysis of these cells by CDC and ADCC depending on isotype. This makes CD19 specific monoclonal antibodies particularly interesting as potential anti-cancer drugs in the treatment of B cell lymphomas and leukemias.

Murine antibodies elicit a strong immune response in humans after repeated use in vivo, and it is necessary to reduce the immunogenicity of the antibody molecules. We are therefore "humanizing" the antibodies by fusing mouse immunoglobulin variable region genes with human constant region genes. The resulting hybrid genes have been introduced in lymphoid cells by electroporation and functional antibodies have been produced. The hybrid antibodies need to be assessed regarding their specificity for antigen, affinity, immunogenicity, anti-tumor activity and halflife in the human organism.

U 211 REGULATION OF Ia EXPRESSION BY MONONUCLEAR PHAGOCYTES: DIFFERENTIAL EXPRESSION LINKED TO THE Bcg GENE, Bruce S. Zwilling, Linda Vespa, Jyotsna Nath and William Lafuse, The Ohio State University, Columbus, OH 43210.

Macrophages from different strains of mice will express Class II glycoproteins (Ia) of the murine Major Histocompatibility Complex (MHC) differently. The differences are linked to the Bcg gene which maps on chromosome 1. Treatment of peritoneal macrophages from mice that are resistant to Mycobacterium bovis (Strain BCG) (Bcg^r) with recombinant interferon (rIFN)-gamma induces the persistent expression of Ia. In contrast peritoneal macrophages from Bcg^s mice will transiently express Ia. The differential expression can be induced by treating macrophages from Bcg^r mice with different amounts of rIFN-gamma. Thus the induction of the Bcg gene effect requires the presence of a threshold concentration of rIFN-gamma. Differences in expression of Class II mRNA by macrophages from Bcg^r and Bcg^s mice do not account for the differences in Ia expression; nor do differences in the affinity of IFN-gamma receptors. Persistent expression, which does not require the continued presence of rIFN-gamma, is not the result of continued synthesis of Class II glycoprotein as judged by slot blot analysis of mRNA and metabolic labeling with ^{35}S-methionine. Pulse chase experiments indicate that transient expression may be associated with degradation of the Ia glycoprotein. Supported by AI22249.

Mechanisms of Action and Therapeutic Application of Biologicals in Cancer and Immune Deficiency Disorders

Gene Therapy and Bone Marrow Transplantation, Immunodeficiency Disorders, New Biotherapeutic Approaches

U 300 MODULATION OF NK ACTIVITY BY INTERFERON AND IL-2 IN COMMON VARIABLE HYPOGAMMAGLOBULINEMIA, Mario Clerici, Maria Luisa Villa, Marina Mantovani, Claudio Rugarli, Enrico Clerici, Istituto Nazionale dei Tumori and Chairs of Immunology and Medical Pathology 5th, University of Milano, 20133 Milano, Italy

CVH is a heterogeneous immunodeficiency syndrome defined as the loss of the ability to syntesize physiological amounts of immunoglobulins in the absence of other conditions known to cause hypogammaglobulinemia. We studied a 57 years old male patient who 10 years ago suffered an acute encephalitis of viral origin (EBV ?); at that time he had a normal gammaglobulin level. The patient recovered but, after five years, several recurrent Salmonella infections took place and finally CVH was documented. IgG and IgM were 1.5 and 10 mg/100 ml and IgA were undetectable, altough the B cell population was normal. CD4 and CD8 were 23 and 64% and the CD4/CD8 ratio 0.35. The phagocytic function, as defined by the superoxide anion production, was almost absent (*5.2 vs 75.0 nmol $O_2^-/10^6$ PBMC*), and *the NK activity sharply impaired (2.53 vs 17.37 C.U* $/10^5$ PBMC), as compared to those of PBMC from normal blood donors.
NK activity was reversed to almost normal values by incubating 10^6 patient's PBMC overnight at 37°C with 600 U of recombinant IL-2, but not with 1000 U of INF alfa2a (1 hr at 37°C). This suggests that disfunctions of NK cells, which play a pivotal role in controlling viral infections, may be influential in causing CVH following an EBV infection.

Supported by C.N.R. grant n. 87.01236.44.

U 301 AIM-V, A SERUM-FREE MEDIUM FOR ADOPTIVE IMMUNOTHERAPY USING LYMPHOKINE ACTIVATED KILLER (LAK) CELLS, D.A. Epstein, D.J. Weppner, D.W. Jayme, D.H. Boldt*, and F.J. Darfler+, GIBCO Laboratories, University of Texas Health Science Center at San Antonio*, and Cell Enterprises, Inc.+

Use of human serum-supplemented medium for the adoptive immunotherapy of cancer patients is severely limited by the finite supply of histocompatible serum and by the concern over adventitious contaminants. AIM-V, a defined, serum-free medium, has demonstrated efficacy in the ex vivo activation of human lymphocytes used in LAK cell mediated tumor therapy. As measured in a chromium release cytotoxicity assay with cultured Daudi cells or fresh tumor cells as targets, interleukin 2 (IL-2) induction of LAK activity in human lymphocytes incubated in serum-free AIM-V medium or conventional growth medium containing human serum is comparable. All protein components of AIM-V medium are defined and either of recombinant origin or pasteurized to eliminate adventitious viral contaminants. AIM-V medium, packaged in glass bottles or in large volume media bags compatible with automated LAK cell generating systems, has demonstrated a stability convenient for therapeutic applications. Our results also suggest that AIM-V medium may provide flexibility to optimize treatment cost and therapeutic efficacy: (1) by permitting a four-fold reduction in IL-2 requirement (250 U/ml); (2) by allowing increased cell density (1 X 10^7 cells/ml) and potential reduction in the required volume of medium per patient; and (3) by extending the active period of extracorporeal expansion (1-2 weeks).

U 302 ANTIBODY - C3b CONJUGATES: A NOVEL REAGENT FOR IMMUNOTHERAPY, Zvi Fishelson and Yoram Reiter, The Weizmann Institute of Science, Rehovot 76100, Israel.

Killing of tumor cells by autologous complement is a multi-hit process which require extensive activation of the complement system by heterologous antibodies. This is due to regulatory mechanisms which restrict activation of complement, facilitate removal of the membrane attack complex and promote recovery of the cells from partial damage. To overcome this resistance of tumor cells to complement, we constructed conjugates of monoclonal antibodies and of the human complement component C3b (mAb-C3b). Stoichiometric cross-linking was achieved using N-succinimide-3-(2-pyridyldithio) propionate. The mAb-C3b was stable in normal human serum (NHS) and selectively activated the alternative pathway of complement. The conjugated C3b supported better generation of the C3/C5 convertase and was more resistant to cleavage by Factors H and I than free C3b. Employing mAb to the transferrin receptor, mAb-C3b and NHS produced 70-100% mortality of the human *K562 and HL60 tumor cells and of the murine ALB1 lymphoma cells. The mAb or C3b alone* with NHS effected less than 15% tumor cell lysis. By repeatedly treating K562 with mAb-C3b and NHS (3 cycles) we selected for a complement-resistant subline (K562/CR). A comparative analysis has demonstrated that fewer molecules of C3b and of C9 were deposited on K562/CR than on K562. Furthermore, K562/CR exhibited a higher membrane associated proteolytic activity than K562. Our results suggest that mAb-C3b is a potent tool for cancer immunotherapy. However, they also demonstrate that if misused, immunotherapy may lead to propagation of immunoresistant and possibly more aggressive tumor cells.

Mechanisms of Action and Therapeutic Application of Biologicals in Cancer and Immune Deficiency Disorders

U 303 DEVELOPMENT OF RECOMBINANT SOLUBLE CD4 AS A NOVEL HIV ANTIVIRAL

R. Fisher[1], J. Bertonis[1], B. Chao[1], D. Costopoulos[1], T. Liu[1], J. Maraganore[1], W. Meier[1], R. Flavell[1], V. Johnson[2], B. Walker[2], R. Schooley[2].
[1]Biogen Research Corp., Cambridge, MA; [2]Massachusetts General Hospital, Boston, MA

The T-cell surface glycoprotein, CD4, is the membrane-anchored receptor for HIV, and progression of disease in HIV-infected persons is correlated with depletion of CD4+ T-lymphocytes. It is likely that the T-cell depletion, with ensuing immunological compromise, results from recurrent cycles of HIV replication and/or from cell-mediated spread of the virus. This suggests a therapeutic strategy. We propose that recombinant soluble CD4 (rsT4) will act as a soluble virus receptor, and that it might block virus spread in HIV-infected persons. We have shown that rsT4, purified from the conditioned medium of transfected Chinese hamster ovary (CHO) cell lines, blocks HIV replication and HIV-induced cell fusion in vitro (R. Fisher et al Nature in press). This suggests that rsT4 is a potent inhibitor of HIV/CD4 interactions. We are now determining whether rsT4, when given together with other antiretroviral agents (such as AZT), will act synergistically or additively to block HIV infection in vitro.

U 304 TREATMENT OF RECURRENT SQUAMOUS CELL CARCINOMA OF HEAD AND NECK WITH LOW DOSES OF INTERLEUKIN-2 (IL-2) INJECTED PERILYMPHATICALLY. Guido Forni, Giorgio Cortesina, Mirella Giovarelli, Giovanni P.Cavallo, Inst. Microbiol., Univ. of Turin, Turin Italy.

The help strategy is highly relevant to cellular immunology. A few lymphocytes are endowed with the ability to deliver messages that derepress characteristic behaviour programs in other cells. The helper message can be equated to a timely release of a cocktail of appropriate lymphokines. In the light of these considerations, we examined whether low doses of exogenous IL-2 influence the immune recognition of tumor cells. Day hospital patients with recurrent inoperable squamous carcinoma of the head and neck received daily injections of natural IL-2 obtained from the Jurkat T-cell tumor line, purified by high-pressure liquid chromatography. 200 Units of IL-2 in 0.5 ml were injected around the regional draining lymph nodes of the tumor area, and patients were allowed to rest after 10 day courses of therapy. When possible, courses were repeated at 30-day intervals. Local injection of IL-2 around cervical lymph nodes is occasionally followed by minimal swelling and local-regional pain, but not systemic disturbances of any kind, nor any dose-limiting toxicity. In 6/8 patients complete or partial tumor disappearance was shown by direct observation with optical fiber. By contrast, were functional or radical neck dissection had already been performed, IL-2 was always ineffective, as might have been predicted from the absence of any local reservoir of host lymphoid cells.

U 305 LYMPHOKINE ACTIVATED TUMOR INHIBITION IN MICE. Guido Forni, Mirella Giovarelli, Angela Santoni, Andrea Modesti. Inst. Microbiol., Univ. of Turin; Dept. Exp. Med., Univ. of Rome; R. Margherita Children's Hosp., Turin, Italy.

Daily administration at the tumor challenge site of 10 injections of 10 U of IL-2 induces a limited inhibition of the growth of CE-2, a poorly immunogenic chemically-induced tumor. By contrast, complete inhibition is observed when these injections are performed in mice challenged with CE-2 cells admixed at 1:5 cell ratio with lymphocytes from tumor bearing mice. The host immune system play a fundamental role in this lymphokine activated tumor inhibition (LATI). When the host is sublethally irradiated, or the reactivity of L3T4 and Asialo GM1 lymphocytes is suppressed by in vivo antibody treatment, LATI no longer takes place. Pretreatment of recipient mice with Cyclosporine A has the same effect, indicating that lymphokine release plays an important role in the recruitment of host reactivity. The morphological data show that when LATI is taking place the tumor challenge area is infiltrated by mononuclear cells and eosinophils, which establish close contacts with each other and with tumor cells. Tumor draining lymph nodes display expansion of cortical and paracortical areas. Lymphocyte proliferation, interferon-gamma release and cytotoxicity against CE-2 and YAC-1 target cells are greatly enhanced. Lastly, the growth of a second contralateral CE-2 challenge is impaired during or after LATI, showing that an effective systemic reactivity can be quickly induced.

U 306 HTLV II TRANSACTIVATION OF A DIPHTHERIA TOXIN GENE IN ELECTROPORATED RAJI CELLS,
Gail S. Harrison, Françoise Maxwell, L. Michael Glode and Ian H. Maxwell, University of Colorado Health Sciences Center, Denver, Colorado 80262.

Previous work has shown that expression of diphtheria toxin A chain (DT-A) from the cloned coding sequence, linked with appropriate transcriptional regulatory elements, can selectively kill specific cell types (Maxwell et al.: Cancer Res. 46:4660,1986; Palmiter et al.: Cell 50:435,1987; Breitman et al.: Science, in press). We have initiated attempts to direct toxin expression to transformed or infected cells expressing a viral trans-activator. A plasmid designed to express the DT-A gene from the HTLV II LTR was transfected into Raji cells expressing or not expressing the HTLV II tat product (Rosen et.al.: J. Virol. 57:379,1986). Preliminary experiments suggested only a modest increase in toxin expression in the tat II-producing cells, in contrast to a strong stimulation of expression of the cat reporter from an analogous construct (Sodroski et.al.: Science 225:381,1984). However, these results were poorly reproducible owing to problems of low transfection efficiency (using the DEAE-dextran method) and reporter gene expression. We have now used electroporation to transfect Raji cells reproducibly with high efficiency and have also greatly increased the sensitivity of our transient assays by using a luciferase coding sequence in the reporter plasmid (de Wet et al.: Mol.Cell.Biol. 7: 725, 1987). Using these improved methods, we have detected significant expression of both DT-A and luciferase from the HTLV II LTR in tat$^-$ Raji cells. Results will be presented on comparative expression levels in tat$^-$ and tat$^+$ cells and on attempts to block expression in tat$^-$ cells by placing an oligomer of the SV40 early polyadenylation signal upstream of the LTR in the expression plasmids (in order to truncate putative plasmid-initiated transcripts (Kadesch & Berg: Mol.Cell.Biol. 6: 2593, 1986). If toxin gene expression can be made stringently dependent on specific trans-activation mechanisms, this may eventually find therapeutic applications in eliminating virus-infected cells before production of viral progeny.

U 307 CYTALLENE AND ADENALLENE, ACYCLIC NUCLEOSIDE DERIVATIVES LACKING AN OXACYCLOPENTENE: IN VITRO INHIBITORS OF REPLICATION AND CYTOPATHIC EFFECT OF HUMAN IMMUNODEFICIENCY VIRUS.
Seiji Hayashi, S Phadtare, J Zemlicka, M Matsukura, H Mitsuya, S Broder. National Cancer Institute, Bethesda, MD; Michigan Cancer Foundation, Detroit, MI.

A cytopathic human retrovirus, human immunodeficiency virus (HIV) has been established as the etiologic agent in the pathogenesis of AIDS and related disorders. While several antiretroviral compounds are already known (Mitsuya & Broder, Nature 325:773,1987), very few if any acyclic nucleoside analogues have been reported to exert significant inhibition against HIV in vitro. We tested numbers of acyclic nucleoside derivatives for anti-HIV activity in vitro, and found that two compounds, adenallene [$HOCH_2$-CH=C=CH-adenine: N^9(4'-hydroxybuta-1',2'-dienyl)-adenine] and cytallene [$HOCH_2$-CH=C=CH-cytosine: N^1-(4'-hydroxybuta-1',2'-dienyl)-cytosine] protect various $CD4^+$ T cell lines from the infectivity and cytopathic effect of HIV-1 at concentrations that do not affect functions of normal T-lymphocytes in vitro. The addition of these compounds to susceptible T-lymphocytes (ATH8) in culture following exposure to HIV-1 resulted in a substantial decrease in the amount of viral DNA synthesized in the cells. These compounds also inhibited the in vitro infectivity of another human retrovirus, HIV-2. The other acyclic nucleosides having a four-carbon chain but not two double bonds and 4'-chloro-4'-dehydroxyl-adenallene showed much less or no anti-HIV activity. These data suggest that the two double bonds in the four-carbon chain and 4'-hydroxyl group are critical for anti-HIV activity. Our observations provide new structure/activity relationships for acyclic nucleosides, and may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases.

U 308 Phosphorothioate Analogs of Oligodeoxynucleotide Inhibit Viral Replication of HIV (Human Immunodeficiency Virus): Inhibition of de novo Infection in Un-Infected Cells and Regulation of Viral Expression in Chronically Infected Cells.
Makoto Matsukura[1] Kazuo Shinozuka[1], Gerald Zon[2], Hiroaki Mitsuya[1], Flossie Wong-Staal[1], Jack S. Cohen[1], and Samuel Broder[1] 1) National Cancer Institute, Bethesda, MD 20892 2) Applied Biosystems, Inc., Foster City, CA 94404.

Evaluations of the anti-HIV activity of nuclease-resistant phosphorothioate analogs of oligodeoxynucleotides against HIV were performed in two different systems. In the first system, we tested the capacity of these agents to block HIV replication and cytopathic effect against uninfected target T-cells. We observed that certain phosphorothioate analogs exerted potent anti-HIV effects. These effects did not require a specific ordered sequence complementary to the genes of HIV. Even phosphorothioate homo-oligomers such as oligodeoxycytidine phosphorothioate were protective against the viral cytopathic effect. In the second system, we tested whether such agents could inhibit viral expression in chronically infected cells. We found that certain phosphorothioate analogs could innibit viral expression in such cells, but in this case, an antisense configuration was necessary. An antisense construct against art/trs (a regulatory gene essential for efficient HIV expression) showed strong inhibitory effects against viral protein production (p24 gag antigen) assessed by an antigen capture ELISA assay, but random sequences or non-sense configurations including homo-oligomer failed to inhibit viral expression under these conditions. Thus, phosphorothioate oligomers can show potent anti-HIV activity and could have at least two different mechanisms of inhibiting the life cycle of HIV.

U 309 DEXTRAN SULFATE SUPPRESSION OF VIRUSES IN THE HIV FAMILY: INHIBITION OF VIRION BINDING TO CD4$^+$ CELLS. Hiroaki Mitsuya[1], DJ Looney[2], S Kuno[3], R Ueno[3], F Wong-Staal[2], and S Broder[1]. Clinical Oncology Program[1], Laboratory of Tumor Cell Biology[2], National Cancer Institute, Bethesda, MD; Ueno Fine Chemicals Industry Ltd., Itami, Japan[3]. A cytopathic human retrovirus termed human immunodeficiency virus (HIV) infects CD4$^+$ cells including helper T-lymphocytes, certain B-lymphocytes and macrophages and causes various pathogenic changes in such immunocompetent cells. The first step in the infection of human T-lymphocytes by this virus is attachment to the target cell receptor, which consists of the CD4 binding domains as essential components. This step might be vulnerable to attack by antibodies, chemicals, or small peptides. Dextran sulfate, a long chain polymer of glucose (M.W.~8,000) containing 17-20% sulfur which has been given orally to human beings for more than two decades as anticoagulant or anti-lipemic agent, was found to block the binding of HIV-1 virions to various target T-lymphocytes, inhibit syncytia formation, and exert a potent inhibitory effect against HIV-1 in vitro at concentrations that are likely to be clinically attainable in human beings. In our experiment, dextran sulfate at $\geq$10 µg/ml completely inhibited the binding of ^{3}H-uridine-labeled HIV-1 to CD4$^+$ T-lymphocytes. We have also observed that combinations of dextran sulfate and certain DNA chain-terminating dideoxynucleosides including 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxycytidine suppress HIV-1 replication better than each drug does alone in vitro. This drug also suppressed the in vitro replication of another human pathogenic retrovirus, HIV-2. These observations could have theoretical and clinical implications in strategies to develop new anti-HIV drugs.

U 310 MEDULLOPROTECTIVE EFFECT OF CHOLINE LACTATE IN MEDULLODEPRESSION INDUCED WITH RADIATION, Nistor C.C., Nistor C., Uray Z., Makkay Klara, Mihail N., Cserni Judita*, Cserni S.**, Szabo Susana, Alexei Viorica, Nistor Vectoria, Alexei Cezara, Bldean Ana, Alexei D., Univ. Cluj Napoca, Policilinic nr 2 Timisoara*, Chem.Ind. Solventul Timisoara**, Romania.
One of the factors affecting the success of oncological radiotherapy is medullosuppression induced by radiotherapy. In order to discover an erythroprotective and hepatoprotective adjuvant in radiotherapy of cancer, we tested the effect of choline lactate (CL) on Wistar rats irradiated with 350 rad. The CL was given 3 days before and 5 days after irradiation, 20 mg CL/kg b.w. The animals were sacrificed day 15. Three hours before the sacrifice, they were injected i.p. with 1µ Ci ^{59}Fe citrate, and the medullar, splenic and hepatic captation of radioiron was measured, and compared with normal unirradiated rats (M), to normal rats treated d. 1-3 and d. 5-9 with daily 20 mg CL/kg (MCL), and to irradiated rats which did not receive any treatment (I). The radioincorporation of ^{59}Fe citrate in hematopoietic system: M (872 $\pm$ 115 cpm); I (1016 $\pm$ 116 cpm); MCL (880 $\pm$ 115 cpm); CL (1160 $\pm$ 156 cpm). In MCL and CL radiocaptation of radioiron was unmodified. A compensatory restoration of erythropoiesis was observed by elevated values of medullar captation. In CL, the restoration of erythropoiesis is very big in comparison with I. Our experimental results recommend the use of CL as an adjuvant in antitumoral radiotherapy.

U 311 SPECIFIC ACTIVE IMMUNOTHERAPY WITH VACCINIA MELANOMA ONCOLYSATE (VMO): EXTENSION OF DISEASE-FREE SURVIVAL, Marc K. Wallack, Jerry A. Bash and Alfred A. Bartolucci, Mount Sinai Medical Center, Miami Beach, FL 33140 and The University of Alabama, Birmingham, AL.

Specific active immunotherapy is based upon the injection of "vaccines" containing tumor-associated antigens (TAA) which are sufficiently immunogenic in the host to elicit an effective response against residual or incipient tumor. An oncolysate prepared from pooled vaccinia-infected allogeneic melanoma cell lines (VMO) was used to treat 39 early stage melanoma patients. Each patient received a smallpox booster, followed one week later by the first of 13 weekly intradermal injections of 2.0 mg VMO. Subsequent injections given at biweekly intervals were continued for one year or until recurrence. Treatment was tolerated with minimal toxicity, with less than 5% of reactions in the grade 3 or 4 category, and none severe enough to interrupt therapy. The study was initiated in 12/84. At the most recent follow-up (12/87) 16 patients remain with no evidence of disease. Twenty-one patients have recurred and 13 of these have died. Median survival was 24 months. Comparison of disease-free survival of VMO treated patients in this study with matched historical controls showed a significant advantage of VMO treatment ($p = 0.04$) at 12 months. A multi-institutional prospective randomized study comparing VMO treatment with treatment using vaccinia alone is currently being initiated in an attempt to confirm this result.

U 312 RECEPTOR BINDING REQUIREMENTS FOR IL-2-TOXIN MEDIATED CYTOTOXICITY. C. Waters, P.Schimke*, R.Sanderson, T.Strom§, & J.Murphy*. Seragen, Inc., Hopkinton MA 01748, §Beth Israel Hospital, Boston MA 02215, and *University Hospital, Boston MA 02118.

We have recently described the genetic construction and properties of a chimeric toxin in which the receptor binding domain of diphtheria toxin was replaced with interleukin-2 (IL-2). The fusion protein has been designated IL-2-toxin. We report here studies on the nature of the IL-2-toxin: IL-2 receptor (IL-2R) interaction required for productive entry of the ADP-ribosyl transferase component of IL-2-toxin into target cells. Using cell lines bearing native and structural variants of the IL-2R, we have found that high affinity IL-2R are obligatory for the entry of the ADP-ribosyl transferase into the cytosol. In p55+p75+ cells, picomolar quantities of IL-2-toxin are sufficient to inhibit protein synthesis by 50% (IC50≅100pM). Cells which express only the p55 or p75 subunit of the IL-2R are resistant to IL-2-toxin. Resistance does not appear to be related to a less affinate interaction with the IL-2R since preliminary Scatchard analysis suggests that the binding constant for IL-2-toxin is not significantly different from that of native IL-2 itself. Preliminary data also suggest that IL-2-toxin may internalize with rates comparable to those reported for IL-2. These studies suggest that the route of IL-2-toxin bound to the p75 subunit of the IL-2R may differ from that of IL-2-toxin bound to high affinity IL-2R.