CYTOKINES AND THEIR RECEPTORS: FROM CLONAL TO CLINICAL INVESTIGATION

Organizers: Steven Gillis and James Watson

April 1-7, 1991

Plenary Sessions Page

Poster Sessions

Cytokines and Their Receptors: From Clonal to Clinical Investigation

Cytokine Regulation of Early Events in Hematopoiesis - I

P 001 CYTOKINE REGULATION OF PROLIFERATION OF HEMOPOIETIC STEM CELLS IN VITRO, Makio Ogawa, VA Medical Center and Department of Medicine, Medical University of South Carolina, Charleston, SC.

Studies using clonal cell culture methods indicate that hemopoietic proliferation is controlled by a cascade of growth factors, each directed at specific stages of hemopoietic development. In this model, several factors appear to regulate the proliferation of hemopoietic progenitors stem cells which, in the steady state, are dormant in the cell cycle state. Earlier, interleukin-3 (IL-3) and IL-4 were found to support proliferation of multipotential progenitors. Studies using a blast cell colony assay developed in our laboratory indicated that these factors support the proliferation of multipotential progenitors but do not trigger their exit from G_0. Subsequent studies in our laboratory identified several factors that appear to shorten the dormancy (G_0) period of early hemopoietic progenitors, including IL-6, granulocyte colony-stimulating factor (G-CSF), IL-11 and the ligand for c-kit. We have now used stem cell enrichment techniques in order to study the effects of these cytokines on dormant hemopoietic progenitors. Both murine and human early progenitors were enriched by combinations of techniques such as density gradient separation, panning, immunomagnetic bead cell separation and sorting. Studies using the highly enriched dormant progenitors indicate that entry into cell cycle of the hemopoietic stem cells appears to be dependent on these synergistic factors.

Cytokine Regulation of Early Events in Hematopoiesis - II

P 002 CHARACTERISTICS OF THE HEMATOPOIETIC STEM CELL COMPARTMENT OF ADULT MICE, Gerald J. Spangrude, Laboratory of Persistent Viral Diseases, NIAID, NIH, Rocky Mountain Laboratories, Hamilton, MT 59840

The process of hematopoiesis involves interactions between stromal elements in hematopoietic organs and a variety of multipotent hematopoietic stem cells. The most primitive of these stem cells must be capable of providing progenitors for all hematopoietic lineages over extended periods of time, leading to the suggestion that the stem cells must self-renew as well as differentiate. If, however, a heterogeneous stem cell compartment is responsible for maintaining hematopoiesis, the self-renewal aspect may not be apparent at a single cell level but may be a reflection of a limited capacity for proliferation and differentiation prior to the loss of multipotentiality. Using monoclonal antibodies and flow cytometry, a rare population of mouse bone marrow cells can be isolated which possesses many characteristics of hematopoietic stem cells. This population of cells can be subdivided into "resting" and "activated" subsets based on uptake of the fluorescent vital dye rhodamine 123. Injection of these populations of cells into thymic lobes of irradiated animals allows colonies of progeny to develop which are derived from individual cells. Although this development occurs in the thymus, which is thought to be a specific microenvironment for promoting T lymphocyte development, most of the thymic colonies contain T lymphocytes as well as neutrophils which are derived from the injected stem cells. This is true for both the resting and the activated populations of stem cells. In contrast, long-term repopulation of irradiated mice is mediated only by the resting population. These results argue that the multipotent characteristic of hematopoietic stem cells can be separated from the self-renewal characteristic, and suggest that self-renewal may reflect the number of cell divisions that a stem cell can undergo prior to the loss of multipotency.

P 003 SOLUBLE CYTOKINE RECEPTORS AS IMMUNE RESPONSE MODIFIERS.
M.B. Widmer, K.H. Grabstein, C.A. Jacobs, D.H. Lynch, C.R. Maliszewski, K. M. Mohler, W.C. Fanslow. Immunex Corp. Seattle, WA 98101.
Molecules comprising the extracellular portions of the cell surface receptors for IL-1, IL-4, IL-7 and TNF have been isolated and purified from the supernatants of cell lines expressing cDNAs encoding them. Such soluble cytokine receptors behave functionally as cytokine antagonists, as they retain ligand binding acvtivity and can compete for ligand binding to the the receptors expressed on the surface of cytokine-responsive cells. We have used these molecules to probe the involvement of the corresponding cytokines in a series of in vitro and in vivo models of immune and inflammatory responses. The data to be presented indicate that soluble cytokine receptors are effective inhibitors of the biological activities of cytokines and may be used to modify immune responses.

Cytokines Involved with Lymphocyte Regulation and Activation - I

P 004 IN VIVO ADMINISTRATION OF RECOMBINANT HUMAN INTERLEUKIN-4 TO PATIENTS WITH CANCER. Michael T. Lotze, M.D., Departments of Surgery, Molecular Genetics and Biochemistry. University of Pittsburgh, 497 Scaife Hall, Pittsburgh, PA 15261

Interleukin-4 (IL-4) is one of three described T-cell growth factors supporting the growth and expansion of human T-cells. Recombinant yeast derived IL-4 has been administered alone or in conjunction with IL-2 to 73 cancer patients in 84 treatment courses in the Surgery Branch of the NCI. The maximal tolerated dose of IL-4 administered thrice daily appears to be 20 ug/kg and in conjunction with IL-2 at 7.2 x 10^5 IU/kg to be 6-20 ug/kg. IL-4 administration is also associated with the development of a vascular leak syndrome with end organ dysfunction (kidney) presumably secondary to edema similar to that observed with IL-2. The weight gain observed with IL-2 or IL-4 administration alone appears to be increased when both of these cytokines are given together. (Median weight gain 8.6 - 9.1% with high dose IL-4 alone, 7.5% with high dose IL-2 alone and 15.2% with IL-4 and IL-2). Unusual side effects occurring prominently during IL-4 treatment include mild to severe nasal congestion and endoscopically proven gastritis and antral gastric ulceration (12 episodes). We observed no responses (partial or complete) in 48 patients receiving IL-4 alone during 56 treatment courses including 23 patients with melanoma or renal cell cancer previously untreated with IL-2. Five patients in this group with stable disease were retreated safely, and received 4 courses (one patient), 3 courses (1 patients) and two courses (3 patients) of therapy. Responses have been noted in patients previously failing IL-4 on subsequent IL-2 based regimens at pulmonary, hepatic, nodal, intramuscular, cutaneous, and mediastinal sites in patients with breast cancer, melanoma and renal cell cancer. Further studies will evaluate the role of tumor infiltrating lymphocytes (with or without gene marking with the neomycin resistance gene) given in conjunction with IL-2 and IL-4. Studies also evaluating intensive treatment with IL-2 or IL-2 and alpha interferon prior to high dose IL-2 and IL-4 treatment are being planned.

For the Cytokines and Their Receptors: From Clonal To Clinical Investigation.

P 005 REGULATION OF SIGNAL TRANSDUCTION IN THE IL-2 SYSTEM, Tadatsugu Taniguchi*, Masanori Hatakeyama*, Takeshi Kono*, Naoki Kobayashi*, Atsuo Kawahara*, Steven D. Levin§, Roger M. Perlmutter§, *Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamadaoka, Suita-shi, Osaka, 565, Japan, §Department of Immunology, University of Washington, Seattle, Washington 98195, USA.
Interleukin-2(IL-2) is one of the first identified lymphokines and plays a major role in the clonal expansion of T lymphocytes (T cells) by interacting with specific cell surface receptor(IL-2 receptor). To understand the molecular mechanism of IL-2 mediated T cell growth, elucidation of the structure as well as the expression mechanism of IL-2 and its receptor complex are essential. The functional, high-affinity form of interleukin-2 receptor(IL-2) is composed of two receptor components, IL-2Rα(p55) and IL-2Rβ(p70-75) chains. We have recently cloned the human and murine IL-2Rβ cDNAs. Unlike the IL-2Rα chain, the IL-2Rβ chain contains a large cytoplasmic domain which shows no obvious tyrosine kinase motif. The IL-2Rβ is structurally related to many of the newly discovered cytokine receptors, particularly the erythopoietin, IL-3, IL-4 receptors. More recently, we established a system in which the cDNA-directed human IL-2Rβ allows growth signal transduction in a murine IL-3-dependent cell lines. In this system, the IL-2-mediated cell proliferation signal(s) seems to be derived primarily via the high-affinity IL-2R which consists of the endogenous mouse IL-2Rα and the cDNA-directed human IL-2Rβ. In fact, the presence of antibodies specific to both IL-2 chains efficiently inhibited the IL-2-mediated cell growth. Utilizing this system, we have identified a critical cytoplasmic region for the growth signal transduction. Furthermore, we have provided evidence for the physical association of IL-2Rβ with 56^{lck}. Using the transient cDNA expression system, we have also identified the regions responsible for such interaction between the two molecules. The functional significance of such association may be profound in understanding the regulatory mechanism of cytokine-induced signal transduction.

P 006 MOUSE INTERLEUKIN-2 MUTANT PROTEINS WITH DEFECTS IN INTERACTION WITH SPECIFIC SUBUNITS OF THE RECEPTOR COMPLEX. Gerard Zurawski, Sandra M. Zurawski, Ellen Doyle and Jean-luc Imler. Department of Molecular Biology, DNAX Research Institute, 901 California Ave., Palo Alto, California 94304-1104.
Substitution analysis of the 149 residue mouse interleukin-2 (mIL-2) protein has revealed a subset of residues that are critical for interactions with specific subunits of the receptor complex (IL-2R). Changes at several residues resulted in proteins that interacted normally with intermediate affinity receptors (composed of the β chain and a component or components called γ) yet failed to bind to the α chain. Many IL-2-responsive cell types have high affinity (αβγ-type) IL-2R. On most such cell types, α-defective proteins gave reduced biological activities. However, α-defective proteins were normally active on some cell types with intermediate affinity (βγ-type) IL-2R. Most changes at Asp34 resulted in proteins with deficiencies of interaction with αβγ, αβ, and βγ-type IL-2R, but with normal binding to the α chain. Such β-defective proteins gave greatly reduced biological activities in most IL-2 assays examined. All substitutions at Gln141 resulted in proteins with deficiencies of interaction with αβγ and βγ-type IL-2R, but with normal binding to α and αβ-type IL-2R. Defective binding to αβγ and βγ-type IL-2R of such proteins appeared to result from increased off-rate. Many such γ-defective proteins had partial agonist (reduced maximal responses) activities. The degree of reduction in maximal response by these proteins varied widely between responding cell types. In several IL-2 assay systems, some γ-defective proteins were effective antagonists. The potential usefulness of this class of IL-2 antagonist for immune suppression can be examined in mouse models. Moreover, α, β, and γ-defective mIL-2 proteins could function as selective agonists. The mouse will be a useful model to examine the possible utility of such classes of agonists for selective activation of antitumor responses.

Cytokines Involved with Lymphocyte Regulation and Activation - II

P 007 CYTOKINE REGULATION OF IMMUNOGLOBULIN ISOTYPE SELECTION *IN VIVO*. Fred D. Finkelman*, Joseph F. Urban, Jr.†, Antonela Svetic@, William Gause@, Ian Gresser#, Joanne M. Holmes*, Paul Trotta$, William E. Paul§, and Ildy M. Katona*•. Depts of *Medicine, @Microbiology, and •Pediatrics, USUHS, Bethesda, MD, 20814; †Helminthic Diseases Lab, U.S. Dept of Agriculture, Beltsville, MD, 20705; #Lab of Viral Oncology, ER CNRS, Villejuif Cedex, France; $Schering Research, Schering-Plough Corporation, Bloomfield, NJ 07003; §Lab of Immunology, NIAID, NIH, Bethesda, MD, 20892. At least three cytokines, IL-4, IFN-α, and IFN-γ, play major roles in the regulation of murine Ig isotype selection *in vivo*. IL-4 is required for the generation of primary IgE responses. Anti-IL-4 mAb and anti-IL-4 receptor (R) mAb each completely inhibits anti-IgD antibody and nematode parasite-induced primary IgE responses. Secondary TNP-KLH- and nematode-induced IgE responses are also completely inhibited by these anti-IL-4 and anti-IL-4R mAbs. However, a secondary IgE response induced by injecting anti-IgD antibody-primed mice with anti-mouse IgE antibody is not convincingly inhibited by even a combination of anti-IL-4 and anti-IL-4R mAbs. Since this latter response is presumably derived from B cells that have already switched to IgE expression, IL-4 appears not to be required for IgE-committed B cells to differentiate into IgE secreting cells. Thus, the IL-4 requirement for the generation of secondary IgE responses to defined antigens and parasites suggests that these responses are derived from B cells that are not initially committed to IgE expression. IFN-γ and IFN-α have inhibitory effects on the generation of IgE responses *in vivo*. Either cytokine, injected into anti-IgD antibody-immunized mice, will inhibit IgE secretion, while antibodies against either cytokine can enhance *in vivo* IgE responses. Both IFN-α and IFN-γ exert direct inhibitory effects on B cell differentiation into IgE secreting cells *in vitro*. In addition, IFN-α indirectly inhibits IgE responses *in vivo* by diminishing steady-state levels of IL-4 mRNA in anti-IgD-treated mice. This effect of IFN-α is selective, since IFN-α has no effect on IFN-γ mRNA levels in these mice. IL-4, IFN-γ, and IFN-α also affect the production *in vivo* of Ig isotypes other than IgE: IL-4 can enhance IgG1 production and inhibit IgG2a production, while IFN-γ can have the opposite effect. IFN-α can enhance IgG2a production but has no independent effect on IgG1 responses. IL-4 and IFN effects on the production of these isotypes, however, vary from system to system and are always less marked than their effects on the IgE response. Thus, IgE responses can be selectively suppressed by agents that neutralize IL-4, block the IL-4R, downregulate IL-4 mRNA levels, or directly inhibit IL-4 effects at the B cell level.

P 008 Multiple Forms of the Human IL-7 Receptor, L. S. Park, W. C. Fanslow, D. J. Friend, C. M. Pleiman, R. G. Goodwin, S. F. Ziegler and R. J. Armitage, Immunex Corporation, Seattle, WA 98101. Human IL-7 exhibits biological effects at different stages of both B-cell and T-cell lymphopoiesis through interaction with specific cell-surface receptors. Cloning of the human IL-7 receptor (IL-7R) revealed that in addition to a membrane-bound form, cDNA clones for a putative secreted form of the receptor existed. Analysis of IL-7R gene structure has revealed that this soluble form is generated by differential splicing which deletes exon 6, containing the transmembrane domain. mRNA for the soluble form is found in a number of cell types, however it is generally present at much lower levels than mRNA for the membrane-bound form. Analysis of culture supernatants from the human fibroblast line WI-26, which has particularly high levels of mRNA for the secreted form, revealed the presence of soluble IL-7R protein capable of binding IL-7 with high affinity. Current biological studies of the effects of the soluble form of the IL-7R will be discussed. Analysis of binding of radiolabeled and biotinylated IL-7 to a range of human primary cells and cell lines indicated heterogeneity may also exist in membrane-bound forms of the IL-7R. Some cell types exhibit biphasic IL-7 binding with both high and low affinity sites, while others express high levels of low affinity sites only. Further analysis revealed that the presence of mRNA for the previously cloned IL-7R correlated with the presence of high affinity binding sites, and was lower or absent in cell types exhibiting only low affinity binding. In addition, monoclonal antibodies raised against the cloned IL-7R protein failed to recognize cell surface molecules on cells which bind IL-7 with only low affinity. These data indicate that in addition to membrane bound and soluble forms of the IL-7R derived from a single gene by differential splicing, a distinct low affinity IL-7 binding protein also exists.

P 009 MOLECULAR CHARACTERIZATION OF THE TYPE II IL-1 RECEPTOR. J. E. Sims, C. J. McMahan, J. Slack, B. Mosley, S. Lupton, D. J. McKean,## N. A. Jenkins,+ N. A. Copeland,+ K. Huebner,++ D. Benjamin,# M. K. Spriggs, D. Cosman, and S. K. Dower. Immunex Research and Development Corporation, Seattle, WA 98101. ##Mayo Clinic, Rochester, MN 55905. +NCI, Frederick Cancer Research and Development Center, Frederick, MD 21701. ++Fels Institute for Cancer and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140. #University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

A novel receptor for interleukin 1 has been cloned from a human B lymphoblastoid cell line, using a direct expression method which is substantially improved over that used previously to clone the type I IL-1 receptor (Sims et al., Science 241, 585, 1988). Like the type I receptor, the extracellular portion of the type II receptor possesses three immunoglobulin-like domains, although there is only 28% amino acid identity between the two receptors in this portion. The type I and type II receptors map to the same chromosomal location in both mouse and man. Soluble versions of the type I and type II receptors probably bind the same region of IL-1, as evidenced by complete competition of the two receptors for ligand binding. In contrast to the 213 amino acid cytoplasmic portion of the human type I IL-1R, the human type II IL-1R has a cytoplasmic domain of only 29 amino acids. Overall, the human type II receptor is predicted to be 384 amino acids after signal peptide cleavage; with 5 potential sites for N-linked glycosylation, the molecular weight would be expected to be around 57kd, similar to the 60 - 68kd size estimated for cross-linked receptor from various B cell lines as well as from cells transfected with the recombinant receptor plasmid. Northern blot analysis of mRNA expression indicates that while some cells express exclusively type I or type II IL-1R mRNA, most cell lines and tissues examined express both. Type II IL-1R mRNA can be strongly induced in some cell types, including human peripheral blood T cells and mouse type 2 helper T cell clones.

Cytokines in Myelopoiesis - I

P 010 PERSPECTIVE ON ERYTHROPOIETIN CLINICAL RESEARCH, John W. Adamson, M.D., New York Blood Center, New York, NY 10021

Recombinant human erythropoietin (rHuEpo) has proved to be extremely successful in correcting the anemia of patients with end-stage renal disease (ESRD). Since the kidney is the organ primarily responsible for Epo production, and anemia is an almost constant consequence of ESRD, it is not surprising that this group of patients was the first targeted for rHuEpo treatment. Treatment in this group of patients represents a form of hormone replacement therapy and the response rate approaches 100%. However, the effectiveness of rHuEpo therapy is now being demonstrated for a number of other indications including the anemia associated with AIDS (with or without AZT therapy), in increasing the amount of predeposited blood available for autologous use in surgery, for the anemia of prematurity, for the anemia associated with malignancies (both hematological and non-hematological), and to augment fetal hemoglobin synthesis in patients with hemoglobinopathies. As wider indications for rHuEpo therapy are found, the response has become less predictable. Consequently, it will be important to develop tests which predict early response to rHuEpo in order to minimize time and cost of the use of the drug. As a model for prediction of early marrow response to rHuEpo, we have employed measurements of acute changes in plasma levels of transferrin receptor protein (TRP). Circulating levels of TRP correlate directly with total erythropoiesis. Within a week of beginning rHuEpo therapy in patients with ESRD, changes of as much as 300% in plasma levels of TRP have been observed, before any change in hematocrit would be evident. We are applying this test to the acute response in anemic patients with myelodysplasia, a group of patients who could clearly benefit from rHuEpo therapy but whose initial response rate is estimated to be 20-25%. Should this test proved to be predictive of an effective response to rHuEpo, its wide application would promote the use of rHuEpo by allowing accurate clinical decisions regarding the effectiveness of the drug and appropriate dosing.

P 011 THE LEUKEMIA INHIBITORY FACTOR, Nicos A. Nicola, The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

Leukemia Inhibitory Factor (LIF) was first purified and cloned on the basis of its capacity to induce terminal differentiation of the murine myelomonocytic leukemic cell line, Ml. It is a glycoprotein of Mr 22,000 with three disulfide bonds and extensive sites of N-glycosylation. Unlike some other differentiation inducers, LIF has no direct colony-stimulating activity on hemopoietic progenitor cells though small numbers of LIF receptors are detected on monocytic cells, megakaryocytes and a small subset of lymphoid-like cells. However, it has been recently shown that LIF, like another differentiation-inducer, IL-6, synergizes with IL-3 in stimulating megakaryocyte colony formation in vitro and stimulates platelet production in vivo. The parallels with IL-6 action are further reinforced by their common action in inducing acute-phase responses in liver hepatocytes but LIF has additional actions that distinguish it from IL-6 including the stimulation of osteoblasts to increase bone turnover, the induction of neurotransmitter switching in certain neurones, the inhibition of lipoprotein lipase activity and, paradoxically,the inhibition of differentiation of embryonic stem cells. These pleitropic actions of LIF all appear to be mediated directly through interaction with a common high affinity LIF receptor on these cells. There is evidence also for a low affinity LIF receptor and that this form can be converted to a high affinity receptor. It remains to be resolved how LIF mediates its very different biological effects on different cells through common receptors and how its production and accessibility to different organs might be regulated.

Cytokines in Myelopoiesis - II

P 012 MOLECULAR ANALYSIS OF THE IL-3 AND GM-CSF RECEPTORS, A. Miyajima[1], T. Kitamura[1], K. Hayashida[1], N. Itoh[1], J. Schreurs[1], D. Gorman[1], H.-M.Wang[1], T. Ogorochi[1], S. Yonehara[2], I. Yahara[2], T.Yokota[3] and K. Arai[3], [1] DNAX Research Institute, Palo Alto, CA 94304. [2] Tokyo Metropolitan Insititute of Medical Sciences, Tokyo, Japan. [3] Institute of Medical Science, University of Tokyo, Tokyo, Japan.

IL-3 and GM-CSF stimulate the development of various lineages of hemopoietic cells. By using the anti-Aic2 antibody, which recognizes the mouse IL-3 receptor, we isolated two kinds of mouse cDNA, AIC2A and AIC2B. The AIC2A cDNA encodes a mature protein of 120 kDa which binds IL-3 with low affinity when expressed on fibroblasts. The AIC2A protein has common structural features of the recently identified cytokine receptor family. Although IL-3 rapidly induces protein tyrosine phosphorylation, AIC2A has no consensus sequence for a protein kinase. Purification of the high affinity IL-3 receptor has revealed that the AIC2A protein is a component of the high affinty IL-3 receptor. These results indicate that the functional high affinity IL-3 receptor is composed of multiple subunits. The second cDNA, AIC2B, is 95% identical to AIC2A at the nucleotide level, yet the AIC2B protein does not bind any cytokines including IL-3 and GM-CSF. The AIC2B protein retains the common structural features of the cytokine receptors and is coexpressed with AIC2A in various hemopoietic cells. We isolated a human cDNA, KH97, which is homologous to mouse AIC2 genes. The KH97 protein by itself did not bind any cytokines including IL-3 and GM-CSF. Interestingly, however, it conferred high affinity GM-CSF binding when coexpressed with the 80 kDa low affinity human GM-CSF receptor. The KH97 protein was required not only for the formation of high affinity receptor but also for signal transduction. Reconstitution of a high affinity receptor for human GM-CSF has suggested that either AIC2A or AIC2B, or both may be involved in the formation of a high affinity receptor for mouse GM-CSF.

P 013 A NOVEL PROTEIN INHIBITOR OF IL-1 AND TNF PRODUCTION BY HUMAN BLOOD CELLS, P. Ralph, I. Nakoinz, A. Sampson-Johannes, S. Fong, M. Doyle, D. Lowe, K. Elrod, and L. Lin, Departments of Cell Biology, Immunology, Fermentation Development and Protein Chemistry, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608

Human body fluids and cell lines were screened for inhibitors of cytokine production. Blood mononuclear cells and monocytes were induced with 0.5 ng/ml LPS for IL-1b, and 10 ng/ml LPS plus 100 U/ml IFNγ for TNF production. Day 1 supernatants were assayed by specific ELISAs. T cell lines HSB and Mo spontaneously secreted an activity that inhibited in these assays. PMA or PHA stimulation of the cell lines did not increase the titers of the factor. The activity was stable for months at 4°C but was sensitive to boiling. It had the properties of a 35 kd protein which bound to an anion exchange column above pH 8 and could be eluted with salt or decreasing pH. It did not bind to Con A or WGA lectin columns. The activity was not associated with TGFβ and was not inhibited by a neutralizing antibody to IL-4. Some of these properties are similar to the recently described murine IL-10/CSIF that inhibits T cell cytokine production by an action on antigen-presenting macrophages (Fiorentino et al., J. Exp. Med. 170: 2081, 1989).

P 014 PHYSIOLOGICAL ROLE OF IL-3 John W. Schrader, Ehud Razin, Melanie Welham, Ian Ponting, and Kevin Leslie Biomedical Research Centre, 2222 Health Sciences Mall, UBC, Vancouver, B. C., Canada V6T 1W5

IL-3 stimulates growth and differentiation of a broad range of hemopoietic stem and progenitor cells, as well as certain mature effector cells such as mast cells, basophils, megakaryocytes and macrophages. It is an important component of the hemopoietic systems response to stress, linking the immune system and the hemopoietic system. IL-3 is produced in vivo during the immune responses by activated T-cells and probably by mast cells activated by binding of antigens to cell-bound IgE. However there is no good evidence that IL-3 has a role in steady-state hemopoiesis or indeed is present in normal, unstressed animals. There are however some data linking IL-3 and two molecules, c-kit and its ligand, that are involved in steady-state hemopoiesis. Thus IL-3 as well as GM-CSF and erythropoietin, but not IL-4, down-regulate levels of c-kit mRNA and protein. Cytokines involved in the stress response may thus disengage mechanisms that regulate steady-state hemopoiesis. Other observations have suggested that mast cells, upon contact with fibroblasts, upregulate levels of IL-3 mRNA. It is possible that some actions of the kit ligand on hemopoietic stem and progenitor cells may be mediated through an indirect, autocrine mechanism involving the production of factors like IL-3 and GM-CSF.

Other Factors Involved in Hematopoiesis

P 015 GROWTH FACTORS AND EARLY HEMOPOIETIC DEVELOPMENT, Frank Lee, Richard Murray, Terrill McClanahan, Choy-Pik Chiu, Department of Molecular Biology, DNAX Research Institute, 901 California Avenue, Palo Alto, California 94304-1104. A large number of cytokines have been identified which regulate the growth and development of hemopoietic cells. We are interested in the potential role of these cytokines during embryonic development and particularly their possible function in the development of pluripotent hemopoietic stem cells. For this reason we have begun an analysis of cytokine and cytokine receptor gene expression in the mouse embryo, focusing on the period leading up to the first appearance of hemopoietic stem cells in the fetal yolk sac (embryonic day 7). Secondly, we are using pluripotent embryo derived stem (ES) cells which under certain conditions can undergo differentiation *in vitro* as embryoid bodies. These embryoid bodies give rise to a variety of differentiated cell types characteristic of the early embryo including blood islands similar to yolk sac blood islands with mature hemopoietic cells. We are analyzing lineage commitment and differentiation of hemopoietic cells within embryoid bodies. Finally, by homologous recombination and gene disruption in ES cells we are working to produce mutant mice deficient in the production of several growth factors. These mice will be useful for determining the role of these factors during normal development.

P 016 IL-5 AND ITS RECEPTOR: THEIR EFFECT IN B CELL DIFFERENTIATION,
Kiyoshi Takatsu, Satoshi Takaki, Yasumichi Hitoshi, Eiichiroh Sonoda, Seiji Mita, Naoto Yamaguchi and Akira Tominaga. Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, Kumamoto 860, JAPAN.

Interleukin 5 (IL-5), is secreted by helper T cells following activation with antigens or mitogens and plays an important role in the growth and differentiation of B cells and eosinophils including other hematopoietic cells. It has been shown that IL-5 acts on various cells via specific cell-surface receptors with high-affinity binding sites for IL-5. We also reported that T88-M cells (IL-5-dependent murine early B lineage cell line) express a relatively large number of IL-5 receptor with both high- and low-affinity. Chemical cross-linking studies with radiolabeled IL-5 yielded two cross-linked complexes (a major and a minor species). Moreover, we generated an H7 mAb which blocks the binding of IL-5 to T88-M cells under both high- and low-affinity conditions. Immunoprecipitation studies revealed that H7 mAb precipitates one major component of approximately 60 kD from the cell lysates of T88-M cells.

In this studies, we have cloned a cDNA encoding murine IL-5 receptor by using expression cloning methods. Murine IL-5 receptor cDNA encodes a glycoprotein of 415 amino acids with a single transmembrane segment. We also isolated cDNAs coding for the secreted forms of murine IL-5 receptor. Analysis of the deduced amino acid sequence of the cDNA revealed that murine IL-5 receptor is unique protein, although it contains a common motif of a cytokine receptor gene family. COS cells transfected with murine IL-5 receptor cDNA expressed binding sites for IL-5 with low-affinity. We will present data showing that recombinant IL-5 receptor expressed on the cell-surface of myeloid cells is functional. We will discuss about molecular and functional properties of associated molecule(s) with IL-5 receptor.

P 017 IDENTIFICATION AND CHARACTERIZATION OF A LIGAND FOR THE C-*KIT* PROTO-ONCOGENE, Douglas E. Williams, Dirk M. Anderson and Stewart D. Lyman, Departments of Experimental Hematology and Molecular Biology, Immunex Research and Development Corporation, Seattle, WA 98101.
Mice with mutations at the dominant spotting (*W*) and Steel (*Sl*) loci of the mouse are characterized by defects in hematopoiesis, gametogenesis, and pig-mentation. Recent studies have shown that the *Sl* locus encodes a ligand for the c-*kit* proto-oncogene (the product of the *W* locus). The ligand, termed mast cell growth factor (MGF), is made as a membrane bound growth factor but can also be cleaved from the cell membrane by an unidentified protease to yield a soluble version. Soluble recombinant MGF has been produced in yeast and purified to homogeneity. This growth factor promotes the proliferation of purified hematopoietic stem cells, T cell precursors, and B lymphoid precursors. Synergy between MGF and various CSFs and interleukins has been demonstrated on all of the aforementioned cell populations. In addition, MGF has biological effects on melanocytes and germ cells, as predicted by the *Sl* and *W* defects.

Cytokines and the Effector arm of the Immune Response - I

P 018 MACROPHAGE DEACTIVATION: COMPARISON OF MACROPHAGE DEACTIVATION FACTOR AND TGF-β. Carl Nathan, Yoram Vodovotz, Subita Srimal, and Christian Bogdan, Dept of Medicine, Cornell University Medical College, New York, NY 10021.

Major advances in understanding of macrophage (mϕ) activation include the elucidation of two biochemically defined, cytokine-inducible antimicrobial pathways, the enzymatic generation of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI); the identification of cytokines capable of inducing these antimicrobial pathways and enhancing mϕ antimicrobial function; the demonstration of the utility of mϕ activating factors in the prevention and treatment of infectious diseases in man; and the discovery of the phenomenon of mϕ deactivation. Two cytokines, mϕ deactivation factor (MDF) and transforming growth factor-β (TGF-β), can each suppress all 3 major cytotoxic pathways of mϕ: production of ROI, RNI and tumor necrosis factor-α. MDF further resembles TGF-β in suppressing the proliferation of lymphocytes stimulated by concanavalin A, PMA + ionomycin, or specific antigen. Finally, MDF and TGF-β comparably stimulate proliferation of normal rat kidney fibroblasts and inhibit proliferation of mink lung epithelial cells. Despite similarity in their bioactivities, MDF is distinct from TGF-β. MDF purified from medium conditioned by P815 mastocytoma cells has a different Mr (13 kD reduced or nonreduced) than TGF-β (12.5 kD reduced, 25 kD nonreduced), and is unrecognized by 7 polyclonal neutralizing anti-TGF-β abs or by cell surface receptors for TGF-β. The reliance of mϕ antimicrobial defense on molecules of high chemical reactivity and correspondingly low specificity may have generated evolutionary pressure for tight immunologic control over their production-- involving both induction (activation) and repression (deactivation). MDF appears to be a novel cytokine with the remarkable property that it is physicochemically and immunochemically distinct from TGF-β, yet shares with TGF-β a variety of bioactivities on cells of diverse lineages. Both cytokines not only interfere with mϕ activation directly, but may also do indirectly, by suppressing the ability of T cells to produce mϕ activating factors.

P 019 PROPERTIES OF THE INTERLEUKIN 8 (INTERCRINE) FAMILY. Joost J. Oppenheim, Ji Ming Wang, Atsushi Hishinuma, Andrew Lloyd, and Kouji Matsushima. Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21702-1201

The intercrine family of homologous cytokines exhibits a number of unique characteristics. The human intercrine genes are located closely together on either human chromosome 4 (intercrine subset α) or chromosome 17 (intercrine ß subset). The genes code for basic heparin-binding small (8-10 kDa) polypeptides. The amino acid sequence includes four cysteines, two of which are adjacent in the intercrine ß, subfamily, which includes huMCAF, huLD78, huACT II, huRANTES, muTCA3, muJE, muMIP 1α and muMIP 1ß, or are separated by one amino acid in the intercrine α subfamily which includes huPF4, hußTG, huIL 8, ch9E3, huGRO, huIP10, and muMIP 2. Structural analysis reveals that these cysteines are important for the tertiary structure and receptor binding of the intercrines. Of the intercrines tested only huGRO, muMIP2, and to a limited extent NAP-2 competitively inhibit the binding of radiolabeled IL 8 to receptors on a human myelocytic cell line, U937. In contrast, muMIP1, huCTAP III, huLD 78, huACT-2, huMCAF and huPF4 did not compete for IL 8 binding sites suggesting that most of the intercrines use receptors distinct from IL 8. All the members of the intercrine family that have been functionally characterized appear to be involved in proinflammatory and/or restorative functions. The intercrine members exhibit considerable overlap in activities within the group and with other cytokines. The idea that they may be more specialized than the classical cytokines can be challenged by observations that IL 8 influences the activities of at least six distinct target cells (e.g., neutrophils, T-lymphocytes, basophils, keratinocytes, monocytes and melanoma cells). These cytokines may be physiologically unique in that various intercrine members may be more available in certain in vivo compartments and micro-environments (e.g., PF4 and ßTG at sites of platelet activation and aggregation), they may vary in half life, time of production and distribution, and some do have clearly specialized functions. Many experimental studies remain to be performed to delineate the pathophysiological roles of this family of novel cytokines and to establish the therapeutic utility of various agonists and antagonists.

P 020 PLEIOTROPIC ACTIONS MEDIATED BY THE COLONY-STIMULATING FACTOR-1 RECEPTOR, Charles J. Sherr, Hitoshi Matsushime, Gary V. Borzillo, Sheila A. Shurtleff, Jun-ya Kato, James R. Downing, and Martine F. Roussel, Department of Tumor Cell Biology and Howard Hughes Medical Institute, St. Jude Children's Research Hospital, Memphis, TN 38105

Colony-stimulating factor-1 (CSF-1), or macrophage CSF (M-CSF), supports the proliferation, differentiation, and survival of cells of the mononuclear phagocyte lineage during hematopoiesis and potentiates the effector functions of mature macrophages during the inflammatory response. The pleiotropic actions of CSF-1 are mediated through its binding to a single class of high affinity receptors (CSF-1R) encoded by the c-*fms* proto-oncogene. Receptor aggregation induced by CSF-1 activates CSF-1R protein tyrosine kinase (PTK) activity, resulting in the cross-phosphorylation of receptor monomers and triggering tyrosine phosphorylation of cellular messengers that relay ligand-induced signals. The phosphorylation of CSF-1R itself on tyrosine induces conformational changes within the PTK domain that enable the receptor to directly bind to cytoplasmic effectors. Mutation of different "autophosphorylation" sites generates CSF-1R variants that retain PTK activity but are unable to interact with particular cytoplasmic effector proteins, thereby selectively disrupting communication between the receptor and certain signal transduction pathways. One basis for the pleiotropic response to CSF-1 therefore reflects the ability of CSF-1R to activate multiple second messenger pathways that act combinatorially in signal-response coupling.

Introduction of the c-*fms* gene into naive cells confers CSF-1 responsiveness and enables target cells to proliferate in CSF-1 in lieu of other growth factors. For example, transduction of the human c-*fms* gene into mouse NIH/3T3 fibroblasts enables their normal requirements for PDGF and IGF-1 to be replaced by purified, recombinant human CSF-1. Expression of transduced CSF-1R can also facilitate differentiative responses. Introduction of the human c-*fms* gene into murine B-lymphoid cells gave rise to early pre-B cell lines that underwent lineage switching to macrophages in response to CSF-1. Their trans-differentiation was absolutely dependent on human (but nor murine) CSF-1, but could be inhibited by IL-7, a lymphoid-specific cytokine that ensured self-renewal of the early pre-B cells even in the presence of CSF-1. Once the cells became committed to differentiation in the macrophage lineage, they lost their ability to respond to IL-7 and up-regulated endogenous receptors for murine CSF-1. Thus, PTK signals emanating from CSF-1R appear to couple to different effector pathways depending on cell context. These experiments underscore the ability of different cytokines – in this case CSF-1 and IL-7 – to cooperate in mediating hematopoietic lineage commitment.

P 021 MOLECULAR CLONING AND CHARACTERIZATION OF HUMAN INTERLEUKIN-11. Yu-Chung Yang[1], Tinggui Yin[1], Denise McKinley[1], Terresa Yang-Feng[2], Steve Paul[3], Frann Bennett[4], Barbara Sibley[4], JoAnn Giannoti[4], Steve C. Clark[4]. [1]Indiana Univ. School of Med., Indianapolis, IN 46202, [2]Yale Univ. School of Med., New Haven, CT 06510, [3] Childrens' Hospital, Boston, MA 02115, [4]Genetics Institute, Cambridge, MA 02140

A cDNA clone encoding a novel growth factor activity produced by a primate bone marrow-derived stromal cell line was isolated by expression cloning based on its ability to stimulate the proliferation of an IL-6-dependent mouse plasmacytoma cell line[1]. The sequence of this cDNA was shown to have no homology with known cytokines and was designated as interleukin-11 (IL-11). The cDNA and genomic sequence of human IL-11 was subsequently isolated using primate IL-11 cDNA as the probe. We have recently determined the entire genomic sequence and the gene consists of five exons and four introns. We have also mapped the gene to the long arm of human chromosome 19 at band 19q13 by in situ hybridization. Preliminary biological analysis have indicated that human IL-11 can synergize with IL-3 in supporting megakaryocyte colony formation in both human and mouse systems. This cytokine can also stimulate the T-cell dependent development of immunoglobulin-producing B cells[1] and act synergistically with IL-3 to shorten the Go period of early progenitors[2]. These data provided evidence that this cytokine may play an important role in hematopoiesis. Since many of the biological properties of human IL-11 overlapped with those of IL-6, we tested the possibility that these two cytokines may utilize similar signal transduction pathway or may even share common receptor(s). Scatchard plot analysis was performed on T10 cells, a mouse plasmacytoma cell line dependent on IL-6 or IL-11 for growth. The results revealed that these two cytokines do not compete for receptor binding on T10 cells. Biochemical properties of IL-11 receptor(s) on T10 cells are currently being characterized. The cloning of human IL-11 gene will now allow us to study the regulation of IL-11 gene expression in different cell types. The availability of the purified recombinant IL-11 protein will enable us to test other in vitro biological activities, evaluate its biological effects in vivo and study the signal transduction mechnism utilized by this cytokine.

[1] Paul et al. (1990) Proc. Natl. Acad. Sci. USA 87: 7512-7516.
[2] Musashi et al. (1991) Proc. Natl. Acad. Sci. USA, in press.

Cytokines and the Effector arm of the Immune Response - II

P 022 CLINICAL DEVELOPMENT OF INTERFERON GAMMA, Susan M. Kramer and Howard S. Jaffe, Departments of Research Collaborations and Medical Affairs, Genentech, Inc., South San Francisco, CA 94080

Clinical trials with recombinant interferon gamma (IFN-γ) began in 1983 with cancer as the primary target. Subsequently, an increasing number of biological activities for IFN-γ have been described as a result of *in vitro* and *in vivo* preclinical studies. A clinical program designed to address four major biological activities has evolved as a result of these observations. The areas under investigation include infectious disease, allergy, fibrotic disorders and cancer. Evidence for clinical benefit associated with IFN-γ therapy has been reported in all four of these target areas, the best characterized being the anti-infective activity of IFN-γ in combination with antibiotics for treatment of patients with chronic granulomatous disease (CGD). A randomized, double-blind, placebo controlled study showed that IFN-γ (50 mcg/m^2) three times weekly was capable of significantly decreasing the number of serious infections experienced by CGD patients. These findings have implications for the immunomodulation and microbicidal activation properties of IFN-γ in other immunosuppressed/infectious disease clinical targets.

P 023 STRUCTURE AND FUNCTION OF SOLUBLE AND MEMBRANE-BOUND FORMS OF RECEPTORS FOR TUMOR NECROSIS FACTOR, Craig A. Smith, Terri Davis, Dirk Anderson, Wenie Din, Rita Jerzy, and Raymond G. Goodwin. Immunex Corp., Seattle, WA. 98101. The isolation of expressible cDNA clones encoding human type I (1) and II (2,3) TNF receptors show these two forms are integral membrane proteins with only modest sequence homology (27%) restricted to an extracellular region (~170 aa) containing cysteine rich, four-fold pseudo-repeats. This structural motif defines a new superfamily of at least 12 membrane proteins. Murine TNFrI and II cDNAs, isolated by cross hybridization with corresponding human cDNAs, also show limited homology to each other, but reveal strong homology (65% at the protein level) to their human analogues.through the entire sequence. Duplication of the primordial TNFr gene therefore predates speciation. All four receptors bind both TNFα and β with multiple affinity classes. Soluble form of huTNFrI (containing only extracellular segments) also binds ligand, and inhibits the cytolytic action of TNF in vitro. A novel form of soluble TNFrI is encoded by the T2 ORF from the Shope Fibroma virus, which we demonstrated specifically binds TNFα and β (4). This is the first such virally-encoded (host acquired) cytokine receptor identified.

Northern analysis of human TNFrI and II from various cell lines reveal that most cells show multiple transcripts for both p60 and p80. Prominent transcripts at 7.2 kb and 2.4 kb hybridize with a TNFrII probe; some cells show both 7.2 and 2.4 kb transcripts, some only one or the other, and two show both. Probes for TNFrI on these same cells show 5.0kb and 4.0kb transcripts, again with variability. Similar results are obtained in the murine system.

Structural studies using recombinant TNF receptors will be discussed, as well as the signal transducing capabilities of both forms.

(1) C. Smith et al., Science 248, 1019 (1990).
(2) T. Schall et al., Cell 61, 361 (1990)
(3) H. Loestscher et al., Cell 61, 351 (1990)
(4) C. Smith et al., Lymphokine Res. 9, 584 (1990)

Regulation of Cytokine Gene Expression

P 024 Regulation of the human interleukin 3 gene.

B. Mathey-Prevot, K. Davies, S. G. Kreissman. The Dana-Farber Cancer Institute and The Children's Hospital, Boston MA 02115.

The expression of human interleukin 3 (IL-3) is restricted to activated T lymphocytes and natural killer cells. The regulation of cell-specific IL-3 expression was studied in a gibbon T cell line, MLA 144 . These cells were chosen for their ability to produce high levels of IL-3 following activation. A modified genomic clone of human IL-3 that contained varying upstream sequences was used as a reporter gene. Experiments in which these constructs were transiently transfected into MLA 144 cells identified the regulatory sequences to be within 300 base pairs of the IL-3 start site. These sequences contain two distinct enhancer regions. The first, ACT-1, maps between nucleotides -139 and -155. It is absolutely required for T cell expression of the IL-3 gene, and comprises two contiguous but distinct motifs. One motif, which shows sequence homology to the ATF/CRE element, confers transcriptional activation when placed upstream of a minimal promoter reporter gene. In the native promoter, however, transcriptional activation conferred by this site requires the presence of a second motif, ATGAATAA, immediately upstream of it. This site binds a T cell and activation-specific factor, but it is unable to promote transcription on its own. A second regulatory region maps between nucleotides -249 and -300. It consists of two sites: One site binds a ubiquitous protein, NIP, which is a strong repressor of IL-3 expression. The second motif is an activator site which binds an AP-1 complex. In the absence of the AP-1 site, the NIP-binding site completely silences the transcriptional activation of IL-3 mediated by ACT-1. In the presence of the AP-1 site, the repression by NIP is not only abolished, but maximal levels of IL-3 are achieved. However, the AP-1 site itself is not sufficient to promote IL-3 expression, as deletion of ACT-1 from the IL-3 promoter abolishes transcription. These findings suggest that human IL-3 expression is mediated by two enhancer regions which must be simultaneously activated. Since each enhancer is tightly regulated in an independent manner, it appears that elaborate controls have evolved to allow IL-3 expression only when the requirements of the two enhancer regions are fulfilled.

Cytokines as Models for Signal Transduction

P 025 CYTOPLASMIC EVENTS IN IL-1 SIGNALLING, Timothy A. Bird, Steven K. Dower and John E. Sims*, Department of Biochemistry and *Molecular Biology, Immunex Corporation, 51 University Street, Seattle WA 98101. There are at least two types of cell surface receptor for IL-1, encoded by distinct genes, which are capable of binding two forms of IL1 (α and ß) and an antagonist polypeptide (IL1ra). Only the first type of ligation appears to result in cellular activation. Molecular cloning of the T cell/fibroblast IL-1R (type 1 receptor) gave few clues as to how it might function as a signalling molecule; it does not, for example, possess a tyrosine kinase domain. There are sequence similarities between type 1 IL1R cytoplasmic domain and certain members (N-Ras, src, cAMP-dependent protein kinase) of the nucleotide-binding protein superfamily. There are also motifs typical of nuclear-localization sequences. We also note the presence of a 14 amino-acid stretch which is homologous to sequences in a number of growth factor receptors and IGFII receptor that have been recently proposed to mediate interaction with G proteins. We have constructed receptors bearing mutations involving some of these domains in order to test their involvement in signal transduction. The type 2 IL1R, expressed predominantly, but not exclusively, on B cells is characterized by a very short cytoplasmic domain. There are two short regions of homology with the type 1 receptor within this domain. There is little else to suggest that the two cytoplasmic domains possess similar signalling functions. We have conducted a series of experiments to ask if ancillary proteins can be demonstrated to interact with the type 1 receptor. Using radiolabelling, immunoprecipitation and crosslinking techniques we have been unable to convincingly demonstrate such an association. This finding is consistent with the observation that stable expression of the single 80 kDa chain of type 1 IL-1R is alone sufficient to augment the IL-1 signal. Truncated IL-1R, lacking the cytoplasmic domain, is non-functional. Similar experiments are underway with the type II receptor. Type I receptor can be phosphorylated upon ligation of IL-1. This appears to happen with low stoichiometry, however, and cannot be demonstrated in all cell types. The murine IL-1R is phosphorylated at a threonine residue near the C-terminus in cells treated with PMA. The human receptor lacks this residue and is not phosphorylated: phosphorylation alters neither ligand binding or internalization; its significance is therefore obscure. Treatment of various cell types with IL1 leads to the rapid serine/threonine phosphorylation of a number of proteins. These include, in peripheral blood mononuclear cells, L-plastin and , in fibroblasts, the 27 kDa heat-shock protein, EGF receptor, and talin, a component of focal adhesions. The last two phosphorylation events can be correlated with functional alteration of the substrates and will be described in detail; our evidence suggests that neither protein kinase A or C is involved. To characterise the IL-1 -stimulated protein kinase activity we have recently developed a cell free assay using a peptide substrate.

P 026 CYTOKINE REGULATION OF PROTEIN KINASE ACTIVITY. William L. Farrar[1], Dennis Michael[1], Gonzalo Garcia[1], Gerald Evans[1], Alan D'Andrea[2] and Diana Linnekin[1]. [1]Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21702-1201. [2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142

A feature central to all of the members of the new hematopoietin receptor family is the apparent lack of any known catalytic domains intrinsic to the receptor structure. The lack of catalytic domains suggests that these receptors may couple to signal transduction systems by novel biochemical mechanisms not previously observed with other growth receptors which contain protein kinase catalytic activities. We have compared the receptor-mediated activation of protein kinases by several members of the cytokine network including IL 2, IL 3, GM-CSF, and EPO. A direct comparison of kinases associated with the IL 2R p70 chain and the EPO receptor revealed that a 97 kDa tyrosine protein kinase was activated by the respective ligands and tightly associated with the principal receptor molecule. In addition, another phosphotyrosyl protein of approximately 55-58 kDa had some of the characteristics of tyrosine kinases. Using EPO-R cytoplasmic deletion mutants we have been able to directly correlate the association of the 97 kDa tyrosine kinase with specific cytoplasmic regions and the functional ability of the receptor to transmit signals leading to EPO regulated cellular growth. Furthermore, murine cells transfected with the EPO-R and the gp55 envelope glycoprotein of Friend Spleen Focus Forming Virus had constitutively activated 97 kDa protein kinase associated with the EPO-R suggesting that the autocrine transformation of these cells by gp55 uses the identical signal transduction pathway controlled by EPO. In contrast to the typical or characteristic protein kinases activated by the growth ligands for myeloid and lymphoid cells, we have discovered a serine kinase activity that is uniformly associated with the growth cessation of these cells. This 55 kDa serine kinase is down-regulated by growth factors such as IL 2 and IL 3 and is upregulated by substances which trigger terminal differentiation or growth arrest. The identification and further characterization of these kinases will be important in understanding their role in the control of cellular growth and oncogenesis.

P 027 GM-CSF AND IL-3 ACTIVATE MULTIPLE CELLULAR KINASES. James D. Griffin, Keiko Okuda, Yuzuru Kanakura, and Brian Druker. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

Human GM-CSF and IL-3 are members of a family of growth factors which stimulate the proliferation, differentiation, and function of hematopoietic cells. The actions of both factors are known to be mediated through binding to specific, high affinity, cell surface receptors. However, the post-receptor signal transduction pathways are largely unknown. The human myeloid cell line M07 requires either GM-CSF or IL-3 for proliferation. Treatment of MO7 cells with either GM-CSF or IL-3 rapidly induces tyrosine phosphorylation of a number of proteins, including two cytosolic proteins, p93 and p70, in a dose-dependent manner at either 37°C or 4°C. The tyrosine phosphorylation of p93 and p70 is transient, with maximal phosphorylation 5-15 min after addition of growth factor to factor-deprived cells. The activation of tyrosine kinase activity by GM-CSF does not require receptor internalization. GM-CSF- stimulated tyrosine phosphorylation is enhanced by an inhibitor of protein tyrosine phosphatase (PTPase), sodium orthovanadate; and inhibited by two activators of protein kinase C, PMA and Bryostatin-1. GM-CSF and IL-3 also rapidly stimulate serine phosphorylation of Raf-1, a 74 kD cytoplasmic serine/threonine protein kinase, and activate Raf-1 kinase activity. These results suggest that proliferation of M07 cells may be regulated by a cascade of CSF-induced protein kinases with both tyrosine and serine specificity. The role of these kinases in the growth of human leukemic cells will be discussed.

P 028 TNF SIGNAL TRANSDUCTION, Martin Krönke, Gracia Kruppa, Albrecht Meichle, and Stefan Schütze. Klinische Arbeitsgruppe, Max-Planck-Gesellschaft, 3400 Göttingen, Germany

The cloning of cDNAs encoding two types of TNF receptors has provided substantial information as to the intracellular domains of these membrane-associated molecules. While several phosphorylation sites could be defined, no resemblance to the catalytic domains of tyrosine or serine/threonine-specific protein kinases was found in any of the two types of TNF receptors. Thus, the pathways of TNF receptor signal transduction across the membrane are still poorly understood. Like other cytokines, TNF causes profound changes in cellular lipid metabolism. We have recently obtained evidence that TNF receptors initiate within seconds the activation of a phospholipase C (PLC) that hydrolyzes phophatidylcholine (PC) to generate phosphocholine and, more importantly, 1,2 diacylglycerol (DAG). DAG in turn is a well established activator of protein kinase C (PKC). Indeed, we could demonstrate with several human leukemic cell lines that TNF triggered a rapid and transient increase of PKC activity in the membranes, which was accompanied by a long-term loss of cytosolic PKC activity. The activation of PKC presumably represents one crucial link in the TNF-induced activation of nuclear transcription factors such as NF*k*B and AP-1. However, while TNF-mediated activation of NF*k*B can be efficiently inhibited at the receptor level, that is, by means of a monoclonal antibody specific for the 60 kDa TNF receptor species, TNF readily induces NF*k*B in the presence of PKC inhibitors as well as in PKC depleted cells. Thus, PKC-independent pathways may mediate TNF activation of NF*k*B. The effects of various other inhibitors of specific cellular signalling systems will be discussed with respect to their potential to dissect the multiple and redundant pathways that mediate TNF signals from the membrane to the transcriptional apparatus in the cell nucleus.

Late Abstract

T-CELL GROWTH FACTOR ACTIVITY AND EXPRESSION OF P40/IL9, Van Snick, J., Ludwig Institute for Cancer Research, Brussels, Belgium

P40/IL9 is a recently described T-cell-derived cytokine endowed with growth factor activity for certain T helper lines, for mast cells and for erythroid precursor cells.

A puzzling feature of the T-cell growth factor activity of IL9 is its unique specificity for a restricted set of mouse T helper clones . Strong evidence has now been obtained to indicate that responsiveness to IL9 is not an intrinsic character of particular T cell clone but an acquired phenotype that develops gradually in culture. Studies with multiple T helper clones indeed show that T cells proliferating in response to IL9 have partly escaped from normal control mechanisms and will eventually develop into fully transformed T cells with strong tumorigenic potential. These results suggest that IL9 may be implicated at some stages in the development of T-cell tumors. Support for this hypothesis was recently obtained with mutagen-induced DBA/2 thymomas.

Analysis of the mechanisms regulating IL9 expression in normal T cells has shown that two signals are required for the induction of IL9 message : one is a T-cell receptor-mediated signal, that can be mimicked by phorbol esters, the other is delivered through the IL2 receptor. None of the known cytokines, including IL4 and IL7, can replace IL2 in this respect. This finding suggests the existence of an IL2-responsive element in the IL9 promoter.

Cytokine Regulation of Early Events in Hematopoiesis

P 100 INTERLEUKIN 7-INDUCED EXPRESSION OF T CELL RECEPTOR GENES IN THE MURINE FETAL LIVER IN VITRO. Pierette M. Appasamy, Pittsburgh Cancer Institute and Department of Pathology, University of Pittsburgh, School of Medicine, Pittsgurgh, PA, 15213.
The mechanisms controlling proliferation and maturation of pre-thymic T cell precursors remain unknown. Since interleukin 7 (IL7) stimulates growth of double negative ($CD4^-$, $CD8^-$) "immature" thymocytes, we explored whether IL7 may function as a growth and/or differentiation factor for pre-T cells in the murine day 14 fetal liver (FL). We found that unseparated FL cells from CBA/J mice proliferate in response to human recombinant IL7 (a gift of Dr. Steven Gillis, Immunex Corp.) in a dose-dependent manner, as measured by ^{3}H-thymidine incorporation. Significant effects of 10 and 100 ng/ml IL7 on growth were observed after 7 days of culture. Further, we found that culture of FL cells with 10 ng/ml IL7 induced expression of alpha, beta, gamma and delta T cell receptor (TCR) mRNA, as determined by Northern blot analysis. The pattern of expression of specific genes was dependent upon the culture period (1-7 days). The size of TCR transcripts indicates that IL7 may induce rearrangement and expression of gamma and delta but only expression of unprocessed alpha and beta. The percentage of cells expressing Thy 1 antigen was also increased after 3 days of culture with 10 ng/ml IL7 (30% Thy 1+) versus medium alone (15% Thy 1+). These data, together with expression of TCR genes, are consistent with the hypothesis that IL7 promotes proliferation and/or differentiation of pre-T cells; thus, IL7 may be used as a tool to address molecular and biochemical events regulating pre-T cell development. Experiments designed to determine the relationship between FL cells induced to proliferate with IL7 and cells induced to express TCR genes are presently underway.

P 101 DISCRETE ACTIVATION OF LATE G-1 GENES SUPPORTS IL-3 PROLIFERATION OF HUMAN HEMOPOIETIC CELLS, Giancarlo Avanzi, Maria F. Brizzi, Pierluigi Porcu, Arturo Rosso, Giovanni L. Pagliardi, Dario Ghigo, Amalia Bosia, Mariagrazia Aronica, Giuliana Cavalloni and Luigi Pegoraro, Dipartimento di Scienze biomediche ed Oncologia Umana, Sezione Clinica (G.C.A., M.F.B., P.P., A.R., M.G.A., G.C., L.P.), Dipartimento di Genetica, Biologia e Chimica Clinica (D.G, A.B.), Istituto di Medicina Interna (G.L.P.) Università di Torino, Torino, Italy.
Both normal hemopoietic progenitors and growth factor (GF) dependent lines require a continuous supply of GF for in vitro survival and proliferation. We have recently established a subclone named M-07e, strictly dependent on the presence of IL-3 or GM-CSF for survival and proliferation from a human megakaryoblastic cell line bearing antigen determinants (CD13, CD33, CD34) common to multilineage hemopoietic precursors. Withdrawal leads to a sharp fall in its proliferative activity to a nadir within 24 hours after GF deprivation. However, after 48 hours of deprivation, cell viability is only reduced by about 30% and GF restoration fully restores proliferation. To elucidate the mechanisms by which IL-3 supports the proliferation of human hemopoietic cells we investigated the GF-dependent regulation of several cell cycle related genes in M-07e cells. No transcriptional activation of early (c-myc), mid (ODC) or mid-late G-1 (p53, c-myb) cell cycle genes was detected on restoration of IL-3 in deprived cells. The fact that only late G-1/S-phase genes (cyclin, TK, H3) are modulated by IL-3 suggests that this factor controls human cell proliferation by acting at the G-1/S boundary.
(Supported by AIRC grant).

P 102 ENHANCED INTERLEUKIN 3 SENSITIVITY OF CIRCULATING HEMATOPOIETIC PROGENITORS DURING TREATMENT OF DIALYZED CHILDREN WITH RECOMBINANT ERYTHROPOIETIN.
V Bier, H-G Hoffmann, DE Müller-Wiefel, A Bosch, R Ludwig, K Scherer, KM Debatin, Depts. of Pediatrics, Immunology/Oncology and Nephrology, Univ. of Heidelberg, Germany.
The effect of recombinant erythropoietin (EPO) treatment on circulating hematopoietic progenitors was investigated in 10 pediatric patients with end-stage renal disease treated by regular hemodialysis. During the 30-week observation period, hemoglobin levels rose from a median pretreatment level of 6.7 g/dl to the target hemoglobin level of 10 g/dl in all patients. After one week of EPO treatment, the median number of circulating burst-forming units erythrocyte (BFU-E) increased 1.2-fold, followed by a subsequent decline. At week 30, BFU-E numbers had decreased to 35% of pretreatment values in spite of stable hemoglobin levels >10 g/dl. Concomitantly, the number of granulocyte-macrophage colony forming cells (GM-CFC) decreased below pretreatment values in all patients. All circulating BFU-E cultured before EPO treatment showed in vitro sensitivity to grading doses of EPO. However, in vitro dose-response curves of BFU-E were not predictive for in vivo EPO dose requirement. After two weeks of EPO treatment, patients' BFU-E growth could be enhanced by a factor of 2.2 relative to pretreatment and untreated controls when interleukin 3 (IL3) was added to cultures with conditions optimized for BFU-E growth. The enhanced sensitivity to IL3 declined subsequently, but could be demonstrated until week 30 of therapy. These findings suggest (1) a broader range of in vivo effects of EPO than expected from in vitro data, and (2) dysregulation of early hematopoietic progenitor cell recruitment in patients with renal anemia, possibly involving a relative deficiency of IL3.

P 103 REGULATION OF STROMAL CELL FUNCTION BY IL-4, Linda G. Billips, Debra Petitte, Robert A. Yapundich, and Kenneth S. Landreth. Department of Microbiology and Mary Babb Randolph Cancer Center, West Virginia University Health Sciences Center, Morgantown, WV 26506.
IL-4 exposure differentially affects both myelopoiesis and lymphopoiesis. Early stages of myeloid cell development are inhibited by IL-4, but later stages are potentiated. Although IL-4 potentiates the expression of sIg and proliferation of newly formed B cells, our studies also demonstrate that IL-4 abrogates pre-B cell formation. Bone marrow stromal cell line S17 potentiates the differentiation of pre-B cells from pro-B cells in vitro. Exposure of this cell line to increasing concentrations of IL-4 resulted in failure to support pre-B cell formation and this inhibition could be reproduced with conditioned medium from IL-4 treated S17 cells. However, inhibition of pre-B cell differentiation required at least one additional regulatory cell in vitro. This regulatory component could be removed from the marrow by depletion with anti-L3T4 antibody and was absent from marrow of Nu/Nu mice. Addition of syngeneic splenic T cells or a T helper cell line to Nu/Nu marrow resulted in reconstitution of IL-4 inhibition. These results strongly suggest that T cells play a role in regulation of primary B lymphopoiesis. Although the stromal derived cytokine responsible for eliciting cytokine production from the T cells is not yet known, it does not appear to be IL-1α, IL-1ß, or IL-6.

P 104 IL-7 SYNERGIZES WITH LMW BCGF ON STIMULATION OF ACUTE LYMPHOBLASTIC LEUKEMIAS, Heidi Kiil Blomhoff, Bjørn K. Erikstein, Erlend B. Smeland, Cecilia Skjønsberg, Lab. of Immunology, Inst. for Cancer Research, The Norwegian Radium Hospital, 0310 Oslo 3, Norway

Il-7 has been shown to stimulate the proliferation of both normal and neoplastic B-cell precursors. We have compared the effects of recombinant human Il-7 (Immunex), low molecular weight B cell growth factor (lmw BCGF) (Cellular products) and the combination of Il-7 and lmw BCGF on highly purified cells (contaminating cells removed by immunomagnetic beads) from 20 cases of common acute lymphoblastic leukemias (cALL). Il-7 (1000 U/ml) stimulated the DNA synthesis and cell cycle progression of cells from 13 cases of cALL whithout inducing differentiation. Lmw BCGF (10%) alone greatly stimulated the DNA synthesis of these cells, and of note was the pronounced potentiation of the Il-7 effects when the two compounds were administered together. BCGF did not induce differentiation. In the three cases of cALL where Il-7 inhibited the DNA synthesis, BCGF had only marginally stimulatory effect. BCGF did, however, seem to potentiate the effect of Il-7 on differentiation of these cells.

As BCGF is a partially purified product known to contain also other cytokines, we are currently examining the effects of recombinant lmw BCGF.

P 105 GM-CSF AND PHORBOL ESTERS MODULATE GM-CSF RECEPTOR EXPRESSION BY INDEPENDENT MECHANISMS, Maria F. Brizzi, Carlo Arduino, G. Carlo Avanzi, Giovanni L. Pagliardi, Federico Bussolino and Luigi Pegoraro, Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione Clinica, (M.F.B., G.C.A., L.P.), Dipartimento di Genetica, Biologia e Chimica Medica (F.B.), Istituto di Medicina Interna (G.L.P.) and Istituto di Farmacologia e Terapia Sperimentale (C.A.), Universitá di Torino, Torino, Italy. (Supported by AIRC grant; M.F.Brizzi is a fellow of AIRC).
Human granulocyte-macrophage colony-stimulating factor (GM-CSF) (0.1 nM) down-modulates its receptor in IL-3/GM-CSF dependent M-07e cells, in KG-1 cells and in normal granulocytes, whereas phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) (2 nM) down-modulates GM-CSF receptor in M-07e cells and granulocytes but not in KG-1 cells. As shows data analysis by non-linear regression, the decreased binding ability depends on a reduction of the binding sites with no significant change of their dissociation constant. To gain insight into the mechanisms involved in the GM-CSF receptor regulation we investigated the role of protein kinase C (PKC). GM-CSF, unlike TPA, was unable to activate PKC in all the cells studied. Moreover, unlike TPA, GM-CSF was still able to down-modulate its receptor in cells where PKC was inhibited by 1-(5-isoquinolonesulphonyl)-2-methylpiperazine (H7) and staurosporine or in cells where PKC was exhausted by prolonged incubation with 1 uM TPA. Finally receptor re-expression rate was accelerated by protein kinases inhibitors. These results, taken together, indicate the presence of a PKC-dependent and -independent down-modulation mechanism and a negative role of the endogenous protein kinases in GM-CSF receptor re-expression.

P 106 POTENTIATION OF EARLY MYELOPOIESIS BY TNFα

Christophe Caux, Isabelle Moreau, Isabelle Durand, Valerie Duvert, Sem Saeland and Jacques Banchereau, Schering-Plough, Laboratory for Immunological Research, BP11, 69571 Dardilly Cedex, France.

We have recently shown that TNFα potentiates IL-3 and GM-CSF induced growth of $CD34^+$ hematopoietic progenitor cells (HPC), while it has very marginal proliferative effect per se (Caux et al, 1990, Blood 75, 2292). Limiting dilution studies have shown that TNFα increases both the frequency and average size of IL-3 dependent clones. Following a 48h preculture of $CD34^+$ HPC in IL-3, sorted transferin receptor (TfR) positive HPC, but not TfR^- HPC, were found to proliferate in response to IL-3 as measured by cell counts and CFU assays. Interestingly the TfR^- HPC were found to strongly proliferate in response to the combination of IL-3 and TNFα. Moreover limiting dilution experiments indicated that the clones generated from TfR^- HPC in the presence of TNFα+IL-3 have a larger size than those generated from TfR^+ HPC in response to IL-3 with or without TNFα. Additionally, preculturing $CD34^+$ HPC for 36-72h in the presence of TNFα resulted in a subsequent enhancement of their response to IL-3 as measured by cell expansion and CFU generation. Taken together, our results indicate that TNFα recruits a subpopulation of $CD34^+$ HPC which is not directly responsive to IL-3 but has a high proliferative capacity.

P 107 THE EFFECT OF RECOMBINANT MAST CELL GROWTH FACTOR (MGF) ON PURIFIED MURINE HEMATOPOIETIC STEM CELLS

Peter de Vries, Ken A. Brasel and Douglas E. Williams, Department of Experimental Hematology, Immunex Corporation, Seattle, WA 98101

A widely applicable, relatively fast and simple multiparameter flow cytometric purification procedure for murine hamatopoietic stem cells (day-14 CFU-S) was developed. This procedure was used to study the effect of MGF alone or in combination with IL-3 or IL-1α on purified stem cell suspensions in liquid cultures for various periods of time. MGF alone caused proliferation of purified day-14 CFU-S as measured by a ^{3}H-thymidine incorporation assay and also caused an increase of nucleated cells. MGF alone did not stimulate production of day-14 CFU-S but maintained their survival for up to 14 days. However, a 2-12 fold net production of day-14 CFU-S was observed in cultures stimulated with MGF + IL-3 and with MGF + IL-1α. MGF acted synergisticly with IL-3 and IL-1α. The production of day-14 CFU-S in cultures with MGF + IL-1α began 4 days later than in cultures with MGF + IL-3, indicating heterogeneity of the purified stem cells. Experiments aimed at a further purification of different stem cell subsets on the basis of their expression of hematopoietic growth factor receptors will be presented.

P 108 ERYTHROID COLONY FORMATION IN SERUM FREE ORGAN CULTURE OF CHICK VITELLINE MEMBRANE

Ferdinand P.E. Brown, N. Laidlaw R. and George C, Division of Natural Science and Mathematics, CUNY, Medgar Evers College, Brooklyn, N.Y. 11225

We have developed a new and simple method to obtain an abundance of early hematopoietic progenitors from the blood islands of the chick vitelline membrane. These progenitors after 3-4 days in serum - free Iscove's media give rise to mixed colonies and also typical CFUe colonies as confirmed by positive benzidine staining. Increase in colony formation was obtained with 2% Fetal calf serum, IL-3 and erythropoietin.
That colonies arise in complete absence of any added stimulatory factors suggests the presence of cytokines released from neighboring cells of the vitelline membrane.

P 109 THYMIC STROMAL CELLS SUPPORT THE DEVELOPMENT OF sIgM B CELLS IN VITRO, Sherree L. Friend and Andrew C. Farr, Departments of Immunology and Biological Structure, University of Washington, Seattle, WA 98195

The necessity of adherent bone marrow stromal cells for B cell development has been well documented. In contrast, the role of stromal cells in T cell maturation is less clear. We have isolated stromal cell lines from Balb/c thymus and have begun to assess their role in lymphocyte differentiation in vitro. Some of these stromal cells support the growth of small refractile lymphocytes from day 15 fetal liver depleted of adherent cells. Lymphocytes grown in these co-culture lack T cell markers but express B220 and 6C3 by day 8 of culture. In contrast to long term bone marrow cultures, surface IgM expression in thymic stromal cultures becomes virtually 100% over time. A population of Lyt-1+ B cells transiently occurs in the co-cultures. The lymphocytes grow both in intimate contact with the stromal cells and as non-adherent cells floating in the dish. Conditioned media from some stromal cell clones are able to support the growth of the non-adherent lymphocytes. This effect is probably not due to IL-7 since the conditioned medium does not support the IL-7 dependent cell-line 2B nor does rIL-7 support the non-adherent cells from the co-cultures. Thymic stromal cells may play a role in developing the small population of thymic B cells in vivo or provide a maturational signal that is common to both B and T lymphocytes. (This material is based upon work supported under a National Science Foundation Graduate Fellowship.)

P 110 DISTURBANCES IN THE ESTABLISHMENT OF LONG TERM BONE MARROW CELL CULTURES FROM SYRIAN HAMSTERS DURING *Leishmania donovani* INFECTION. M.I. Gaspar Elsas, M. Senna Salerno, E. D. Madeira and P. Xavier Elsas, Instituto Oswaldo Cruz and Instituto de Microbiologia UFRJ, Rio de Janeiro, Brazil.

Leishmania donovani, the agent of kala-azar, survives inside macrophages, leading to widespread changes in spleen and bone marrow (BM), due both to intracellular parasitism and to disturbances of the cytokine/CSF network. We studied the initial establishment of long term BM cultures (LTBMC) from normal and from chronically (56 days) infected Syrian Hamsters of the LHC strain, which closely reproduce features of human infection. Between days 0-21, LTBMC of normal animals become well established, with a stromal layer of fibroblasts, fat cells, large flattened cells and macrophages, to which many hemopoietic clusters adhere. LTBMC of 56 day-infected animals have a delay in stromal establishment, an increase in macrophage numbers and a paucity of stroma-associated hemopoietic cells. In short term adherence assays, significant differences between BM cells of normal and infected animals were found, suggesting a defect at an early step of LTBMC development. We also found decreased numbers of GM colonies in BM from infected animals relative to normal controls, when supernant of Con A-activated Hamster spleen cells was used as a source of GM-CSF. In contrast, no differences between normal and infected animals were found in long term spleen hemopoietic cultures. The defect found in chronically infected animals develops gradually in the course of infection (days 0-45), and defects in stromal establishment and GM colony formation are first detectable at different time points.

P 111 GENERATION OF PRIMITIVE RAT LYMPHOID PRECURSOR CELLS IN VITRO IS MEDIATED BY A SOLUBLE FACTOR(S) DERIVED FROM MOUSE BONE MARROW STROMAL CELLS. Irving Goldschneider and Sean D. McKenna. Department of Pathology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030.

We have previously demonstrated that the selective long term *in vitro* generation of rat bone marrow (BM) lymphoid precursor cells in the absence of detectable pluripotent hemopoietic stem cells is dependent upon the presence of a mouse BM adherent cell feeder layer. These culture-generated lymphoid cells are phenotypically undifferentiated, express the enzyme terminal deoxynucleotidyl transferase (TdT), and are capable of reconstituting both the T- and B-cell (but not the myeloid or erythroid) compartments of irradiated rats. Recently, we have used microporous culture-well inserts and conditioned medium to show that the lymphostimulatory activity in our culture system is due, at least in part, to the release of a soluble factor(s) by the mouse BM adherent cells. We now demonstrate that this activity is produced by mouse BM stromal cells, several lines of which have been established by serial passage *in vitro*. Upon ultrafiltration, the active component of the conditioned medium is associated with the 50-100 kD apparent MW fraction. Moreover, this activity can be generated under serum-free conditions, suggesting that the high MW of the responsible factor is not due to complexing with albumin or other serum proteins. The *in vitro* lymphopoietic response induced by this factor is time- and dose-dependent and is accompanied by the appearance of numerous lymphoblastic and mitotic cells. Additionally, the responsible factor maintains the growth of the precursors of the TdT^+ BM cells ("pre-TdT" cells) *in vitro*. Thus, we have identified a soluble factor produced by mouse BM stromal cells that appears to selectively stimulate the development of rat lymphoid stem and/or progenitor cells *in vitro*. (Supported in part by NIH Grant # GM 38306.)

P 112 ESTABLISHMENT AND CHARACTERIZATION OF A NOVEL TYPE OF CLONES FROM HUMAN FETAL LIVER, Toshiyuki Hori, Brian W. Duncan*, Michael R. Harrison*, Maria Grazia Roncarolo, and Hergen Spits, Department of Human Immunology, DNAX Research Institute of Moleculr and Cellular Biology, and *Department of Surgery, University of California at San Francisco.

We have established clones of a novel cell type from human fetal liver which have some characters of putative early T cell precursors. Fesh 14- to 18-week fetal liver cells were first cultured in bulk with irradiated PBL, PHA, and IL-2. After 14 days of culture, $CD2^-$ $CD3^-$ cells were sorted by FACS and cloned by limiting dilution in the presence of a feeder cell mixture of irradiated PBL, irradiated JY cells (an EBV-transformed B cell line), and PHA. Two stable $CD2^{low}$ $CD3^-$ clones were obtained and characterized in detail. Although these clones derived from different donors, they were similar to each other and shared a common phenotype of $CD2^{low}$ $CD3^-$ $CD4^-$ $CD8^-$ $CD7^+$ $CD5^-$ $CD6^-$ $CD11b^+$ $CD16^-$ $CD56^{low}$. These clones were demonstrated to express cytoplasmic CD3δ and ε antigens, indicating that they were committed to the T cell lineage. Consistent with this, they showed no or very low levels of cytotoxic activity agianst K562, JY or Daudi. Southern blot analysis revealed that the TCR β genes and the TCR γ genes of these clones were in the germ-line configuration. The most conspicuous feature of these clones was that they responded not only to IL-2, IL-4 or IL-7, but also to IL-3 with proliferation. GM-CSF had no effect on them. At present it is not clear whether they actually represent early T cell precursors and can differentiate into mature T cells. Nevertheless, the establishment of these clones may provide a novel insight into early events in human T cell development.

P 113 A DEVELOPMENTAL SWITCH IN THYMIC LYMPHOCYTE MATURATION POTENTIAL OCCURS AT THE LEVEL OF HEMATOPOIETIC STEM CELLS, Koichi Ikuta, Tatsuo Kina, Ian MacNeil, Nobuko Uchida, Bruno Peault, Yueh-hsiu Chien, and Irving L. Weissman, Howard Hughes Medical Institute, Beckman Center for Molecular and Genetic Medicine, Stanford University Medical Center, Stanford, CA 94305

Hematopoietic stem cells (HSC's) isolated from mouse fetal liver like adult HSC's, are $Thy\text{-}1^{lo}$ Lin^- $Sca\text{-}1^+$. Donor-derived $V\gamma3^+$ T cells were detected in fetal thymic lobes repopulated in vitro with fetal liver HSC's, but not in those with adult bone marrow HSC's. Single clonogenic fetal HSC's gave rise to thymic progeny that include $V\gamma3^+$, other $\gamma\delta^+$, and $\alpha\beta^+$ T cells. No $V\gamma3^+$ T cells were detected in adult thymus injected intrathymically with either fetal or adult HSC's. These results support a hypothesis that only fetal HSC's have the capacity to differentiate into $V\gamma3^+$ T cells in the fetal thymic microenvironment, and that the developmental potential of HSC's may change during ontogeny.

P 114 CYTOKINE REGULATION OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) RECEPTOR EXPRESSION ON PURIFIED NORMAL HEMATOPOIETIC PROGENITOR CELLS. [1]S.E.W. Jacobsen, [2]F.W. Ruscetti, [3]C.M. Dubois and [4]J.R. Keller, [1,2]LMI-BRMP-, [3,4]BCDP-PRI/Dyncorp, NCI-FCRF, Frederick, MD.

A hierarchial model of CSF receptor modulation on whole bone marrow has previously been proposed to explain proliferative effects of different cytokines on hematopoietic progenitor cells. However, since progenitor cells are in a low frequency in unfractionated bone marrow, the regulation of GM-CSF receptor expression on purified progenitor cells was examined. Mature hematopoietic and lymphoid cells were removed from murine bone marrow by positive selection with antibodies and magnetic beads. The remaining 1 % of the marrow is enriched for progenitor cells and designated lineage negative (Lin-). As previously described IL-3 and GM-CSF down-modulated the expression of GM-CSF receptors on unfractionated bone marrow by 24 hours by 40 and 50 % respectively. In contrast, IL-3 induced a 350 and GM-CSF a 120 % increase in GM-CSF receptors on Lin- cells by 24 hours. In addition, 24 hour treatment of Lin- cells with IL-1 and G-CSF resulted in a 120 and 310 % increase in GM-CSF receptors respectively. Cytokine induced up-regulation of GM-CSF receptors preceeded the induction of lineage specific cell surface antigens as well as changes in cell morphology. Thus, unfractionated bone marrow represents an incomplete model to study GM-CSF receptor regulation on hematopoietic progenitor cells.

P 115 EFFECTS OF HEMATOPOIETIC GROWTH FACTORS ON HIGHLY ENRICHED HUMAN PROGENITOR CELLS: GROWTH AND DIFFERENTIATION STUDIED BY PROLIFERATION ASSAY, IMMUNOPHENOTYPING AND LIMITING DILUTION. J.M. Kerst[1], W. Algra[1], I.C.M. Slaper-Cortenbach[1], C.E. van der Schoot[1] and M.H.J. van Oers[2]. [1] Central Laboratory of the Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam and [2] Department of Hematology, Academic Medical Center, Amsterdam, The Netherlands.
We studied the effects of growth factors, alone and in combination, on CD34 enriched human bone marrow cells. Colony forming capacity in semisolid medium was compared with the growth factor induced proliferation and differentiation of the progenitor cells in liquid medium. It was found that GM-CSF synergizes with G-CSF in the formation of colonies. Immunophenotypically this synergism was revealed in strong and exclusive expression of mature granulocytic antigens CD15 and CD67. As to proliferation, limiting dilution analysis showed the synergism to be based on an increased frequency of responding cells. Data on IL-6, M-CSF and TGFß in this system will be presented.

P 116 STRUCTURAL STUDIES OF STEM CELL FACTOR. K.E. Langley*, H.S. Lu*, D.A. Yphantis†, J.W. Lary†, L.O. Narhi*, C.L. Clogston*, E.A. Mendiaz*, J. Wypych*, K.M. Zsebo*, T. Arakawa*. *Amgen Inc., Thousand Oaks, CA and †Dept. of Molecular and Cell Biology, Univ. Connecticut, Storrs, CT.

We have recently reported the identification, purification, characterization, and cloning of an early-acting hematopoietic factor, termed stem cell factor (SCF) [Zsebo et al., Cell 63, 195 (1990); Martin et al., Cell 63, 203 (1990)]. The amino acid sequence encoded by the gene includes a transmembrane region, whereas the isolated natural protein represented a soluble form lacking this region. The factor is the product of the steel locus in the mouse, and is a ligand for the c-kit tyrosine kinase type receptor [Zsebo et al., Cell 63, 213 (1990)]. Soluble forms of the human stem cell factor (huSCF) have been recombinantly expressed in procaryotic and eucaryotic hosts. Natural and eucaryotic-expressed SCF are heavily glycosylated, with both N-linked and O-linked sugars. By gel filtration and equilibrium sedimentation analyses, it appears that SCF is a non-covalently associated dimer in solution and the glycosylated form has about 30% carbohydrate. Circular dichroism (CD) studies indicate extensive α-helix and β-sheet structure. Fluorescence emission spectra suggest that the single Trp residue of the molecule is located in a hydrophobic environment. The CD and fluorescence analyses are not affected by the presence or absence of carbohydrate. The four Cys residues are all involved in disulfide linkage, and the linkages have been identified.

P 117 [^{125}I]LABELLED INTERLEUKIN-6 APPLICATIONS IN RECEPTOR STUDIES. S.D. LEWIS, P. TOWERS, A.P. DAVENPORT*, L.M.P. PROUDFOOT.
Assay Research and Development, Amersham International plc, White Lion Road, Amersham, Buckinghamshire, England, HP7 9LL.
*Clinical Pharmacology Unit, University of Cambridge, Addenbrookes Hospital, Cambridge, England.
A [^{125}I] Interleukin-6 (IL-6) ligand has been labelled and purified to retain activity. Binding sites were mapped using quantitative tissue autoradiography and image analysis. In addition a homogenous competitive receptor binding assay based on Scintillation Proximity Assay (SPA) technology[1] has been established. In this assay the Auger electrons of ^{125}I must be in close proximity to the bead in order to emit light, otherwise the radioactive energy is dissipated by the aqueous medium. Cell membrane preparations and solubilized receptors[1,2] have been linked to fluomicrospheres (a support matrix consisting of a bead impregnated with fluor). This assay strategy removes the need to physically separate bound and free ligand. Samples are incubated in a medium that contains anti-mouse antibody covalently linked to a fluomicrosphere, a mouse monoclonal antibody directed to the soluble portion of the IL-6 receptor and the solubilized receptor preparation[3]. A saturable binding curve with over 90% specific binding (defined as displacable by 250nm unlabelled IL-6) was obtained.

References
1) BOSWORTH, N., and TOWERS, P. **Nature**, 341, pp.167-168, 1989.
2) TOWERS, P. *et al* J. **Endocrinol.Suppl.**, 124 No. 36, 1990.
3) YASUKAWA, K., *et al*, **J.Biochem**, **108**, pp.673-676, 1990.

P 118 DOUBLE NEGATIVE (L3T4$^-$, Lyt2$^-$) THYMOCYTES OF C57Bl/6 lpr/lpr MICE ARE RESISTANT TO DOWN REGULATION OF DNA SYNTHESIS BY A 70 kD THYMIC STROMAL CELL PRODUCT, John M. Lubinski, Jeffrey C. Chung and Takashi Makinodan, Geriatric Research, Education and Clinical Center (GRECC), VA Medical Center West Los Angeles, Los Angeles, CA 90073, and Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

The murine, autosomal recessive gene, lpr, induces a progressive lymphadenopathy and lupus-like autoimmune syndrome characterized by the accumulation of immature Thy 1$^+$, L3T4$^-$, Lyt2$^-$, T cells in peripheral lymphoid organs. Although the precise nature of the gene defect is not entirely known, thymic replacement studies point to the thymic microenvironment as crucial in the development of the abnormal cells. *In vitro* culture of thymic stromal elements in defined, serum free medium produces a 70 kD product that down modulates DNA synthesis of double negative thymocytes from normal C57Bl/6 mice. The cultured lpr stroma also produce the 70 kD factor affecting normal L3T4$^-$, Lyt2$^-$ thymocytes but the lpr double negative thymocytes continue to proliferate when exposed to the factor derived from either C57Bl/6 or lpr thymic stroma. The FACS profile of the affected thymocytes is Thy 1$^+$, TCR$\alpha\beta^+$, L3T4$^-$, Lyt2$^-$, B220(CD45R)$^+$, J11d$^+$, the phenotype of T cells present in lpr peripheral lymphoid organs. This work was supported by the Electric Power Research Institute (8000-8) and by VA Medical Research Funds.

P 119 EXPRESSION OF IL-10 IN MURINE THYMUS, Ian A. MacNeil, Takashi Suda, Chrysa M. Daley, Melissa B. Fischer and Albert Zlotnik, DNAX Research Institute, 901 California Ave., Palo Alto, CA 94304

Interleukin 10 (IL-10) a novel cytokine whose cDNA was recently isolated at our institution, induces proliferation of mature and immature thymocytes in the presence of IL2 and IL4. Both adult and day 15 fetal thymocytes (CD4$^-$8$^-$, CD4$^+$8$^-$, CD4$^-$8$^+$) proliferate strongly in the presence of IL2, IL4 and IL-10. IL-10 alone does not stimulate thymocyte proliferation. Further studies have shown that day 15 fetal and adult CD4- and CD8- thymocytes cultured in the presence of IL-10, IL2 and IL4 remain CD4$^-$ and CD8$^-$ but exhibit increased CD3 expression. The cDNA for IL-10 is strongly homologous to the Epstein-Barr virus gene BCRFI(vIL-10), and both recombinant proteins have Cytokine Synthesis Inhibitory Factor (CSIF) activity. In contrast to IL-10, BCRFI does not exhibit detectable thymocyte-stimulating activity, suggesting the existence of at least 2 functional epitopes on the IL-10 molecule.

IL-10 is secreted by day 15 fetal thymocytes, adult thymocytes and adult splenocytes when stimulated via their T cell receptor. IL-10 mRNA can be detected in unstimulated fetal but not adult thymocytes using a polymerase chain reaction (PCR) suggesting a physiological role for IL-10 in thymic development.

P 120 **EICOSANOID PRODUCTION IN CULTURED THYMIC NURSE CELLS**, James E. McCormack, Philippa Marrack, John W. Kappler and Jay Y. Westcott, Howard Hughes Medical Institute and Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, and Cardiovascular Pulmonary Research Laboratory, Department of Medicine, Department of Biochemistry, Biophysics and Genetics and Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80206

We have cultured freshly isolated thymic nurse cell complexes (TNC) from BALB/c mice, and assayed the resulting culture supernatants for the presence of eicosanoids. TNC spontaneously and continuously produce PGE_2 and PGI_2 (prostacyclin) under these conditions. Levels of thromboxane B_2, PGD_2, and leukotrienes B_4, $C_4/D_4/E_4$ were also assayed, but no significant production of these arachidonic acid metabolites was noted. PGE_2 was produced at levels up to 70 ng/ml/10^5 TNC clusters in 24 hr, while PGI_2 was produced at up to 12 ng/ml per 10^5 TNC in a 24 hr period. This eicosanoid production could not be accounted for by contaminating cells. This observation raises the possibility that PGE_2 and/or PGI_2 may play an important role in defining a particular microenvironment within the thymus, and may thereby have an effect on thymocyte development.

P 121 POST-TRANSCRIPTIONAL REGULATION OF THE ERYTHROPOIETIN RECEPTOR (EpoR) GENE, AND NOT GATA-1 EXPRESSION, CORRELATES WITH ERYTHROID DIFFERENTIATION, Anna Rita Migliaccio, Giovanni Migliaccio, Laura Ruzzi, Silvia Crotta, Stefania Nicolis, Sergio Ottolenghi and John W. Adamson. Laboratory of Hematopoietic Growth Factors, NY Blood Center, NY, NY 10021 and Dipartimento di Genetica e di Biologia dei Microrganismi, Università di Milano, Milano, Italy.
During erythroid differentiation, cells undergo a series of coordinated changes enabling them to respond to Epo and to express erythroid-specific genes. To understand the molecular basis of erythroid differentiation, we have analyzed the expression and function of EpoR and of GATA-1, a transcription factor which possibly activates several promoters and enhancers of erythroid-specific genes, such as EpoR and globin. These studies employed factor-dependent subclones of the IL-3-dependent cell line 32D. Epo-, GM-CSF, G-CSF- and IL-3-dependent cells showed erythroid, myelomonocytic, granulocytic or mast cell phenotypes, respectively. EpoR and GATA-1 mRNA (by RNAase protection analysis) and protein (by immunoprecipitation with EpoR-specific antibody or by electrophoretic mobility shift assays specific for GATA-1 consensus sequences) were detectable not only in erythroid cells but also in the original 32D and in the GM-CSF-dependent cells. In contrast, only the erythroid 32D Epo cells expressed functional EpoR by receptor binding analysis grew in Epo and expressed chloramphenicol acetyltransferase (CAT) activity when transfected with a CAT gene under the control of erythroid GATA-1 cognate sequences ($^{-175}\gamma$ promoter). GATA-1 mRNA, but not protein, was found in the G-CSF-dependent subclone. GATA-1 expression correlated with the level of EpoR mRNA detected in the different cell lines (Epo>IL-3>GM-CSF>>G-CSF). Therefore, functional EpoR, and not EpoR mRNA or protein, correlates with a cell's ability to express the erythroid phenotype. Post-transcriptional regulation of the expression of the EpoR gene, possibly involved in the transport of EpoR to the cell surface, may be important in the control of the erythroid differentiation program. These data suggest that GATA-1 expression is not confined to erythroid cells and that expression of functional EpoR determines the erythroid phenotype.

P 122 RECOMBINANT RAT AND HUMAN STEM CELL FACTOR (SCF) INDUCES PROLIFERATION AND DIFFERENTIATION OF PRIMITIVE HEMATOPOIETIC PROGENITORS CELLS (HPC) IN SERUM-DEPRIVED CULTURES, Giovanni Migliaccio, Anna Rita Migliaccio, Jay Valinsky*, Kriztina N. Zsebo*, Jan W.M. Visser* and John W. Adamson. Laboratory of Hematopoietic Growth Factors, New York Blood Center, New York, NY 10021 and Amgen, Thousand Oaks, CA, USA and TNO, Rijswijk, The Netherlands.
SCF is a novel growth factor which is the ligand for c-kit. We analyzed the action of SCF, alone and with IL-3, on the proliferation of highly purified murine and human HPC in suspension culture. Based on their ability to bind the mitochondrial dye, rhodamine 123 (Rh), murine HP were divided into two groups, Rh^+ and Rh^-. Human HPC were partially purified from perinatal cord blood by density gradient separation and selective panning with wheat-germ agglutinin and anti-CD34. Purified murine and human HPC failed to form colonies in semisolid cultures and died within 2 days in suspension culture in the absence of growth factors. Rh^+ HPC proliferated in the presence of SCF and/or IL-3. Maximal proliferation was observed with SCF and IL-3 and resulted in the accumulation of BFU-E, CFU-GM and CFU-Mix with peak numbers at day 5 of culture. Under this condition, the frequency of progenitor cells increased from 1% to 18%. Rh^- cells and human HPC required the presence of both IL-3 and SCF to proliferate in culture. In contrast to the results with Rh^+, cultures of Rh^- cells failed to yield colony-forming cells (CFC) before day 10 of culture and CFC peaked at day 21-28, representing 30-50% of all cells present. These results suggest that SCF permits the proliferation of primitive murine and human HPC.

P 123 *IN VITRO* EFFECT OF THE *EVI-1* GENE EXPRESSION IN HEMATOPOIETIC CELLS.
Kazuhiro Morishita, Evan Parganas, Wendy Lumm, and James N. Ihle. Department of Biochemistry, St. Jude Children's Research Hospital, Memphis TN. 38115

The *Evi-1* gene was identified in murine myeloid leukemias by retroviral insertions in *Evi-1* or *fim-3/CB-1* common viral integration sites. The *Evi-1* gene product is a 145 kd nuclear DNA binding protein with ten zinc finger repeats. Retroviral insertion within this locus activates transcription of the *Evi-1* gene, which would be expected to contribute to the transformation of myeloid cells by regulating transcription of specific genes. To study the effect of *Evi-1* gene expression in hematopoietic cells, we infected a IL-3 dependent cell line 32Dcl3, and normal primary bone marrow cells, with an *Evi-1* retroviral expression vector. *Evi-1* infected 32Dcl3 cells continued to require IL-3 for growth, but lost their viability in media with G-CSF. In contrast, parental 32Dcl3 cells maintained their viability and differentiated to granulocytes after a week in culture. Culturing *Evi-1* retroviral infected primary bone marrow cells in IL-3 media for one month gave rise to over 90% basophilic and eosinophilic population of cells, along with a few immature hematopoietic cells. After three months in culture, all basophils and eosinophils died leaving only a few immature cells which continued to grow slowly. Following six months in culture, these cells remained immature on the basis of phenotype, were IL-3 dependent, showed a slight response to erythropoietin and did not respond to G-CSF. These experiments demonstrate that *Evi-1* gene expression can alter the differentiation pathways of immature myeloid cells as a step in myeloid transformation, yet appear to require secondary events for overt leukemogenesis. *In vivo* effects of *Evi-1* gene expression is currently being addressed.

P 124 KIDNEY CELL LYSATES CONTAIN AN ACTIVITY THAT STIMULATES MATURE ERYTHROID BURST-FORMING-UNIT (mBFU-E) PROLIFERATION, Guy Mouchiroud, Julien Royet, Sylvie Arnaud, Thierry Oddos, and Jean-Paul Blanchet, Centre de Génétique Moléculaire et Cellulaire, UMR CNRS 106, Université Claude Bernard, 69622 Villeurbanne Cedex, France.

In the mouse system, the burst-forming-unit-erythroid (BFU-E) give rise, within 7 days, to large multicentric erythroid colonies, in semi-solid cultures, called bursts; the colony-forming-unit- erythroid (CFU-E) form colonies of 8 to 64 cells within 48 hours. Erythropoietin (Epo) is required for the proliferation and the differentiation of CFU-E whereas burst-promoting-activity (BPA) induces the development of BFU-E. This activity is shared by interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4).

We report here the presence of an erythropoietic activity in murine kidney cell lysates that predominantly stimulates the proliferation of mature BFU-E progenitors (mBFU-E) giving rise to small bursts of fewer than 15 erythroid subcolonies in semi-solid cultures established in the presence of saturating concentrations of Epo (0.5 U/ml). This activity was found to be respectively enhanced and decreased in kidneys from anemic and polycythemic mice. The disappearance of activity in kidney-cell extracts during long-term polycythemia correlated with an accumulation of mature BFU-E in the spleen and bone marrow of polycythemic mice. This activity was not abolished in the presence of neutralizing anti-IL-3, anti-GM-CSF or anti-IL-4. Moreover, it was demonstrated by using relevant biological assays that this activity was different from Epo, Stem Cell Factor (c-kit ligand), IL-9 and HILDA/Leukemia Inhibitory Factor (LIF).

In conclusion, we have detected in kidney cell extracts a novel erythropoietic activity that acts on a particular class of progenitors, the mBFU-E, and that appears to be regulated in vivo by the erythropoietic status of the donor.

P 125 INTERACTION OF THE KIT LIGAND (KL) WITH C-KIT: EFFECTS OF ANTIBODIES DIRECTED AGAINST C-KIT AND KL. Karl H. Nocka, Jochen Buck, Eric Huang, Norma B. Lerner, Ester Levi, Leonie Ashman and Peter Besmer, Depts. of Molecular Biology, Immunology, and Pediatrics, Memorial-Sloan Kettering Cancer Center, New York, NY, 10021 and Dept. of Microbiology and Immunology, University of Adelaide, Adelaide, S. Australia.

The c-kit proto-oncogene encodes a transmembrane receptor which maps to the W locus of the mouse. Recently we have purified a hematopoietic growth factor which is a ligand of c-kit (KL) and have mapped KL to the SL locus of the mouse. Based on the phenotype of mice with mutations in W and Sl loci, c-kit/KL are thought to play important functions in hematopoiesis, gametogenesis, and melanogenesis. Previously, we have generated a number of polyclonal antisera against murine c-kit and have also characterized a murine monoclonal antibody (YB5.B8) which recognizes human c-kit. In addition an antisera which precipitates murine KL has also been raised. These antibodies have been used to study a complex which is formed following binding of KL to c-kit on mast cells. They have also been tested for their ability to affect the binding of KL to c-kit. Antibodies raised against KL or which recognize extracellular epitopes of murine or human c-kit were found to inhibit the binding of KL to its receptor. These experiments further define the receptor/ligand relationship between the c-kit/W and Sl gene products and may be of use in further characterizing their expression and function in various cellular systems.

P 126 STIMULATION OF PRECURSOR B CELL PROLIFERATION IN MOUSE BONE MARROW BY IN VIVO ADMINISTRATION OF RECOMBINANT INTERLEUKIN-1 AND INTERLEUKIN-7, Dennis G. Osmond, Lucy Fauteux and Homer Valenzona, Department of Anatomy, McGill University, Montreal, Quebec, H3A 2B2 Canada

In C3H/HeJ mice given interleukin-1 (IL-1) or IL-7, immunofluorescence labeling of terminal deoxynucleotidyl transferase (TdT), B220 glycoprotein and μ chains has been used to quantitate 3 populations of pro-B cells lacking μ chains (TdT^+B220^-, TdT^+B220^+, TdT^-B220^+), cytoplasmic μ-bearing pre-B cells and surface μ-bearing B lymphocytes. Proliferative activity was indicated by the number of cells arrested in metaphase after giving vincristine. Assays 1d and 6d after ip injection of recombinant murine IL-1 or IL-7 (Immunex Corp., Seattle) in low-endotoxin mouse serum albumin (MSA) established optimal doses for precursor B cell responses, compared with MSA-injected controls. Daily assays after 250 ng IL-1 ip revealed a delayed increase in the proliferation and number of large pre-B cells and in the number of post-mitotic small pre-B and B cells. 125 ng IL-7 ip produced a wave of increased number and proliferation of late pro-B cells and pre-B cells at 1-3d with expansion of the populations of small pre-B and B cells. The results demonstrate that IL-1 and IL-7 can modulate primary B cell genesis in vivo, suggesting that these cytokines could play a role in the previously observed effect of activated splenic macrophages in stimulating the proliferation of early precursor B cells in the bone marrow. (Supported by NCI Canada).

P 127 THE ROLE OF CYTOKINES IN THE GROWTH AND DIFFERENTIATION OF PURIFIED SUBSETS OF IMMATURE THYMOCYTES, Howard T. Petrie and Ken Shortman, The Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia.
T-lymphocyte differentiation in the thymus can be characterized into two general phases. The first involves extensive proliferation, which leads to the production of large numbers $CD3^{lo}4^{+}8^{+}$ typical cortical cells. The second is characterized by stringent positive and negative selection events, which act on the products of the first phase. T-lymphocytes at various stages of development can be categorized into a number of phenotypically distinguishable subsets, which form a single developmental stream. The factors which regulate progression along this stream are poorly understood. However, cytokines are likely to play a large part in this process, particularly during the initial proliferative phase. We have identified populations of cells which have limited differentiation capacity in culture without added cytokines; we consider these cells to be 'preprogrammed.' Other populations do not differentiate in simple culture medium, but do so intrathymically. We are attempting to identify factors which can induce these to proliferate and/or differentiate. Three types of studies are being carried out. The first involves microscopic examination of cell growth in Terasaki trays, using either single cells or mini-bulk cultures (20-500 cells/well) with various combinations of cytokines. The second type of study involves Northern blotting of mRNA from purified populations of thymocytes, to probe for the expression of known cytokine receptor genes. The final approach being used involves coculture of immature thymocytes with purified stromal cells or stromal cell lines. Any stromal cells which successfully support thymocyte growth will subsequently be analyzed for the production of known cytokines by Northern blotting. Together, these studies should help to identify the factors responsible for the intrathymic maturation of T-lymphocytes.

P 128 Tyrosine Phosphorylated Substrates Induced in 32D Cells Transfected with Either Tyrosine Kinase-Containing or Hematopoietin Receptors: Correlation with Proliferation or Monocytic Differentiation. Jacalyn H. Pierce, Mohammad Heidaran, Jennifer Artrip, Warren Leonard, Hiro Otani, Stella Zannini, Michael Pangelinan, Jin Chen Yu and Stuart A. Aaronson. NIH, Bethesda, MD
The IL-3-dependent myeloid progenitor cell line, 32D, was transfected with expression vectors containing human cDNAs for EGF, PDGF or CSF-1 receptor tyrosine kinases. Exposure of the 32D transfectants to their respective ligands triggered proliferation coupled with partial monocytic differentiation. Expression of receptors lacking tyrosine kinase domains in 32D cells induced long-term proliferation without monocytic differentiation. These receptors included those for erythropoietin (Epo), IL-2 and GM-CSF. Analysis of cellular substrates which became tyrosine phosphorylated in response to specific ligands revealed that identical phosphoproteins with molecular weights of 70 and 93 kd were induced in response to Epo, IL-2, IL-3, and GM-CSF. In contrast, EGF, CSF-1 and PDGF triggered the tyrosine phosphorylation of multiple and overlapping substrates. These results suggest that activation of receptors lacking tyrosine kinase domains may mediate proliferation by coupling with a specific tyrosine kinase which induces the phosphorylation of a common set of intracellular proteins. In contrast, tyrosine kinase-containing receptors may induce monocytic differentiation by regulating tyrosine phosphorylation of a distinct set of cellular substrates.

P 129 IL-11: IN VITRO ACTIVITY ON HEMATOPOIETIC PROGENITORS
Valerie F.J. Quesniaux, S.R. Paul, Steve C. Clark, and Barbara Fagg, Sandoz Pharma, 4002 Basel Switzerland, Dana-Farber Cancer Institute, Boston MA 02115, Genetics Institute, Cambridge MA 02140
IL-11 was recently cloned from a primate bone marrow stromal cell line (Paul et al., PNAS 87:7512, 1990) and is a candidate growth factor for primitive hematopoietic progenitors. We investigated the effect of IL-11 on different hematopoietic progenitors either directly in colony assays or by pretreating murine bone marrow in suspension culture and then testing the survival of primitive pluripotent precursors in several in vivo and in vitro assays:
1) The most differentiated erythroid precursors (CFU-E) were detected in a 2 day colony assay. 2) Precursors commited to the erythroid, granulocyte-macrophage, and megakaryocyte lineages and pluripotent erythroid-myeloid precursors (CFU-mix) were tested in a 9 day colony assay. 3) Suspension culture used prior to colony assay allowed the detection of early pluripotential erythroid-myeloid precursors which are able to undergo self-renewal (pre-CFU-mix). 4) Long-term and short-term repopulating stem cells were assayed in vivo by competitive bone marrow reconstitution using $Gpi\text{-}I^{a/b}$ markers.
The ability of IL-11, alone or in combination with other factors such as IL-1, IL-3, IL-6 or erythropoietin, to allow the multiplication of these different hematopoietic progenitors in suspension culture or to directly stimulate colony formation will be discussed.

P 130 A MODEL STROMAL CELL: FIBROBLASTS EXPRESSING INTERLEUKIN-7 SUPPORT THE LONG TERM GROWTH OF FEEDER DEPENDENT BONE MARROW CELLS

Benjamin E. Rich and Philip Leder, Department of Genetics, Harvard Medical School, Boston, MA 02115

Interleukin-7 (IL-7) is a cytokine that has been implicated in the early development of B lymphocytes and has been shown to be responsible for some of the properties of the stromal cells of murine bone marrow cultures. Although the molecular basis of the cell-cell contact in bone marrow cultures is not well understood we reasoned that it is important for growth stimulation by IL-7. Thus we have engineered an NIH3T3 cell line to secrete IL-7 and used it as a feeder cell in bone marrow cultures. This cell line is called NAIL-7. When feeder dependent lymphocytes from a bone marrow culture are co-cultured with NAIL-7 cells they proliferate vigorously and to higher densities than they do if grown on primary stromal cells, while the same lymphocytes co-cultured with the parental NIH3T3 cells show very little growth. The ready availability of feeder cells and the ability of lymphocytes to be serially passaged onto fresh NAIL-7 plates allow for rapid expansion and cloning of cell lines. We have used NAIL-7 feeder cells to establish a number of cloned B lineage cell lines from mature bone marrow cultures as well as directly from fresh marrow.

P 131 ISOLATION OF MURINE HEMATOPOIETIC PROGENITOR SUBSETS RESPONSIBLE FOR IL-3-INDUCED HISTAMINE SYNTHESIS, Elke Schneider*, Rob E. Ploemacher** and Michel Dy*, *INSERM U 25, CNRS UA 122, Hôpital Necker, Paris, France, **Department of Immunology and Immunohistology, Erasmus University, Rotterdam, The Netherlands

We have characterized herein the murine bone marrow cells responsible for increased histamine synthesis in response to IL-3. After excluding the participation of differentiated end cells or stromal componants in this biological activity, we have analyzed different hematopoietic subsets for their capacity to synthesize histamine after exposure to IL-3. FACS sorting procedures were carried out on Ficoll-fractionated or lineage-depleted progenitor-enriched bone marrow populations. Primitive stem cells with marrow repopulating activity (MRA) were separated from other progenitors on the basis of rhodamine retention, whereas CFU-S were purified in the cell subsets with high wheatgerm agglutinin (WGA) affinity. Using these techniques, the targets of IL-3 in terms of histamine production could be defined as relatively large and granular cells, whose incidence increases with increasing rhodamine retention, reflecting enhanced mitochondrial activity. In contrast, the most primitive rhodamine-dull cells do not synthesize histamine in response to IL-3 and histamine-producing cells do not copurify with CFU-S day-7 and day-12 in the WGA-bright fractions. Together with the observation that the enrichment for histamine-producing cells is associated with increased frequencies of cells forming colonies in methylcellulose (CFU-G/M), our data are consistent with a predominant involvement of these partially lineage-restricted progenitors in IL-3-induced histamine synthesis.

P 132 TRANSFORMING GROWTH FACTOR-B (TGF-B) INHIBITS THE SYNERGISTIC EFFECT OF IL-6, IL-1 and G-CSF ON IL-3 INDUCED GROWTH OF ISOLATED HEMATOPOIETIC PROGENITORS. [1]K.T. Sill, [2]F.W. Ruscetti, [3]J. R. Keller. [1,3] BCDP-PRI/DYNCORP, [2] LMI-BRMP, NCI-FCRDC, Frederick, MD. Interleukin-6 (IL-6),interleukin-1 (IL-1) and granulocyte stimulating factor (G-CSF) have been shown to act synergistically with IL-3 to promote the growth of primitive hematopoietic progenitors. We have shown that TGF-ß can directly inhibit the IL-3-induced growth of highly purified progenitor cells. To determine if the synergistic effect was direct or indirect on isolated cells, and if this could be inhibited by TGF-B, bone marrow was purified by positive selection with antibodies and magnetic beads to enrich for progenitor cells (lineage negative, Lin-) and then FACS separated for Thy-1 antigen expression enriching for the most immature progenitors, Lin- Thy 1+. Single cells were plated in Teraski wells, plus and minus factors, and colonies greater than 10 cells per well were scored. The average frequency of cells responding to IL-3 alone was 1 in 8. The addition of TGF-B dropped the frequency of cells responding to IL-3 to an average of 1 in 40. In the presence of IL-3 plus G-CSF, IL-6 or IL-1, the frequency increased above IL-3 alone to an average or 1 in 3 and this response was inhibited by TGF-B, dropping the frequency of cells responding to both signals to 1 in 30. These results demonstrate that the synergistic effect of IL-3 plus IL-6, or IL-1 or G-CSF is direct on Lin-Thy+ progenitor cells and that TGF-ß directly inhibits this effect.

P 133 IL-7 DIFFERENTIATES A SUBGROUP OF ACUTE LYMPHOBLASTIC LEUKEMIAS, Cecilia Skjønsberg, Bjørn Erikstein, Erlend B.Smeland, Steinar Funderud, Heidi Kiil Blomhoff, Lab. of Immunology, Inst. for Cancer Research, The Norwegian Radium Hospital, 0310 Oslo 3, Norway.

The bone marrow stromal cell derived growth factor Il-7 is known to stimulate growth of normal human B-cell precursors. Thus, it was of interest to examine the effect of recombinant Il-7 on their neoplastic counterparts. Leukemic cells from 20 patients with common acute lymphoblastic leukemia (c-ALL) were highly purified by removing T-cells and monocytes by rosetting with immunomagnetic beads. Il-7 markedly reduced the DNA synthesis in leukemic cells from 3 patients. This inhibition was accompanied by maturation of the cells as analysed by expression of the differentiation antigens CD19, CD20, CDw75, surface μ-chain and terminal deoxynucleotidyl transferase. By examining G1 parameters, such as MYC, 4F2-, and transferrin receptor-levels as well as RNA and Ki67, it appeared that the cells were inhibited late in G1. That the Il-7 responding cells were B-lineage cells was confirmed by co-staining the cells with CD19 and Ki67. Our results may be an example of an altered response to growth factors by malignant cells compared to their normal counterparts or it may reveal a hitherto unknown normal response to Il-7 in a subgroup of human B-cell precursors.

P 134 HEMODYNAMIC EFFECTS OF IL-1α ADMINISTERED TO CANCER PATIENTS (PTS) IN A PHASE I TRIAL. John W. Smith II, Dan L. Longo, Ronald G. Steis, John E. Janik, William H. Sharfman, Robert G. Fenton, Augusto Ochoa, Kevin C. Conlon, Mario Sznol, Langdon Miller, Stephen P. Creekmore, John Vitarello, Joost Oppenheim, Masanao Shimuzu, Juan Ochoa, Walter J. Urba. BRMP, DCT, NCI; PRI/DynCorp, FCRDC; FMH, Frederick, MD 21701; Dainippon, Osaka, Japan.

IL-1 is potentially useful in the treatment (tx) of cancer because it has antiproliferative, immunological, and hematological effects. One of the dose-limiting toxicities noted in our phase I trial of IL-1α was severe hypotension requiring blood pressure (BP) support with intravenous (IV) pressors (phenylephrine/dopamine). Five pts treated with 0.3 or 1.0 μg/kg of IL-1α were invasively monitored in the intensive care unit in order to characterize the cardiovascular effects of IL-1α. Systolic BP rose during the first 30 min. after tx but fell consistently thereafter reaching 90 mm Hg between 3 to 5 hrs after tx. The hypotension was caused by a significant (50%) drop in systemic vascular resistance. No differences were noted between patients treated with or without indomethacin. Reflex tachycardia and an increase in cardiac output occurred in all pts; however, from 3 to 8 hrs. after tx, the cardiac output failed to rise in response to IV fluid loading that resulted in a significant increase in the pulmonary capillary wedge pressure. The latter observation indicates ventricular dysfunction and is similar to what is noted with endotoxin administration to human volunteers. The sera of pts showed significant increases in nitrate levels suggesting that the mechanism for the hypotension is related to the release and formation of nitric oxide.

P 135 **HEMATOPOIETIC DEVELOPMENT OF EMBRYONIC STEM CELLS IN VITRO: CYTOKINE AND RECEPTOR GENE EXPRESSION.**

Ralph Snodgrass, Regina M. Schmitt, & Eddy Bruyns. Lineberger Comprehensive Cancer Center, University of North Carolina Chapel Hill, NC 27599.

We describe a novel system in which stable totipotent tissue culture maintained embryonic stem (ES) cells are capable of differentiating in vitro into hematopoietic precursors for many of the colony forming cells found in normal bone marrow. We examined the genetic expression of cytokines, their receptors, β-globins, and hematopoietic cell surface markers throughout the time course of ES cell differentiation and correlated these results with hematopoietic development. Our results indicate that there is a strong transcriptional activation in a well defined temporal order of most of these genes including erythropoietin, CSF-1, IL-4, IL-6, β-globins, as well as the receptors for erythropoietin, CSF-1, and IL-4. In addition IL-3 and GM-CSF were not expressed before or during the peak of hematopoietic development. These data strongly support the conclusion that IL-3 and GM-CSF are not critical to the early hematopoietic development. Furthermore, these results are consistent with the conclusion that Epo, IL-4, and IL-6 are important during the early stages of ES cell differentiation. This system offers a unique in vitro model to study hematopoietic development at the earliest stages, and may be useful as an in vitro source of hematopoietic cells.

P 136 HUMAN CD3$^-$ CD4$^-$ CD8$^-$ THYMOCYTES DIFFERENTIATE INTO CD3$^-$ CD4$^+$ CD8$\alpha^+\beta^-$ CELLS IN VITRO IN THE PRESENCE OF IL-7, Hergen Spits, James Cupp, Nicholas Wrighton*, Frank Lee* and Toshiyuki Hori, Department of Human Immunology and *Department of Molecular biology, DNAX Research Institute of Molecular and Cellular Biology

Human CD3$^-$ CD4$^-$ CD8$^-$ (triple negative) thymocytes were highly purified from post-natal thymi by using magnetic bead columns and cell sorting. Proliferation experiments showed that these cells responded much better to IL-7 than to IL-2 or IL-4. IL-7 but not IL-2 or IL-4 could maintain the cell viability for up to 14 days. We followed the phenotypic change of triple negative thymocytes during the culture with IL-7. After 7 to 14 days of culture with IL-7, a considerable proportion became CD4$^+$ CD8$^+$ (double positive). In contrast to common cortical double positive thymocytes which express low levels of CD3 and both α- and β-chains of CD8, these double positive cells were found to be CD3$^-$ CD4$^+$ CD8$\alpha^+\beta^-$. There were no CD3$^+$ cells in this culture, indicating that human triple negative thymocytes can not differentiate into TCR $\alpha\beta^+$ or TCR$\gamma\delta^+$ cells under these culture conditions. By using four color immunofluorescence and four parameter cytofluorometric analysis, we identified a minor subset with the phenotype of CD3$^-$ CD4$^+$ CD8$\alpha^+\beta^-$ in fresh human thymocytes. These results suggest that the CD3$^-$ CD4$^+$ CD8$\alpha^+\beta^-$ subset physiologically exists in the thymus and may represent an intermediate stage between triple negative and common cortical double positive thymocytes.

P 137 STRUCTURE-FUNCTION ANALYSIS OF HUMAN INTERLEUKIN-6, EVIDENCE FOR THE INVOLVEMENT OF THE CARBOXYTERMINUS IN BIOLOGICAL FUNCTION

Tanja Stoyan, Alex Krüttgen, Stefan Rose-John, Berthold Wroblowski, Axel Wollmer, and Peter C. Heinrich
Institut für Biochemie der RWTH-Aachen, Pauwelstrasse, 5100 Aachen, FRG

Interleukins are a group of signaling molecules involved in the communication of cells. They are produced and secreted by many different cell types, particular by those of the immune system. IL-6 elicits a wide spectrum of biological functions via specific surface receptors. To understand the interaction of IL-6 with its receptor, it is important to get some information on structural features essential for its biological function. Secondary structure prediction of human IL-6 resulted in 58% helix, 14% ß-structure and 28% turn and coil, respectively. The circular dichroism of recombinant IL-6 was measured in the near and far UV light. Evaluation of the latter in terms of secondary structure gave 67% helix, 15% ß-structure and 18% turn and coil. As a first approach to define regions of IL-6 important for its function, we constructed IL-6 mutants lacking various portions of the carboxyterminus. Removal of only one amino acid resulted in a 80% loss of biological activity of IL-6. The truncation of three residues completely abrogated biological activity. IL-6 polypeptides lacking up to 48 carboxyterminal amino acids were also inactive. These studies show that the carboxyterminus of IL-6 is probably involved in the ligand-receptor interaction.

P 138 IL-7 MAINTAINS THE T CELL PRECURSOR POTENTIAL OF CD3$^-$CD4$^-$CD8$^-$CD25$^+$ MURINE THYMOCYTES *IN VITRO*, Takashi Suda, and Albert Zlotnik, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304

IL-7 was originally described as a pre-B cell growth factor, but has since been shown to induce proliferation of various subsets of thymocytes including CD4$^-$CD8$^-$ adult thymocytes as well as fetal thymocytes at day 15 of gestation. These results suggested that IL-7 might also be a pre-T cell growth factor, because both adult CD4$^-$CD8$^-$ and day 15 fetal thymocytes contain early T cell precursors which can reconstitute a T cell-depleted thymus. However, we recently observed that among adult CD4$^-$CD8$^-$ thymocytes, only the CD3$^+$ subset proliferated in response to IL-7. Yet, the viability of the CD3$^-$CD4$^-$CD8$^-$ triple negative (TN) thymocytes, especially of the CD25 (IL-2 receptor p55) positive subset, was strongly enhanced by IL-7. Interestingly, the CD25$^+$TN thymocytes that survived *in vitro* in the presence of IL-7 retained the ability to reconstitute lymphoid cell-depleted (2-deoxyguanosine-treated) fetal thymus organ culture. In contrast, other cytokine combinations that induced proliferation of adult CD25$^+$TN thymocytes (IL-4 (in the presence of PMA), IL-2+IL-4+IL-10) did not maintain their T cell progenitor potential. Our results indicate that IL-7 maintains the T cell progenitor potential as well as the viability of adult CD25$^+$TN thymocytes *in vitro*, but does not support their self-renewal by itself.

P 139 INHIBITORS OF HEMOPOIETIC COLONIES ARE PRODUCED BY CERTAIN RAT FIBROBLASTOID CELL LINES AND ARE MODULATED BY CORTICOSTEROIDS, Arthur K. Sullivan and Hong Wang, McGill Cancer Centre, 3655 Drummond St., Montreal, Canada H3G 1Y6, and the Department of Medicine, Division of Hematology, Royal Victoria Hospital, Montreal, Canada.

Both stimulatory (CSA) and inhibitory (INH) factors may contribute to hemopoietic regulation, but little is known about how their physiologic balance is maintained. Previously we have shown that antigen-defined fibroblastoid cells cultured from rat lung ($ST3^-/ST4^+$, by the antibodies ST3 and ST4) constitutively produce INH, and marrow-derived FB ($ST3^+/ST4^-$) respond to macrophage cytokines to release both CSA and INH into their conditioned media (CM). Here we show that this pattern was maintained in cell strains ("ST3" and "ST4") propagated from the primary cultures, and that the presence of CSA was measured in "ST4" CM if the inhibitory >100 kDa fraction was removed. Two subclones of "ST3" (2A and 9D) selected for high or low expression of the ST3 antigen both produced CSA, but only 9D produced the >100 kDa inhibitor. Culture of cells in the presence of hydrocortisone blocked the appearance of INH but not CSA. These results further demonstrate that the appearance of inhibitory activity in the growth media differs among FB subpopulations, and that it can be modified by natural regulators such as corticosteroids.

P 140 MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF IL-1 RECEPTOR TYPE II Aldo Tagliabue°, Paolo Ghiara, Giuseppe Scapigliati, Stefano Censini, Doretta Armellini, Sandra Nuti, Paola Bossù° and Diana Boraschi.
Sclavo R&D, Siena and °Dompe' R&D, Milan, Italy.
An 80 kDa glycoprotein expressed by T cells, fibroblasts, keratinocytes, epithelial and endothelial cells binds both IL-1α and IL-1β and, after molecular cloning, has been defined as IL-$1R_I$. However another 60-68 kDa glycoprotein present on B and myelomonocytic cells which binds IL-1β better than IL-1α has recently been described. We have cloned human B cell lymphoma RAJI cells to select an IL-$1R_{II}$-hyperexpressing clone (1H7). The IL-$1R_{II}$ has been purified to homogeneity from membranes of dexamethasone (DXM)-stimulated 1H7 cells by gel filtration and affinity chromatography and used for obtaining N-terminal sequence. Monoclonal antibodies raised against purified membrane preparations from DXM-stimulated 1H7 cells recognize a 65 kDa protein in 1H7 membranes, block binding of IL-1 to IL-$1R_{II}$, but not to IL-$1R_I$, stain in IL-1-inhibitable manner 1H7 and other IL-$1R_{II}$-expressing cells, but not EL4-6.1 cells (IL-$1R_I$+), nor DAUDI cells (IL-1R-). At single cell level, only a fraction of B lymphoma cells could express IL-$1R_{II}$. Cells were thus enriched in different phases of cell cycle by counterflow centrifugal elutriation and analyzed for IL-1β binding: cells in G_o-G_1 accounted for most of the IL-1 binding capacity, whereas cells in G_2-M were unable to bind IL-1. This provides a better characterization of IL-$1R_{II}$.

P 141 **ROLE OF IL-7 IN THE GROWTH AND DIFFERENTIATION OF IMMATURE THYMOCYTES,** Fumio Takei, Dolores Saunders, and Christine Vissinga, Terry Fox Laboratory, B.C. Cancer Research Centre and Department of Pathology, University of British Columbia, Vancouver, B.C., Canada
The effects of recombinant murine IL-7 on subpopulations of $CD4^-CD8^-$ (DN) thymocytes from young adult mice were studied in vitro. When highly purified $CD3^-$ DN thymocytes were incubated with IL-7 for overnight, up to 30% of them became $CD4/CD8^+$, but they remained mostly $CD3^-$. Longer incubations (2 days) resulted in the expression of CD3 as well as CD4/CD8. In the absence of IL-7, the expression of CD4/CD8 proceeded less efficiently and the viability of the cells was quite low, whereas the presence of IL-7 greatly enhanced cell viability and the expression of CD4, CD8 and CD3. No cell proliferation was detected in these cultures as measured by thymidine incorporation assays. Therefore, the detection of $CD4/CD8^+$ cells in the IL-7 stimulated cultures did not seem to be due to the contamination of mature cells which preferentially proliferate. On the other hand, IL-7 vigorously stimulated $CD3^+$ DN thymocytes to proliferate and this stimulation was independently of CD3 antibody. However, IL-7 stimulated $CD3^+$ DN thymocytes did not express CD4 or CD8. These results implicate important roles of IL-7 in the growth and differentiation of immature thymocytes. It promotes the differentiation of $CD3^-$ DN thymocytes without supporting their growth, and it also stimulates the proliferation of $CD3^+$ DN thymocytes without inducing their differentiation.

P 142 REGULATION OF C-KIT RECEPTOR EXPRESSION AND IDENTIFICATION OF TYROSINE PHOSPHORYLATED SUBSTRATES. Melanie J.Welham, Ian Clark-Lewis and John W. Schrader. The Biomedical Research Centre, 2222 Health Sciences Mall, UBC, Vancouver, British Columbia, V6T 1W5, CANADA.

The c-*kit* transmembrane protein tyrosine kinase is the receptor for a novel haemopoietic growth factor, which stimulates mast cell proliferation and survival of progenitor cells, amongst other actions. We have shown that c-*kit* receptor expression is modulated by several haemopoietic growth factors, notably IL-3, GM-CSF and Epo. Optimal levels of these growth factors lead to down-regulation in the levels of c-*kit* mRNA and protein. This occurs at the mRNA level 2 - 4h after administration of factor, remaining depressed for 18-24 h. However, IL-4 does not exert the same effects and in combination with IL-3, IL-3 acts in a dominant manner to down-regulate c-*kit* expression. This mechanism of receptor modulation could be an important cross-talk link between the effectors controlling steady state haemopoiesis and those such as IL-3 involved in the stress-related responses.

C-*kit* signalling pathways are largely uncharacterised. Analyses are underway to identify and characterise the proteins which become phosphorylated on tyrosine in response to activation of c-*kit* by its ligand. An active form of the ligand has been chemically synthesised and used to demonstrate rapid receptor autophosphorylation occuring on tyrosine after incubation of mast cells with the ligand. Immunoblot analyses have shown the phosphorylation of a number of cellular proteins on tyrosine after stimulation with the kit ligand. Interestingly, a cell line which expresses c-*kit* receptors, but fails to proliferate in its presence, exhibits phosphorylation of only a small number of these proteins. Further analyses are underway to characterise these proteins in more detail and draw correlations with other systems.

P 143 HEMOPOIETIC CYTOKINE PRODUCTION BY PRIMARY LYMPHOCYTE-SUPPORTING STROMAL CELLS, Pamela Witte, Phillip Funk, Muriel Hergott, and Robert Becker, Departments of Cell Biology and Microbiology, Loyola Univ. Med. Cnt., Maywood, IL and Connaught Laboratories, Swiftwater, PA.

Studies of Whitlock-type bone marrow cultures have revealed the necessity for at least two types of cells for B-lymphopoiesis: a stem cell and a stromal cell. To define the actual functions of stromal cells in vivo, we have focused on two populations of primary, unpassaged stromal cells and the transcription and secretion of IL-7, M-CSF and GM-CSF. One stromal population is isolated directly from Whitlock cultures by FACS sorting; the other is enriched from fresh bone marrow. Sorted, cultured stromal cells alone supported pre-B cell growth and supernatants contained CSF that gave rise mostly to macrophage colonies. Indeed, PCR amplification of cDNA from purified RNA revealed the presence of mRNA for IL-7 and M-CSF, but not GM-CSF. The minor fraction of fresh bone marrow cells found within small aggregates are an enriched source of uncultured lymphopoietic stromal cells. In contrast to cultured stromal cells, PCR analysis of marrow aggregates did not reveal RNA for M-CSF, but IL-7 RNA was detectable especially in young mice. However, stromal-depleted marrow (deaggregated or G-10 passed) also expressed IL-7 RNA. Our current comparisons of fresh, primary cultured, and cloned stromal cell lines suggest that: 1) primary stromal cells differ from some stromal lines by absence of GM-CSF; 2) M-CSF production is enhanced or activated upon culture; 3) one or more marrow cells other than stromal cells transcribe the IL-7 gene.

P 144 INHIBITION OF INTERLEUKIN 4 RECEPTOR EXPRESSION ON HUMAN LYMPHOID CELLS BY FK 506, Gaëtane Woerly, *Brian M.J. Foxwell & Bernhard Ryffel, Drug Safety Assessement, Sandoz Pharma Ltd., Basel/Switzerland, and *Charing Cross Sunley Research Center, London W6 8LW/England.

The effect of the immunosuppressant macrolide FK 506 on the expression IL-4 membrane receptors on human peripheral blood mononuclear cells (PBMC) was investigated after cell activation by anti-CD3 antibody, IL-2 or IL-4. Previous studies with ^{125}I-IL-4 identified on resting lymphocytes a trimolecular complex consisting of 65/70 kD doublet and a 110 kD protein with ~ 300 high affinity binding sites (Kd 100 pM) and with ~ 9000 low affinity binding sites (Kd 30 nM). Upon cell activation by anti-CD3 antibody both low and high affinity binding sites increased about threefold concomitant with upregulation of all the crosslinked proteins. FK 506 inhibited anti-CD3 antibody induced upregulation of IL-4R associated proteins as well as the expression of high affinity binding sites. However, the growth promoting effect of IL-4 on activated, IL-4R positive T cells was FK 506 resistant. Since FK 506 inhibits the synthesis of IL-4, exogenous IL-4 was added to the cultures and it partially reversed the inhibitory effect of FK 506 on cell proliferation as well as on IL-4R expression. It is concluded that the inhibitory effect of FK 506 on IL-4 receptor expression may contribute to the immunosuppressive effect of the drug.

P 145 BIOLOGICAL CHARACTERIZATION OF RECOMBINANT RAT AND HUMAN STEM CELL FACTOR, Krisztina M. Zsebo, Neal C. Birkett, Kent A. Smith, Joan Egrie, Phil McMahon, and Keith E. Langley, Stem Cell Biology, AMGEN, Thousand Oaks, CA 91320-1789

Recombinant rat and human stem cell factor (SCF), c-kit ligand, has been purified to homogeneity and tested in vitro and in vivo. Rat SCF treatment of steel (Sl/Sl^d) mice leads to a reduction in the severity of their macrocytic anemia, reconstitution of CFU-S_{12} content, and the appearance of mast cells at the injection site. Serum levels of EPO and G-CSF have been measured in control and rat SCF treated Sl/Sl^d mice. Serum EPO levels are inversely proportional to the severity of the anemia (anemic animals have higher EPO levels). The levels of G-CSF are lower in Sl/Sl^d mice than in +/+ controls. When bone marrow or spleen cells are transplanted from normal mice which have received rat SCF in vivo into W/W^v recipients, the number of cells required for engraftment of donor cells is reduced by 10-100 fold. Human SCF synergizes with other colony stimulating factors to increase the size and number of colonies in semi-solid cultures. The addition of human SCF to bone marrow and peripheral blood cultures in the presence of colony stimulating factors overcomes the inhibition of BFU-E formation by AZT but not inhibition of CFU-GM by TGF-β_1, IFN-γ, or TNF.

P 146 REGULATORY SEQUENCES REQUIRED FOR THE IL-9 GENE EXPRESSION IN HTLV-I-TRANSFORMED HUMAN T CELLS. Yu-Chung Yang and Wen Luo. Departments of Medicine and Molecular Biology and Walther Oncology Center, Indiana University School of Medicine, Indianapolis IN 46202

We have recently determined the human IL-9 genomic sequence and mapped the gene to the long arm of human chromosome 5 at 5q31-32, a region found to be deleted in a number of patients with acquired 5q- abnormalities and hematological disorders[1]. The 5'-regulatory region of the human IL-9 gene contains sequences identified in the 5'-flanking regions of other cytokine genes mapped to the long arm of human chromosome 5 including IL-3, IL-4, IL-5 and GM-CSF and other T cell growth factor genes including IL-2 and IL-6. The IL-9 gene is constitutively expressed in the HTLV-I-transformed human T cells and the expression can be further induced by TPA. The presence of recognition sequences for several TPA-inducible activation proteins in the 5'-flanking may account for the TPA inducibility of IL-9 in these transformed cells. Transient transfection analysis using plasmids containing various portions of IL-9 5'-flanking region upstream from the firefly luciferase reporter gene have revealed that different control sequences are involved in the constitutive and activated expression in the HTLV-I-transformed T cells. The constitutive expression of IL-9 in HTLV-I-transformed cells does not appear to be mediated by HTLV-I encoded transactivator, tax, alone. Other cis- and trans- elements required for IL-9 gene expression are currently being investigated.

[1] Kelleher et al. (1991) Blood, in press.

Cytokine Regulation of Lymphocyte Activation

P 200 EFFECT OF TGF-BETA ON THE PROLIFERATION OF B CELL LINES AND EBV INDUCED TRANSFORMATION OF B CELLS, G. Aysin Altiok, Maria T Bejerano, Frank Ruscetti, Ender Altiok,Eva Klein. Department of Tumor Biology, Karolinska Institutet, S-104 01 Stockholm, SWEDEN.

We tested the effect of transforming growth factor (TGF)-beta 1 and -beta 2 on the proliferation of human B cell lines. The panel was selected to give information whether 1) their origin 2) their phenotype 3) their Epstein-Barr virus (EBV) carrier state, influence their responsiveness. The growth of lymphoblastoid cell lines (LCL) was not inhibited by TGF-beta 1. The EBV carrying Burkitt lymphoma (BL) lines, Daudi, Jijoye, Rael but not Raji were inhibited. Three EBV negative BL lines and the majority of their converted sublines were sensitive. The cell lines tested expressed TGF-beta receptors and TGF-beta 1 transcripts. The proliferation of EBV infected B cells was inhibited by TGF-beta, their sensitivity decreased however after 3 days. The results suggest that the activation state of the B cells is decisive for TGF-beta sensitivity and EBV influences it indirectly by changing the cell phenotype.

P 201 **EFFECTS OF IL-4 ON GROWTH AND MATURATION OF HUMAN B CELL PRECURSORS,** Jacques Banchereau, Dominique Pandrau, Valérie Duvert and Sem Saeland, Schering-Plough, Laboratory for Immunological Research, BP 11, 69571 Dardilly Cedex, France

We investigated the role of IL-4 in the proliferation and maturation of normal and leukemic human B cell precursors (BCP). $CD10^+s\mu^-$ BCP were isolated from mid-term normal fetal bone marrow. IL-7, and, to a lesser extent, IL-3 induced significant cell proliferation within this population. Moreover, potentiating effects were observed when these two cytokines were used in combination. Addition of IL-4 to proliferating cultures resulted in inhibition of cell growth. This dose-dependent effect of IL-4 reached a maximum at 10 U/ml. Comparable concentrations of IL-4 were also found to inhibit spontaneous proliferation in cultures of fresh B lineage acute lymphoblastic leukemia cells (BCP-ALL). This inhibitory effect was found in the majority, but however not all cases, of BCP ALL tested. We did not observe induction of expression of sIg on BCP ALL cells cultured in IL-4. Also, IL-4 did not increase the proportion of $CD10^+s\mu^-$ normal BCP spontaneously acquiring sμ expression when cultured in medium alone. IL-4 was found to induce expression of CD20 in a number, but not all, cases of BCP-ALL. However, IL-4 failed to induce CD20 on normal $CD34^+CD20^-$ BCP. Taken together, our data suggest that IL-4 induced inhibition of proliferation plays an important regulatory role in human B cell ontogeny.

P 202 LYMPHOKINES RELEASED BY ACTIVATED OR HTLV INFECTED T CELLS INDUCE GROWTH OF NORMAL MESENCHYMAL CELLS AND OF CELLS DERIVED FROM KAPOSI'S SARCOMA LESIONS OF AIDS PATIENTS, Giovanni Barillari, Luigi Buonaguro, Valeria Fiorelli, Robert C. Gallo and Barbara Ensoli, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Mitogen-activated primary T cells release a number of lymphokines (TNF-β, TGF-β, GM-CSF, the interleukins, etcetera) which function on the immune, vascular, and hematopoietic systems. Here we show that cells transformed/infected by HTLVs (I and II) release the same lymphokines, and the levels of each type in conditioned media (CM) is approximately the same. The HTLV-I tax gene product is capable of trans-activating cellular gene expression by inducing NFκB activity in infected cells, and expression of several lymphokines is activated by this pathway (TNF, TGF, GM-CSF, etcetera). Therefore, infection by HTLVs may mimic the normal T cell activation following antigenic or mitogenic stimulation of T cells. Furthermore, CM from both types of cells induce proliferation of normal mesenchymal cells (smooth muscle, endothelial cells, and fibroblasts) and of cells derived from Kaposi's sarcoma (KS) lesion of AIDS patients (AIDS-KS cells). Similarly, a synthetic CM (containing the same concentration of lymphokines as calculated on the average concentration of several CM preparations) induces very similar levels of cell growth. Because most of these lymphokines and the CM from both types of activated T cells are also capable of inducing activation of the HIV-1 LTR directed gene expression, we conclude the T cell activation (by antigenic stimulation or HTLV infection) may represent a predisposing cofactor with HIV-1 in the development of KS in HIV-1 infected individuals, and/or for other HTLV-or HIV-associated malignancies.

P 203 STEROID INDUCIBLE EXPRESSION OF A PREGNANCY ASSOCIATED CYTOKINE

K.D. Beaman, J.A. Chang, C.K. Lee, R.C. Hoversland* & A. Gilman-Sachs Dept. of Micro/Immun. UHS/The Chicago Medical School, N. Chicago, IL 60064, & *Indiana University, Fort Wayne, IN

A human cDNA clone encoding a pregnancy associated cytokine (PAC) was identified by screening an expression cDNA library with a mouse PAC cDNA clone. The cDNA clone was approximately 86% identical at the nucleic acid level and 94% identical at the protein level to the murine cDNA clone. A recombinant PAC was expressed in COS cells. The recombinant protein inhibited the one way MLR in both murine and human systems. The expression of PAC both *in vivo* and *in vitro* was induced by progesterone or prednisolone but estrogen has no effect. As we have previously shown, antibody to PAC will ablate murine pregnancy when administered at or during the time of implantation. The addition of monoclonal antibodies to PAC reversed the effects of progesterone when added to an one way mixed lymphocyte culture. This effect on an MLR was measured by both thymidine incorporation and flow cytometry. The addition of progesterone to an MLR reduced the number of lymphoblasts and $CD8^+$ cells to near 0%. This effect was totally reversed by adding monoclonal antibody to PAC. The expression of PAC mRNA in an MLR was further examined by dot blot analysis and was shown to be induced by the addition of progesterone and prednisolone but not estrogen in both human and murine systems. This was further substantiated by *in vivo* studies which showed that progesterone, and not estrogen, induced the expression of PAC in the decidual lymph nodes which drain the uterus of the mouse. The addition of monoclonal antibody to PAC reversed the effects of progesterone *in vivo* and *in vitro* in the mouse and *in vitro* in man but had no effect on the expression of PAC mRNA. These data demonstrate that PAC is an immunoregulatory protein which supports successful pregnancy and may have a potentially important role in immunotherapy.

P 204 HUMAN T LYMPHOCYTES ACTIVATED VIA THE CD2 + CD28 PATHWAYS EXPRESS MONOKINES.

Françoise Birg, Chantal Cerdan, Hervé Brailly*, Régis Costello, Yves Martin, Marianne Courcoul, Claude Mawas and Daniel Olive, INSERM U 119 and *Immunotech SA, 13009 Marseille (France).

Beside activation via the TCR complex, resting purified T cells can be activated to proliferate by mAb directed against the adhesion molecules, CD2 and CD28. CD2 + CD28 T cell activation is a monocyte-independent, prolonged (> 3 weeks) and autocrine proliferation. We investigated whether T cells express the genes for the cytokines involved in the regulation of their activation, IL-1α and β, TNF-α, IL-6 and CSF-1, usually produced by monocytes. Cells activated via either CD2 or CD28 expressed the CSF-1 and TNF-α genes ; activation with CD2 + CD28 induced higher levels of expression of both genes. In contrast, only the CD2 + CD28 combined activation induced the expression and secretion of IL-1α. Neither IL-1β nor IL-6 genes were expressed. Secretion of such monokines by activated T cells could play a role in T-B cell cooperation under physiologic as well as pathologic circumstances (autoimmune diseases). Expression of these cytokines is differentially regulated, at the transcriptional as well as at the mRNA stability levels. Evidence for the presence of a new CSF-1 transcript will also be presented.

P 205 AGGREGATION OF HUMAN B CELLS AFTER ACTIVATION BY IL-4 AND ANTI-IgM,

Pia Björck, Bernt Axelsson and Staffan Paulie, Dept. of Immunology, Stockholm University, Stockholm, Sweden

When activated, leukocytes tend to adhere to each other resulting in the formation of aggregates or cell clusters. This process, which is thought to be an important phase in activation, is dependent on adhesion molecules present on the cell surface. B cells, as most other leukocytes, express the adhesion molecule LFA-1 (CD11a/CD18) as well as its ligand ICAM-1 (CD54). We have here studied the effect of different cytokines (IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-6 and interferon-γ) on homotypic aggregation and cell proliferation of resting or anti-IgM primed tonsillar B cells. Of these cytokines only IL-4 and to a lesser extent IL-2 were able to cooperate with immobilized anti-IgM antibodies in promoting cell aggregation. In the case of IL-4 this led to the formation of large, dense aggregates which were macroscopically visible after three to four days in culture. When antibodies to the α- or ß-chain of LFA-1 were added to IL-4 or IL-2 stimulated cultures, aggregation was more or less completely inhibited, indicating the importance of LFA-1 /ICAM-1 interaction. IL-4 and IL-2 also induced proliferation of the anti-IgM-treated cells. Addition of LFA-1 antibodies either did not affect or increased the proliferation of these cells. Furthermore, FACS analysis showed that the expression of both LFA-1 and ICAM-1 increased after activation with IL-4 and anti-IgM, but not when these agents were used alone. Taken together, the findings indicate that homotypic aggregation of B cells is primarily dependent on LFA-1 and ICAM-1 interaction and that aggregation and proliferation may be uncoupled events.

P 206 MICRONUTRIENT SUPPLEMENTS AND SERUM INTERLEUKIN-2 RECEPTOR LEVELS IN THE ELDERLY
John D. Bogden, Francis Kemp, Kay Bruening, Suzanne Katz, Adrianne Bendich, and Zhengang G. Yang, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2714
Prior evidence suggests that IL-2 production is decreased, but serum IL-2 receptor levels are increased, in the elderly; this may be a factor in the decline of cellular immunity with age. Although zinc and other essential micronutrients have been reported to influence IL-2 production, there have been no reports of the effects of micronutrient supplements on cellular or circulating IL-2 receptors. The results of a previous study from our laboratory suggested that delayed dermal hypersensitivity in elderly subjects could be enhanced by daily supplementation for 6-12 months with an over-the-counter formulation containing 22 essential vitamins and trace elements. We gave the same formulation to 21 healthy elderly subjects, aged 60-89, for 6 months; a placebo was given to 18 age-matched control subjects. Values of serum IL-2 receptor levels at 6 months were significantly correlated with initial values ($r=0.87$, $p < 0.0001$.) Mean ($\pm$ SE) values for the placebo group at 0 and 6 months did not differ significantly (437 $\pm$ 34 vs. 449 $\pm$ 33 U/ml); mean values for the supplemented group at 0 and 6 months also did not differ significantly (455 $\pm$ 41 vs. 461 $\pm$ 47 U/ml.) However, 47.4% of all values exceeded the upper limit of normal (447 U/ml) for the method used. The results verify that older people have high serum IL-2 receptor levels, and suggest that these levels are fairly constant over a period of several months in the absence of disease. The data further suggest that micronutrient supplementation of the elderly is not associated with alterations in serum IL-2 receptor levels. (Supported in part by grants from the New Jersey State Commission on Cancer Research and Hoffmann-La Roche Inc.)

P 207 MURINE IL-4 PRODUCTION IN RESPONSE TO IMMOBILIZED ANTI-CD3 IS IL-2 DEPENDENT. ANALYSIS OF IL-4 mRNA.
Jean-Louis Boulay, Robert A. Seder and William E. Paul, Laboratory of Immunology, National Institutes of Allergy and Infectious Diseases, National Institutes of Health BETHESDA MD 20892

Purified T cells from naive donors and from donors recently activated with anti-IgD antibodies produce IL-4 in response to immobilized anti-CD3. This IL-4 production is enhanced by IL-2 and inhibited almost completely by monoclonal anti-IL-2, either alone or together with anti-IL-2 receptor antibodies (Ben Sasson *et al.*, 1990).

We have analyzed IL-4 mRNA levels in response to anti-CD3 using RNase protection and PCR techniques. Our results demonstrate that T-cells harvested from anti-IgD injected donors have substantial levels of IL-4 mRNA within two hours of stimulation by anti-CD3 and by addition of IL-2. By contrast, anti-IL-2 completely inhibits the appearence of IL-4 transcripts. Studies aimed of determining the mechanisms through which IL-2 enhances steady state IL-4 mRNA levels in normal T cells are now under way.

P 208 MEL-14, THE LYMPH NODE HOMING RECEPTOR, IS EXPRESSED ON NAIVE PRECURSOR BUT NOT RESTING MEMORY CD4+ HELPER T CELLS.
L.M. Bradley, G.G.Atkins, G. Huston, and S.L. Swain, Department of Biology, UCSD, Cancer Center, La Jolla, CA 92093

MEL-14, the receptor for lymph node high endothelial venuoles, is expressed on T cells just prior to their exit from the thymus. T cells may lose MEL-14 after activation, and about half of splenic $CD4^+$ T cells are MEL-14^+. Because MEL-14 delineates two subsets of $CD4^+$ T cells, we investigated its association with naive and memory phenotypes and function. MEL-14 positive and negative subsets of CD4-enriched T cells from mice primed 3 months previously with KLH were isolated by magnetic separation. The two populations, as well as unseparated cells and mixtures of the separated cells, were evaluated in a series of functional assays. Antigen-specific proliferation, lymphokine production, and helper activity for a B cell antibody response were found exclusively in the MEL-14^- population. Both populations proliferated in response to polyclonal activation. However, the MEL-14^+ population secreted only IL-2 in response to stimulation, while the MEL-14^- population secreted IL-4, IFNγ, and IL-3 in addition to IL-2. This lymphokine profile is characteristic of long-lived $CD4^+$ T cells that are present 20 weeks after adult thymectomy, and express high levels of Pgp-1, low levels of CD45RB, and include memory T cells. We have previously shown that short-lived $CD4^+$ T cells are depleted from thymectomized mice and give rise in vitro to effector cells which secrete high titers of lymphokines. This precursor activity was contained exclusively in the MEL-14^+ population. The data suggest that MEL-14 expression distinguishes naive from memory helper T cells and that these T cells recirculate via distinct pathways.

P 209 A FORM OF LYMPHOTOXIN IS EXPRESSED ON THE SURFACE OF ACTIVATED T-CELLS J.L. Browning*, M.J. Androlewicz# and C.F. Ware*, *Biogen Inc. Cambridge, Ma. 02142, #Division of Biomedical Sciences, University of California, Riverside, Ca. 92521

Tumor necrosis factor is a secreted cytokine that can also exist on the surfaces of activated macrophages in a membrane bound form retaining the leader sequence (sTNF). The biological relevance of surface forms of TNF and other cytokines remains unknown. We have exploited a T-cell hybridoma, II-23.D7, to identify a surface form of lymphotoxin (sLT) using both immunofluorescence and immunoprecipitation techniques with both polyclonal and monoclonal anti-r-LT antibodies. The expression of sLT on this cell type was only observed following activation by PMA. Human CTL clones displayed sLT following antigen or anti-CD3 stimulation and leu 19^+ LAK cells were found to express sLT. Hence, sLT expression is a characterisitic of activated T-cells, whereas sTNF is primarily observed on activated macrophages. sLT appears to be identical to secreted LT on the basis of size, CNBr peptide mapping and glycosylation state and unlike sTNF, sLT is not anchored to the surface via its leader sequence. No evidence was obtained for a lipid-like linkage. sLT may be only peripherally associated with the membrane, but sLT could not be chemically cross-linked to either the 60 or 80 kD TNF receptor chains. An apparently unique 33 kD surface glycoprotein was immunoprecipiated with sLT. The cys/met ratio of p33 was inconsistant with it being a cleaved form of one of the TNF receptor chains. Peptide mapping showed this protein to be distinct from either LT or TNF. The possibility exists that the 33 kD protein is bound to sLT. Membrane forms of LT may have distinct biological activities mediating precise local events.

P 210 IL-4 HAS A DECISIVE INFLUENCE ON TH SUBSET DIFFERENTIATION IN MICE INFECTED WITH *LEISHMANIA MAJOR*. Rene Chatelain, Kari Varkila and Robert L. Coffman, DNAX Research Institute, Palo Alto, CA 94304.

Infection of mice with the protozoan parasite *Leishmania major* results, depending on the genetic background of the mice, in either a disseminating, fatal disease or a localized healing disease. The CD4 T cell response of healer C3H mice is largely a TH1 response, characterized by high IFNγ production, but no detectable IL-4 or IL-5. In contrast, nonhealer BALB/c mice respond with a TH2 pattern with their TH cells secreting IL-4 and IL-5 but only little IFNγ. Our attempt was to study the role of cytokines as inducers of the development of TH responses in *L. major* infections. A single injection of an anti-IL-4 antibody (11B11) given at the time of infection with the parasite enables the nonhealer BALB/c animals to control the infection and heal the disease and confers protection to reinfection. The cytokines produced by these anti-IL-4 treated mice show a TH1 pattern (high IFNγ, no IL-4 or IL-5). This TH response conversion by 11B11 into a TH1 response can be observed as early as four days after infection. This result suggested that IL-4 is required for the development of a TH2 response to *L. major* in BALB/c mice. To further study the role of IL-4 in TH differentiation, C3H mice were injected with rIL-4 at the time of infection with *L. major*. A single dose of rIL-4 caused a 90% reduction in IFNγ production from draining lymph node cells restimulated 4 days later. Multiple injections of rIL-4 abolished the IFNγ secretion almost completely and resulted in the production of IL-4 and IL-5 from cells restimulated in vitro. These results demonstrate that IL-4 can have a decisive influence on TH subset differentiation during the primary response of mice to *L. major* infection.

P 211 EFFECT OF MUTATION OF O- AND N-LINKED GLYCOSYLATION SITES ON EXPRESSION AND ACTIVITY OF HUMAN INTERLEUKIN 5, Chuan-Chu Chou, Jacqueline Carter, James Anagnost, Satwant Narula and Paul Zavodny, Schering-Plough Research, Bloomfield, New Jersey, 07003

Human interleukin 5 (hIL5) is a lymphokine secreted from T cells which has been shown to specifically stimulate the production of eosinophils in bone marrow cultures and to have no effect on production of other myeloid cells. Such findings have implicated IL5 in the pathogenesis of respiratory diseases such as asthma, where eosinophils augment bronchial hyperreactivity and cause destruction of respiratory epithelium. Recombinant human IL5 produced in Cos cells has been shown to undergo post-translational modification (Yokota et al., 1987. PNAS **84**, 7388). The structure of recombinant hIL5 produced in Chinese Hamster Ovary cells has recently been reported, with glycosylation at Thr-3 and Asn-28 (Minamitake, Y. et al., 1990. J. Biochem **107**, 292). In the current study, individual single amino acid substitutions at Thr-3 and Asn-28 confirmed glycosylation of hIL5 at those sites in Cos cells, as determined by Western Blot analysis. Mutation of the two glycosylation sites, either alone or in pairs, had no apparent effect on the biological activity of recombinant hIL5 as determined by proliferation assay using a human proerythroleukemia cell line. In contrast to the wild-type hIL5 and the Thr-3 mutant, both the Asn-28 mutant and the double mutant showed a marked decrease in the level of accumulation in the conditioned media, although the proteins were apparently fully biologically active. Therefore, while neither O- nor N-linked glycosylation of hIL5 appears to be essential for bioactivity, N-linked glycosylation at Asn-28 appears to be important for either secretion or stability of the molecule.

P 212 DEMONSTRATION OF μ–γ1 DNA-DELETIONAL CLASS SWITCH IN RESPONSE TO LPS AND IL-4 BY A NOVEL DIGESTION/CIRCULARIZATION PCR (DCPCR) ASSAY. Charles C. Chu, William E. Paul, and Edward E. Max. NIAID, NIH, and Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

IL-4 directs LPS-stimulated murine B cells to switch from expression of IgM to expression of IgG1 and IgE. To study effects of IL-4 on the isotype switch recombination at the DNA level independent of expression, we have developed a novel assay that allows detection of recombined DNA in bulk cultures. In this "digestion/circularization PCR" (DCPCR) assay, genomic DNA is digested using a restriction enzyme with recognition sites that flank the recombined composite switch region, and the DNA is then religated at low concentrations to favor the formation of circles. This ligation brings the 5' and 3' ends of each restriction fragment into apposition, and thus makes it possible to amplify across the ligated restriction site using appropriate primers and the polymerase chain reaction. Using a DCPCR assay designed to detect recombination between μ and γ1 we show that no recombined product can be detected in splenic B cells cultured with LPS alone for up to 6 days, but that in cells cultured with LPS plus IL-4, a μ–γ1 DCPCR product is detectable on day 3, increasing on days 4 and 5. With suitable modifications to make the assay quantitative, it will be possible to determine whether switch recombination precedes or follows membrane IgG1 expression in this system. With appropriate choice of primers the circular reciprocal deletion product of the isotype switch recombination should also be detectable. Such studies are currently in progress.

P 213 HYPERSECRETION OF IFN-γ AND TNF-α BY STIMULATED lpr and gld $CD4^+$, $B220^-$ BUT NOT CD4 $DULL^+$ $B220^+$ or $B220^+$ DN T CELLS. Wendy Davidson[a], Catherine Calkins[b], Ambros Hugin[c] and Kevin Holmes[d]. Laboratory of Genetics, NCI[a]; LIP, NIAID[c]; BRB, NIAID[d], NIH, Bethesda, MD 20892. Dept. of Microbiology, Thomas Jefferson Univ., Philadelphia, PA 19107[b]

Mice homozygous for lpr and gld develop profound lymphodenopathy characterized by the expansion of two unusual T cell subsets, a predominant Ly-5$(B220)^+$ $CD4^-$ $CD8^-$ ($B220^+$ DN) population and a minor CD4 $dull^+$ Ly-5$(B220)^+$ population. To assess the contribution of growth factors to lymphoproliferation, unfractionated LN cells and isolated T cell subsets from C3H-lpr, gld and +/+ mice were compared for spontaneous and induced production of a spectrum of lymphokines. No significant spontaneous production of lymphokines was observed with any cell population. Following stimulation with immobilized anti-TCR α/β mAb, lpr and gld LN but not $B220^+$ DN T cells secreted high levels of IFN-γ, TNF-α and GM-CSF and low levels of IL-3, -4 and -6. Despite a 5-10 fold deficit in $CD4^+$ and $CD8^+$ cells, lymphokine secretion by lpr and gld LN exceeded that of +/+ LN. Further studies of $CD4^+$ T cells showed that following stimulation both +/+ and lpr $CD4^+$ $B220^-$ T cells proliferated strongly but only lpr cells produced significant levels of IFN-γ. The lpr $CD4^+$ T cells also produced higher levels of TNF-α than +/+ T cells. Stimulated lpr CD4 $dull^+$ $B220^+$ T cells secreted neither IFN-γ nor TNF-α. Both lpr and gld $CD4^+$ T cells expressed markedly higher levels of CD44 than +/+ $CD4^+$ T cells. Since elevated CD44 expression is indicative of T cell activation and only previously activated +/+ $CD4^+$ T cells produce high levels of IFN-γ, our data suggest that lpr and gld $CD4^+$ T cells contain an abnormally high proportion of activated or memory cells that potentially may hypersecrete factors in vivo.

P 214 **HUMAN IgD^+ B CELLS ACTIVATED THROUGH CD40 SWITCH TO IgA FOLLOWING EXPOSURE TO BCRF1 AND TGFß**, Thierry Defrance, Béatrice Vanbervliet, Francine Brière, Kevin Moore*, Françoise Rousset and Jacques Banchereau, Schering-Plough, 69571 Dardilly Cedex, France; *DNAX Palo Alto, California 94304

The Ig response of tonsillar IgD^+ and IgD^- B cells stimulated via their surface Igs, or CD40, or both was investigated. The positive and negative populations displayed the phenotypic features of mantle zone B cells (IgD^+, IgM^+, $CD23^+$, $CD39^+$) and germinal centre B cells (IgD^-, $IgM^{+/-}CD23^-$, $CD39^-$ and $CD10^+$) respectively. In the absence of exogenous factor, no Ig production was observed in both B cell subsets upon stimulation with SAC or anti-CD40. In contrast, concomittant activation of B cells with SAC and anti-CD40 induced synthesis of IgM from IgD^+ B cells and of IgM, IgG and IgA from IgD^- B cells. In costimulation with anti-CD40, IL-4 stimulated IgE production from IgD^+ B cells. IL1 to IL7, TNFα, TGFß and IFNγ failed to induce IgG or IgA synthesis from IgD^+ B cells whatever mode of activation. The EBV-encoded protein BCRF1 which is homologous to IL10 (Hsu et al, Science 1990, 4982, 830) elicited a moderate IgA production from IgD^+ B cells which was strongly potentiated by TGFß. In contrast, the BCRF1-induced IgM, IgG and IgA synthesis in IgD^- B cells were suppressed by TGFß, suggesting that TGFß exerts its stimulatory effect on the IgD^+ B cells. Our results imply that IgD^+ B cells triggered through CD40 and surface Igs are able to switch in response to cytokines.

P 215 IL-2 INHIBITS THE DIFFERENTIATION OF NORMAL AND MALIGNANT B CELLS, Dieter Dennig and Michael K. Hoffmann*, Memorial Sloan-Kettering Cancer Center, New York, NY, and New York Medical College, Valhalla, NY*.
An increase in IL-2R expression has been demonstrated on activated B cells and IL-2 has been shown to sustain proliferation of B cells expressing this receptor. We show here that IL-2 has an inhibitory effect on the differentiation of B cells. IL-2 was previously found to enhance the antibody production if given after induction of differentiation. IL-2 suppresses the antibody production when administered prior to the induction of differentiation by IL-1 and cAMP. The antagonistic effect might be attributed to the ability of IL-2 to block the IL-1 and cAMP induced expression of differentiation markers. This hypothesis was tested in tissue culture using the cloned pre-B cell line 70Z/3. In the presence of IL-1 cAMP induces an increase in IL-2R expression on 70Z/3 cells. IL-2 blocks the effect of IL-1 and cAMP when given prior to cAMP. The relevance of these findings was further explored for the T cell mediated activation of B cells. In the presence of IL-1 antigen-primed T helper cells stimulate the production of antibodies. IL-2 abrogates this T helper effect unless its addition is delayed. The inhibitory effect of IL-2 is neutralized by cAMP. The data are consistent with the assumption that the differentiation of B cells proceeds through two phases, an early phase which is inhibited by IL-2 and a late phase which is supported by IL-2.

P 216 CD23-MEDIATED RELEASE OF INFLAMMATORY MEDIATORS FROM INTERLEUKIN-4-ACTIVATED HUMAN MONOCYTES, B. Dugas, J. P. Kolb[1], N. Paul-Eugène, V. Lagente, S. Picquot, J. Gordon[2], M. Sarfati[3], G. Delespesse[3], J. M. Mencia-Huerta, and P. Braquet
Institut Henri Beaufour, Les Ulis, France ; [1]INSERM U196, Institut Curie, 75006 Paris, France ; [2]University of Birmingham, Birmingham, England ;[3]Notre-Dame Hospital, Montreal, Canada.
There is increasing evidence that the low affinity receptor for IgE (FcεRII/CD23) plays an important regulatory role during allergic manifestations. Interleukin-4 (IL-4) has been shown not only to induce the expression of FcεRII/CD23 on freshly isolated normal human monocytes but also to reduce the capacity of these cells to generate IL-1, IL-6 and TNF-α. However, after a 48-h incubation period with 30 U/ml IL-4, human monocytes released the lysosomal enzyme, ß-glucuronidase, when incubated with two different anti-CD23 monoclonal antibodies (34 ± 9 nmol/10^6 cells for the 4.3 antibody, 39 ± 5 nmol/10^6 cells for the MHM6 antibody, 2 ± 0.3 nmol/10^6 for unstimulated cells). In contrast, human monocytes that have been not preincubated with IL-4, and thus did not express FCεRII/CD23, did not release ß-glucuronidase when stimulated with either 4.3 or MHM6 antibodies. When IL-4-stimulated monocytes were successively incubated with IgE, and then with anti-IgE to form IgE/anti-IgE complexes, a 45 ± 11 nmol/10^6 cells ß-glucuronidase release was observed. In control experiments, monocytes not preincubated with IL-4 and that expressed low level of FcεRII/CD23 (5 ± 2 % versus 35 ± 5 % for IL-4-incubated monocytes) failed to release ß-glucuronidase upon stimulation with IgE/anti-IgE complexes. IL-4-incubated monocytes expressing FcεRII/CD23 produced high quantities of Platelet-Activating Factor and thromboxane following challenge with IgE/anti-IgE complexes or with 10 μg/ml of either 4.3 or MHM6 antibodies. This inducing effect of 4.3 and MHM6 antibodies was dose-dependent, a significant effect being observed at 100 ng/ml and a maximum was noted at the 10 μg/ml final concentration. For both antibodies such productions of Platelet-Activating Factor and thromboxane were not observed upon stimulation of monocytes that were not preincubated with IL-4. These results indicate that the modulation of FcεRII/CD23 expression may regulate IgE-dependent monocyte functions.

P 217 PHENOTYPIC CHARACTERIZATION OF T-COLONY FORMING CELLS AND THEIR ACTIVATION BY THE COMMON PATHWAY INVOLVING Ti-CD3 AND CD2, Elfenbein GJ, Castle K, Popken PD, Greene VM and Zucalli JR. Bone Marrow Transplant Program, Moffitt Cancer Center, University of South Florida, Tampa, FL 33612
The formation of T cell colonies (CFU-T) by resting human peripheral blood (PB) lymphocytes in response to phytohemagglutinin (PHA.M) requires red blood cells (RBC) and, in the absence of accessory cells, interleukin-2 (IL-2). The mechanism of activation involves both the T-cell antigen receptor complex Ti-CD3 and the alternative CD2 activation pathway through interactions with its natural ligand LFA3 on the RBC membrane. Activation is blocked by monoclonal antibodies to CD3, by the absence of RBC or RBC membranes, by anti-CD2 antibodies, by incubation of RBC with anti-LFA3 antibodies and requires exogenous IL-2 in the absence of accessory macrophages, helper T-cells and B-cells. Previously, we were unable to demonstrate that the T-colony forming cell (T-CFC) was a T-cell, although we have demonstrated that the T-CFC produces mature progeny with CD4 and CD8 phenotypes. We demonstrate here that the T-CFC is not restricted to CD4 or CD8 phenotypes. Antibodies to CD5 do not inhibit CFU-T formation, but ricin A-chain conjugated antibodies extinguish colony formation. Immunomagnetic bead depletion of PB mononuclear cells bearing the CD5 antigen produces an equivalent decrease in CFU-T. We believe that CFU-T formation in response to PHA-M demonstrates that CD5 positive resting T-cells are capable of response to mitogenic stimuli, that this response demonstrates a functional explanation for autologous rosette formation, and that T-cell activation in CFU-T formation involves both CD2 and CD3 antigens. Further, we suggest that T cell differentiation continues extrathymically through the interaction of post-thymic T-cells with adhesion molecules in various hematopoietic and lymphoid microenvironments. Finally, the T-CFC appears to be a cell bearing the antigens CD5, CD2 and CD3 and is, in addition, a resting, naive post-thymic T-cell.

P 218 CHARACTERIZATION OF TIL SUBSETS THAT MEDIATE LYSIS OF IFNγ/TNFα TREATED RENAL CELL CARCINOMAS TARGETS. James Finke, Patricia Rayman, Mark Edinger, Raymond Tubbs, Jeannine Alexander, Edson Pontes, Robert Connelly, and Ronald Bukowski.

We previously reported that the cytolytic response of cultured TIL from renal cell carcinoma (RCC) is mediated mostly by LAK cells, (CD56+CD3- and CD56+CD3+), whereas CD3+CD56- T cells displayed low levels of lytic activity. Several groups have shown that treatment of RCC with interferon γ (IFNγ) and tumor necrosis factor α (TNFα) makes them more susceptible to lysis by the CD3+CD56- TIL. Here we defined the effect cytokine treatment of RCC has on the lytic activity of the various lymphocyte subsets that constitute TIL. In some experiments NK and T cell subsets with LAK activity were isolated by cell sorting (FACStar+) and in other experiments we employed CD3+CD4+ and CD3+CD8+ TIL lines as effectors. Treatment of RCC with cytokines had opposing effects on the lytic response of T cells and NK cells within the TIL culture. IFNγ/TNFα treatment of RCC either had no effect or made them resistant to NK cell (CD3-CD56+) lysis. In contrast, cytokine treated RCC were more susceptible to lysis by CD56+ and CD56- T cells (CD5+), than were the untreated targets. Moreover, most of the lytic response observed with the CD3+CD56- TIL was due to the CD3+CD8+ cells. Comparable results were observed with 5/6 CD3+CD8+ TIL lines that were not specific and with one line that displayed specificity for the autologous tumor. Increased lysis of IFNγ/TFNα treated targets by the specific and nonspecific TIL appeared to involve recognition via TCR/CD3 complex. Modulation of TCR/CD3 complex off the cell surface by overnight incubation with anti-CD3 antibody but not by isotype matched control antibody blocked T cell mediated lysis of treated targets. Cytokine treatment of RCC appeared to have a selective effect on the lytic capacity of CD3+CD8+ TIL but not their ability to proliferate to RCC. These results suggest that IFNγ/TNFα can differentially regulate RCC susceptibility to lysis by NK and T cells and that these changes may not influence other lymphocyte responses to the tumor.

P 219 IN VITRO EFFECTS OF CYTOKINES ON TARGET SURFACE MOLECULES AND SUBSEQUENT RECOGNITION BY LAK EFFECTORS. Didier Fradelizi, Anne Quillet-Mary, Maria L. Gil, Carmen Marchiol-Fournigault, Hélène Conjeaud, URA 1156 CNRS, IGR, 39 rue Camille Desmoulins, 94805 Villejuif. FRANCE

Lymphokine Activated Killer (LAK) cells, which mainly belong to the CD2+ CD3- lymphocyte subset, have been used with some success to treat patients with metastatic cancers. However, the process by which they recognize theirs targets is still unknown. Cytokines are known to induce surface expression of adhesion molecules on lymphocytes, polymorphonuclear, and endothelial cells. We have used this effect to identify molecules on the targets which could be ligands of LAK effector recognition structures. Human lymphoblastoid cell lines, growing in culture, were incubated for 72 hours with various cytokines such as IL-1, IL-6, TNF bêta, IFN gamma and IL-4. These cells were subsequently studied for: a) Expression of adhesion molecules of the integrin and immunoglobulin super family. b) Adhesion properties as reflected by the effector/target conjugates formation. c) Susceptibility to LAK lysis.

The results indicate that pretreatment of target cells increased adhesiveness of these cells as demonstrated by an increased number of conjugates formed with the LAK effectors. This effect was paralleled by an increase susceptibility to LAK lysis. This effect, however, could not be strictly correlated with an increased expression of any specific adhesion molecules, although up regulation of ICAM-1 and LFA3 were occasionally observed. The lysability of the targets, preincubated with the antibodies (anti ICAM-1 and anti LFA3), then washed, before addition of the effector, was not modified. These results suggest that unidentified membrane products on target cells, displaying adhesion properties, upregulated by cytokine, have a major role in the lysis of target cells by CD3- effectors.

P 220 PROLIFERATION OF ACUTE LYMPHOCYTIC LEUKEMIA (ALL) BLASTS IS STIMULATED BY THE COMBINED ACTION OF INTERLEUKIN-7 (IL-7), INTERLEUKIN-3 (IL-3) AND ACCESSORY CELLS. Ganser A., Eder M., Ottmann O.G., Hansen-Hagge T.E., Bartram C.R., Gillis S., and Hoelzer D., Deparment of Hematology, Johann Wolfgang Goethe-University, D-6000 Frankfurt/Main, Germany

IL-7 and IL-3 can induce proliferation of ALL blasts (Eder et al, Leukemia 4:533, 1990). We now investigated the proliferation and maturation inducing effects of blood-derived macrophages and bone marrow fibroblasts, alone and in combination with IL-7 and IL-3, on pre-B-ALL (cALL) cells. In 7-day suspension cultures, IL-7 (50 U/ml) stimulated DNA-synthesis as defined by ^{3}HTdR incorporation in 2/9 cases (stimulation index (SI)>5, max. SI=10.1), while IL-3 (10 ng/ml) induced proliferation (SI>5, max. SI=6.6) in 4/9 cases. In co-culture experiments, irradiated (20 Gy) macrophages enhanced DNA-synthesis of ALL blasts up to 90 fold, to be further enhanced by IL-7 and IL-3 in 5/9 cases. Irradiated fibroblasts also induced proliferation of ALL blasts but were less effective. No evidence of lymphoid maturation could be detected by immunophenotyping using flow cytometry. Southern blot analysis of Ig and TCR gene rearrangements prior to and after culture proved clonal proliferation of leukemic cells in 3 cases, while expression of myeloid or T-lymphoid antigens after culture was invariably associated with a polyclonal growth pattern. These results demonstrate that stromal cells can induce proliferation in cALL cases unresponsive to IL-7 or IL-3 alone.

P 221 EXPRESSION OF LARGE AMOUNTS OF MURINE IL-10 IN E. COLI AND ITS MODULATING EFFECT ON THE CELLULAR IMMUNE RESPONSE TO L. MAJOR André Gessner and Martin Röllinghoff, Institut für Klinische Mikrobiologie der Universität Erlangen-Nürnberg, FRG.

The pattern of lymphokine expression is important for the clinical outcome of an infection of mice with the protozoan Leishmania major. In resistant C57BL/6 mice predominantly $CD4^+$ T cells of the Th1 type are activated to produce IFN-γ and IL-2, whereas in BALB/c mice, which succumb the infection, large numbers of antigen specific Th2 type T cells, producing IL-4, can be found. Clones of the latter type are known to produce IL-10, formerly known as cytokine synthesis inhibitory factor (CSIF) (Moore et al., 1990). This lymphokine was initially characterized as a factor which suppresses IFN-γ production by Th1 cells (Fiorentino, Bond, and Mosmann, 1989).
To study the function of this cytokine with regard to its effect on the modulation of the $CD4^+$ cell driven cellular immune response to L. major large amounts of the protein were expressed in E. coli as glutathion S-transferase-fusion protein, purified and digested with thrombin to remove the N-terminal part, yielding functional active murine IL-10 . Results concerning in vivo and in vitro effects of this recombinant product during experimentally induced leishmanisis will be presented.

P 222 UNRESPONSIVENESS OF CBA/N "xid" B CELLS TO INTERLEUKIN-10 INDUCED CLASS II MHC ANTIGEN EXPRESSION, Ning Fei Go, and Maureen Howard, Immunology Department, DNAX Research Institute, Palo Alto, CA 94304, USA

We have shown that interleukin-10 (IL-10) upregulates the expression of class II MHC antigen on small splenic B cells from normal strains of mice but not on B cells from CBA/N "xid" mice. CBA/N "xid" B cells are not totally refractory to IL-10 activity: IL-10 enhances in vitro B cell viability on both CBA/N "xid" mice and normal mice; IL-10 augments the IL-2/IL-4 induced proliferation of thymocytes from both young adult "xid" mice and normal mice; IL-10 inhibits the ability of both "xid" spleen cells and normal spleen cells to present antigen to Th1 T cell clones, thereby suppressing their IFNγ production. In vitro reconstitution experiments indicated that coculture of "xid" B cells with either total splenocytes or peritoneal cells from normal mice did not render them responsive to IL-10 in terms of upregulated class II MHC antigen expression. These data suggest that the unresponsiveness of CBA/N "xid" B cells to the class II antigen inducing activity of IL-10 is not due to the lack of a population of accessory cells which is normally present in normal mice, nor to the absence of functional IL-10 receptors. It is likely that the unresponsiveness reflects an intrinsic defect of the "xid" B cells.

P 223 CLONING AND EXPRESSION OF CYTOTOXIC LYMPHOCYTE MATURATION FACTOR (CLMF), A HETERODIMERIC LYMPHOKINE THAT POTENTIATES NK, LAK and T-CELL RESPONSES

U. Gubler, D. Schoenhaut, *N. Nabavi, R. Chizzonite, #A. Stern, *M. Gately
Departments of Molecular Genetics, *Immunopharmacology, #Protein Biochemistry, Hoffmann La Roche, Inc., Nutley, NJ 07110, USA

Cytotoxic lymphocyte maturation factor (CLMF) is a disulfide bonded heterodomeric lymphokine that was identified on the basis of its ability to synergize with IL-2 in the activation of cytotoxic lymphocytes in vitro. The cDNAs encoding the two CLMF subunits (40Kd and 35Kd) were recently cloned, using N-terminal amino acid sequence information obtained from purified protein. These subunits represent distinct gene products whose mRNAs are coordinately regulated upon induction of human NC-37 B-lymphoblastoid cells; however, steady state mRNA levels for the 40Kd CLMF subunit were severalfold higher than for the 35Kd subunit. Coexpression of both subunits in COS cells is required for the secretion of biologically active CLMF. Recombinant CLMF caused the activation of NK cells, induced the generation of LAK cells, and augmented allogeneic CTL responses. It caused proliferation of activated T-cells independent of IL-2 and enhanced Il-2 induced proliferation of resting peripheral blood lymphocytes (PBL); however, CLMF by itself did not cause resting PBL's to proliferate.

P 224 SELECTION OF A HUMAN T_H1 LIKE T CELL SUBSET BY *MYCOBACTERIUM LEPRAE* ANTIGENS,
John B.A.G. Haanen*, René de Waal Malefijt†, René de Vries* and Hergen Spits†, Dept. of Immunohematology & Blood Bank*, University Hospital Leiden, The Netherlands, DNAX†, Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304
Tuberculoid leprosy is characterized by high cellular immunity to *M. leprae* and immunopathology due to delayed type hypersensitivity (DTH) reactions, Skin lesions of patients suffering from this disease have the characteristics of DTH reactions in which macrophages and CD4+ T lymphocytes predominate. In animal models it has been shown that DTH responses are associated with the presence of a particular subset of CD4+ T cells (T_h1) that secrete only certain cytokines like IL-2, IFN-γ but no IL-4 or IL-5. We studied the cytokine release of activated *M. leprae* reactive CD4+ T cell clones from three tuberculoid leprosy patients. We found that these patient derived T cell clones exhibit a T_h1 like cytokine secretion pattern with very high levels of IFN-γ and neglegibel to normal levels of IL-4 and IL-5. This cytokine secretion pattern was also observed for anti-mycobacterial T cell clones from healthy individuals, but not for T cell clones from the same individuals reactive with non-mycobacterial antigens.
We also studied the effect of a novel cytokine, human IL-10, on these T_h1 like T cell clones. In mice IL-10 is produced by a T_h2 subset. IL-10 may have a negative effect on the outgrowth of T_h1 subset. Our preliminary results show that human recombinant IL-10 inhibits antigen specific proliferation of human T_h1 like cells when antigen is presented by monocytes.

P 225 PROLIFERATIVE RESPONSES OF T & B CELLS IN RFM/(T6XRFM)F1 CHIMERAS WITH HOST VERSUS GRAFT (HVG) DISEASE. Richard C. Hard, Jr., Mona Osman & James Montour, Medical College of Virginia/VCU, Richmond, VA 23298
HVG disease is the fatal immunodeficiency syndrome induced by the perinatal inoculation of F1 hybrid spleen cells into susceptible strains of related inbred mice. Evidence of T-cell depletion and B-cell hyperplasia with mainly IgG1 hyperglobulinemia are features. The aim of these studies is to test T & B cell proliferative responses as the disease progresses. Spleen and node cells from RFM/(T6xRFM)F1 chimeras & RFM littermate controls were incubated with conconavalin A (Con A), a T-cell mitogen, or with lipopolysaccharide (LPS), a B-cell mitogen, or with irradiated (2Gy) RFM, (T6XRFM)F1 or A/J spleen cells. Uptake of 3H-thymidine was measured. T-cell responses to Con A in HVG nodes averaged 23% control values (23%C) at day 25, & 8%C in HVG spleens. B-cell responses were severely decreased in HVG spleens (5%C) and moderately in the nodes (47%C). The results of the mixed lymphocyte reactions indicated that most HVG mice were hyperreactive to RFM, (T6xRFM)F1 and A/J cells, and unexpectedly promoted the growth of fibroblasts/endothelial cells present in the cultures. Taken together, these results are consistent with data that suggests that there is an early increase, then loss of IL-4 producing T-cells, and that TGF(Beta) & IL-1 may play roles more important than previously expected.

Supported by monies from the Privat and Carnes Memorial Funds.

P 226 THE T-CELL-SPECIFIC MOLECULE CD27 IS RELEASED UPON STIMULATION OF THE T-CELL RECEPTOR/CD3 COMPLEX, Rogier Hintzen[1,2], Erik Hack[2], Chris Polman[1], Wil Loenen[3], Jannie Borst[3], René van Lier[2]. [1]Dep. of Neurology, Free University Hospital, Amsterdam; [2]Central Lab. Netherlands Red Cross Blood Transfusion Service & Lab. Exp. Clin. Immunol. of the University of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam; [3]The Netherlands Cancer Institute, Amsterdam. The Netherlands.

The T-cell differentiation antigen CD27 is expressed on medullary thymocytes and on 75% of both CD4+ and CD8+ peripheral blood cells. On resting T cells, CD27 is expressed as a disulphide-linked homodimer with chains of 55 kD mol mass. Sequencing of the CD27 encoding cDNA has revealed that the molecule belongs, together with the nerve growth factor receptor, the tumor necrosis factor receptor and the B-cell-specific molecule CD40, to a new family of membrane receptors. Upon activation of T lymphocytes via the T cell receptor/CD3 complex, membrane expression is strongly enhanced and an additional component of 32 kD is found on the cell surface.
The activation-specific CD27-32kD polypeptide is present in the supernatant of TCR/CD3-activated T cells. We could demonstrate its presence in serum, urine and synovial fluid from reumatoid arthritis patients and in cerebrospinal fluid from patients suffering from multiple sclerosis.

P 227 INTERLEUKIN-7 DIRECTLY STIMULATES THE PROLIFERATION OF A NOVEL TCR $\alpha\beta^+$ CD^-4 $CD8^-$ T CELL POPULATION IDENTIFIED IN THE SPLEEN OF NORMAL MICE.
Andrea D. Ingram, Dolores J. Saunders and Fumio Takei, Terry Fox Laboratory, B.C. Cancer Research Center and Department of Pathology, University of British Columbia, Vancouver, B.C., Canada
A unique class of T lymphocytes has been identified in the spleen of normal mice. These splenic T cells express the αβ T cell receptor associated with the CD3 antigen but lack the CD4 and CD8 accessory molecules and are therefore phenotypically identical to the splenic T cell population which is massively expanded in the spleen of the autoimmune MRL-lpr/lpr mice. This TCR $\alpha\beta^+$ $CD4^-$ $CD8^-$ splenic T cell population is thought to be functionally mature as suggested by their ability to be stimulated by anti-CD3 crosslinking. In addition they do not express the immature T lymphocyte marker, Heat Stable Antigen, as recognized by the M1/69 antibody. Three day co-culture with Interleukin-7 induces the vigorous proliferation of this splenic T cell population as determined by the ^{3}H-thymidine incorporation assay. However, unlike mature T cells and total thymocytes, this responsiveness to IL-7 does not require the presence of other co-stimulatory factors and is therefore similar to the response of TCR $\alpha\beta^+$ $CD4^-$ $CD8^-$ thymocytes to this lymphokine. The function of these IL-7 responsive TCR $\alpha\beta^+$ $CD4^-$ $CD8^-$ splenic T cells with respect to cytokine production and cytolytic activity is currently being studied.

P 228 PURIFICATION OF MONOCLONAL ANTIBODIES ON PROTEIN A-SORBENTS. IMPLICATIONS FOR INTRACELLULAR CALCIUM CHANGES AND PROLIFERATION OF HUMAN B LYMPHOCYTES. Eva Jakobson, Bernt Axelsson and Staffan Paulie, Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden
It is well known that the binding of antibodies to e.g. growth factor receptors may in some cases mimic the effects of a natural ligand. Such agonistic antibodies have become a very useful tool when trying to establish the functional properties of different cell surface molecules. However, during studies of the effects on B cells of agonistic antibodies to the CD40 and CD43 molecules, we made some observations that we think are of general interest, and which emphasize the need for some precaution when using antibodies purified on Protein A-sorbents. Thus we found that soluble Staphylococcal protein A (SpA), present as a minor contaminant in purified IgG preparations, could by itself elicit changes in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), as well as promote proliferation of phorbol ester activated B cells. Pure SpA in nanogram quantities was shown to elevate the $[Ca^{2+}]_i$, as a consequence of mobilization of Ca^{2+} from internal stores, and an influx of Ca^{2+} across the plasma membrane. A similar effect on cytosolic Ca^{2+} levels was also noted for the Streptococcal protein G, whereas the effect of this agent on proliferation was less pronounced. Moreover, the effects of contaminating SpA or Protein G could be easily removed by absorption of the IgG-preparations on columns prepared with human or rabbit IgG. This study shows that trace amounts of contaminating SpA or Protein G, resulting from the antibody purification procedure, may complicate the evaluation of cellular effects of agonistic B cell antibodies.

P 229 SELECTIVE ENDOTHELIAL BINDING PROPERTIES OF IL-2 ACTIVATED T CELLS ORIGINATED FROM DIFFERENT ORGANS OF THE BODY. Sirpa Jalkanen[1], Kaisa Granfors[1], Marjatta Leirisalo-Repo[3], Martti Hämäläinen[4], Richard MacDermott[5], Rauli Leino[2], Marko Salmi[1]. Depts of Med Microbiol[1] and Medicine[2], Turku Univ, Turku, Finland; Dept of Medicine[3], Univ of Helsinki, Helsinki, Finland; Rheumatism Foundation Hospital[4], Heinola, Finland; Dept of Medicine[5], Univ of Pennsylvania, PA
Lymphocyte-endothelial cell recognition and binding is an essential step in lymphocyte migration to lymphoid tissues and to sites of inflammation. Since clinical usefulness of T cell therapy is critically dependent on the endothelial cell binding capacity and homing of T cell lines and clones used, we wanted to study high endothelial venule (HEV) binding properties of T cell lines from different tissue sources. IL-2 dependent T cell lines were originated from human appendix, lamina propria, inflamed synovium, synovial fluid, peripheral lymph nodes, and their phenotype and capacity to bind to peripheral lymph node, mucosal and synovial HEV were analyzed. In spite of great individual variations between different cell lines, some common features in their phenotype and binding capacity were observed. Cell lines originated from mucosal sites were almost entirely of cytotoxic phenotype and bound well to mucosal and synovial HEV, but not to peripheral lymph node HEV. Synovial cell lines also were mainly CD8 positive, and two binding patterns were seen: some cell lines bound only to synovial HEV, and some bound both to synovial and mucosal HEV. In contrast, cell lines from peripheral lymph nodes had high percentage of CD4 positive cells and showed preferential binding to peripheral lymph node HEV despite the lack of Leu-8 defined peripheral lymph node homing receptor expression. No significant differences were seen in the expression of other homing-associated molecules (CD44, LFA-1 and VLA-4) between the lines from different tissue sources. These findings support the idea that the tissue origin of IL-2 activated T cells determines their specificity for endothelial cell recognition, and besides the known homing-associated molecules some other molecules may also mediate the binding between these T cells and HEV.

P 230 METABOLISM OF IL2 RECEPTOR ALPHA CHAIN, R. P. Junghans, T. A. Waldmann, Metabolism Branch, National Cancer Institute, Bethesda, MD 20892.
Activation of T lymphocytes is accompanied by markedly increased surface expression of CD25, the alpha chain of the IL2 receptor (IL2Rα, "Tac"). This molecule has been used as a target for antibody-directed therapy in allograft settings to suppress T cell activation, and in malignant T cell disorders to suppress tumor cell proliferation. Yet this receptor is also shed by T cells (soluble Tac or sTac), which can accumulate to high levels in the serum, and has been used as a marker of graft rejection and tumor burden. Further, the presence of sTac can interfere with anti-Tac antibody therapy by competing with Tac+ cells for antibody binding. We have investigated the metabolism of sTac in humans and in mice, and studied its interaction with exogenously administered antibody. The following conclusions have been obtained. 1) sTac is produced by actively metabolizing Tac+ T cells over a wide range with a modal surface shedding $t_{\frac{1}{2}}$ of about 10-20 hours; 2) the whole body $t_{\frac{1}{2}}$ of sTac was 1-2 hours with 90% of catabolism occuring in the kidney, with less than one percent of the material excreted as intact protein; 3) the presence of excess anti-Tac antibody markedly prolongs the $t_{\frac{1}{2}}$ of sTac with a consequent elevation of sTac levels in the body; 4) to overcome the competing effects of sTac, large quantities of anti-Tac antibody may need to be administered. These observed alterations in catabolism compromise the value of sTac as a measure of T cell activation and rejection in renal allograft settings where renal failure itself will elevate serum levels of sTac. However, these data permit rational predictions that assist in the design of antibody therapies for Tac+ malignant and non-malignant disorders, and is a model for receptor-directed antibody therapies generally.

P 231 TREATMENT WITH MONOCLONAL ANTI-CD3 ANTIBODY PROTECTS AGAINST LETHAL SENDAY VIRUS INFECTION BY LYMPHOKINE INDUCTION OF NK CELLS, WM Kast[1], JA Bluestone[2], MHM Heemskerk[1], J Spaargaren[1], AC Voordouw[1], JDI Ellenhorn[2], CJM Melief[1]. [1]The Netherlands Cancer Institute, Plesmanlaan 121 1066 CX Amsterdam, The Netherlands, [2]University of Chicago, USA.
B6 mice are protected from a lethal pneumonia caused by Sendai virus when treated with low doses of monoclonal antibody directed to the CD3 antigen. The protective mechanism is not due to an accelerated Sendai virus-specific T helper cell, CTL or antibody response but to a strong NK cell response via the in vivo induction of lympokines. Antibodies directed against the NK1.1 and asialo GM1 marker totally reversed the protective effect of anti-CD3 treatment. In vivo treatment with recombinant IL-2 also induced NK activity and induced antiviral protection. Treatment with anti-CD3 protects when given in a narrow time window (1 day before until 1 day after Senday virus inoculation), indicating that NK activity is protective in the early phase of virus infection.

P 232 A GAMMA-DELTA T CELL RECEPTOR IS ESSENTIAL IN SPONTANEOUS SECRETION OF CYTOKINES, Gary E. Kikuchi, Kevan Roberts, Ethan M. Shevach and John E. Coligan, Biological Resources Branch and Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, MD 20892

The Vδ6 Cδ, Vγ1.1Cγ4-bearing murine dendritic epidermal T cell T195 has the unusual phenotype of spontaneous secretion of the cytokine IL-4. Previous studies have demonstrated that this spontaneous secretion of IL-4 by T195 can be blocked by monoclonal antibodies (MAb) specific for CD3-ε or the γδTCR clonotype. In addition, the vitronectin receptor (VNR) is a requisite accessory molecule for T195. Both the synthetic peptide RGDS (which binds VNR) and MAb against the VNR can also block spontaneous secretion of IL-4 by T195. In these cells, recognition of a self antigen by the T cell receptor (TCR) has been proposed to cause self-activation and spontaneous secretion of IL-4.

We have isolated the cDNAs for the γ and δ TCR chains of T195. These cDNAs were cloned into an expression vector and transfected into the cell line TG-40, a variant of the cytochrome c-specific hybridoma 2B4 that was previously mutated to eliminate endogenous TCR chain expression. The transfectant, TG524, expresses the same γδTCR as T195 as determined by staining with pan-γδ and anti-clonotypic MAb. The γδ transfectant was found to spontaneously secrete the cytokine IL-2, just as 2B4 secretes IL-2 upon activation. Spontaneous secretion of IL-2 by TG524, like spontaneous secretion of IL-4 by T195, could be blocked by MAb specific for CD3-ε, anti-clonotypic MAb, MAb specific for VNR, and the VNR ligand RGDS. These studies demonstrate that this Cγ4 Vδ6 TCR is essential in spontaneous secretion of cytokine by these T cells.

P 233 IL-2 CONJUGATED LIPOSOMES-A DRUG DELIVERY SYSTEM FOR THE IL-2 R POSITIVE CELLS, Paula J. Konigsberg and Reneé A. Godtel, Vestar, Inc., San Dimas, CA 91773

A novel liposome has been developed which selectively delivers an antineoplastic drug to aberrantly activated IL-2 receptor positive T-cells present during graft rejection. Small unilamellar vesicles (SUVs-50 nm diameter) with human recombinant IL-2 externally coupled (SUV-IL-2) have been shown to specifically bind to the high affinity IL-2 receptor on mitogen stimulated human PBL and to IL-2 receptors on the murine CTLL-2 line (receptor positive cells). Proliferation of both types of cells in culture occurs with liposomal IL-2 as the sole source of IL-2. Methotrexate encapsulated in SUV-IL-2 (SUV-MTX-IL-2) is targeted to activated PBL and inhibits their growth in a dose-dependent manner after 72 hours in culture. Complete inhibition of proliferation by SUV-MTX-IL-2 was observed with 0.18-45.8 μg/ml of the encapsulated drug. PBLs that are not mitogen stimulated, do not proliferate to any appreciable degree in the presence of SUV-IL-2. Moreover, non-induced PBL are not killed by SUV-MTX-IL-2. Jurkat cells (receptor negataive), nonselectively engulf SUV-MTX-IL-2 or encapsulated MTX (SUV-MTX) and their growth is inhibited with a three fold higher drug concentration. In the presence of SUV-MTX-IL-2 PBL are first primed to grow with the targeting ligand (IL-2) and then killed during proliferation, due to internalized liposome encapsulated MTX. Intracellular delivery of an immunosuppressive or antineoplastic drug to a specific group of lymphocytes can be achieved with this approach.

P 234 DOWN-REGULATION OF Th1-RESPONSES TO MYOGLOBIN DURING *SCHISTOSOMA MANSONI* INFECTION, **Marika C. Kullberg[1], Edward J. Pearce[2], Alan Sher[2] and Jay A. Berzofsky[1],** [1]Metabolism Branch, NCI, and [2]Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892

S.mansoni infection in mice is characterized by a mixed Th1 (IL-2, IFNγ) and Th2 (IL-4, IL-5) response to parasite antigens early in infection. After egg-deposition occurs, however, the Th1 response is down-regulated, and a predominantly Th2 response is seen. Since the same lymphokine pattern is observed after mitogen stimulation, our studies focused on whether *S.mansoni* infection might also influence the outcome of immune responses to other foreign antigens. BALB/c mice, 8 wk after infection with cercariae, were inoculated s.c. with sperm whale myoglobin in CFA, used as a model antigen. Ten days later, spleen and draining lymph nodes (LN) were removed and cytokine production measured after *in vitro* stimulation of cells with antigen. Myoglobin-specific production of IL-2 and IFNγ by spleen cells was decreased (64%±26 and 87%±8, respectively) in schistosome infected as compared to non-infected mice. Although the percent CD4+ cells was decreased in infected animals, the observed reductions in Th1 response were also apparent when the data was normalized for the total number of CD4+ cells present. Decreased Th1 cytokine responses (IL-2, 79% and IFNγ, 34%) were also evident in LN cells from infected vs non-infected mice. Myoglobin-specific production of IL-4 could be detected in splenic but not LN cell populations from both infected and non-infected animals. Amounts of IL-4 were variable, although on average, the infected mice gave higher levels of this cytokine. No myoglobin-specific IL-5 response was measurable. One mechanism which could explain the observed decrease in Th1 cytokine response to myoglobin is the production of IL-10 by Th2 cells. This lymphokine inhibits the synthesis of Th1 derived cytokines and is known to be produced by T-cells from *S.mansoni* infected mice after the onset of parasite egg production. Thus, *in vivo*, *S.mansoni* infection might lead to an increased susceptibility to pathogens for which protective immunity requires a strong Th1 response.

P 235 **INTERLEUKIN 2 (IL2) INDUCES TUMORICIDAL ACTIVITY IN RAT KUPFFER CELLS THAT EXPRESS CONSTITUTIVE IL2 RECEPTOR (IL2r). PS Latham, GW Cox, AM Pilaro, L Varesio. Laboratory of Molecular Immunoregulation and Laboratory of Experimental Immunology, BRMP, NCI-FCRDF, Frederick MD 21702**

Expression of the IL2r and IL2-responsiveness are characteristic features of PHA-activated lymphocytes and natural killer (NK) cells. Recently, IL2r has been demonstrated in activated monocytes by immuno-fluorescence. We now report that recombinant human IL2 alone can induce cytotoxicity of rat KC for N1S1 rat hepatoma cells which increases with dose (1-300 U/ml IL2) and effector:target ratio. IL2-induced cytotoxicity persisted after NK cell depletion of KC fractions. Affinity binding and crosslinking of [^{125}I]IL2 to KC resulted in protein bands specific for IL2r with one band at Mr 70-75 kD, consistent with the p55 IL2r-α chain, and a pair of bands at 82-93 kD, consistent with p70-75 IL2r-β chain. The IL2r protein bands appeared in untreated rat KC. These results demonstrate that IL2 alone can induce tumoricidal activity in rat KC in vitro. In contrast to lymphocytes, rat KC show a constitutive expression of IL2r. The ability of KC to respond to low doses of IL2 and the appearance of protein bands consistent with α and β chain IL2r suggest that the IL2r expressed on KC is a functional receptor.

P 236 INTERRELATIONSHIPS OF GLUTATHIONE, LYMPHOKINES, AND DIFFERENTIATION OF PRIMARY LYMPHOCYTES, Shu-Mei Liang, Chi-Ming Liang, Myrthel E. Hargrove, and Chou-Chik Ting, CBER, FDA and Division of Cell Biology and Diagnosis, NCI, NIH, Bethesda, MD 20892 By activating murine lymphocytes with anti-CD3 antibodies for 1-2 days, we generated a subset of activated killer (AK) cells, namely CD3-AK$^-$ cells. CD3-AK$^-$ cells mediated the slow lysis of allogeneic P815 but had little, if any, effect on syngeneic HFL/b cells. Addition of IL-2 or PMA in assay medium induced the cytolytic activity of CD3-AK$^-$ cells on HFL/b. The activating effect of murine IL-2 and PMA on CD3-AK$^-$ cells was decreased by anti-murine IL-2 monoclonal antibodies (mAbs). Although anti-murine IL-4 mAb alone did not show any effect, it enhanced the inhibitory effect of anti-IL-2 mAb. Incubation of CD3-AK$^-$ cells with L-buthionine-(S,R)-sulfoximine (BSO), an inhibitor of de novo GSH synthesis, decreased the cellular GSH and inhibited cytolytic activity of CD3-AK$^-$ cells concentration-dependently. This inhibitory effect of BSO was not primarily due to general cytotoxic effect and was positively correlated with responsiveness of CD3-AK$^-$ cells to IL-2. Incubation of CD3-AK$^-$ cells with GSH or 2-ME which increased the level of cellular GSH reversed the inhibitory effect of BSO. These results suggest that cellular GSH may regulate the effect of lymphokine(s) such as IL-2 and affect the differentiation of activated primary cytotoxic lymphocytes.

P 237 INHIBITION OF DNA REPAIR BY T-CELL FACTOR, Peter Marquardt*, Horst Röschlau +, Dietmar Schiffmann + , *Institute of Pathology, + Institute of Toxicology, University of Würzburg, D-8700 Würzburg, F.R.G.

There is now direct evidence that somatic mutation occurring in and around genes coding for the V-regions of the Ig-molecules contributes to the generation of antibody diversity, but potentially to the generation of autoreactivity also. Therefore, a strict control of this process has to be postulated, but so far, the mechanisms of somatic hypermutation and its regulation are unknown.

We have found a T-cell factor which in vitro, in two different assay systems, almost completely inhibits DNA repair, suggesting a T-cell-mediated control of this critical pathway of acquired (T-cell-dependent) immunity. From our results we conclude that there might be another major physiological problem inherent in the phenomenon of somatic mutation: the permission of cell transformation and/or the generation of tumor cell diversity in areas where there are activated T-cells (inflammatory diseases, intratumorous lymphoid infiltrates). This might be an important mechanism by which immune effector cells may produce tumor initiation and/or tumor progression.

Apart from the basic and tumor immunological aspects of our finding, this system may be applicable as a general method to considerably improve the sensitivity of genotoxicity assessment in toxicological studies.

P 238 EFFICACY OF CYTOTOXIC T LYMPHOCYTES WITH IL-2 AGAINST ADENOVIRUS-INDUCED TUMORS, CJM Melief, WM Kast, The Netherlands Cancer Institute, Plesmanlaan 121 1066 CX Amsterdam, The Netherlands.

Cytotoxic T lymphocytes (CTL) against virus-induced tumors are highly effective mediators of tumor specific immunity in vivo. CTL bearing the surface molecule CD8 recognize small (approximately 10- amino-acid) viral peptides, that are presented in association with major histocompatibility (MHC) class I molecules at the surface of tumor cells. In the case of mouse tumors induced by the early regio (E1) of human Adenovirus type 5 (AD5), cloned CD8$^+$ CTL directed against a peptide derived from the viral nuclear oncogene product E1A can rapidly eradicate large established tumors (up to 10 cm^3) implanted s.c. in T-cell deficient (nu/nu) mice, provided that interleukin-2 (IL-2) is given simultaneously. IL-2 given as a deposit injection was much more efficient than IL-2 given i.v. or i.p. IL-2 alone had no effect. The fine CTL specificity requirements for this type of therapy will be reported. In this model only combination of specific CTL therapy and IL-2 is effective in eradication of large established tumors. Conceivably, adoptive therapy with CD8$^+$ CTL and IL-2 could be extended to tumors that originate in other ways, including those arising through activation or mutation of cellular oncogenes.

P 239 RELATIONS OF CYTOKINE GENE EXPRESSION TO DEVELOPMENT OF A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY SYNDROME, H.C. Morse III, M. Makino, R. Gazzinelli, A. Cerny, C. Cheung and P.M. Pitha, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892 and Johns Hopkins University Oncology Center, Baltimore, MD 21231

Massive activation of CD4+T cells results from infection of susceptible strains of mice with a mixture of murine leukemia viruses, termed LP-BM5 MuLV. T cell activation has been shown to be associated with marked alterations in the expression of cytokine genes that affect the function of the immune system. Studies of mRNA prepared from spleen or lymph node cells of infected mice showed that infection results in constitutive expression of IL-1, TNF and IFN-gamma while completely inhibiting induction of IFN-alpha or-beta by Newcastle Disease Virus. At the protein level, infection with these viruses also induced expression of IL-5 and IL-6. While mRNA or protein for IL-4 was not detected, IgE levels following infection increased 30-fold and Ia levels on B cells were increased, suggesting that expression of this gene was also induced by infection. These alterations in cytokine gene expression appear to be central to the development of lymphoproliferation and immunodeficiency as treatment of infected mice with cyclosporin A greatly inhibits the progression of these abnormalities.

P 240 S-CD23 CLEAVAGE OF CD23 IS ESSENTIAL FOR CD23 MEDIATED HOMOTYPIC ADHESION, Kevin Moulder, Aisling Barton and Beverley Weston, Immunotherapeutics Programme, SmithKline Beecham Pharmaceuticals, Great Burgh, Surrey KT18 5XQ, United Kingdom

CD23 (FcE RII) is expressed by cells of lymphoid and myelomonocytic lineages, and is differentially regulated by a variety of cytokines. Membrane bound CD23 is a low affinity receptor for IgE, a cell adhesion molecule and is implicated in antigen presentation in the generation of an immune response. CD23 is capable of autoproteolysis to yield a soluble form (S-CD23). In its soluble, or cytokine form S-CD23 sustains an ongoing IgE response, and is present at high levels in the serum of allergic individuals. Cell: cell interactions have been shown to be important in antigen presentation in the generation of an IgE response, here we show that CD23 mediated homotypic adhesion by B cells and monocytes is dependent upon protease activity. Homotypic adhesion is induced by chymotrypsin, elastase or S-CD23. All three proteases were found to degrade CD23 to similar molecular weight fragments. These data suggest S-CD23 and select proteases play an important role in the regulation of cell:cell interactions. Furthermore, cleavage of membrane bound CD23 is essential for homotypic adhesion.

P 241 PARTIAL PURIFICATION OF A LOW MOLECULAR WEIGHT LYMPHOPOIETIC COFACTOR WITH PROLIFERATIVE AND DIFFERENTIATING ACTIVITY, Michael J. Muirhead and Thomas J. Waldschmidt, Departments of Medicine and Pathology, University of Iowa College of Medicine and Veterans Administration Medical Center, Iowa City, IA 52242.

We have previously reported a low molecular weight activity of about 450 daltons which is comitogenic with Interleukin 7 (IL-7) for many feeder layer dependent mouse pre-B cell lines. We will call this activity Dialyzable Lymphopoietin (DL). DL is present in the medium conditioned by a smooth muscle cell line (3E) derived from mouse bone marrow, and we have purified it approximately 1,000 fold, using 3 different gel filtration columns (Sephadex G10, Biogel P2, and the HPLC column Synchropak GPC60) followed by cation exchange on the Ultropac SP-5PW HPLC column. DL shows only marginal retention on a C18 reversed phase column, eluting shortly after the salt in 0.1% TFA without acetonitrile. Thus, it has very little hydrophobicity.

The Immunex pre-B cell line 2B is absolutely dependent on IL-7 for growth and long-term viability. We found that clone 2B cells could be passaged in the presence of low concentrations of IL-7 (50 Units/ml) if a 3E feeder layer was also present. At high concentrations of IL-7 (1000 U/ml), with or without a feeder layer, clone 2B cells express surface μ but not κ chain. In contrast, in the presence of a 3E feeder layer and permissively low concentrations of IL-7 (50 U/ml), clone 2B cells express low levels of surface κ. These cells were then removed from the feeder layer and maintained in 50 U/ml IL-7 in the presence and absence of DL for 48 hours. In IL-7 alone they continued to express low levels of κ, but in IL-7 plus DL surface κ was augmented 10-fold.

In summary, DL is neither a lipid nor a protein. In the presence of permissively low concentrations of IL-7, DL sustains growth as a co-mitogen and drives a neoplastic pre-B cell clone toward B cell differentiation.

P 242 The Stromal Cell Line S17 Supports the Growth of CBA/N B Cell Colonies in vitro.
A.Narendran, A.Cumano and C.Paige. Division of Cellular and Molecular Biology, The Ontario Cancer Institute, 500, Sherbourne Street, Toronto, Ontario, Canada M4X 1K9.

The X linked defect in the CBA/N strain of mice has been found to manifest in a wide spectrum of immune abnormalities. These include their greatly reduced ability to respond to many of the common B cell mitogens. One intriguing in vitro manifestation of this condition is the virtual inability of CBA/N B cells to form colonies in LPS containing semisolid agar cultures. In this report we show evidence that the colony forming ability of CBA/N spleen cells can be effectively restored by the bone marrow stromal derived cell line S17. Spleen cells from 4-5 weeks old homozygous CBA/N female mice were grown in double layer agar cultures containing S17 feeder layers. Control cultures contained the fibroblast like cell line 95.17 or treated with medium alone. It was found that at an input cell concentration of 10^4 cells per plate, CBA/N colony formation was increased from a frequency of 1 in 4000 to 1 in 100 total splenic cells. When purified sIg^+ cells were used, 1 in 50 cells formed colonies but no colony growth was seen with sIg negative cells. The CBA/N colonies formed were dependent on the presence of LPS and secreted detectable amounts of IgM. This indicates that LPS responsive clonable B cells are present in these animals and the factor(s) provided by S17 either helps to overcome the deficiency of a critical accessory cell or an autocrine factor, or helps bypass a defect in the relevant biochemical pathways imposed by the genetic abnormality.

P 243 STRESS PROTEINS INDUCED BY CHRONIC LOW DOSE IONIZING RADIATION (LDR) IN VIVO IS CORRELATED WITH AUGMENTATION OF MITOGENIC RESPONSE IN MURINE SPLEEN CELLS, Mari Nogami, John M. Lubinski and Takashi Makinodan, Geriatric Research, Education and Clinical Center (GRECC), VA Medical Center West Los Angeles, Los Angeles, CA 90073, and Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Cells respond defensively to abrupt environmental stress by producing stress proteins, enabling them to adapt to lethal effects of the stress. It is possible, therefore, that the enhancing effect of LDR *in vivo* on the proliferative activity of murine T cells observed previously could be associated with the production of stress proteins. Stress protein production by murine T cells treated *in vivo* with LDR was therefore determined by Northern blot and 2-D gel electrophoresis analysis. The results showed that 0.04 Gy/day x 20 days (total, 0.8 Gy) irradiation (a) elevated the synthesis of HSP 70 proteins by resting splenic T cells and (b) enhanced their production of HSP 70 mRNA and proteins and their proliferative activity in response to mitogenic stimulation *in vitro*. In contrast, 0.10 Gy/day x 20 days (total, 2 Gy) irradiation had no enhancing effect on either HSP production or T cells proliferative activity. Stress proteins could therefore play an important role in LDR-induced potentiation of T cell proliferative activity. This work was supported by the Electric Power Research Institute (8000-8).

P 244 PURIFICATION, CHARACTERISATION AND SEQUENCE ANALYSIS OF THE APO-1 ANTIGEN, A MEMBRANE PROTEIN THAT TRIGGERS PROGRAMMED CELL DEATH,
Alexander Oehm[1], Werner Falk[1], Gernot Maier[2], Susan Richards[1], Gerard Garcin[1], Jens Dhein[1], Bernhard C. Trauth[1] and Peter H. Krammer[1]; [1]Institute for Immunology and Genetics, [2]Institute for Cell- and Tumorbiology, German Cancer Research Center, Heidelberg, FRG.

Binding of the monoclonal antibody anti-APO-1 to a protein antigen termed APO-1 leads to growth inhibition of the human B lymphoblast cell line SKW6.4 and a variety of other cells by induction of programmed cell death, apoptosis. Apoptosis is the most common form of eukaryotic cell death. The APO-1 antigen was purified from membranes of SKW6.4 cells by solubilization with sodium deoxycholate, affinity chromatography on anti-APO-1 and (RP)-HPLC. For detection and quantification of APO-1 the purification was followed by ELISA. The purified APO-1 antigen was found to be a glycoprotein with apparent Mr of 48000 as determined by SDS-PAGE analysis and western blotting. Futhermore it was shown to block anti-APO-1 induced apoptosis of SKW6.4 cells in vitro. Purified APO-1 was digested with endoproteinase Asp N to generate peptides that were seperated by C8 (RP)-HPLC and sequenced on a gas phase sequencer. The obtained sequences were not found in any other polypeptides listed in protein or nucleotide databases. APO-1 therefore, is a novel membrane molecule. Cloning of the APO-1 cDNA may provide further information with regard to the role of APO-1 in the regulation of lymphocyte growth.

P 245 ROLE OF CYTOKINES IN MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY MODEL (MAIDS), Paula M. Pitha, Sau C. Cheung, and Judith M. Pozsgay, The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD 21205

A syndrome characterized by severe immunodeficiency and lymphoproliferation develops in susceptible strains of mice infected with a mixture of viruses (LP-BM5) containing a replication defective virus (BM5d) (the etiologic agent) and replication competent helper virus (BM5e), that is required for cell to cell spread of the BM5d in the host. We have studied the expression of BM5d and BM5e in tissues of infected mice at various times after inoculation in relation to the expression of cytokine genes that may contribute to the pathogenesis of this disorder. Infection with the virus mixture was associated with constitutive expression of TNF, IL-1β, and IFN-γ in a variety of tissues, including peritoneal macrophages, while no expression of IL-3, IL-4, IL-5, and IL-6 was detected either at early or late stages of infection. Furthermore, macrophages infected with BM5d virus responded differently to stimulation either by NDV or LPS. These results suggest that aberrant expression of certain cytokine genes may play a role in immunopathogenic condition in MAIDS. The role of cytokines in strains of mice which are resistant to the development of disease will be discussed.

P 246 MOLECULAR CHARACTERIZATION OF A NOVEL CYTOKINE: CYTOTOXIC LYMPHOCYTE MATURATION FACTOR, Frank J. Podlaski, Venkata B. Nanduri, Jeffrey D. Hulmes, Yu-Ching E. Pan, Phyllis M. Quinn, Aimee G. Wolitzky, Richard Chizzonite, Maurice K. Gately and Alvin S. Stern, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110-1199

Cytotoxic Lymphocyte Maturation Factor (CLMF) is a novel cytokine purified to homogeneity from a human B-lymphoblastoid (NC-37) cell line that was induced to secrete lymphokines by culture with phorbol ester and calcium ionophore. The purified protein was shown to synergize with low concentrations of interleukin 2 in causing the induction of lymphokine-activated killer cells. In addition, purified CLMF stimulated the proliferation of human phytohemagglutinin-activated lymphoblasts by itself and exerted additive effects when used in combination with suboptimal amounts of interleukin 2. The protein is a heterodimer composed of 40-kDa and 35-kDa subunits. Amino acid sequence analysis confirmed predicted sequences from the cloned cDNAs of each subunit. N-glycanase digestion of the heterodimer demonstrated that the 40-kDa and 35-kDa subunits contain 5% and 20% N-linked carbohydrate respectively. Chemical modification revealed that the disulfide linked association of the two subunits is essential for bioactivity. Interestingly, the 40-kDa subunit of CLMF was identified by SDS/PAGE and confirmed by immunoblotting as being present in NC-37 cell supernatant solutions in relatively large amounts free of the 35-kDa subunit. However, in contrast to the heterodimer, the 40-kDa subunit alone caused little or no proliferation of activated human T lymphocytes or enhancement of the cytolytic activity of human LAK cells.

P 247 IMMUNOMODULATORY EFFECTS OF NEUROTROPHIC FACTORS, Glenn Rice, Jim Borree, Randy Johnson, Arnon Rosenthal, Qiao Yan, Rebecca Flynn and Michael Palladino. Departments of Immunology Research and Assay Technology, and Molecular Biology, Genentech, Inc. , S. San Francisco, CA 94080

The mechanisms involved in bidirectional signal transduction pathways of neuro-endocrine-immune interactions are poorly understood. Nerve growth factor (NGF), Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin-3 (NT-3) are neurotrophic factors necessary for the survival and functional maintenance of various neuronal cell populations. In addition, D factor (DF; also known as leukemia inhibitory factor) has been shown to possess cholinergic differentiating activity for neonatal sympathetic neurons. Both freshly isolated and cultured human leukocytes were used to test the effects of these agents on immune response and function. T and B lymphocytes and monocytes (but not neutrophils) rapidly increased $[Ca]i^{++}$ after treatment with NGF, BDNF, NT-3 and DF. This effect was dose responsive and potentiated with cAMP pretreatment and decreased with pretreatment with protein kinase inhibitors. The precise nature of the signal transduction mechanism is under investigation. Using multiparameter flow cytometric analysis, all of these factors were shown to increase IL-2 and low affinity NGF receptor expression in both T and B-cell subsets following a 3 day incubation. The functional significance of these and other changes in expression patterns of immunoregulatory molecules following treatment with these agents is under investigation. In conclusion, NGF, BDNF, NT-3 and DF are cytokines that may play an important role in the regulation of various neuroimmune interactions.

P 248 EXPRESSION OF LARGE AMOUNTS OF MURINE IL-10 IN E. COLI AND ITS MODULATING EFFECT ON THE CELLULAR IMMUNE RESPONSE TO L. MAJOR André Gessner and Martin Röllinghoff, Institut für Klinische Mikrobiologie der Universität Erlangen-Nürnberg, FRG.

The pattern of lymphokine expression is important for the clinical outcome of an infection of mice with the protozoan Leishmania major. In resistant C57BL/6 mice predominantly $CD4^+$ T cells of the Th1 type are activated to produce IFN-γ and IL-2, whereas in BALB/c mice, which succumb the infection, large numbers of antigen specific Th2 type T cells, producing IL-4, can be found. Clones of the latter type are known to produce IL-10, formerly known as cytokine synthesis inhibitory factor (CSIF) (Moore et al., 1990). This lymphokine was initially characterized as a factor which suppresses IFN-γ production by Th1 cells (Fiorentino, Bond, and Mosmann, 1989).
To study the function of this cytokine with regard to its effect on the modulation of the $CD4^+$ cell driven cellular immune response to L. major large amounts of the protein were expressed in E. coli as glutathion S-transferase-fusion protein, purified and digested with thrombin to remove the N-terminal part, yielding functional active murine IL-10 . Results concerning in vivo and in vitro effects of this recombinant product during experimentally induced leishmanisis will be presented.

P 249 IN VIVO ACTION OF CYTOKINES, F.M. Rollwagen and Shahida Baqar
Naval Medical Research Institute, Bethesda, MD 20889 and GEOCENTERS, INC.
Cytokines incorporated into agarose blocks and implanted subcutaneously into mice establish an in vivo gradient which can be used to mimic a local inflammatory process. We have characterized a model in which cellular influx into cytokine impregnated blocks parallels the normal cellular reaction to infection or wounds. Initial experiments, using supernatant from Con A activated rat spleen cells (CAS) reveal that PMNs are the first cells to arrive (within 4 hours) and are followed in 24 hours by lymphocytes (LC). FACS analysis of these LC reveals 13-20% T and 78-82% B cells are present, and this ratio is maintained until day 10. All T-cells were L3T4 negative. Macrophages arrive by 24-36 hours, and initially are quiescent, but by day 6-7 exhibit active membranes and increased phagocytic capacity. Electron microscopic examination of these cells reveals massive rough endoplasmic reticulum, indicative of secretory cells. Further experiments using recombinant material show that rIL-1 attracts LC (50% T-cells) to the block, rIL-2, macrophages and PMNs, rIL-4 attracts mostly macrophages, and rIL-5 all three cell types.

P 250 PROSTAGLANDIN E PROMOTES IL-4 INDUCED CLASS SWITCHING TO IgE AND IgG1. R. L. Roper, D. H. Conrad, D. M. Brown, G. L. Warner, and R. P. Phipps. Department of Microbiology & Immunology, University of Rochester, Rochester, NY 14642.
Our laboratory has been studying the effects of macrophage secreted prostaglandins (PG) on murine B lymphocyte activation and immunoglobulin synthesis. As previously reported, PGE2 and PGE1, but not PGF2α, significantly *enhance* IL-4 induced IgE and IgG1 synthesis up to 26 fold (*J. Immunol 145:2644*). PGE induces a rise in intracellular cAMP and we have shown, using cAMP inducing pharmacologic agents, that PGE acts via a cAMP dependent mechanism. In addition to enhancing IgE and IgG1 synthesis, PGE inhibits the production of IgM and IgG3, suggesting that PGE promotes IL-4 induced class switching. We now present further evidence supporting this hypothesis. PGE does not promote IgE synthesis in IgE secreting hybridomas (which have already undergone class switching), suggesting that PGE is not simply enhancing the rate of IgE production. Data from IgE spot ELISA show that PGE increases the frequency of IgE secreting cells, even though proliferation is mildly inhibited. The enhancing effect of PGE on IgE and IgG1 synthesis is in marked contrast to its numerous inhibitory effects on immune functions. In addition to PGE inhibiting B cell proliferation and IgM and IgG3 production, we have found that E series prostaglandins (PGE) specifically prevent *early* B cell activation events including enlargement and upregulation of class II MHC and FcεRII antigens on the cell surface. Thus, PGE prevents certain B cell responses and may instead channel lymphocytes into differentiative pathways and promote isotype switching.
Research supported by grants CA42739, CA-11198, and T32-AI07285.

P 251 THE EBV PROTEIN BCRF1/vIL-10 IS A POTENT GROWTH AND DIFFERENTIATION FACTOR FOR HUMAN B LYMPHOCYTES, Françoise Rousset, Eric Garcia, Thierry Defrance, Catherine Péronne, Di-Hwei Hsu*, Robert Kastelein*, Kevin Moore*, Jacques Banchereau, Schering-Plough, Laboratory for Immunological Research, BP 11, 69571 Dardilly Cedex, France; *DNAX Research Institute, Palo Alto, California 94304

The mIL-10 protein displays 70% identity, at the amino acid level, with the EBV open reading frame BCRF1/vIL-10 and, as mIL-10, recombinant BCRF1/vIL-10 is able to inhibit the synthesis of IFNγ by activated mononuclear cells (Hsu et al, Science 1990, 4982, 830). Anti-CD40 Mabs presented in a crosslinked fashion by transfected mouse Ltk$^-$ cells stably expressing human FcγRII/CDw32 were able to induce human resting B lymphocytes to enter into sustained proliferation. In combination with IL-4, this approach allowed us to generate factor dependent long term human B cell lines, which were constantly negative for Epstein-Barr viral infection (Banchereau et al, Science, in press). BCRF1/vIL-10 and crosslinked anti-CD40 also induced long lasting B cell proliferation as well as production of high levels of IgM, IgG and IgA. The combination of crosslinked anti-CD40, BCRF1/vIL-10 and IL-4 was the most potent in terms of both B cell proliferation and Ig production (IgM, IgG, IgA and IgE). BCRF1/vIL-10 also enhanced the proliferation and Ig production by B cells stimulated with EBV or Staphylococcus aureus. We are presently exploring whether BCRF1/vIL-10 contributes to the immortalization of B cells by Epstein Barr Virus.

P 252 EFFICACY OF BCG AND CYCLOPHOSPHAMIDE IMMUNOPRIMING PRIOR TO CYTOKINE MEDIATED ADOPTIVE IMMUNOTHERAPY, Marvin Rubenstein, Sergey Muchnik, Michael Shaw, Alvin Dubin and Patrick Guinan, Department of Research Biochemistry, Hektoen Institute for Medical Research, Chicago, IL 60612

Adoptive immunotherapy is dependent upon the leukocytic subsets isolated as tumor infiltrating lymphocytes (TIL) prior to their in vitro expansion with interleukin-2. To favorably influence T-cell subset representation in TIL the efficacy of preoperative injection ("immunopriming") of bacillus Calmette Guerin (BCG) and cyclophosphamide (CTX) was evaluated in rats bearing Dunning R3327 AT-3 prostatic tumors. When assessed by immunohistochemistry, both agents significantly ($p < 0.001$) increased helper-T cell and decreased suppressor-T cell representation in TIL.

		RESULTS			Helper/
Group	N	Total-T	Helper-T	Suppressor-T	Suppressor
Control	9	50.5±3.8	24.4±3.2	33.8±4.2	0.73±0.11
BCG	6	54.3±7.4	38.0±5.5	20.4±4.8	1.93±0.39
CTX	6	40.9±3.3	31.8±4.6	22.6±2.6	1.40±0.25

Immunopriming might enhance the effectiveness of adoptive immunotherapy by favorably influencing both T-cell distribution and the activity of leukocytes isolated as TIL prior to in vitro cultivation. BCG may be particularly useful since it enhances the activities of both NK and cytotoxic T cells predominantly found in TIL. These agents are also being used to "immunoprime" thoracic duct cells for adoptive immunotherapy.

P 253 REGULATION OF *IN VIVO* MURINE IgE RESPONSES BY INTERLEUKIN-4 AND INTERFERON-γ Huub F.J. Savelkoul and Robert L. Coffman*. Dept. Immunology, Erasmus University, Rotterdam, The Netherlands and *DNAX Research Inst., Palo Alto, CA, USA.

The cytokines IL-4 and IFN-γ are thought to regulate IgE responses by directing isotype switching and influencing the activation of Th1 and Th2 cells antagonistically. BALB/c, SJL and SJA/9 mouse strains differing in their ability to mount IgE responses were therefore investigated for the role of IL-4 and IFN-γ in these responses. In IgE high responder BALB/c mice given anti-IL-4 treatment was found to inhibit primary IgE responses (induced by TNP-KLH/alum) >95% and secondary only partially (± 70%) as measured by serum IgE levels. This suggests a large IL-4 dependent and a minor IL-4 independent part of the secondary IgE response. SJL mice display a marked suppression of antigen-specific IgE production later during the response. IL-4 treatment (by implanting an IL-4 transfected cell line encapsulated in alginate) reversed this suppression as did anti-IFN-γ treatment, while IFN-γ itself induced a complete inhibition of the IgE production. IgE non-responder SJA/9 mice are normally unable to mount detectable IgE responses. This defect was shown not to be caused by overproduction of IFN-γ or a defective state of activation of Th2 cells, since IL-5 driven blood eosinophilia could be induced in these mice. Evidence for IL-4 production *in vivo* was found by the presence of IL-4 mRNA in T cells of spleens and lungs of SJA/9 mice infected with Nippostrongylus brasiliensis. The defective Nb-induced IgE production was not restored until administration of IL-4. These data show that the outcome of IgE responses in BALB/c, SJL and SJA/9 mice are mainly directed by the amounts of IL-4 and IFN-γ present in these mice.

P 254 A CYTOSTATIC PROTEIN ISOLATED FROM NATURAL KILLER CELL GRANULES, T.J. Sayers, R. Sowder, T. Wiltrout, J. Wine, L. Henderson, R.H. Wiltrout and M.J. Smyth, Biological Carcinogenesis and Development Program, and AIDS Vaccine Development Program, PRI/DynCorp, and Laboratory of Experimental Immunology, NCI-FCRDC, Frederick, MD 21701
The granules of natural killer cells are known to contain a number of proteins including perforin (cytolysin), various serine esterases, and some less well defined cytostatic or cytotoxic proteins. We have partially purified a protein from the granules of the rat NK cell leukemia (RNK) which is cytostatic to a variety of tumor cells. The cytostatic factor was purified by extracting the granules with salt followed by sequential chromatography on a sizing column (ACA54), a heparin agarose affinity column and reverse phase HPLC. The protein has a mol. wt. of 30-32 kD, binds fairly strongly to heparin, is inactivated by heating at 70°C for 5 min but is stable to trypsin treatment. This protein is not species specific since it inhibits tumor lines of mouse, rat and human origin. The effect on sensitive cells is predominantly cytostatic as opposed to cytotoxic and solid tumors seem particularly sensitive. Treatment of sensitive cells results in a rounding of the cells followed by aggregation into large clumps of cells. These morphological changes still occur even if the cells have been adhered to the culture dish prior to addition of the factor. This factor is unable to hydrolyse Benzyloxycarbonyl-L-lysine-thiobenzylester (BLT) and is quite distinct from perforin. Attempts to purify the protein to homogeneity for N-terminal amino acid sequencing are in progress.

P 255 EFFECTS OF RANTES/SIS CYTOKINES ON DISTINCT POPULATIONS OF LYMPHOCYTES, Thomas J. Schall, Kevin Bacon*, Caroline Hébert and David V.Goeddel, Molecular Biology Department, Genentech, S. San Francisco, CA 94080, and St. Thomas's Hospital, London, UK*
Lymphocyte trafficking is an essential process in immune and inflammatory functions that can be thought to contain two main components: homing and migration. Adhesion molecules, known as homing receptors, are known to mediate the passage of leukocytes as a class from the blood to the tissues via the endothelium, but the chemoattractant substances responsible for the migration of specific subsets of lymphocytes to sites of infection or inflammation are largely unknown. Our studies show that a newly emerging family of hematopoietic cytokines, the RANTES/SIS family, have differential attractive effects on specific subsets of lymphocyte effector cells. Using an *in vitro* chemotactic assay, we show that the cytokine RANTES causes the selective migration of T helper cells of the memory phenotype ($CD4^+/CD45RO^+$), while not affecting T cells of other phenotypes. In direct contrast, the cytokine HuMIP-1β preferentially attracts lymphocytes of the naive T helper phenotype ($CD4^+/CD45RA^+$). Interestingly HuMIP-1α, though closely related to HuMIP-1β in primary structure, has a broader range of concentration-dependent chemoattractant specificities. HuMIP-1α attracts B cells and T cells of the cytotoxic and helper phenotypes at low relative concentrations, while at higher concentrations T helper phenotype cells respond in the absence of B cells or cytotoxic T cells . Thus, this one small family of related cytokines has differential and specific activities for many of the major subsets of immune effector cells, and some of these activities can be controlled by differences in cytokine concentration. These findings suggest that this family of cytokines may have a key role in lymphocyte trafficking.

P 256 BOVINE UTERINE LUMINAL PROTEINS INHIBIT THE BINDING OF IL-2 TO THE IL-2 RECEPTOR OF T-CELLS, Edward C. Segerson and David W. Libby, Department of Animal Science, North Carolina A&T State University, Greensboro, NC 27411
Inhibition of IL-2 mediated T-cell activation within the uterus may be requisite for fetal allograft survival in ruminant species. Bovine uterine luminal protein (ULP) components, ≥248 kDa (H-ULP) and 21 kDa (L-ULP), have been shown to suppress the proliferation of PHA and IL-2 treated T-cells. H- and L-ULP (32 - 64 µg/ml) resulted in >90% suppression of ^{3}H-thymidine incorporation into DNA within $2X10^4$ cells. This laboratory hypothesized that suppressor activity resulted from blocking the IL-2 receptor (IL-2R) by ULP, binding of ULP to IL-2, or both. In ensuing experiments, further purification (DEAE and Sepharose CL-6B chromatography) of H-ULP resulted in a 1.76 X 10^6 Da component. The 1.76 X 10^6 Da and 21 kDa components inhibited the binding of ^{125}I-IL-2 (7.0 pM) to the IL-2R of 5 X 10^6 bovine and human mononuclear cells. For bovine cells, percentage ligand binding for both protein components declined from 52 to 18% between 10 and 50 µg protein, respectively. In parallel experiments, anti-Tac binding to human cells was not affected by ULP, suggesting that suppressor activity was not associated with p55α of IL-2R. Experiments were then conducted to determine the binding capacity of ULP (200 µg) to ^{125}I-IL-2 (140 pM). Although the 21 kDa component failed to bind ^{125}I-IL-2, the 1.76 X 10^6 Da component bound 11.7% of the radioligand. In conclusion, two protein components within the bovine uterus suppress T-cell activation by IL-2. The high molecular weight component blocks IL-2R recognition of IL-2 and binds IL-2; whereas, the low molecular weight component blocks the IL-2R.

P 257 **Decreased Interferon α/ß responsiveness in variant T cells not expressing the T cell antigen receptor.**

By M. Tarek Shata[1], George K. Lewis[2], Connie R. Faltynek[3], and Roberta M. Kamin-Lewis[2]. [1] Department of Microbiology, School of Medicine, Assuit University, Assuit, Egypt, [2] Department of Microbiology and Immunology, Universtiy of Maryland Medical School, Baltimore, MD 21201, [3] BCDP-Program Resources Inc., National Cancer Institue-Frederick Cancer Research Facility, Frederick, MD, 21701[4].

In this study, we have investigated the relationship between T cell antigen receptors (CD3/Ti) expression and the inhibitory signal delivered by IFN α/ß. A series of antigen specific, class II restricted, TH1 T cell clones and their receptor negative variants were used in these studies. Addition of either murine IFN-ß or a recombinant human hybrid IFN-α [IFN-α (A/D)] to wild-type cells inhibited tritiated thymidine incorporation in a dose-dependent fashion when the cells were stimulated with any of the following; a. IL2, b. anti-CD3 antibodies, or, c. antigen plus antigen-presenting cells. By contrast, the IFN effect was decreased by at least 166-fold in parallel cultures when the CD3/Ti negative T cell clone F7' was stimulated with IL2. Consistent with the loss of responsiveness, we also found a loss of functional IFN α/ß receptor expression on clone F7'. The decrease of responsiveness to IFN α/ß in CD3/Ti negative variant places the IFN α/ß receptors in a category of cell surface molecules that include Thy-1, CD2, and Ly-6/TAP the functions of which are decreased or lost in CD3/Ti negative variant. Several mechanisms that explain this association are considered. Supported by NIH grants AI-25862 and NS-26665 and ACS grant FRA-254.

[4]Present address; department of Immunopharmacology, Sterling Research Group, Malvern, PA 19355.

P 258 **INDUCED SECRETION OF TNF AND IL6 FROM HIGHLY ENRICHED NON-HODGKIN'S LYMPHOMA CELLS**

Erlend Smeland[1], Heidi K. Blomhoff[1], Terje Espevik[2], Werner Lesslauer[3], Bjørn Erikstein[1], [1]Lab. of Immunology, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 OSLO 3, Norway. [2]Institute for Cancer Research, Univ. of Trondheim, N-7006 TRONDHEIM, Norway. [3]Central Research Units, F. Hoffman-La Roche AG, 4002 BASEL, Switzerland.

A number of cytokines have been shown to potently influence B cell growth and differentiation in vitro. We have shown that extensively purified normal human B cells will, upon activation with various stimuli, produce IL6, TNF-α and -ß. The combination of ionomycin and phorbol ester was particularly potent in inducing the production of these cytokines. Notably, IL4 alone selectively induced production of IL6 and not TNF-α or TNF-ß production. None of several other cytokines tested were able to induce IL6 production when given to resting cells.

In this study we have tested cytokine production in cell suspensions from lymph node biopsies from patients with non-Hodgkin's lymphomas after removing contaminating T cells and monocytes by negative depletion with immunomagnetic beads. The cells were >99% B cells, and showed light chain restriction (>95% of the cells expressed one light chain). The majority of the lymphoma samples produced IL6 and/or TNF in response to ionomycin/TPA or anti-μ/IL4. No significant cytokine production was observed in medium alone. Some of the lymphomas also showed a modest proliferation in response to TNF, suggesting a possible autocrine loop. Using specific antibodies, we found that most lymphomas expressed 55 and/or 75 kD TNF-receptors, but to a variable degree.

P 259 CYTOKINE GENE EXPRESSION DURING A PRIMARY *IN VIVO* IMMUNE RESPONSE.

Antonela Svetić[1], Fred D. Finkelman[2], Carl W. Dieffenbach[3], Dorothy E. Scott[4], Alfred D. Steinberg[4], and William C. Gause[1]. Departments of Microbiology[1], Medicine[2], and Pathology[3], USUHS, Bethesda, MD 20814; [4]ARB, NIAMS, National Institutes of Health, Bethesda, MD 20892.

Cytokines are important mediators of effector lymphoid cell function during an immune response, but their expression during an in vivo immune response has not been well documented. We analyzed the kinetics of cytokine gene expression during the course of an in vivo primary immune response to GaMδ antibody. Total RNA was purified from spleens taken from freshly killed BALB/c mice 1 hour and 1-7 days after immunization. The reverse transcriptase polymerase chain reaction (RT-PCR) was used to evaluate the expression of seven cytokine genes, all of which encode cytokines that are secreted by T cells and are important in T and/or B cell activation and differentiation. These were: IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-9, and IL-10. IL-10 increased markedly within 1 hour after immunization, decreased to baseline levels by day 2 and then increased again at day 3, peaking by day 6. IL-2 and IL-9 exhibited elevations at days 2-3 and sharply declined thereafter. IL-4, IL-6, and IFN-γ were increased by day 3, but remained high, peaking as late as days 5-6. IL-5, in contrast, showed little change during the course of the immune response, exhibiting a similar pattern to the housekeeping gene, HPRT. Our results demonstrated a specific and highly reproducible cytokine gene expression pattern during the course of an in vivo primary immune response, which was marked by an absence of a clearcut Th1/Th2 dichotomy.

P 260 IL-4 INCREASES IL-2 PRODUCTION BY T CELLS IN RESPONSE TO ACCESSORY CELL-INDEPENDENT STIMULI, Toshio Tanaka, Shlomo Z. Ben-Sasson and William E. Paul, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892

Highly purified T cells failed to produce IL-2 in response to soluble anti-CD3 or Con-A and produced small amounts of IL-2 in response to immobilized anti-CD3 or anti-TCR αβ chain antibody. IL-2 production in response to soluble anti-CD3 or Con-A required the presence of accessory cells. In contrast, the addition of IL-4 strikingly enhanced the production of IL-2 and anti-IL-4 antibody inhibited IL-2 production by immobilized anti-CD3-stimulated T cells in the absence or presence of accessory cells, indicating that endogenously produced IL-4 was important for IL-2 production under such conditions. The effect of IL-4 on enhancing or anti-IL-4 on inhibiting IL-2 production was achieved by augmenting or diminishing the expression of IL-2 mRNA, respectively. Half-lives of IL-2 mRNAs from anti-CD3-stimulated T cells in the absence or presence of IL-4 or anti-IL-4 were similar, suggesting that IL-4 may regulate transcription of the IL-2 gene. The effect of IL-4 on enhancing IL-2 mRNA was inhibited by cyclohexamide, indicating that it depended on protein synthesis. Experiments to examine the detailed mechanism through which IL-4 augments IL-2 gene expression are now in progress. These results indicate that IL-4 plays an important role in accessory cell-independent IL-2 production.

P 261 ANTI-CD3 ANTIBODY-INDUCED ACTIVATED KILLER CELLS: CYTOKINES AS THE ADDITIONAL SIGNALS FOR ACTIVATION OF KILLER CELLS IN EFFECTOR PHASE TO MEDIATE SLOW LYSIS, Chou-Chik Ting, and Myrthel E. Hargrove. NCI, National Institutes of Health, Bethesda, MD 20892.

This study examines the role of cytokines in activating the killer cells at effector phase to mediate slow lysis. Previous study showed that after initial activation of splenocytes by αCD3, further culturing the cells in the absence of αCD3 resulted in the generation of activated killer cells ($CD3\text{-}AK^-$) to mediate slow lysis which required 16-20 h to complete the lytic reaction. In contrast to fast lysis which was not affected by a PKC inhibitor H-7, slow lysis was inhibited, suggesting that a PKC-dependent activation phase preceded the lytic phase in slow lysis. Further studies were performed to explore the mechanism for activating the lytic machinery of slow lysis at effector phase. First, it was found that αIL-2 or an αIL-2/αIL-4 combination inhibited slow lysis but had no effect on fast lysis. Secondly, IL-2, IL-4 or TNFα converted a noncytolytic $CD3\text{-}AK^-$ cells to mediate slow lysis, but they did not augment fast lysis. Exogenous IL-6 and INF (α, β, γ) did not affect the cytolytic activity of the killer cells. Treatment with cycloheximide for 24 h abrogated the cytolytic activities of CD3-AK cells, suggesting that a cytotoxic factor(s) was continuously synthesized, stored and was catabolized within 24 h. Our results indicated that in the effector phase of slow lysis, after activating the $CD3\text{-}AK^-$ cells by the first signal (appropriate target cells), IL-2 and or IL-4 appeared to be the second signal to initiate a cascade of events which triggered the release of other cytokines (e.g TNF). These events lead to full activation of the killer cells to convert the preformed cytotoxic factors into active form to inititate the lytic reaction and completed the lytic process.

P 262 CORRELATION BETWEEN SERUM ANTIBODIES TO MALARIAL ANTIGENS AND INDUCTION OF IL-4 RELEASE IN T-CELLS FROM HUMANS PRIMED TO P.f. MALARIA BY NATURAL INFECTION, Marita-Troye Blomberg and Peter Perlmann, Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden.

Immunity to P.falciparum malaria is complex and involves both humoral and cellular components. Thus, immunogens to be included in a subunit malaria vaccine should contain both B- and T-cell activating sites to assure anamnestic responses following reinfection as well as antibody-independent cellular immunity. The P.falciparum antigen Pf155/RESA is a candidate for a vaccine against the asexual blood stages. We have recently shown that the invariant amino acid repeat regions of this molecule, known to contain some of its immunodominant B-cell epitopes also include some of its important T-cell epitopes. More detailed functional analysis of these defined epitopes revealed that they induced expression of IL-4 mRNA, IFN-γ, proliferation and B-cell help. In individual donors the different T-cell activities were not correlated, indicating that they were displayed by different cells. However, in individual donors, expression of IL-4 mRNA correlated with the occurrence of elevated serum antibodies for the peptide used for T-cell activation, suggesting the involvement of IL-4 producing T-helper cells in the induction of Pf155/RESA specific antibodies in people who have acquired functional immunity to malaria after long term natural exposure to the parasite. Taken together, our data suggest the existence of functionally distinct CD4+ T-cells in humans similarly to what has been described in mice.

P 263 CYTOKINES INVOLVED IN THE INDUCTION OF CHRONIC GRAFT-VERSUS-HOST (GVH) DISEASE, Shelby P. Umland, Shad Razac and D. Kyle Nahrebne.
Department of Allergy and Immunology, Schering Corp., Bloomfield, NJ 07003.

The cytokines involved in the induction phase of chronic autoimmune GVH disease ($DBA \rightarrow B_6D_2F_1$) have been studied by measurement of serum Ig classes and subclasses and ELISA assay of γ-IFN, IL-4, IL-5, and IL-6 in supernatants of unstimulated GVH spleen cells. These parameters were also measured in GVH mice treated in vivo with purified anti-IL-4 or anti-γ–IFN antibodies. Production of IL-6 only was observed in cultures of GVH unstimulated spleen cells. The involvement of IL-4 is indicated by increases in serum IgE (100-300x) and IgG_1 (10-20x). In vivo anti-IL-4 treatment decreased IgE and G_1 levels, and delayed the onset of proteinuria and death. Anti-γ–IFN treatment increased IL-4, 5, and 6 but not γIFN secretion by GVH spleen cells, increased IgE and G_1 levels, and accelerated proteinuria and death. These results suggest that a T_H2-like pathway mediates chronic autoimmune GVH disease.

P 264 LYSIS OF MELANOMA CELLS BY AUTOLOGOUS CTL IS ENHANCED BY IFN-γ AND TNF-α. Elly van de Wiel-van Kemenade[1], Annemiek de Boer[1], Erik Danen[1], Peter Schrier[2], Lucie Peltenburg[2], Cornelis Melief and Carl Figdor. [1]The Netherlands Cancer Institute, Plesmanlaan 121 1066 CX Amsterdam, [2]Academic Hospital Leiden, Leiden, The Netherlands.
Human peripheral blood T lymphocytes from melanoma patients expanded in autologous mixed lymphocyte/tumor cell cultures in the presence of IL-4 develop cytotoxic activity against autologous melanoma cells. IL-4 enhanced the outgrowth of CD8 positive T cells. Culture of the autologous melanoma cells with 500 U IFN-γ and/or 500 U TNF-α per ml during 48 hours strongly increased the sensitivity for lysis by these CTL and CTL clones cultured with 100 U IL-2/ml. In contrast, the sensitivity for lysis by IL-2 activated lymphocytes (LAK cells) remained unchanged. IFN-γ and/or TNF-α increased the expression of MHC class I and MHC-class II antigens, ICAM-1 and VLA-4. Antibodies against LFA-1 or ICAM-1 could block lysis of the target cells. Furthermore, antibody blocking studies indicated that lysis of autologous melanoma cell line cells was not MHC-restricted. In addition there was no correlation between the sensitivity of melanoma cells for lysis and their level of MHC class I and MHC class II expression.
From these data we conclude that IFN-γ and TNF-α enhance the sensitivity for lysis by autologous CTL. The nature of this enhanced cytotoxic activity by these autologous CTL remains unclear. The LFA-1/ICAM-1 adhesion pathway is involved in both the autologous and allogeneic cytotoxic activity.

P 265 *IN VIVO* INTERACTIONS BETWEEN IFN-γ-PRODUCING CELLS AND TNP-SPECIFIC ANTIBODY-FORMING CELLS DURING A TI-2 IMMUNE RESPONSE. Alfons J.M. Van den Eertwegh,* Huub F.J. Savelkoul,# and Eric Claassen*. *Department of Immunology, TNO Medical Biological Laboratory, P.O. Box 45, 2280 AA, Rijswijk, The Netherlands; and #Department of Immunology and Immunohistology, Erasmus University, Rotterdam, The Netherlands.
We studied IFN-γ producing cells (IFN-γ-PC) *in situ* and *in vitro* during a thymus independent type 2 (TI-2) immune response (day 0-21). After TNP-Ficoll immunization we found a significant increase both *in vivo* (immunohistochemistry) and *in vitro* (ELISA-spot assay) of the number of IFN-γ-producing / secreting cells in spleens of mice on day 6 and 7. Double staining of cells in spleen sections during the peak of the immune response revealed that, ± 40% of the IFN-γ-PC were CD4-cells, ± 50% CD8-cells and 10% NK-cells. The IFN-γ-PC and the TNP-specific antibody-forming B cells (TNP-AFC) displayed similar kinetics. Double staining experiments revealed that IFN-γ-PC were located in the same compartment (PALS, around the terminal arterioles and red pulp) as TNP-AFC. A large proportion of IFN-γ-PC were found in juxtaposition to TNP-AFC. These results suggest that *in vivo* IFN-γ-PC, mostly T-cells, play a regulating role in the immune response to TNP-Ficoll. Moreover, our data suggest that regulation of this TI-2 immune response is mediated by direct cell-to-cell interaction between TNP-AFC and IFN-γ-PC.

P 266 INTRAARTERIAL AND ENDOVESICAL RECOMBINANT INTERLEUKIN-2 (rIL-2) DIFFERENTLY AFFECTS SYSTEMIC IMMUNE RESPONSE IN BLADDER CANCER PATIENTS (Pts), Francesca Velotti, Antonella Stoppacciaro*, Andrea Tubaro°, Anna Giuffrida, Stefania Morrone, Pia C. Bossola°, Luigi Ruco*,Lucio Miano° and Angela Santoni, Depts. of Exp. Med. and *Biopathol., University "La Sapienza",00161 Rome; °Clin. of Urol., Dept. of Surgery, L'Aquila University, 67100 L'Aquila, Italy.

We report the effects on local recurrences and immunological response induced by rIL-2 administered through intraarterial or endovesical route in Pts with low stage (Ta-T1, No,Mo) bladder cancer. The following two phase IB trials have been performed: 1) 12 Pts were treated with intrahypogastric artery infusion of escalating doses of rIL-2 ($18X10^3$ to $18X10^6$ IU/m^2/d) for two 5-day courses, prior to surgery. 2) 12 Pts were treated with10 daily endovesical instillation of escalating doses of rIL-2 ($6X10^6$-$18X10^6$ IU/d), before surgery and with maintenance cycles ($6X10^6$ IU/d) every 4 months. Cystoscopy was performed bi-monthly. 2/12 and 0/12 Pts developed local recurrences after intraarterial and endovesical treatment respectively, during a median follow up of 6.5 months. Peripheral blood immunological analysis, performed in Pts undergoing intraarterial IL-2 treatment, shows an increase of CD14+, CD16+CD3+ cells and DR, Tfr, surface-associated and soluble CD25 antigens mainly on T lymphocytes; no changes of the above mentioned parameters have been observed during endovesical IL-2 treatment. These data indicate that the observed therapeutic effects appear to be independent from the modulation of the described immunological parameters. Studies are ongoing to analyze affection of local immune response by both IL-2 treatments.

P 267 TRANSDUCTION OF IL-2 BY INFECTION OF MURINE TUMOR CELLS WITH RECOMBINANT RETROVIRUS ACTIVATES BOTH NK AND T-CELL RESISTANCE,
MJW Visseren, M Koot, HJ Schoenmakers, L Gravestein, JAC Voorthuis, CJM Melief. The Netherlands Cancer Institute, Plesmanlaan 121 1066 CX Amsterdam, The Netherlands.

The murine IL-2 gene was productively introduced into C57BL/6 (B6) murine EL-4 thymoma cells by a retroviral vector (pLJ). Inoculation of graded doses of live IL-2 producing tumor cells (EL-4pLJ-IL-2) and non-IL-2 producing tumor cells (EL-4plJ) in syngeneic recipients revealed a 1000-fold difference in cell dose required for tumor take. This difference is largely mediated by NK resistance. When B6 mice were treated with anti-asialo GM1 antibodies or anti-NK 1.1 antibodies, the day before s.c. injection of the tumor cells, the difference in tumor take rates between the IL-2 producing tumor cells and non-IL-2 producing tumor cells was dramatically reduced. By this protocol no T-cell memory was detectable.

However, i.p. immunization with large numbers of irradiated IL-2 producing EL-4 tumor cells once a week during 3 weeks resulted in strongly reduced tumor take rates of subsequently implanted live EL-4 cells, indicating T-cell memory.

It is now investigated whether vaccination with large numbers of irradiated IL-2 producing EL-4 tumor cells can induce regression of already established non-IL-2 producing EL-4 tumor cells.

P 268 INVOLVEMENT OF LFA-1 IN THE REGULATION OF IL-2 MEDIATED CELL-CELL AGGREGATION BY IL-4,
Florry A. Vyth-Dreese, Trees A.M. Dellemijn, Yvette van Kooyk, Cornelis J.M. Melief, Carl G. Figdor. Division of Immunology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.

One of the features of IL-2 mediated T-cell activation is its induction of cell-cell aggregation. During investigation of the involvement of adhesion molecules in this cell-cell interaction, it was noted that IL-4 (which is known to inhibit several IL-2-mediated functions i.e. cell proliferation, Ig synthesis, IL-2 receptor expression, generation of lymphokine activated killer cell activity), reduced cell-cell aggregation early during IL-2 culture of resting PBL. This reduced cellular adhesion correlated with a decreased expression of the activated form of LFA-1 recognized by mAb NKI-L16. In addition IL-4 reduced the IL-2 enhanced expression of CD2, CD54 (the LFA-1 ligand ICAM-1) and the VLA-5 antigen. No effect of IL-4 was observed on lymphocytes that were preactivated by IL-2. Such cells show elevated expression of the NKI-L16 epitope and are not blocked in their IL-2 induced functions by IL-4. Since LFA-1 was found to be specifically required for optimal IL-2 induced proliferation of resting PBL, our data may provide a clue for the IL-4 mediated inhibition of IL-2 responsiveness.

P 269 RAPAMYCIN RESTORES CONCANAVALIN A RESPONSIVENESS AND INTERLEUKIN-2 PRODUCTION BY MRL/lpr SPLENOCYTES, L.M. Warner, T.A. Cummons, L.M.Adams, S.N. Sehgal, and J.Y. Chang, Wyeth-Ayerst Research, Experimental Therapeutics, CN 8000, Princeton, NJ 08543.

The MRL/MpJ/lpr/lpr (MRL/l) mouse is a model for systemic lupus erythematosus (SLE), an autoimmune disease in man. The autosomal recessive allele, lpr (lymphoproliferation) is associated with many pathological changes including development of autoantibodies, glomerulonephritis, splenomegaly and severe lymphadenopathy of double negative ($L3T4^-$, $Lyt\text{-}2^-$) lymphocytes (Kotzin et al., 1988), which have lost the normal T cell responsiveness to concanavalin A (Con A) and the ability to produce Interleukin-2 (IL-2) (Cameron et al., 1986). As the disease progresses, mitogenic responsiveness and IL-2 production continue to decrease.

Rapamycin (RAPA), a potent immunosuppressive macrolide natural product, is 10 fold more inhibitory to murine thymocyte proliferation than Cyclosporin A (CsA) (Adams et al., FASEB 1990). Since activation and proliferation of T cells is thought to be important in the progression of SLE, we investigated the effect of RAPA and CsA in 6 week old, female MRL/l mice. Twelve mice per group, were dosed for 2 months with either RAPA or CsA at 12.5 mg/kg or 25 mg/kg 3x/week p.o. Controls consisted of naive and vehicle dosed MRL/l groups, and an MRL wild type naive group. When the animals were sacrificed, spleens were weighed and lymph node diameters were measured. Con A stimulated splenocyte proliferation ex vivo was measured by ^3H Thymidine uptake, and IL-2 production ex vivo was measured by an CTLL-2 bioassay. RAPA at 12.5 mg/kg and 25 mg/kg significantly reduced spleen (32%, 50%) and lymph node (inguinal: 46%, 44%; axillary: 61%, 59%) size compared to MRL/l vehicle control, and restored Con A responsiveness (43%, 60%) and IL-2 production (66%, 61%). Unlike CsA, RAPA maintained immunocompetence at levels approaching the wild type MRL strain, and also significantly prolonged survival.

RAPA could be acting in this model to inhibit the lymphadenopathy of the double negative phenotype, therefore maintaining the lymphocytes' ability to produce IL-2. Restoration of IL-2 production may in part contribute to the delayed onset of other lupus pathologies, by facilitating lymphocyte regulation and maintaining immunocompetence.

P 270 A NOVEL LOW MOLECULAR WEIGHT CYTOKINE IMPORTANT IN AUTOCRINE HUMAN B CELL GROWTH, Richard J. Warrington and W. John Rutherford, Departments of Medicine and Immunology, University of Manitoba, Winnipeg, Manitoba R3A 1M4

A cloned human B cell line JR2.B10 was generated from an IgG producing EBV-transformed B cell line. The B10 cell line differs from its parent phenotypically, being a small round cell that does not form aggregates, lacks expressed CD23 and CD23 message and does not produce IgG. It lacks EBV genome as detected by a Bam HI G fragment used as probe. The cell line does express CD71 and 4F2 and grows specifically in response to polyclonal and recombinant T cell-derived LMW-BCGF (latter kindly provided by Dr. S. Sharma, Brown University). It also grows in the presence of polyclonally activated B cell-derived growth factors. It does not respond to any other known recombinant cytokine, alone or in combination. But JR2.B10 does proliferate in response to a soluble factor produced by an autocrine mechanism. This cytokine can be obtained from the B10 cells by washing at 4°C, and under low protein conditions has an apparent M.W. <10 kd (by ultrafiltration). The growth factor is sensitive to heat at 56°C for 1/2 hr, is destroyed by pH <2 >11 and is completely destroyed by neuraminidase. It is resistant to ribonuclease but sensitive to protease. This autocrine growth factor from the B10 cell line appears therefore to be a low moleular weight glycoprotein that may be shed from the cell membrane, perhaps in a similar way to CD23, which also has B cell growth factor activity, but which is not expressed by B10 cells.

P 271 HIGH AFFINITY IL-2 RECEPTOR-EXPRESSING HUMAN LYMPHOCYTES ARE SELECTIVELY ELIMINATED BY DAB_{486}IL-2 (IL-2 TOXIN). C. A. Waters*, C. E. Snider*, K. Itoh,* L. R. Poisson*, J. C. Nichols*, T. B. Strom* and J. R. Murphy.* (Intr. by J. Bartholomew) Seragen, Inc., Hopkinton, MA, M. D. Anderson Hospital, Houston, TX, Beth Israel Hospital, Boston, MA and University Hospital, Boston, MA.

DAB_{486}IL-2 is a diphtheria toxin-related IL-2 gene fusion protein constructed by replacing the 50 amino acid C-terminal receptor binding domain of diphtheria toxin with human IL-2 sequences. When added to either 72 h PHA activated human PBMC or malignant human T cell lines which constitutively express high affinity IL-2 receptors (IL-2R), [^{125}I] DAB_{486}IL-2 binds to the IL-2R on these cells with a K_d of 748 pM and is rapidly internalized with a $t_{1/2}$ of 11-14 min. By comparison, human rIL-2 binds with a K_d of 28 pM and is internalized with a $t_{1/2}$ of 5 min. Endocytosis of bound ligand under conditions in which half the available IL-2 binding sites are saturated results in half-maximal inhibition of protein synthesis in approximately 4.6 h for both normal and tumor cells. Continuous exposure to DAB_{486}IL-2 is not obligatory, however. A cell contact period of approximately 30 minutes is sufficient for subnanomolar doses to half-maximally intoxicate. Despite the 25-fold alteration in K_d, the IC_{50} for DAB_{486}IL-2 intoxication of activated normal PBMC and tumor cells is ~ 4×10^{-11}M, suggesting that in contrast to IL-2, only a small fraction of available IL-2 binding sites need be occupied by DAB_{486}IL-2 to achieve its biologic effect. Furthermore, action of the fusion protein is IL-2R specific and is inhibited by co-culture with rIL-2 or monoclonal antibodies recognizing either the p55 or p75 subunit of the IL-2R. However, resting PBMC with natural killer cell activity which express only the p75 chain of the IL-2R are relatively resistant to intoxication. The extent to which protein synthesis is impaired in high affinity IL-2R bearing cells correlates with inactivation of elongation factor 2, the intracellular target of diphtheria toxin. These results indicate that DAB_{486}IL-2 mediated protein synthesis inhibition occurs as a result of a classical diphtherial intoxication mechanism. Our studies underscore the potential utility of DAB_{486}IL-2 as a selective immunosuppressive agent both for IL-2R expressing hematologic malignancies as well as in transplantation and autoimmune applications.

P 272 HEMATOLOGICAL AND ANTIMETASTATIC EFFECTS OF rhIL7 IN MICE, R.H. Wiltrout, G. Damia, M. MacPhee, T.A. Gregorio, F.W. Ruscetti, C.R. Faltynek, and K.L. Komschlies, Biological Response Modifiers Program, NCI-FCRDC, Frederick, MD 21702-1201, and Sterling Drug Inc., Malvern, PA 19355 and BCDP, PRI/DynCorp, Frederick, MD 21702-1201
The administration of $\geq$ 5 μg rhIL7 twice per day for at least 5 days to C57BL/6 or BALB/c mice resulted in a >90% reduction in the number of single lineage CFU-c and multi-lineage CFU-GEMM that could be cultured from the bone marrow, but a 5-10 fold increase in the number of CFU-c and CFU-GEMM that could be cultured from the spleen. This regimen of rhIL7 administration also increased by 2-5 fold the total number of leukocytes that could be obtained from the peripheral blood, spleen, and lymph nodes, and increased the spontaneous proliferation of cells obtained from the spleen and lymph nodes in a dose-dependent manner. The leukocytosis was largely a result of an increase in the total number of early B cells. There was also a disproportionate increase in $CD8^+$ T lymphocytes that caused a significant decrease in the T4/T8 ratio. Subsequent studies demonstrated that the administration of 5-10 μg rhIL7 for 5-10 days reduced the number of pre-existing experimental B16 melanoma and Renca murine renal carcinoma metastases in the lungs by about 50%. Further, the antimetastatic effects of rhIL2 against Renca were enhanced by the co-administration of rhIL7. These results demonstrate that rhIL7 has significant hematological and antitumor effects in mice.

P 273 CYTOKINE PATTERNS IN LEPROSY LESIONS. Masahiro Yamamura and Robert L. Modlin. Division of Dermatology, UCLA School of Medicine, Los Angeles, CA 90024.
The clinical spectrum of leprosy provides a model to study immune responses to an infectious pathogen. Tuberculoid leprosy patients have few skin lesions containing rare bacilli and strong CMI responses to M. leprae, but lepromatous leprosy patients have many skin lesions containing numerous bacilli and are specifically unresponsive to M. leprae antigens. In order to investigate the cytokines which regulate these immune responses, we identified cytokine mRNA derived from biopsy specimens by PCR. mRNA coding for IL-2, IFN-gamma, and lymphotoxin were striking in tuberculoid lesions, virtually absent in lepromatous lesions. In marked contrast, IL-4 and IL-5 mRNA was detected at higher levels in lepromatous than tuberculoid lesions. Furthermore, mRNA for IL-1ß, TNFα, TGFß1 and IL-6 were more abundant in tuberculoid lesions. These results define two different patterns of cytokine production which characterize resistant versus susceptible responses to an infectious pathogen.

P 274 THE SYNERGISTIC ANTI-TUMOR ACTIVITY OF rTNF AND rIL-2 IS rTNF SPECIES SPECIFIC, Robert J. Zimmerman, Lisa Langlois, Jeffery L. Winkelhake, Stacey Gauny and Laurie Owen-Schaub. Dept. Pharmacology, Cetus Corp., Emeryville, CA 94608, and Dept. Tumor Biology, MD Anderson Cancer Center, Houston, TX 77030

Treatment of murine tumors with the combination of human rTNF and human rIL-2 results in synergistic anti-tumor activity. In addition, hrTNF must be given at the maximally tolerated dose (MTD) for 3 days prior to IL-2 to obtain the best anti-tumor efficacy. Clinical testing of hrTNF and hrIL-2 at low doses of hrTNF given at the same time as hrIL-2 has produced evidence of anti-tumor activity. Follow-up preclinical studies have now surprisingly demonstrated that the same protocols which resulted in the most synergistic activity using hrTNF and hrIL-2 do not produce the same synergy with mrTNF and hrIL-2 in the same murine tumor models. The results showed that while mrTNF and hrIL-2 treatment was significantly better than either agent alone, the combination produced equivalent anti-tumor activity at the MTD of mrTNF and at up to 10-fold below the MTD when they were given on a schedule similar to that used clinically. However, when hrTNF was tested at these lower doses on the clinical schedule, less anti-tumor activity was observed when compared to the original protocol. We hypothesize that these differences between human and murine rTNF are related to the known species specificity of TNF for activation and proliferation of T cells. Human rTNF activates murine T cells, but does not induce proliferation, whereas both effects occur in homologous systems.

Cytokine Regulation of Myelopoiesis

P 300 EFFECTS OF RECOMBINANT INTERLEUKIN-8 ON NEUTROPHIL CELL SHAPE AND F-ACTIN CONTENT. Camille N. Abboud, Maureen C. Kempski. Department of Medicine, University of Rochester School of Medicine, Rochester, NY 14642.
We and other have shown that colony-stimulating factors, tumor necrosis factor alpha and interleukin 1 affect neutrophil function and chemotaxis. In these studies we show that recombinant human interleukin-8 (IL-8), is a very rapid activator of neutrophils causing universal cell activation as determined by scanning electron microscopy and increased F-actin polymerization determined by NBD-phallacidin staining and flow cytometry. As little as 10 ng/ml of IL-8 was associated with a 1.4 fold increase in F-actin and a sustained increase in the coefficient of variation (CV) of the forward light scatter parameter. The maximal response was obtained at 500 ng/ml and resulted in a two fold increase of F-actin at 30 seconds. The role of inhibitors of protein kinase C (H7 and staurosporine), and calmodulin kinase (W13) in this system will be presented. Unlike IL-8, interleukin-1 alpha and beta had no effects on neutrophil cell shape and F-actin content.

P 301 Novel receptor tyrosine kinases expressed in human leukemia cell lines

Riitta Alitalo, Juha Partanen, Elina Eerola, Jaana Korhonen, Tomi Mäkelä and Kari Alitalo
Transplantation laboratory and Departments of Pathology and Virology, University of Helsinki, 00290 FINLAND

Receptor tyrosine kinases (RTKs) have important roles in the control of hematopoietic cell growth and differentiation. Several oncogenic mutations can convert RTKs to potent leukemia-inducing genes. We have recently cloned five novel RTKs from the K562 chronic myeloid leukemia cell line (Partanen et al., PNAS, in press). One of these genes is expressed in the HEL and KG-1 erythroleukemia and Dami megakaryoblastic leukemia cell lines as well as in a hybrid endothelial cell linebut not in other leukemia cell lines or cell lines from a variety of solid tumors. This pattern of expression is interesting, because the structure of the RTK suggests that it participates in cell-cell interactions. We also cloned two novel members of the FGFR family, named FGFR-3 and FGFR-4, expressed as a 4.4 and 3.0 kb mRNAs, respectively, in several human leukemia and solid tumor cell lines. Their expression patterns are distinct from that of *flg* and*bek*, the two previously characterized FGFRs. The finding of FGFR expression in leukemia cell lines is interesting because of the recent recognition of the regulatory role of FGFs in hematopoiesis.

P 302 MATERNAL ADMINISTRATION OF G-CSF STIMULATES FETAL RAT MYELOPOIESIS. J. Andresen*, D. Kaplan*, M. Cecchini*, T. Ulich, J. Del Castillo, and E. Medlock*. AMGEN Inc.*, Thousand Oaks, CA, and the University of California, Irvine.
Pregnant rats were treated with 50 ug/kg of recombinant human granulocyte colony stimulating factor (G-CSF) subcutaneously twice daily for 2, 4, or 6 days prior to parturition. G-CSF crossed the placenta and peak serum concentrations were detected in the fetal circulation 4 hours after administration. Fetal serum levels were approximately 1000 fold less than levels detected in the dam. Hematopoietic effects of G-CSF were assessed by histological and cytological analysis of neonatal liver, spleen, bone marrow, thymus, and blood at birth. The white blood cell count was increased 2 to 4 fold in neonates from all treatment groups. This increase was entirely due to increased numbers of circulating neutrophils. Administration of G-CSF for 4 to 6 days induced a striking myeloid hyperplasia in the neonate femoral marrow. A diffuse marrow hypercellularity and significant increase in hypersegmented neutrophils was observed. Hypersegmented neutrophils were also detected in the fetal circulation after 4 days of treatment. There was a significant increase in the number of postmitotic neutrophils in the spleens of all treatment groups. However, no change was detected in the number of splenic lymphocytes or monocytes. No effect of G-CSF was detected in the neonatal liver or thymus. These results demonstrate that maternally administered colony stimulating factors, such as G-CSF, may cross the placental barrier and alter the hematopoietic status of the developing fetus. The significant myelopoietic effects of G-CSF at low concentrations in the fetus suggest an exquisite degree of developmental sensitivity to this cytokine.

P 303 A MURINE MONOCLONAL ANTIBODY TO THE HUMAN *c-kit* PROTO-ONCOGENE PRODUCT (SCF RECEPTOR): EFFECT ON THE GROWTH OF HAEMOPOIETIC PROGENITOR CELLS *in vitro* AND EXPRESSION OF *c-kit* IN AML. Leonie K. Ashman, Department of Microbiology and Immunology, The University of Adelaide, Adelaide, South Australia 5001.

Monoclonal antibody YB5.B8 was raised against a cell surface antigen on human acute myeloid leukaemia (AML) cells. The antigen was detected on ~3% of bone marrow cells and tissue mast cells but not on other normal cell types. In recent collaborative experiments (Lerner et al, manuscript in preparation) we have demonstrated that YB5.B8 binds to the *c-kit* proto-oncogene product, a member of the receptor tyrosine kinase family for which the ligand ('Stem cell factor') was recently identified. These experiments involved cell distribution studies (immuno-fluorescence/Northern analysis) and sequential immunoprecipitation using a polyclonal antiserum to the *v-kit* protein.
MAb YB5.B8 recognises a functionally important conformational epitope on the extracellular domain of the receptor: we have used it in the purification of haemopoietic progenitor cells, and have shown that it inhibits factor-dependent colony formation *in vitro*. Expression of elevated levels of the receptor by blast cells from patients with AML at diagnosis is strongly associated ($P < .001$) with failure to achieve remission in response to standard therapy.

P 304 USE OF RECOMBINANT GROWTH FACTORS IN MURINE ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANTATION: VALUE OF COMBINATIONS, EFFECT ON GVHD AND SURVIVAL, AND TOXICITY OF INTERLEUKINS 1 AND 4, Kerry Atkinson, Christina Matias, Anne Guiffre and James C. Biggs, Department of Haematology, St. Vincent's Hospital, Sydney, Australia and Steven Gillis, Immunex Corporation, Seattle, USA
We have assessed the effects of 4 recombinant cytokines on the rate of engraftment and the severity of graft-versus-host disease (GVHD) in a murine model of allogeneic haemopoietic stem cell transplantation. The cytokines used were recombinant human (rh) G-CSF, recombinant murine (rm) GM-CSF, rh IL 1-α and rm IL 4, both alone and in combination. BALB/c mice ($H2^b$) were given 10 Gy total body irradiation followed by the intravenous injection of 10^7 bone marrow and 10^6 spleen cells from C57BL/6 donors ($H2^d$). The day 7 absolute circulating neutrophil count (ANC) was utilised as the parameter of the rate of engraftment. To date, all cytokines tested either alone or in combination, produced significantly higher ANC's compared to control (saline) injections ($0.07 \pm 0.05 \times 10^9$/L) at day 7, with the most effective combination being 100 ng G-CSF + 50 ng IL 1-α (2.4 ± 1.6, $p<0.025$) given twice daily by intraperitoneal (IP) injections. The use of 100 ng G-CSF alone twice daily led to a significantly higher ANC at day 10 (6.9 ± 2.1, $p<0.01$) when compared to control (3.5 ± 1.4), and its effect was further enhanced when used in combination with 200 ng GM-CSF (7.5 ± 2.3, $p<0.05$). GVHD morbidity and mortality was not increased by twice daily injection of 100 ng G-CSF or 200 ng GM-CSF. Indeed, survival was significantly enhanced by administration of GM-CSF ($p<0.05$) and a similar trend was seen with the use of G-CSF. In contrast, IL 1-α at doses beyond 50 ng twice daily was associated with consistent early mortality and IL 4 500 ng twice daily appeared to result in a decreased survival rate by increasing the severity of GVHD. These data show that combinations of haemopoietic growth factors are likely to be more effective than single agents alone; that neither G-CSF nor GM-CSF adversely effect the incidence or severity of GVHD; that IL 1-α has early dose limiting toxicity and that IL 4 has late toxicity probably by exacerbation of GVHD.

P 305 EX VIVO AND IN VIVO INFUSIONS OF CYTOKINES IN MURINE RECIPIENTS OF T-CELL DEPLETED BONE MARROW ALLOGRAFTS, Bruce R. Blazar, Michael B. Widmer, Daniel A. Vallera, Departments of Pediatrics and Therapeutic Radiology, University of Minnesota, Mpls., MN 55455, and Department of Immunology, Immunex Corporation, Seattle , WA 98101
Our laboratory has tested the effect of cytokines on survival, hematopoiesis, and chimerism in the context of irradiated murine recipients of T-cell depleted fully allogeneic bone marrow grafts. Over 1100 mice were transplanted for these cytokine studies. In the first strategy, a one hour preincubation at 37°C (to allow receptor saturation) of T-cell depleted allografts with varying amounts of recombinant cytokines was performed to determine the effect of donor marrow graft preactivation with cytokines. Recipients of GM-CSF (13 μg/ml) pretreated allografts had significant increases in mean donor cell percentages (44% vs. 23% in controls) 2 months post-transplantation. Neither hematopoiesis nor survival were affected. In contrast, recipients of IL-3 (40-400 units/ml) pretreated allografts had decreases in mean donor cell percentages (25% vs. 9%) and leukocyte recovery (43% of controls) on days 14 post-transplantation. Pretreatment of donor grafts with IL-1 (up to 10 μg/ml) had no effect on engraftment, although survival was significantly greater (96% vs. 80% in controls) in the IL-1 pretreated group. In the second strategy, cytokines were delivered by continuous subcutaneous infusion at a dose of 1 μ/day for 14 days. Survival was significantly increased in recipients of GM-CSF, G-CSF, or IL-1. Mean percentages of donor cells were significantly greater in recipients of IL-1 (69% vs.36%), not changed in recipients of G-CSF (70% vs. 69%) and decreased in recipients of GM-CSF (51% vs.75%). Leukocyte recovery analyzed 14 days post-BMT was 24-fold greater with IL-1, 3-fold greater with G-CSF, and not significnatly higher with GM-CSF. We conclude that donor graft preincubations and in vivo infusions of cytokines can alter survival, hematopoiesis, and chimerism post-transplantation of T-cell depleted allogeneic marrow with beneficical effects noted on one or more of these processes with GM-CSF ex vivo or GM-CSF, IL-1, or G-CSF in vivo.

P 306 CORRELATION BETWEEN PRODUCTION OF MONOCYTE CHEMOTACTIC PROTEIN (MCP/JE) AND MACROPHAGE INFILTRATION IN MURINE SARCOMA LINES.Barbara Bottazzi, Sabine Walter, Deanna Govoni, Francesco Colotta, Alberto Mantovani. Istituto M. Negri, Via Eritrea, 62, Milano, Italy.

The regulation of macrophage infiltration into tumors is complex and involves several factors. Tumor-derived chemotactic factors have been identified that can play a role in determining the levels of tumor-associated macrophages (TAM). Recently a human monocyte-specific chemoattractant (monocyte chemotactic protein, MCP) has been purified and cloned from normal and tumor cells. Here we describe our results on the characterization of two clones (5B11 and 1D3) selected from a murine fibrosarcoma cell line for the different expression of JE mRNA, the murine counterpart of MCP.The two clones had similar growth rate in vitro. 1D3 clone showed a high expression of JE mRNA while 5B11 did not express appreciable levels of JE. Moreover culture supernatants from 1D3 had high chemotactic activity on monocytes while supernatant from 5B11 is inactive. When trasplanted in vivo, tha 1D3 clone showed a sustantially greater level of infiltration by macrophages (28% TAM) than the 5B11 clone (15%) The two clones transplanted into syngeneic mice showed different growth properties in that 5B11 grew twice faster as 1D3. The difference in in vivo growth rate and macrophage infiltration between 1D3 and 5B11 were also evident upon transplantation in nude mice. This data confirm and extend previous observations from our group showing the existance of a correlation between production of chemotactic factors, expression of mRNA for the chemotactic protein and levels of TAM.

P 307 SEQUENTIAL CYTOKINE STIMULATION OF NEWBORN RAT HEMATOPOIESIS WITH IL-6 AND G-CSF: IN VIVO IL-6 ENHANCES NEWBORN MYELOPOIESIS AND THROMBOPOIESIS, Mitchell S. Cairo, J. Michael Plunkett, Steven Clark, Richard Dana, Eva Knoppel, Jeffrey Busby and Carmella van de Ven, Genetics Institute, Cambridge, MA, and Children's Hospital of Orange County, Orange, CA.

Disregulation of hematopoiesis, especially granulopoiesis, is a hallmark finding in neonatal host defense. Previously, we demonstrated the modulation of newborn granulopoiesis by prolonged (7 day) administration of RhG-CSF in the newborn rat (Cairo, et al, Blood 76:1788, 1990). Recently, Gardner et al (Blood 2150, 1990) has demonstrated that RhIL-6 has profound in vitro stimulating effect on fetal progenitor proliferation. We therefore examined the in vivo hematological effects of either IL-6, G-CSF or sequential combinations in the newborn rat. Sprague Dawley newborn rats (≤ 24 hrs) were injected intraperitoneally with either RhIL-6 5µg/kg/d (kindly provided by Steven Clark, Genetics Institute) or RhG-CSF 5µg/kg/d (Amgen) in the following combinations: IL-6 x 14 d, G-CSF x 14 d, IL-6 x 7 d and G-CSF x 7 d, IL-6 x 7 d and BSA x 7d, BSA x 7 d and G-CSF x 7 d, and BSA x 14 d. Daily CBC, platelets, and day 14 BM and L/S NSP, NPP, CFU-GM, and CFU-GM proliferative rates were assessed after aseptic surgical removal of femurs, livers and spleens. The peripheral ANC on days 10 and 14 were significantly increased in the IL-6/G gp vs. IL-6 or PBS/BSA (4728 ± 24 vs 1239 ± 465 and 1104 ± 96, p<.001, and 5733± 845 vs. 997 ± 109 and 1260 ± 500, p <.03). The day 14 platelet count was significantly increased with IL-6 or IL-6/G vs PSA/BSA (981 ± 47 and 922 ± 54 vs. 692 ± 29/mm^3)(p<.04). Additionally, IL-6/G significantly increased the BM NSP vs. IL-6 (9873 ± 882 vs. 3564 ± 159) (p<.01) and increased BM CFU-GM vs. PBS/BSA and PBS/BSA/G-CSF (85 ± 3) vs (71 ± 4 and 69 ± 2) (p<.04 and P<.01). This preliminary data suggests that IL-6 induces significant in vivo effects relative to neonatal neutrophilia and thrombocytosis and that the sequential combination of IL-6/G may be synergistic in modulating myelopoiesis and thrombopoiesis in the newborn rat.

P 308 G-CSF DRIVEN TUMOR INHIBITION AND TUMOR REGRESSION REQUIRE A DIFFERENT HOST IMMUNOLOGICAL PERFORMANCE, Mario P. Colombo, Division of Experimental Oncology D, Istituto Nazionale Tumori, 20133 Milano, Italy.

It has been shown that several cytokines can exert anti-tumor activity if delivered at the site of tumor growth, we tested whether G-CSF has any anti-tumoral activity and the role of the host immune system. A retroviral mediated G-CSF transduction in murine colon adenocarcinoma C-26 inhibits tumorigenicity of this tumor in both syngeneic BALB/c and *nu/nu* mice. Injection of C-26 transducing G-CSF into sublethally irradiated BALB/c mice (600 Rad) and *nu/nu* mice (400 Rad) gave rise to tumors that grew progressively and eventually killed *nu/nu* mice but that, after the initial outgrowth, regressed in syngeneic mice. Regression started after normalization of leukocytes counts, 30 days after irradiation of BALB/c mice. Histological and immunocytochemical examination of tumor infiltrate revealed that neutrophilic granulocytes are the major leukocyte sub-population involved. These results indicate that 1) G-CSF released at the tumor site causz an inhibition of C-26 tumor growth; 2) that neutrophilic granulocytes are responsible of such inhibition; 3) that to eradicate an already formed tumor, a cooperative involvement of granulocytes and T cells is needed.

P 309 CRYSTAL STRUCTURE OF RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR, William J. Cook, Steven E. Ealick, Mark R. Walter & Charles E. Bugg, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, AL 35294 and Paul Reichert, Gerald S. Hammond, Hung V. Le, Tattanahalli L. Nagabhushan & Paul P. Trotta, Schering Corporation, Bloomfield, NJ 07003. Crystals of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) have been grown using solutions of sodium/potassium phosphate. The protein used in this study was derived from *E. coli* and is similar to naturally-occurring human GM-CSF. The crystals are orthorhombic, space group $P2_12_12_1$; the axes are a = 45.5(1), b = 58.7(1) and c = 127.3(1) Å. Although the molecule exists as a monomer in solution, it crystallizes with two molecules in the asymmetric unit. Based on a molecular weight of 14,477 daltons as predicted from the cDNA, this corresponds to a solvent volume fraction of 59%. The crystals are stable to x-rays at room temperature for at least three days, and x-ray diffraction data for the native crystals have been collected to 2.8 Å resolution using an area detector. Crystallographic analysis of the structure of GM-CSF is in progress, and several derivatives have been identified. Current structural results will be presented.

P 310 **PERIPHERAL MOBILIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS IN CYTOKINE TREATED MICE**, David A. Crouse, Babu Rajendran, Changnian Liu, Anne Kessinger and J. Graham Sharp, Departments of Anatomy, Radiology and Medicine, University of Nebraska Medical Center, Omaha, NE 68198.

Circulating stem cells are being used increasingly for hematopoietic reconstitution of cancer patients following high dose cytoreductive therapy. Because of the normal low frequency of stem cells in the blood, mobilization following cyclophosphamide or recombinant cytokines can be employed to increase the relative frequency of circulating stem and progenitor populations. Since little is known about the biological properties of such mobilized cells, this study was conducted to determine the potential of using a mouse model to evaluate the qualitative and quantitative characteristics of circulating stem cells collected after single or combination cytokine treatment of the donor. In these pilot studies, we have used partially purified mixed cytokines prepared from the conditioned medium (CM) of either the WEHI3b or the KLN205 cell lines grown in vitro. BDF_1 mice were treated for 7 days by sc injection of 0.2 ml of the CM or carrier. At sacrifice, the CM treated mice exhibited a moderate lymphopenia as well as a significant granulocytosis, splenomegaly and thymic atrophy. Upon assay, granulocyte-macrophage progenitor (GM-CFC), high proliferative potential progenitor (HPP-CFC) or spleen colony forming (CFUs) content of the spleen (both CMs) and peripheral blood (KLN205 CM) were significantly increased in both relative (per 10^5 cells) and absolute (total number) terms. The same populations in bone marrow were relatively unchanged. The specific cytokines responsible for this mobilization are currently being evaluated. These data support the use of a mouse model to further investigate the efficiency and optimal sequencing of cytokines to mobilize stem and progenitor cell populations in intact as well as recipients exposed to prior cytotoxic chemotherapy. (Supported by NIH Grant CA 46686).

P 311 **PREVENTION AND THERAPY OF S. AUREUS INFECTIONS WITH RECOMBINANT CYTOKINES.** *M. Daley, T. Williams, P. Coyle, R. Dougherty, G. Furda, P. Hayes & P. Johnston. Immunology Group, American Cyanamid Co., Princeton, NJ 08543-0400.*

Recombinant cytokines have been investigated as potential therapeutics for a variety of diseases. Pleiotropic effects and toxicity have limited their wide acceptance as therapeutics. Targeted therapy of closed organ systems may minimize systemic toxicity and maximize therapeutic efficacy by focusing the immunomodulatory effects. Bacterial infections are common in the bovine mammary gland causing $ 2-4 X 10^9 loss to the dairy industry annually, and current therapy is by direct intramammary infusion of antibiotics which is often ineffective for S. aureus. Polymorphonuclear neutrophils (PMNs) are central in the pathophysiology of mastitis, and cytokines can precisely regulate both the quality and quantity of phagocytic cells. We evaluated the effects of recombinant bovine interleukin-1_b, interleukin-2 and granulocyte/macrophage colony stimulating factor (r-BoIL-1, r-BoIL-2 & r-BoGM-CSF, IMMUNEX, Corp.) on milk PMNs after intramammry infusion. Cytokine infusion protected glands (20-100%) from subsequent challenge with S. aureus. Treatment with r-BoGM-CSF had no effect on the numbers of PMNs in the mammary gland, but increased the percentage of PMNs which ingested fluorescent beads by 2-3 fold. Treatment with r-BoIL-2 increased the number of PMNs in the gland, as well as phagocytic ability and inducible oxygen radical formation. A total of 52% of the quarters responded to therapy and 32% of the treated glands remained cured. In contrast, r-BoIL-1, increased the number of PMNs in the gland with no positive effect on phagocytosis. While 75% of r-BoIL-1 treated quarters responded to therapy, 22% of the treated glands remained cured. Modulation of quantity and quality of phagocytic cells by cytokines show great promise as alternatives or in combination with antibiotics as preventative and therapeutic agents which may have applications in therapeutic modalities in human infectious and genetic diseases.

P 312 IRREVERSIBLE OCCUPANCY OF THE NUCLEOTIDE BINDING SITE OF RECOMBINANT MURINE GRANULOCYTE/MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF) MODULATES ITS BIOLOGIC ACTIVITY. Michael Doukas, Ashouk Chavan, Susan Campbell, Boyd Haley. Lucille P. Markey Cancer Center, University of Kentucky Medical Center, Lexington, KY 40536.
GM-CSF is a hematopoietic cytokine important in the proliferation of myeloid progenitors. We have detected specific nucleotide binding sites on several cytokines, including GM-CSF, utilizing azido-photoaffinity analogues. Labeling by [γ-^{32}P]-8-azido diadenosine 5',5'' - P_1, P_4 - tetraphosphate (N_3AP_4A) occurs with an apparent Kd (half maximal saturation) of 0.7μM with specificity demonstrated by appropriate saturation effects and competition studies with naturally occurring nucleotides. Efficiency of photoincorporation at saturable levels of N_3AP_4A is on the order of 40-50%. Photoincorporation is optimal at 0°C in phosphate buffer, pH 4.5. Following covalent photoaffinity labeling of N_3AP_4A to its binding site on GM-CSF, we were able to purify N_3AP_4A : GM-CSF using boronate affinity chromatography. Bioactivity of the modified molecule as assessed by tritiated thymidine uptake studies with FDCP-1 cells revealed a 2-log or greater reduction of biologic activity. The physiologic role of this nucleotide binding site is unknown but the abrogation of biologic activity by covalent occupancy suggests a modulatory role for AP_4A.

P 313 **DIETHYLDITHIOCARBAMATE (DDTC) INDUCED INCREASE IN COLONY STIMULATING FACTOR (CSF) PRODUCTION IN HUMAN BONE MARROW CULTURES IS MEDIATED VIA TUMOR NECROSIS FACTOR.** Christopher J. East[1], Camille N. Abboud[2], and Richard F. Borch[1,3], Departments of Pharmacology[1], Medicine[2], and the Cancer Center[3], University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642.
DDTC has been shown to protect bone marrow progenitors from the cytotoxicity of a variety of cancer chemotherapeutic agents in a murine model. The following data suggest that this generalized protection is due to increased production of CSFs. Long-term human marrow (Dexter) cultures were established and at day 17 treated with 30 μM DDTC for 1 hr, after which DDTC was removed and replaced by complete medium. Conditioned medium was then collected 6, 12, 24, and 48 hr later and analyzed for the presence of cytokines. A time dependent increase in GM-CSF (12-fold), G-CSF (66-fold), IL-6 (3-fold), IL-1β (161-fold), and TNF-α (25-fold) resulted. The maximum increase for the factors other than TNF-α was at 24-48 hr post-treatment. TNF-α, however, peaked as early as 6 hr post-DDTC. When conditioned medium from these cultures was tested in a GM-CFC assay, a time dependent increase in colony formation resulted; this correlated very well with the increase in CSF secretion. The data are consistent with a mechanism whereby DDTC stimulates TNF-α release, which subsequently induces an elevation in the levels of the other CSFs. Studies to determine the level at which TNF-α production is being affected are ongoing. Supported by Grant CA34620.

P 314 IL-5 MODULATES EOSINOPHIL ACCUMULATION IN ALLERGIC GUINEA PIG LUNG, R.W. Egan*, A.R Gulbenkian*, X. Fernandez*, H. Jones*, W. Kreutner*, T. Kung*, F. Payvandi†, L. Sullivan†, J.A. Zurcher* and A.S. Watnick*, Departments of Allergy* and Cell Biology†, Schering-Plough Research, Bloomfield, NJ 07003
During antigen-induced anaphylaxis, eosinophils accumulate in the lungs of guinea pigs, in similar fashion to the intense eosinophilia observed during human pulmonary inflammation. It has also been reported that IL-5 is necessary for eosinophil maturation. Using a monoclonal antibody to IL-5 secreted by hybridoma TRFK-5 cells, we have examined whether IL-5 is necessary for eosinophil accumulation in lung tissue and in BAL fluid during allergic responses to ovalbumin (OVA) in guinea pigs. Eosinophil levels were measured 24 hours after OVA challenge when the serum anti-IL-5 level was detectable. When the anti-IL-5 was injected i.v. two hours before OVA challenge, it caused dose-dependent inhibition of eosinophil accumulation at total doses as low as 10 μg. Neither denatured nor isotype control antibodies attenuated eosinophil infiltration. The anti-IL-5 was also delivered by entrapping viable TRFK-5 cells in alginate, injecting the gel into the peritoneal cavity and releasing antibody in situ over a 4 day period. This method also blocked eosinophil accumulation at antibody serum levels of 3 μg/ml. IL-5 is, therefore, necessary for eosinophil infiltration into the lungs of allergic guinea pigs, suggesting that anti-IL-5 agents could be useful for treating pulmonary inflammation.

P 315 EXPRESSION OF THE HEMATOPOIETIC CELL KINASE (*HCK*) GENE IN THE MYELOID CELL LINE 32DCL3 BLOCKS GRANULOCYTIC DIFFERENTIATION IN RESPONSE TO G-CSF, B. Keith English and Ryne W. Brown, Department of Pediatrics, University of Tennessee Memphis Health Science Center, Memphis, TN 38103.

The *hck* gene, a member of the *src* family of tyrosine kinases, is expressed in human cells of the monocyte/macrophage and granulocyte lineages, and its expression appears to increase with differentiation in human cell lines that serve as models of differentiation (e.g. U-937, HL-60). 32DCL3 is an early murine myeloid cell line that depends on IL-3 for growth but will differentiate into granulocytes when incubated in IL-3 free media with G-CSF. By Northern blotting, murine *hck* transcripts could not be detected in 32DCL3 cells, even after differentiation to granulocytes in G-CSF. We used a retroviral vector to express wild type or "activated" (Tyr 501-Phe) *hck* cDNAs in 32DCL3 cells. 32DCL3 cells containing the *hck* constructs were morphologically and phenotypically similar to parental cells, and continued to require IL-3 for growth. However, these cells remained viable in the absence of IL-3 for much longer than parental cells, and did not differentiate into granulocytes when stimulated with G-CSF. In addition, unlike parental cells, the cell lines expressing hck did not up-regulate expression of the myeloperoxidase gene after stimulation with G-CSF. These data demonstrate that expression of the *hck* kinase in early murine myeloid cells interferes with granulocytic differentiation.

P 316 **IL-5 ACTIVITIES *IN VIVO* IN NORMAL AND T-CELL DEFICIENT MICE**, Fattah D.I., Wright J., O'Malley R., Page K. & Champion B.R., Immunoregulation Group, Biochemistry Department, Glaxo Group Research Ltd., Greenford, Middlesex, UB6 0HE, UK.

BALB/c mice infected with the helminthic parasite *Mesocestoid corti* develop a marked eosinophilia, detectable in the blood, bone marrow and peritoneal cavity (site of infection). Injection of the IL-5-specific monoclonal antibody TRFK5 at the time of infection completely blocked the development of eosinophilia. Furthermore, TRFK5 was also able to completely reverse an established eosinophilia when injected 2 weeks after parasite infection. Thus, although other cytokines can induce eosinophil differentiation *in vitro*, the *in vivo* response induced by parasite infection is completely dependent on IL-5 for both its induction and maintenance. Parallel studies have shown that *M. corti* infection also leads to eosinophilia in athymic BALB/c mice, which lack $\alpha\beta$ T cell receptor-bearing T cells. This response was also completely inhibitable by TRFK5, implying that either $\gamma\delta$ T cells or non-T cells are also able to make IL-5 in response to parasite infection. The cellular source of IL-5 in athymic mice is currently under investigation.

Direct injection of either IL-5 or IL-2 (both recombinant human proteins) was also able to elicit a prominent eosinophil response, with IL-2 being 5-10-fold more potent than IL-5. Both responses were completely blockable by TRFK5. The effect of IL-2 is therefore indirect, acting via the stimulation of IL-5 production. Serum immunoglobulin levels were also affected by cytokine administration. IL-5 induced a marked 10-20-fold increase in IgG_1, modest 2-3-fold increases in IgA but no effect on IgG_{2a} levels. In contrast, IL-2 injections (at similar concentrations) had no effect on IgA or IgG_{2a}, and only slightly increased the levels of IgG_1.

Thus, our results indicate that, like its *in vitro* effects, IL-5 stimulates both B cell and eosinophil differentiation *in vivo*.

P 317 **RETROVIRAL MEDIATED G-CSF EXPRESSION INHIBITS TUMORIGENICITY OF A MURINE ADENOCARCINOMA *IN VIVO*.** Giuliana Ferrari, Antonella Stoppacciaro*, Fulvio Mavilio and Mario Colombo^. Istituto Scientifico H.S. Raffaele, Milano, Italy; *Dipartimento di Biopatologia Umana, Sezione di Immunopatologia, Università di Roma, Roma, Italy; ^ Divisione di Oncologia Sperimentale D, Istituto Nazionale Tumori, Milano, Italy.

Growing evidence indicates that several cytokines (IL-2, IL-4, IFN-γ) may have antitumor activity, most effectively if they are delivered at the site of tumor growth. We focused our attention on the possible antitumor activity of G-CSF, which is known to act specifically by stimulating production of PMN neutrophilic granulocytes and by enhancing their migration to the cytokine production site. We used a retroviral vector to express human G-CSF in a poorly immunogenic murine colon adenocarcinoma (C26), which was then injected in both syngeneic and athymic mice to test the effect of G-CSF synthesis at the site of tumor-host interaction. C26 cells transduced with the huG-CSF were unable to develop tumors in 100% of injected mice, although huG-CSF was expressed and produced at low level, as estimated by Northern blot and ELISA assay. These results indicate that G-CSF can exert anti-tumor effect at physiological doses. Implication of G-CSF as mediator of tumor inhibition was proven by reversing the non-tumorigenic phenotype of G-CSF-producing cells with anti-huG-CSF mAb. Tumors were formed by injecting a mixture of infected and uninfected C-26 cells, although critical delay in tumor formation occurred when infected cells were ten times more represented in the mixture. Hystological examination of tissue surrounding the site of injection showed infiltration of PMN neutrophilic granulocytes whose number correlated with that of G-CSF-producing C-26 cells in the injected mixture. These results indicate that G-CSF may have a potent antitumor activity when released, even at low doses, at the tumor site. The antitumor effect is mediated by recruitment and targeting of neutrophilic granulocytes to G-CSF-releasing cells.

P 318 THE CULTURE OF HUMAN MONOCYTES IN GMCSF RESULTS IN INCREASED INTERFERON-gamma (IFNg) RECEPTORS (IFNgR) WITH DECREASED RESPONSIVENESS, D. S. Finbloom, A. Larner, Y. Nakagawa, D. L. Hoover*, Food and Drug Administration, Bethesda, MD 20892, and *Walter Reed Army Institute of Research, Washington, DC 20307
The monocyte (Mo) plays a central role not only in immune responsiveness (e.g. antigen presentation) but also in the initiation and promulgation of chronic inflammation through the elaboration of IL-1, TNF, and IL-6 and other inflammatory mediators (e.g. PGE_2) most of which are influenced by IFNg. Since GMCSF is probably present at inflammatory foci and has important effects on Mo, GMCSF has the potential to alter Mo responsiveness. We studied this possibility by culturing Mo purified (>95%) by elutriation in DMEM supplemented with 10% human serum with or without 40 U/ml GMCSF for 6 days. After 6 days in culture, GMCSF treated cells were round and generally non-adherent whereas cells in serum only were mostly adherent and spread out. Mo cultured both ways bound substantially more molecules of Bolton-Hunter labelled ^{125}I-IFNg (6-8 fold over fresh monocytes). Although the binding of IFNg to the cultured Mo was blocked by anti-IFNgR, immuno-precipitation of ^{35}S-methionine labelled cultured Mo revealed the presence of a band at 62kD not seen with fresh monocytes. In addition, when cultured Mo were exposed to IFNg and the expression of IP10 RNA (a member of the PF4/IL-8 gene family) was monitored, marked decreased responsiveness occurred only in the cells cultured with GMCSF. These cells also had a blunted response to IFNg as measured by enhanced FcRI (IgG) receptor expression. Therefore, culture in GMCSF appears to have resulted in the dissociation between increased IFNgR expression and IFNg responsiveness.

P 319 RADIATION PROTECTION OF HUMAN AND MURINE MYELOID HEMATOPOIETIC PROGENITOR STEM CELLS *IN VITRO* WITH BASIC FIBROBLASTIC GROWTH FACTOR. Vincent S. Gallicchio, Nedda K. Hughes, Ben C. Hulette, Lynette N. Hoblett, Hematology/Oncology Division, Departments of Clinical Sciences, Medicine, and Microbiology and Immunology, Lucille P. Markey Cancer Center, University of Kentucky Medical Center, Lexington, KY 40536
We have previously demonstrated that basic fibroblastic growth factor (B-FGF), a hormone-like protein which belongs to a class of heparin binding growth factors, which is synthesized and released to circulate in the blood, is an effective *in vitro* hematopoietic modulatory agent for CFU-S, CFU-GM, CFU-Meg, and BFU-E. B-FGF can be recognized by target cells through specific high-affinity plasma membrane receptors. We report here further studies that demonstrate B-FGF is also an effective radioprotective agent *in vitro* for myeloid progenitor stem cells, CFU-GM. Normal human and murine bone marrow cells were prepared as usual following harvest. Single-cell marrow suspensions were plated in radiation survival studies (0.5 - 5 Gy) in the presence of escalating doses of recombinant B-FGF (0.1-100 ng/ml) and recombinant GM-CSF (optimal dose, 25 units/ml). Control cultures consisted of plates containing only GM-CSF. At concentrations > 1 ng/ml, B-FGF induced enhanced radiation survival compared to controls (e.g., at 1 Gy, control 50% survival, compared in the presence of B-FGF, 68% survival). In the presence of protamine sulfate (100 µg/ml), a receptor antagonist for B-FGF, no radiation protection was observed. These results demonstrate that B-FGF influences hematopoietic stem cells and their responsiveness to agents such as radiation, and therefore should be included in the family of CSF molecules that are effective modulators of hematopoiesis.

P 320 REGULATION OF M-CSF RECEPTOR EXPRESSION DURING MYELOID LINEAGE RESTRICTION. Brian C. Gliniak, and Larry R. Rohrschneider, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Development of specialized myeloid cells is mediated by the actions of a hierarchy of hematopoietic growth factors and the coordinated expression of their receptors. To examine the regulation of M-CSF receptor (c-*fms* proto-oncogene) expression during myeloid lineage restriction, we have isolated a murine myeloid precursor cell line (FDC-P1/MAC) that simultaneously expresses receptors for multi-CSF, GM-CSF, and M-CSF. FDC-P1/MAC cells express high levels of c-*fms* mRNA and protein when grown in M-CSF, whereas growth in multi-CSF or GM-CSF caused a dramatic reduction in both c-*fms* glycoprotein and mRNA. Nuclear run-off assays demonstrated that c-*fms* transcription was not growth factor dependent and the regulation occurred posttranscriptionally. This regulation occurs through the selective destabilization of c-*fms* mRNA in GM-CSF stimulated cells. Factor switching experiments have shown that both GM-CSF and multi-CSF act dominantly to suppress c-*fms* expression and that the extent of suppression is determined by the concentration of the factor present. Removal of these factors is sufficient for the re-expression of c-*fms* and this can occur in the absence of new protein synthesis. Therefore, the protein factor(s) governing the stability of the c-*fms* mRNA are apparently modified posttranslationally by GM-CSF and multi-CSF. *In vitro* agar assays of bone marrow cells grown in the presence of GM-CSF and M-CSF, individually and in combination, support the concept that GM-CSF can act dominantly to prevent monocyte/macrophage development. These results suggest that GM-CSF and multi-CSF can suppress development along the monocyte/macrophage lineage through the trans-modulation of c-*fms* expression and offer a simple stochastic mechanism governing myeloid lineage restriction.

P 321 FURTHER CHARACTERISATION OF AN INHIBITOR OF MYELOPOIESIS (GM-CFC) ASSOCIATED WITH IDIOPATHIC APLASTIC ANEMIA.

Mona Hansson, Gunilla Kling and Pavel Pisa, Dept of Immunology, Karolinska Institutet, Box 60 400, 104 01 Stockholm, Sweden.

Based on both clinical and experimental observations, an immuno-mediated suppression of hematopoiesis has been suggested as the ethiological mechanism of idiopathic aplastic anemia (AA). Increased production of the cytokines IFN-τ, TNF-α and other not yet identified cytokines with suppressive activity on hematopoietic progenitor cells in vitro have been reported, but their contribution to the clinical observations is still a matter of debate. We describe here a Colony Inhibitory Activity (CIA) produced by PBMC from AA patients, with inhibitory activity on healthy human and murine in vitro myelopoiesis as studied in a colony forming assay (GM-CFC). This CIA is rapidly produced and detected in the supernatant after stimulation of PBMC with PHA, IL-2 or anti CD3 moab, but not with IL-1 or IFN-τ. It has a strong suppressive effect ($> 50\%$) down to dilutions corresponding to 0.1 % supernatant and its effect is not associated with the presence of either TNF-α, IFN-τ, IL-2 or prostaglandins. The biologically active CIA is of low molecular weight (8-10kD), inactivated by trypsin and stable at 56°C. We suggest that CIA is a novel physiological regulator of myelopoiesis which is being over-produced by the PBMC of idiopathic aplastic anemia patients.

P 322 EVOLUTIONARY PATTERN OF HEMATOPOIETIC GROWTH FACTORS RECAPITULATES THE HIERARCHY OF HEMATOPOIESIS--MOLECULAR BIOGENETIC LAW, Fuchu He, Inst of Radiat Med, 27 Taiping Road, Beijing, CHINA

The amino acid homologies between mouse and human for multi-CSF(IL-3), GM-CSF, G-CSF, M-CSF and EPO are 29%, 58%, 72.6%, 69.5% and 78.8%, respectively. The variation percentage of IL-3(71%) is the highest, GM-CSF's(42%) median, G-(27.4%), M-CSF(30.5%) and EPO's(21.2%) the lowest. The simple interpretation for the various evolution rates is that IL-3 appeared first on the evolution tree, GM-CSF second, G-, M-CSF and EPO last. This order just matches the order of these factors in the hierarchy of hematopoiesis, i.e., IL-3 acts on pluripotent stem cells, GM-CSF on immature non-lineage specific progenitor cells, and G-, M-CSF and EPO on more mature lineage-committed progenitor cells. This consistence appears to suggest that Haeckel's Biogenetic Law is correct not only for creation's morphology but also for molecular structure of its development-related factors.

P 323 INTERLEUKIN-1α (IL-1α) AND MYELOPOIESIS IN VIVO: MODULATION OF COLONY STIMULATING FACTOR RECEPTOR EXPRESSION ON BONE MARROW CELLS AFTER IL-1α IN VIVO. [1]K. Hestdal, [2]S.E.W. Jacobsen, [3]C.M. Dubois, [4]F.W.Ruscetti, [5]J.J. Oppenheim, [6]D.L. Longo, and [7]J.R. Keller, [1,7]Biological Carcinogenesis Development Program, PRI/DynCorp, [2,3,4,5]Laboratory of Molecular Immuno-regulation., [6]BRMP, NCI-FCRDC, Frederick, MD, 21702.

The role of IL-1α in hematopoietic cell growth is not fully understood. IL-1α alone does not promote growth of hematopoietic cells in vitro, while it enhances the proliferative response to other cytokines. Further, IL-1α in vivo protects and promotes myeloid recovery in mice after either lethal irradiation or chemotherapy. This study was initiated to better understand the mechanism of action of IL-1α in vivo. Bone marrow cells were harvested from mice at different time points after IL-1α administration and examined for specific binding of radioiodinated GM-CSF, G-CSF and IL-3. The specific binding of both ^{125}I-GM-CSF and ^{125}I-IL-3 were enhanced (80-100%) after IL-1α administration, maximum effect observed 24 hours after the injection. In contrast ^{125}I-G-CSF binding was decreased. The binding of ^{125}I-GM-CSF to an enriched progenitor population, Lin$^-$ cells, was examined. In vivo IL-1α administration enhanced the GM-CSF binding by 3-4 fold on this cell population, indicating that this enhanced binding was not solely due to an increased differentiation of mature myeloid cells. The in vitro proliferation of Lin$^-$ cells to both GM-CSF and IL-3 was increased both in ^{3}H-thymidine incorporation and colony formation assays after IL-1α administration. Thus, IL-1α may promote myelopoiesis and myeloid cell recovery following chemotherapy or irradiation by increasing the GM-CSF and IL-3 binding ability on hematopoietic progenitor cells.

P 324 ANALYSIS OF THE EFFECTS OF IL-6 AND IL-3 ON EARLY HEMATOPOIETIC EVENTS IN HUMAN LONG TERM MARROW CULTURES, Teruhisa Otsuka, J. Dean Thacker, Connie J. Eaves, Donna E. Hogge, B.C. Cancer Research Centre; Department of Medicine, Pathology and Medical Genetics, University of British Columbia, Vancouver, Canada.
To study the ability of IL-6 and IL-3 to influence hematopoiesis in long-term marrow cultures (LTC), a system which mimics many features of the marrow microenvironment, normal human marrow cells were co-cultivated with feeder layers of murine marrow-derived stromal cells (M2-10B4) genetically engineered to produce human IL-6 and/or IL-3. Feeders stably producing 20 ng/ml IL-6 slightly increased the progenitor output from the LTC, but, did not change the production of mature nonadherent cells as compared to control cultures. Feeders producing 50 ng/ml IL-3 increased progenitor numbers 3 fold and the output of nonadherent cells from LTC 6 fold while those producing 15 ng/ml IL-6 and 25 ng/ml IL-3 also increased both progenitors and nonadherent cells, but to a lesser degree than that seen with IL-3 feeders alone. At the time of weekly half medium change when normal primitive adherent layer progenitors in control LTC were quiescent, such progenitors were actively cycling in the cultures with IL-6, IL-3 and combination IL-3 and IL-6 producing M2-10B4 feeders. The combined presence of IL-6 and IL-3 producing feeders but not either factor alone was able to enhance more than two-fold the maintenance and early differentiation of LTC initiating cells, the most primitive hemopoietic cell detectable in this system. Thus, the provision of a continuous source of IL-6 or IL-3 to hemopoietic cells in LTC will enhance later events in the hematopoietic hierarchy but a combination of the two factors is required to stimulate very early multipotent progenitors.

P 325 TRANSFORMING GROWTH FACTOR-ß(TGF-ß) IS A DIRECT BIDIRECTIONAL MODULATOR OF HEMATOPOIETIC PROGENITORS IN THE PRESENCE OF GRANULOCYTE/MACROPHAGE-COLONY STIMULATING FACTOR. [1]J.R. Keller, [2]K.T. Sill, [a]nd [3]F.W. Ruscetti. [1,2,4]BCDP-PRI/DynCorp, [3L]MI-BRMP, NCI-FCRF, Frederick, MD. We previously demonstrated that TGF-ß is a direct negative regulator of hematopoietic progenitor cell growth. Also, we have shown that TGF-ß can promote the growth of large granulocyte(G) bone marrow colonies in response to GM-CSF -tentatively designated burst forming unit-G (BFU-G). To determine whether this was a direct effect and the progenitors involved, bone marrow progenitors were purified to assay in single cell Terasaki experiments. B and T cells, macrophages and granulocytes were removed from whole bone marrow by positive selection with antibodies and magnetic beads which resulted in an enrichment of progenitors termed lineage negative (Lin-) representing 1% of the starting marrow. In the presence of GM-CSF, 1 in 23 isolated Lin- cells proliferated in Terasaki wells and increased to 1 in 13 cells with the addition of TGF-ß. Further purification of Lin- cells into Thy-1+(immature) and Thy-1-(less mature) finds that 1 in 18 Lin-Thy-1- cells proliferate in response to GM-CSF and is increased to 1 in 7 with the addition of TGF-ß. In contrast, the proliferation of Lin-Thy-1+ cells (5-10% of Lin-) decreased from 1 in 8 cells in the presence of GM-CSF to 1 in 16 cells in the presence of TGF-ß. Thus, the expression of Thy-1 antigen on lin- cells enables one to predict effects of TGF-ß on GM-CSF responsive progenitors.

P 326 THE REGULATION OF IL-8 SECRETION FROM HUMAN ENDOTHELIAL CELLS AND ITS EFFECT ON THE EXPRESSION OF ADHESION MOLECULES ON HUMAN NEUTROPHILS. Jin Kim, Merlyn Alphonso, Gregory Bennett, Mark Hehse and Wai Lee T. Wong. Depts of Medicinal and Analytical Chemistry and Immunology Research and Assay Technology, Genentech, Inc. South San Francisco, CA 94080.
To understand the mechanism of IL-8 secretion from human endothelial cell, we have developed a simple, quantitative ELISA that uses two monoclonal antibodies (mAb 4.1.3 and mAb 5.12.14) specific for IL-8 with a detection limit of 0.1 ng/ml of recombinant human IL-8. Using this ELISA, we investigated the effect of various cytokines on the secretion of IL-8 from endothelial cells after stimulating cells with cytokines for 3 days. We could detect as much as 300-400 ng/ml of IL-8 after the cells (10^4 cells/microwell) were treated with IL-1, TNF and LPS for 3 days but there was no significant level of IL-8 secreted in cultures treated with IL-4, IL-6, GM-CSF or IF. The level of proliferation of endothelial cells did not have any correlation with the level of IL-8 secreted. The regulatory mechanism of IL-8 secretion is under investigation.
To further understand the role of IL-8 secreted from endothelial cells, the expression of adhesion molecules on human neutrophils was examined by flow cytometric analysis. The levels of the expression of LFA-1β and Mac-1 but not LFA-1α and p150/p95 were greatly enhanced by IL-8. The effect of IL-8 on the expression of these molecules was similar to IL-1 but less thanTNF. In conclusion endothelial cells after stimulation by inflammatory agents secrete a significant amount of IL-8 which modulates the expression of adhesion molecules on neutrophils and thereby can play an important role in trafficking of neutrophils.

P 327 APOPTOSIS IN INTERLEUKIN-3-DEPENDENT HEMOPOIETIC CELLS. REGULATORY ROLE OF CALCIUM, Abelardo López-Rivas, Gemma Rodriguez-Tarduchy and * Mary Collins Instituto de Investigaciones Biomédicas CSIC, Facultad de Medicina UAM, Madrid, Spain and * Chester Beatty Laboratories, London, England.

Interleukin-3 (IL-3) is a growth factor that supports the *in vitro* proliferation of multipotent hemopoietic cells as well as many of the progenitor cells already committed to particular lineages. *In vivo* the action of IL-3 seems to be restricted to situations of stress to the hemopoietic system and the main cellular source of IL-3 are T lymphocytes. Thus, IL-3 is probably produced at elevated levels during immune responses and may then serve to recruit additional hemopoietic cells to sustain or amplify these responses. Given the broad spectrum of target cells for IL-3, fine tuning of IL-3 availability and a mechanism for removing the excess of cells is necessary to maintain the homeostasis of the hemopoietic system. We have observed that an immortalized IL-3-dependent progenitor cell line (BAF-3) undergoes programmed cell death (apoptosis) when deprived of IL-3. This program is characterized by an early degradation of DNA into oligonucleosome-length fragments that precedes by several hours the loss of cell viability. In the absence of IL-3, DNA fragmentation and cell death can be prevented by calcium ionophores. This addition of calcium ionophore maintains cell viability while reversibly arresting the cell cycle. Apoptosis by growth factor deprivation is also a mechanism of cell elimination in bone marrow cells removed from the stromal micro-environment, as DNA fragmentation and cell death was shown to take place in primary cultures of IL-3-responsive bone marrow cells after IL-3 removal.

P 328 STUDIES ON THE RECOVERY OF MYELOPOIESIS IN 5- FLUROURACIL TREATED MICE. Judy Lucas,Jeffrey Scott, Carmen Raventos-Suarez, Roseann Petersen, Richard Hernandez and James J. Gibbons, Jr.

We have studied the recovery of circulating neutrophils and neutrophil bone marrow precursors (CFU-GM) in mice after a single injection of 5-Flurouracil (5-FU). Neutrophils reached a nadir 4-5 days after 5 FU and remained suppressed through day 10-11. In bone marrow total CFU-GM were depressed through day 6 and returned toward control levels by day 8-9. During the course of recovery of CFU's and neutrophils we followed the levels of serum IL-3, GMCSF and IL-6 using cytokines dependent cell lines 32Dcl5, FDCP-1 and 7TD1 respectively. Serum IL-3 and GMCSF were undetectable throughout. Serum IL-6 began to rise gradually on days 3-5 and then sharply increased on day 7 after 5 FU. The sharp increase in serum IL-6 on day 7 was accompanied by an increase in serum colony stimulating activity (CSA) as measured by colony forming assay of bone marrow cells in soft agar. The increase of serum IL-6 and CSA correlated with the sharp increase of CFU-GM in the bone marrow. The proliferative capacity of the bone marrow as measured by the % of cells in S phase of the cell cycle was at its lowest on day 2 post 5 FU and began to rise steadily on day 3 concomitant with the increase in serum IL-6. The data suggests that IL-6 may play an important role early in the recovery of myelopoiesis after 5 FU and that it may act in conjunction with circulating CSA to produce the sharp increase in CFU 's which precede neutrophil recovery.

P 329 CHARACTERIZATION OF TYROSYL-PHOSPHORYLATED PROTEINS INVOLVED IN IL-3 SIGNAL TRANSDUCTION. H. Mano, K. Mano, S. Bartholomew, J.T. Stine, O. Miura, R. Isfort, V.A. Fried and J.N. Ihle, Department of Biochemistry, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.

Interleukin-3 (IL-3) is a glycoprotein which acts on a broad range of hematopoietic cells and supports their growth and differentiation. Although little is known about the signal transduction pathway of IL-3, accumulating evidence has suggested that tyrosine-phosphorylation step plays a key role in its mechanism.

By virtue of anti-phosphotyrosine antibody (1G2 antibody; Oncogene Science), we could identify tyrosyl-phosphorylated proteins in a murine myeloid cell line (FDC-P1). A series of proteins (pp140, pp92, pp70, pp56 and pp38) were inducibly phosphorylated on tyrosine in response to IL-3 stimulation, whereas another set of proteins (pp105, pp58 and pp53) were rather constitutively phosphorylated. Using iodinated IL-3, a stable complex containing pp140 and IL-3 could be identified on glycerol gradients after the purification step by 1G2 antibody. These data suggest that pp140 is, at least, a component of IL-3 receptors.

One of the major substrates of phosphorylation is pp58. From the large scale culture of FDC-P1 cells, pp58 was purified and molecullary cloned. pp58 is a novel phosphoprotein which is ubiquitously expressed in the murine system. The predicted amino acid sequence of pp58, named STP-1 (*S*ubstrate of *T*yrosine *P*hosphorylation-1), contains a charged amino acid-rich region and a glutamine-rich region. Interestingly, the latter domain also contains QYQ motifs found at the C-terminus of the *src*-family. Further characterization of STP-1 protein will be presented.

P 330 *Abstract Withdrawn*

P 331 *Abstract Withdrawn*

P 332 THE HUMAN I-309 GENE ENCODES A MONOCYTE CHEMOTACTIC FACTOR, Michael D. Miller and Michael S. Krangel, Division of Immunology, Box 3010, Duke University Medical School, Durham, NC, 27710

The human I-309 gene is transcribed in activated T cells and is known to encode a secreted protein. This protein is a member of a family of inflammatory cytokines including the murine macrophage inflammatory proteins MIP-1α and MIP-1β. The murine homolog of I-309, called TCA-3, is known to induce an inflammatory response when injected into the footpads of mice. In order to examine the biological activities of I-309 protein, we have expressed the gene in CHO cells and purified the secreted products, a doublet of about 15 and 16 kD, to homogeneity. Amino terminal sequence analysis of the secreted material confirms that it is the product of the transfected I-309 gene. A rabbit antiserum raised against the recombinant protein immunoprecipitates an identical doublet from culture supernatants of IDP2, a human T cell line that constitutively expresses the I-309 gene. Both proteins immunoprecipitated by the antiserum are substrates for N-glycanase, and analysis by two-dimensional gel electrophoresis reveals that they carry distinct asparagine-linked oligosaccharides. Preliminary experiments using a 48-well microchemotaxis chamber demonstrate that purified recombinant I-309 protein is chemotactic for human monocytes but not for human neutrophils. In addition, monocytes respond to I-309 protein by increasing their intracellular free calcium concentration, consistent with signalling pathways known to be used by other chemotactic factors. Additional data detailing functions of I-309 in vitro and the expression of I-309 in vivo will be presented.

P 333 HEMATOPOIETIC GM-CSF'S IN SUPPORT OF DOSE INTENSIVE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (C), VP-16 (E), AND CISPLATINUM (P), James A. Neidhart, Christine Stidley, Aroop Mangalik, Julie Ferguson, Dagmar Oette, and Fred Oldham, University of New Mexico Cancer Center, Albuquerque, New Mexico 87131 and Hoechst-Roussel Pharmaceuticals, Inc, Route 202-206 North, Somerville, NJ 08876
Since 1987 we have conducted a series of trials using three cycles of (C) (5000 mg/m^2), (E) (1500 mg/m^2) and (P) (150 mg/m^2) (JCO Oct. 1990). rhG-CSF shortens duration of ANC less than 300/mm^3 from 8.5 to 5.5 days, ($p<0.007$) although duration of hospitalization was not shortened (JCO Nov. 1989). GM-CSF given CIV starting the day post CEP shortens leukopenia from 10.2 to 5.8 days. 500 μg/m^2 is the optimum dose. The duration of severe cytopenia is extended minimally with subcutaneous administration. Durations of hospitalization are shorter with the subcutaneous route for any total daily dose: 375 μg/m^2, 13.2 vs 9.8 days; 500μg/m^2, 18.7 vs 9.6 days; 750 μg/m^2 10.5 vs 9.8 days. Duration of severe thrombocytopenia with platelet counts below 10,000/mm^3 was shorter with the subcutaneous route at all dose levels: 375 μg/m^2, 7.4 vs 4.0 days; 500 μg/m^2, 6.4 vs 4.6 days; 750 μg/m^2, 6.5 vs 2.8 days. Compared to historical controls of this regimen, CM-CSF shortens duration of neutropenia ($p < 0.14$), hospitalization ($p < 0.001$), and thrombocytopenia ($p < 0.05$).

P 334 ROLE OF CYTOKINES IN NATURAL AND LPS-ENHANCED RADIORESISTANCE
R. Neta*, J. J. Oppenheim‡, G. D. Ledney* and T. J. MacVittie*
*Armed Forces Radiobiology Research Institute, Bethesda, MD 20814; and ‡BRMP, National Cancer Institute, Frederick, MD 21701;
Studies of radioresistance and radioprotection provide an excellent in vivo model for dissection of the pathophysiological role of cytokines. The availability of neutralizing antibodies to cytokines has made it possible to assess the contribution of cytokines to host defense and repair processes involved in radioresistance and radioprotection. Administration of anti-IL 1 receptor antibody (35F5) or anti-TNF antibody (TN3 19.12) reduced survival of irradiated CD2F1 mice. These results demonstrate conclusively that natural levels of IL 1 and TNF contribute to radioresistance of normal mice. Furthermore, the radioprotective effect of administered IL 1 was blocked not only with anti-IL 1 receptor antibody but also with anti-TNF antibody. Similarly, the radioprotective effect of TNF was reduced with anti-IL 1 receptor antibody. These data suggest that cooperative interaction of both cytokines is necessary to achieve successful radioprotection. Finally, when LPS was used as a radioprotector, the combined administration of anti-IL 1 receptor and anti-TNF, not only blocked the radioprotection with LPS, but actually revealed LPS to have a radiosensitizing effect. This effect may be due to induction of TGFβ, since administration of this cytokine results in reduced survival of irradiated mice. Therapeutic use of selected cytokines, therefore, may be preferable to the use of immunomodulators, which can induce a battery of cytokines with synergistic as well as opposing effects.

P 335 INTERFERON-γ ABROGATES THE DIFFERENTIATION BLOCK IN v-*myc* EXPRESSING U-937 MONOBLASTS, Fredrik Öberg, Lars-Gunnar Larsson, Rein Anton* and Kenneth Nilsson, Laboratory of Tumour Biology, Department of Pathology, Uppsala University, S-751 85 Uppsala, SWEDEN. * present adress Estonian Biocenter, Tartu, Estonia, USSR.
Extensive studies suggest a role for the *myc* proto-oncogene family in the control of cell proliferation and differentiation in vertebrates. We have utilized the human monoblastic U-937 cells constitutively expressing an exogenous v-*myc* construct to study the role of *myc* during hematopoietic differentiation. The phorbol myristic acid (PMA) induced differentiation in v-*myc* expressing U-937 cells have previously been shown to be inhibited. We show that v-*myc* also inhibits differentiation associated with other inducers, utilizing signal pathways distinct from PMA, i.e. 1α,25-dihydroxy-cholecalciferol (VitD3) and retinoic acid (RA). However, the v-*myc* associated block of PMA, vitD3 and RA induced differentiation can be overcome by interferon-γ (IFN-γ) as a co-stimulatory factor. IFN-γ restores terminal differentiation as evidenced by acquisition of a macrophage phenotype and an irreversible growth arrest in the G_0/G_1 phase of the cell-cycle, but induces only limited differentiation on its own. The differentiation is accomplished without altering the expression or nuclear localization of the v-*myc* protein. Furthermore, the co-stimulatory effect of IFN-γ can be replaced by a combination of interleukin 6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF) indicating that multiple physiologic signals can overcome the differentiation block in v-*myc* expressing U-937 cells.

P 336 CELL SURFACE EXPRESSION OF ICAM-1 (CD54) AND LFA-3 (CD58), TWO ADHESION MOLECULES, IS UP REGULATED ON BONE MARROW LEUKEMIC BLASTS FOLLOWING *IN VIVO* ADMINISTRATION OF HIGH DOSES rIL-2, Daniel Olive[1], Marc Lopez[1], Didier Blaise[1], Patrice Viens[1], Anne-Marie Stoppa[1], Maud Brandely[2], Claude Mawas[1], Patrice Mannoni[1] and Dominique Maraninchi[1], [1]INSERM U.119, Cancérologie et Thérapeutique Expérimentales, Marseille, France ; [2]Société Roussel-Uclaf, Romainville, France.

High dose rIL-2 therapy can induce long term remission in patients with melanoma, renal and colon cancer, presumably as consequence of increased host immunological defenses. More recently, *in vivo* IL-2 therapy was shown to induce remission in some cases of relapsed chemotherapy resistant acute myeloid leukemia. We have investigated the phenotypic modifications of bone marrow cells obtained from 5 patients with acute myeloid leukemia in relapse receiving high dose i.v. rIL-2. We found that, in 6 out of 13 patients, IL-2 could induce, *in vivo*, an increase in the expression of CD54/ICAM-1 and to a lesser extent of CD58/LFA3 and of CD56/NKH1 in 1 out of 3 patients, on bone marrow leukemic blasts. This demonstrates that rIL-2 modifies directly or indirectly the expression of the cell surface molecules of the tumor cells themselves. Up regulation of such adhesion molecules could account for the enhancement of cell interactions between the tumor and effector cells such as T, NK and phagocytic cells as well as being indicators of differentiation signaling.

P 337 **G-CSF-PRODUCTION AND G-CSF RECEPTOR EXPRESSION IN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA,** **Torsten Pietsch, Urte Kyas, Kerstin Mempel, and Karl Welte, Dept. of Ped. Hematology/Oncology, Medical School Hannover, Hannover, FRG**

Severe congenital neutropenia (SCN) is characterized by a maturation arrest of neutrophil precursor on the level of promyelocytes. The hypotheses on the pathophysiology of SCN include a defective G-CSF production or a defect response to G-CSF. We have started a clinical trial with rhG-CSF in 30 SCN patients. All but one patient responded with an elevation of neutrophils above 1000/µl. The aim of this study was to investigate the production of G-CSF by monocytes using Western blot analysis, NFS-60 bioassay, and CFU-GM assays and to determinate receptor numbers on neutrophils of SCN patients using binding assays with ^{125}I-rhG-CSF and subsequent scatchard analysis. We could demonstrate that monocytes from SCN patients are able to produce G-CSF. We also found increased serum levels (up to 0.9 ng/ml) of biological active G-CSF before therapy and normal levels (up to 0.1 ng/ml) during therapy with rhG-CSF. The G-CSF receptor number on neutrophils from SCN patients during rhG-CSF therapy is elevated (2000-3000 rec/cell) compared to neutrophils from healthy donors (500-800 rec/cell). The binding affinity of G-CSF to its receptor is not altered in SCN patients. From this data we conclude that there is no defect in G-CSF production and G-CSF receptor expression in SCN patients. This does not exclude a defect in signal tranduction of the G-CSF receptor or a defect in a yet unknown growth factor or co-factor mandatory for neutrophil maturation.

P 338 EXPRESSION OF ABNORMAL IFNα2 RECEPTORS IN HEMATOLOGIC MALIGNANCIES, LC Platanias, G Reaman, RA Larson, HM Golomb, MO Diaz, and OR Colamonici. University of Chicago, Pritzker School of Medicine, Chicago, IL, and Childrens Hospital, Washington, DC.

We have shown the existence of 2 types of IFNα2 receptors: 1)The IFNα2 receptor expressed in normal lymphocytes, monocytes, and the interferon sensitive cell lines H-929, U-266, and Daudi. This receptor has a multichain structure and is characterized by bands with molecular weights (MWs) of 210, 130, 110, 75 and 55 kD in affinity crosslinking experiments, 2)The abnormal IFNα2 receptor expressed in the monocytic cell line U-937, which is resistant to the antiproliferative effect of IFNα2, characterized by the presence of a novel 180 kD band, loss of the 210 and 110 kD bands, and an enhanced 75 kD band. We studied the structure of IFNα2 receptors in leukemia cells obtained from patients with acute and chronic leukemias by affinity crosslinking of ^{125}I IFNα2 to its receptor. We detected the abnormal receptor complex in 4/6 acute myeloid leukemias (AML), 3/10 acute lymphoblastic leukemias (ALL), 1/2 chronic myelogenous leukemias (CML) in blast crisis, 0/6 hairy cell leukemias (HCL), 0/1 prolymphocytic leukemias (PLL) and 3/26 pediatric acute leukemias (AML/ALL). Studies with the monoclonal antibody IFNαR3 that recognizes the 130 kD protein of the receptor, revealed that the 210 kD band of the normal receptor and the 180 kD band of the abnormal receptor are associations of the 130 kD protein. The novel 180 kD band is homologous to the 210 kD band and is the result of lack of expression of the 55 kD protein in the case of the abnormal receptor. The presence of a unique abnormal IFNα2 receptor, primarily seen in acute leukemias, may be associated with loss of a regulatory mechanism on leukemic cell growth.

P 339 TRANSCRIPTION IS A MAJOR MECHANISM REGULATING THE SYNERGISTIC ACTION OF IL-6 AND IL-1 ON INTERLEUKIN-2 RECEPTOR EXPRESSION DURING MYELOID DIFFERENTIATION, Stefan Ruhl, Roger B. Cohen and Dov H. Pluznik, Division of Cytokine Biology, CBER, FDA, Bethesda, MD 20892

We have recently shown that interleukin-6 (IL-6) induces the expression of the α-chain of the interleukin-2 receptor (IL-2Rα) during the process of differentiation of murine myeloid M1 cells into mature macrophages. In the present study, we show that interleukin-1 (IL-1) enhances the IL-6-induced expression of IL-2Rα, but not that of IL-2Rβ, FcγR, and lysozyme production. Using cytofluorometry and a monoclonal antibody against IL-2Rα (AMT-13), we found that IL-1 amplifies the expression of IL-2Rα induced by an optimal dose of IL-6, while IL-1 by itself does not induce expression of IL-2Rα. By Northern analysis, we found that IL-1 strongly enhances the expression of mRNA for IL-2Rα. Nuclear run-on transcriptional analysis and Actinomycin-D chase experiments show that the enhancement in IL-2Rα mRNA after stimulation by IL-1 is mainly a result of an increase in transcription of the IL-2Rα gene and to a smaller extent of stabilization of mRNA for IL-2Rα. In addition, in gel retardation assays we show that IL-1 induces expression of the nuclear factor κB, which is known to be involved in the regulation of IL-2Rα gene transcription. Thus, our data show that transcription is the major mechanism by which IL-6 and IL-1 synergize to induce IL-2Rα gene expression and that the effect of IL-1 might be mediated by the nuclear factor κB.

P 340 REGULATION OF MHC CLASS II EXPRESSION ON HUMAN MONOCYTES BY GM-CSF AND DEXAMETHASONE, Roya Sadeghi, Catherine Hawrylowicz and Marc Feldmann, Charing Cross Sunley Research Centre, Hammersmith, London W6 8LW.

Dexamethasone is an immunosuppresive agent known to inhibit the synthesis of many immune mediators including IL-1, IL-6 and TNF. In addition the upregulation of human MHC class II molecules or HLA-DR antigen by IFNγ is inhibited in the presence of dexamethasone. Recent reports have described upregulation of MHC class II molecules by GM-CSF. In contrast to the effect of dexamethasone on IFNγ driven class II expression, we have observed that dexamethasone can synergise with GM-CSF to further enhance HLA-DR, DP and DQ antigen expression on the cell surface. This is firstly an indication that GM-CSF may be one cytokine that could overcome the immunosuppresive effects of dexamethasone. Secondly, it suggests that MHC Class II induction on monocytes by either GM-CSF or IFNγ can be differentially affected by other stimuli.

P 341 HIGH LEVEL EXPRESSION OF HUMAN LEUKEMIC INHIBITORY FACTOR (LIF) IN E. COLI AND ITS EFFECTS ON LEUEKMIC CELLS. Babru Samal, Thomas Boone, Joseph Pope, Craig Crandal, Theodore Jones, George Stearns and Sid Suggs. Amgen Inc. Amgen Center, Thousand Oaks, ca. 91320.

The gene for the human leukemic inhibitory factor (hLIF) was chemically synthesized and assembled in vitro. The expression of hLIF from a lambda promoter containing vector in E. coli approached 20-25% of the total cellular protein after thermal induction. Recombinant hLIF in inclusion bodies was solubilized, purified by column chromatography and folded to gain the natural configuration. Biological activity of hLIF was tested on murine and human leukemic cells. Recombinant hLIF at a concentration range of 10 to 20 ng per ml, inhibited the growth of M1 cells and differentiated these towards macrophages. We failed to find any differentiation inducing activity of hLIF on human luekemic cells.

P 342 CATHESPIN-G AND LEUKOCYTE ELASTASE INACTIVATE HUMAN TUMOR NECROSIS FACTOR AND LYMPHOTOXIN, Philip Scuderi, *Patricia A. Nez and Melinda L. Duerr. Cutter Biological, Miles Inc., Berkeley, CA 94710; *University of Arizona, Health Sciences Center, Tucson, AZ 85724

The addition of either cathepsin-G or leukocyte elastase to endotoxin stimulated human peripheral blood monocytes decreased the immunoreactive tumor necrosis factor (TNF) detected in culture supernatants in a concentration dependent manner. Both enzymes also induced a loss of supernatant cytolytic activity as determined on the WEHI-164 target cell line. Incubation of recombinant human TNF and lymphotoxin (LT) with either cathepsin-G or leukocyte elastase resulted in a loss of cytokine bioactivity. Examination of enzyme treated recombinant cytokines by gel electrophoresis revealed that cathepsin-G cleaved TNF and LT into 14.3 and 12.6 kD fragments respectively. Leukocyte elastase fragmented TNF into a 13.9 kD and LT into a 14.1 kD products. Incubating leukocyte elastase with plasma elastase inhibitor alpha-1-antitrypsin prevented the loss of recombinant TNF bioactivity and blocked the degradation of this cytokine. This study suggests that two of the most abundant neutrophil proteases, cathespin-G and leukocyte elastase may be important regulators of TNF and LT bioactivity.

P 343 **EOSINOPHIL PROLIFERATION BY AN INTERMEDIATE FACTOR GENERATED IN THE PLEURAL CAVITY OF PAF-INJECTED RATS.** Sandra A.C.Perez, **Patrícia M.R. Silva**, * Marcia C. El-Cheikh, Marco A. Martins, Renato S.B.Cordeiro and * Radovan Borojevic. Depto Fisiologia Farmacodinâmica/FIOCRUZ, Av. Brasil, 4365, CEP 20010, RJ,* Depto Bioquímica, Instituto de Química/UFRJ, CEP 68021, RJ, Brazil.

Intrathoracic injection of PAF into rats led to an eosinophil accumulation, 24h later, under conditions where no peripheral eosinophilia was noted. The transfer of a cell free 6h-PAF pleural washing from donor to recipient rats induced a selective and delayed pleural eosinophilia, probably due to the generation of a secondary eosinophilic principle. Here, we investigated the ability of the 6h-PAF pleural washing supernatant to stimulate eosinophil proliferation in liquid culture of murine bone-marrow cells. We verified that this fluid produced a sustained eosinophil proliferation, but not differenciation, under conditions where PAF itself had no effect. The phenomenon lasted for 4 days and was maximal on the third day of culture. Treatment with monoclonal antibodies for IL-5 or GM-CSF did not modify this eosinophil proliferation, ruling out the potential involvement of both cytokines in this process. In conclusion, we demonstrated that the rat pleural fluid obtained 6h after PAF induces eosinophil proliferation **in vitro** independently of Il-5 and GM-CSF. Supported by FAPERJ/CNPq.

P 344 MODULATION OF MACROPHAGE CYTOTOXICITY BY TUMOR-DERIVED GM-CSF, Eduardo M. Sotomayor, Yang-Xin Fu, Joaquin J. Jimenez*, Cesar A. Albarracin and Diana M. Lopez, Department of Microbiology and Immunology and Department of Medicine*, University of Miami School of Medicine, Miami, FL 33101.

Downregulation of cytotoxic cells in animals models and human subjects by developing tumors negatively influences the host's defenses against malignant cells. The growth of the murine mammary adenocarcinoma D1-DMBA-3 results in a decreased capacity of peritoneal elicited macrophages (PEM) from these animals, as compared to those of normal mice, to become cytotoxic upon *in vitro* activation with LPS. This effect is observed against both the allogeneic tumor cell lines P-815 and EL-4, and the syngeneic mammary adenocarcinoma cell line DA-3, cloned from the transplantable D1-DMBA-3 tumor. We investigated if factor(s) released by DA-3 tumor cells are involved in the loss of cytolytic activity observed in the macrophage population. The capacity of activated normal PEM to kill a broad range of target cells could be inhibited dose-dependently by pretreatment with DA-3 tumor supernatants. This inhibitory effect occurred even when the PEM were stimulated with high doses of LPS or a combination of LPS and gamma-interferon. Fractionation of the tumor cell supernatant demonstrated that the inhibitory tumor derived factor(s) has a m.w. < 30,000. Since we have recently reported that DA-3 cells constitutively produce GM-CSF (27 kDa), we evaluated the role of this cytokine in the downregulation of macrophage cytolytic capability. When normal PEM were pretreated with murine r-GM-CSF *in vitro*, a strong dose-dependent inhibition of macrophage cytotoxicity was observed at ranges from 10 U/ml to 1000 U/ml. Combined pretreatment of normal PEM with Indomethacin and GM-CSF did not change the inhibition of macrophage cytotoxicity, suggesting a regulating mechanism other than prostaglandin release. Furthermore, *in vivo* treatment of normal mice with r-GM-CSF (20,000 U/mouse/day x 25 days) also affected the capacity of PEM from these mice to kill DA-3 target cells, as compared to those of control animals. These results indicated that tumor derived GM-CSF could be one important factor responsible for the decreased cytolytic capacity of macrophages observed during mammary adenocarcinoma progression.

P 345 HIGHLY MALIGNANT 3LL-R TUMOR CELLS STIMULATE HEMATOPOIESIS AND CONCOMITANT IMMUNE SUPPRESSION THROUGH SECTRETION OF CSF-LIKE FACTORS, E. Torreele, L. Remels, L. Bouwens*, L. Fransen** and P. De Baetselier, Laboratory of Cellular Immunology, 1640 St Genesius Rode, Laboratory of cell biology and histology*, 1090 Jette, Vrije Universiteit Brussel, ** N.V. Innogenetics, 9000 Gent, Belgium.

Subcutaneous tumor growth of the highly tumorigenic and metastatic 3LL-R variant of the murine Lewis Lung Carcinoma in C57bl/6 mice was associated with an increased hematopoiesis and a cell mediated immune suppression in spleen and bone marrow. We found that the mitogen stimulated T- and B-cell blastogenesis of spleen cells from 3LL-R tumor-bearing mice was strongly suppressed. Moreover, both spleen and bone marrow cells from tumor-bearing mice exerted a suppressive activity on mitogen stimulated T- and B-cell blastogenesis of normal spleen cells. The in vivo hematopoiesis consisted mainly of an expansion of myeloid precursors of the neutrophilic lineage (myeloblasts, pro-myelocytes and myelocytes). Spleen cell fractionation experiments indicated that these pre-neutrophilic cell populations mediated the observed immune suppression. The in vivo hematopoiesis and induction of suppressor cells could be the direct effect of colony-stimulating factor secretion by the 3LL-R cells since i) colony-stimulating activity on normal spleen and bone marrow cells was detected in the serum from 3LL-R tumor-bearing mice and in 3LL-R derived conditioned medium (R-CM) ii) normal spleen and bone marrow cells, which were cultured in the presence of R-CM, GM-CSF or IL-3 suppress the T- and B-cell blastogenesis of normal spleen cells iii) the in vitro colony-stimulating activity of R-CM could be partially blocked by adding anti-GM-CSF iv) abundant GM-CSF m-RNA transcription was observed in the 3LL-R tumor cells v) the suppressive capacity of cell fractions from tumor-bearing mice, which were enriched for neutrophil precursors, was significantly enhanced after incubation in R-CM. These results suggest that, during subcutaneous tumor growth, the 3LL-R tumor cells secrete colony-stimulating factors (one of which being GM-CSF), resulting in an increased hematopoiesis and the concomitant appearance of pre-neutrophilic immuno-suppressive cells. These tumor-induced suppressor cells may contribute to the highly malignant behaviour of the 3LL-R tumor cell line.

P 346 MURINE MAST CELL COLONY FORMATION SUPPORTED BY IL-3, IL-4 AND RECOMBINANT RAT STEM CELL FACTOR (SCF), Kohichiro Tsuji, Krisztina M. Zsebo and Makio Ogawa, Department of Medicine, Medical University of South Carolina and VA Medical Center, Charleston, SC and AMGen Corp, Thousand Oaks, CA.

Recently, a novel cytokine designated stem cell factor was isolated from medium conditioned by Buffalo rat liver cells and proved to be the ligand for c-*kit*. We have examined the effects of recombinant rat SCF alone and in various combinations with IL-3 and IL-4 on murine mast cell colony formation in methylcellulose culture. As a source of connective tissue-type mast cells (CTMC), we used peritoneal mast cells. No individual factor supported colony formation. When cells were grown in combinations of two factors, significant mast cell colony growth was seen. When cells were grown in the presence of three factors, not only the number of colonies increased but also the colonies were larger. Mast cells in these colonies contained safranin+/berberine sulfate+ cells, but the proportions of positive and negative cells varied depending on the factor combinations. We then examined the effects of these factors on proliferation of bone marrow-derived mast cells (BMMC) by replating pooled mast cell colonies. As a single factor, only IL-3 supported mast cell colony formation. Combinations of two of the three factors supported mast cell colony formation. However, the most impressive synergism was seen again with the combination of the three factors. Not only was the number of colonies increased, but there was a significant increase in size. These results indicate that SCF is an important factor for the proliferation of both CTMC and BMMC.

P 347 CHARACTERIZATION OF MULTIPLE ISOFORMS OF THE SNOWDROP LECTIN AT THE MOLECULAR LEVEL , Els J.M. Van Damme, Ben Peeters and Willy J. Peumans, Laboratory for Phytopathology and Plant Protection and Department of Biochemistry, University of Leuven, Leuven, Belgium.

The snowdrop (Galanthus nivalis L.) lectin has previously been shown to occur as a very complex mixture of isolectins when analyzed by ion exchange chromatography and isoelectric focusing. Screening of a cDNA library constructed using polyA RNA isolated from young developing snowdrop ovaries resulted in the isolation of five lectin cDNA clones which definitely differ from each other for what concerns their nucleotide sequence and deduced amino acid sequence. The sequence homology for the total coding region at the amino acid level ranged from 69 to 94 % for the different lectin cDNA clones. The lectin is synthesized as a preproprotein which is post-translationally processed. However, whereas the signal peptide sequence of the different clones is highly homologous the sequence of the C-terminal extension which is cleaved of during a second processing step shows some more heterogeneity.

The occurrence of closely related multiple isoforms of a lectin has previously been ascribed to differences in glycosylation (soybean lectin), combination of different protein subunits (Phaseolus lectin) or the simultaneous expression of lectin genes by each of the individual genomes of cereal species. Since all Galanthus nivalis isolectins have the same molecular structure and are not glycosylated, a possible explanation for the complexity of the isolectin pattern could be the occurrence of a gene family.

P 348 AUGMENTATION OF CYTOKINE STIMULATED HUMAN BONE MARROW COLONY FORMATION BY CULTURED HUMAN MARROW ADHERENT CELL EXTRACT. Lisbeth A. Welniak, Shantaram S. Joshi and John D. Jackson. Departments of Pathology & Microbiology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198

The regulation of hematopoiesis is mediated at least in part by stromal cells and adjacent extracellular matrix components. Stromal cell surface molecules appear to play a role in the presentation of regulatory molecules/factors to hematopoietic stem and progenitor cells for their proliferation and differentiation. In this report, we describe the augmentation of in vitro proliferation of hematopoietic progenitor cells by cell surface extracts of adherent cells from human long-term bone marrow cultures. The cell surface molecules were extracted from cultured bone marrow adherent cells using 0.05% n-octyl glucoside, a nonionic detergent. The detergent was removed from the extract by extensive dialysis. Three different concentrations (10 μg, 20 μg and 40 μg) of marrow stromal extract (MSE) were used to determine its effect on the proliferation of normal bone marrow cells as determined by ^{3}H thymidine uptake and CFU-GM colony formation in semi-solid medium. The MSE enhanced the ^{3}H thymidine uptake and colony formation of normal human bone marrow cells when stimulated with plateau levels of IL-3, GM-CSF and G-CSF. These enhancements were not due to endotoxin contamination of the MSE preparation. These results suggest that extracts from adherent bone marrow stromal cells have the ability to augment the proliferation and differentiation of hematopoietic progenitor populations.

P 349 MOLECULAR MECHANISM OF DOWNREGULATION OF TNF PRODUCTION, H.W. Lõms Ziegler-Heitbrock, Patrick Bäuerle, Gert Riethmüller, Jürgen G. Haas, Institute for Immunology, University of Munich, W8000 Munich 2, Germany

Similar to blood monocytes, the human monocytic cell line Mono Mac 6 can be induced by LPS to produce high levels of TNF mRNA and protein. When Mono Mac 6 cells are precultured for 3 days with LPS, they become refractory to a secondary stimulation with LPS. This phenomenon of desensitization to LPS has long been known, but the molecular mechanisms involved remained obscure. Using the Mono Mac 6 cell line, we have analyzed both the induction phase of desensitization and the desensitized, refractory cells (Haas et al, PNAS, in press). The induction of desensitization by LPS can be blocked by inhibitors of cyclooxygenase and by inhibitors of proteinkinase C. Conversely, phorbolesters and also PGE_2 (together with a second signal) can mimick desensitization. The second signal required for PGE_2 mediated induction of desensitization can be provided by phorbolesters and we can, in fact, demonstrate a synergism of PGE_2 and PKC activation. The desensitized Mono Mac 6 cells can be stimulated by LPS to activate the nuclear factor NFKB which is central to TNF gene expression. Still refractory cells produce no TNF protein and little, if any, TNF transcripts, as demonstrated in Northern blot and in nuclear run on. These data suggest that desensitization to LPS is controlled at the level of nuclear factors.

Structure and Function of Cytokine Receptors

P 400 A NOVEL LOW AFFINITY HUMAN INTERLEUKIN-4 RECEPTOR THAT EXISTS IN A SOLUBLE FORM.
R. J. Armitage, K. Clifford, M. K. Spriggs, B. M. MacDuff, S. K. Dower, C. M. Rauch, K. Van Ness, C. J. March and W. C. Fanslow, Immunex Corporation, Seattle, WA98101.
Biotinylated human IL-4 was used to examine IL-4 receptor expression on B-cell lines by flow cytometry. Pre-B lines Nalm6, JM1 and BMB bound $5x10^{-7}M$ biotinylated IL-4 with strong intensity while the more mature B-lymphoblastoid lines (B-LCL) RPMI 1788 and CESS, and Burkitts lymphoma lines Daudi and Raji showed only moderate or weak reactivity. Staining with this concentration of biotinylated IL-4 did not correlate with levels of IL-4R mRNA, which were barely detectable in pre-B lines and high in B-LCL and Burkitts lines, or with ^{125}I-IL-4 binding which was compatible with detected mRNA. From binding curves generated using a titration of biotinylated IL-4, a Ka of $6.3x10^{7}M^{-1}$ was calculated for the binding of IL-4 to JM1 cells. In contrast, murine CTLL-2 T cells transfected with the cloned human IL-4 receptor (hIL-4R$^+$ CTLL) bound IL-4 with a Ka of $1.4x10^{10}M^{-1}$ under the same conditions.
Cross-linking experiments performed with high concentrations of ^{125}I-IL-4 revealed a distinct 75kD protein on the surface of JM1 cells and a 120kD protein on the surface of hIL-4R$^+$ CTLL cells. In addition, a 47-50 kD protein was purified from the supernatant of Nalm6, JM1 and BMB on IL-4-agarose. Partial N-terminal amino acid sequence showed this novel IL-4 binding protein to be identical from the 3 pre-B lines and distinct from other molecules previously reported. The IL-4 binding protein was shown to be biologically active and to effectively inhibit IL-4-induced CD23 induction on tonsil B cells and block IL-4-mediated inhibition of JM1 proliferation. Data will be presented that strongly suggests that this IL-4 binding protein is a soluble form of a novel low affinity human IL-4 receptor.

P 401 THE STRUCTURE AND FUNCTION OF THE MURINE PROLACTIN RECEPTOR, Roland K. Ball, Friedrich Miescher Institute, P.O. Box 2543, 4002 Basel, Switzerland.

The receptors for the cytokines IL-2,3,4,6,7, G and GM-CSF, EPO, growth hormone and prolactin form a family having homologous extracellular domains. There is a tissue-specific expression of seven different prolactin receptor transcripts in the mouse. Transcripts of 1.4, 2.4, 3.5 and 4.2 kb encode a 300 amino acid receptor isoform expressed in the liver, kidney and small intestine. Transcripts of 8.3, 9 and 10 kb encode a 600 amino acid receptor with an extended cytoplasmic tail, expressed in the ovary, mammary gland and kidney. The transcripts are regulated in a complex manner. The expression of the transcripts for the small isoform is elevated in the liver during pregnancy. The expression of the transcripts for the large isoform is elevated in the kidney during lactation, whereas expression in the mammary gland is always constant. The signal transduction pathway of the cloned murine mammary prolactin receptor is being analysed using mutants and receptor chimeras to test the effect upon the regulation of β-casein gene transcription in the mouse mammary epithelial cell line, HC11.

P 402 CLONING AND INITIAL CHARACTERISATION OF MURINE TUMOR NECROSIS FACTOR RECPTOR: A POSSIBLE ROLE FOR THE 55kD RECEPTOR. Kathy Barrett, Andrew Cope, Brian M. J. Foxwell, Patrick W. Gray and Marc Feldmann, Charing Cross Sunley Research Centre, Lurgan Avenue, Hammersmith, London, W6 8LW, England.
The cloning and characterisation of the human tumor necrosis factor (TNF) receptor has demonstrated the existence of two distinct receptor proteins of 55 and 75kD. The two receptors show only a low degree of homology, confined mainly to the extracellular domains. Preliminary investigations have indicated that at least two distinct murine TNF receptors exist. We have cloned the murine homologue of the human p55 TNF receptor and show that the expressed protein binds both murine and human TNF. Using affinity cross-linking and FACS analysis with biotinylated ligands a second murine TNF receptor, the likely homologue of the human p75 TNF receptor has been identified. This receptor only binds murine TNF. The expression of the two different receptors on various murine cell lines and their relationship to the effects of TNF on cells is being investigated.

P 403 INTERACTION OF THE EXTRACELLULAR DOMAINS OF THE ALPHA AND BETA SUBUNITS OF THE HUMAN IL2 RECEPTOR: STUDIES WITH PI-LINKED AND SOLUBLE FORMS OF THE RECEPTOR. Ronald W. Barrett, Erik A. Whitehorn, Emily H. Tate, Gerard Zurawski*, Martha S. Ackerman, Ann M. Olson. Affymax Research Institute, Palo Alto, CA and *DNAX Research Institute, Palo Alto, CA.

Phosphoinositol (PI)-linked forms of the α or β subunit of the human IL2 receptor were created by transfecting CHO cells with chimeric genes in which DNA of the extracellular domain of the receptor subunit was fused to the DNA of a C-terminal signal sequence from human placental alkaline phosphatase (HPAP). FACS was used to select clones expressing high levels of α alone , β alone or both subunits. Greater than 90% of surface-bound receptor was released from cells by treatment with PI -specific phospholipase C. Radioligand binding studies indicated that the PI-linked and soluble form of the α subunit were capable of binding ^{125}I-IL2. Low amounts of saturable binding were detected on cells expressing PI-linked β subunit alone. The binding properties of cells co-expressing PI-linked forms of both subunits were different than for PI-linked α subunit alone - most notably a shift to lower affinity for mouse IL2. Gel filtration studies of the soluble product from PI-PLC treament of cells expressing both subunits indicated the presence of a α /β heterodimer. These data provide evidence that the PI-linked forms of α and β subunits (which lack transmembrane and cytoplasmic regions of the native subunits) can interact on the cell surface and that a soluble heterodimer can be formed in the absence of IL2.

P 404 THE ACTIVATION OF PHOSPHOLIPASES A_2 AND C ESSENTIAL FOR THE SIGNAL TRANSDUCTION OF ERYTHROPOIETIN, Barbara S. Beckman, Meredith-Garcia and Jen-sie Tou, Departments of Pharmacology and Biochemistry, Tulane University Sch. of Med., New Orleans, LA. 70112

Erythropoietin (Epo) is a well-recognized growth factor/hormone that signals erythroid progenitor cells to proliferate and differentiate. Unlike many other growth factor receptors the receptor for Epo does not appear to contain intrinsic tyrosine kinase activity. Instead, Epo receptors are internalized, much like receptors for prolactin, and activate phospholipases A_2 and C to produce early lipid signalling molecules for proliferation and differentiation. One of these signalling molecules is the beta II isoform of protein kinase C which we have identified by immunocytochemistry in murine erythroid progenitor cells isolated from fetal liver at day 13-14 of gestation. In addition to the activation of nuclear protein kinase C activity via phospholipase C there is also an early activation of phospholipase A_2 which results in a rise in the signalling molecules, leukotriene B_4 and in 12-hydroxyeicosatetraenoic acid. Since there is not a significant increase in inositol 1,3 trisphosphate within minutes, and since a rise in calcium occurs gradually up to 30 minutes as determined by indo-1 prelabeling experiments, it is concluded that diacylglycerol is not produced according to the classical scheme involving phosphatidylinositol but may be produced *de novo* or from the breakdown of phosphatidylcholine or phosphatidylethanolamine which are the predominant phospholipids in these cells. In conclusion, the activation of phospholipases A_2 and C occurs early in response to erythropoietin and may represent essential features of the signal transduction scheme for Epo.

P 405 EXPRESSION OF NAP-1/IL-8 RECEPTORS ON HUMAN ALLERGIC EFFECTOR CELLS, Jürgen Besemer, Peter Valent and Peter Bettelheim,
Sandoz Forschungsinstitut, Vienna, Austria, and I. Medical Department, University of Vienna, Austria

NAP-1/IL-8 specific binding sites were characterized on highly purified human eosinophils, human basophils and human basophilic and mast cell lines by means of a radio-receptor assay. Eosinophils expressed 14,000 to 96,000 NAP-1/IL-8 binding sites per cell, and basophils 550 to 9,000. The basophilic human cell line KU812-F had about 1,200, and the human mast cell line HMC-1 2,400 specific NAP-1/IL-8 receptors per cell. NAP-1/IL-8 receptors on each of the characterized cell type were of one single class and of high affinity, K_D values were estimated to be in the few nanomolar range. The distribution of NAP-1/IL-8 receptors will be discussed in the context of functional activation of allergic effector cells by this cytokine.

P 406 T CELL ACTIVATION BY BISPECIFIC MONOCLONAL ANTIBODIES FOR IMMUNOTHERAPY OF CANCER: FUNDAMENTAL, PRACTICAL AND CLINICAL ASPECTS. Reinder L.H. Bolhuis Department of Immunology, Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands. Under physiological conditions T lymphocytes become activated when their T cell receptor (TCR) binds to antigen/MHC complex on another cell, resulting in multiple link between them. This interaction also involves costimulatory molecules on lymfocytes such as LFA-1. For cytotoxic T lymphocytes (CTL) the result is delivery of a lethel hit to its conjugated target cell. Lymphocyte activation and triggering for cytolysis can also be induced by bispecific monoclonal antibodies (bs-mAb) which recognize lymphocyte and target cell structures, respectively and bridge these cells. Such bs-mAb therefore retarget the specificity of CTLs, which is MHC-unrestricted. Our investigations revealed that 1) the combined use of bs-mAb preparations, comprising both, CD3 and LFA-1 costimulator specificities, induce effective lysis of (also ICAM$^-$) tumor cells. ICAM$^-$ tumor cells may escape immunedestruction; 2) tumor cells which do not express the tumor associated antigen (TAA) recognized by the bs-mAb may still be lysed: bystander killing; 3) bs-mAb retargeted lymphocytes become readily inactivated upon interaction with ovarian cancer (OVA-CA) tumor cells; 4)the lymphocyte inactivation process is not due to antibody binding site occupency by soluble TAA nor to shedding of the bs-mAb from the surface of lymphocytes. 5) bs-mAb retargeted lymphocytes can enter multiple lytic cycles provided and become retargeted with readded bs-mAb. The use of bs-mAb allows the biochemical analysis of lymphocyte TCR/CD3 complexes which have been engaged in signal transduction versus "naive" TCR/CD3 molecules. Our findings also have redirected the design of the immunotherapy protocol involving the treatment of ovarium carcinoma patients which in vitro expanded and activated lymphocytes retargeted with bs-mAb. The clinical results on the first group of OVA-CA patients treated intraperitoneally with in vitro expanded mAb retargeted lymphocytes will be presented.

P 407 CLONING AND EXPRESSION OF THE MURINE IL-6 RECEPTOR, Gennaro Ciliberto, Teresa Fiorillo, Carlo Toniatti and Luciana Dente.
I.R.B.M. Via Pontina Km, 30,600. 00040 Pomezia, Rome, Italy

Interleukin-6 (IL-6) is a multifunctional cytokine which acts as growth and differentiation factor on B-cells, T-cells, hepatocytes, macrophages, neural cells and cells of the hematopoietic lineage. Deregulation of IL-6 synthesis is constantly associated to several pathological conditions such as multiple myelomas, rheumatoid arthritis, psoriasis, mesangial glomerulonephritis, etc. In order to better understand the role and the activities of this molecule during embryonal development and adult life we have cloned the murine IL-6 receptor. We have screened a mouse liver cDNA library with a synthetic oligonucleotide reproducing part of the known human IL-6 receptor. One positive clone was obtained.(mIL6R.1) which encodes for a protein of 460 aa with a 53% homology to the human IL-6 receptor. Murine IL-6 does not bind to the human receptor and therefore is not active on human cells. On the other hand, the cDNA from clone mILR6.1, when introduced in a eukaryotic expression vector and transfected into human hepatoma cells, confers the ability to respond to murine IL-6, with the following transcriptional activation of the acute phase C-reactive protein gene.
The murine IL-6 receptor cDNA detects in Northern blots with polyA+ selected RNA a mRNA species of about 5.5 kb which is more abundant in spleen, lung and liver, less represented in kidney and heart and undetectable in brain. During mouse development mIL6R expression peaks at the 7th day of gestation. Studies are in progress to express recombinant receptor molecules and map the aminoacid residues involved in the interaction with IL-6.

P 408 THE ROLE OF THE p55 CHAIN OF THE INTERLEUKIN-2 RECEPTOR IN SIGNAL TRANSDUCTION, Mary Collins, Prupti Malde, Claudia Fleming and Steve Russell, Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, U.K.

We have used recombinant retroviral vectors to transfer the p55 chain of the interleukin 2 (IL2) receptor to an interleukin 3 dependent cell line. The ability of the recipient cells to respond to IL2 is correlated with their level of p55 expression (1). We therefore propose that the functional IL2 receptor is a trimolecular p75/p55/IL2 complex in these cells, the level of which can be varied by altering the p55 concentration. We have also demonstrated that a single amino acid point mutation in IL2, which decreases p75 interaction, yields a molecule which can stimulate one IL2 dependent T cell line equivalently to native IL2, while having no biological effect on a panel of further IL2 dependent cells. The implication of these data for the structure of IL2 receptors on the various cell lines will be discussed.

(1) Collins *et al.* Eur. J. Immunol. (1990) **20**: 573-578.

P 409 CHARACTERIZATION OF IL-6 BINDING PROTEINS BY AFFINITY CROSS-LINKING AND IMMUNOPRECIPITATION STUDIES. Francesco D'Alessandro, Oscar R. Colamonici*, Lucien Aarden# and Richard P. Nordan. Tumor Cell Biology Section, Clinical Pharmacology Branch, NCI, Bethesda, MD 20892, *Department of Medicine, University of Chicago, Chicago, IL 60637 and #Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

IL-6 is a cytokine which displays a broad range of biological activities including the stimulation of immunoglobulin production by activated B-cells, the support of myeloma, plasmacytoma and hybridoma proliferation and the induction of acute phase responses in hepatocytes. Only one IL-6-binding receptor molecule has thus far been described. This receptor, termed p80, has been cloned and consists of an 80 kD IL-6-binding transmembrane glycoprotein. In addition, it has been reported that upon interaction with IL-6, p80 but not IL-6 associates with a 130 kD non-ligand-binding glycoprotein which may be involved in the generation of a transmembrane signal.

Our studies with ^{125}I-IL-6 affinity crosslinking on several cell lines has revealed a 3 band pattern of IL-6/membrane protein complexes corresponding to 100, 120 and 150 kD. This result suggested that the association of IL-6 with its receptor is more complex than previously described and prompted us to identify the membrane components that were associated with IL-6 in the three complexes. We employed an iodinated, photoactivatable and cleavable crosslinker (^{125}I-Denny-Jaffe reagent coupled to IL-6) which allows the transfer of ^{125}I from the ligand to its receptor. Crosslinked samples were analyzed by two-dimensional SDS-PAGE in which the crosslinker was cleaved prior to the second dimension. This analysis revealed that both the 100 kD and 120 kD complexes include an 80 kD membrane glycoprotein whereas the 150 kD complex includes a 130 kD membrane glycoprotein. These results indicated that within the 120 kD crosslinked complex the 80 kD protein is associated with two IL-6 molecules. In addition, the 130 kD protein appears to directly bind IL-6 thus forming the 150 kd complex. HPLC analysis and purification of ^{125}I-IL-6 used in these studies demonstrated that covalent IL-6 dimers did not contribute to the 120 kD complex. Models for the receptor structure are discussed.

P 410 DELINEATION OF THE REGIONS OF THE EPO-RECEPTOR REQUIRED FOR SIGNAL TRANSDUCTION AND GP55 BINDING, Alan D. D'Andrea, Jah-Won Koo, and Leonard I. Zon, Division of Hematology-Oncology, The Children's Hospital, Dana-Farber Cancer Institute, Department of Pediatrics, Harvard Medical School, Boston, MA 02115.

The erythropoietin receptor (EPO-R), a member of a large cytokine receptor superfamily can be activated either by binding EPO or by binding gp55, the retroviral envelope protein encoded by the Friend spleen-focus forming virus. In order to delineate the functional domains of the EPO-Receptor required for EPO- or gp55-binding, we have generated a series of EPO-Receptor truncated mutants and EPO-Receptor/IL-3 Receptor chimeric mutants. The mutants were stably expressed in a murine interleukin-3 dependent cell line, Ba/F3, and EPO-dependent growth characteristics of the isolated Ba/F3 subclones were studied. A secreted form of the EPO-Receptor, generated by PCR, although capable of binding radiolabeled EPO, failed to confer EPO responsiveness on the transfected cells and failed to bind the gp55 envelope protein. We conclude that, while the EPO binding domain exists in the extracytoplasmic region of the EPO-R, signal transduction and gp55 binding requires portions of the transmembrane and cytoplasmic region.

P 411 RELATIONSHIP BETWEEN THE MOUSE INTERLEUKIN-5 RECEPTOR AND THE MOUSE INTERLEUKIN-3 RECEPTOR, Rene Devos, Jose Van der Heyden, Geert Plaetinck and Jan Tavernier, Roche Research Gent, Plateaustraat 22, B-9000 Gent, Belgium.

Monoclonal antibodies were obtained against the mouse Il-5R by immunization of rats with a mouse Il-5R$^+$ and mouse Il-3R$^+$ pre-B cell line, B13. These antibodies fell into two different groups. One group immunoprecipitated a 50 kD protein from a detergent lysate of B13 cells and completely blocked the binding of radiolabeled mouse Il-5 to these cells. These antibodies did not affect the binding of radiolabeled mouse Il-3 or the mouse Il-3 induced proliferation of B13 cells. A second group of antibodies immunoprecipitated a 130-140 kD doublet and partially blocked the binding of both mouse Il-5 and mouse Il-3 to B13 cells. One of these antibodies however immunoprecipitated the 130 Kd band only, and inhibited the binding of mouse Il-3 only. Internal amino acid sequence was obtained from four trypsin-peptides generated from a protein preparation purified by one of these second group antibodies, R52. All peptides were found to be present in the published amino acid sequence of the mouse Il-3R and the mouse Il-3R-like protein deduced from cDNA (Itoh et al. 1990 Science 247:324, Gorman et al. 1990 Proc. Natl. Acad. Sci. USA 87:5459). Our results suggests that both the mouse Il-5R and the mouse Il-3R have a common or homologous polypeptide subunit, and that the specificity of these lymphokine receptors is generated by association with a second ligand polypeptide chain.

P 412 GM-CSF RECEPTOR: PURIFICATION AND SIGNALING, DiPersio, J.F., Raines, M.B., Golde, D.W. University of Rochester, Hematology Unit, Rochester, NY 14642 and UCLA Medical Center, Division of Hematology/Oncology, Los Angeles, CA 90024.

The low affinity binding subunit of the human GM-R has been characterized and the cDNA which encodes this protein identified. We have developed a soluble binding assay and a purification scheme for the human GM-R. The expression of high affinity GM-R and IL-3R were studied on intact AML cells and myeloid cell lines. GM-CSF and IL-3 cross-compete for each other's receptors using radioreceptor binding assays, chemical crosslinking, and FACS analysis. This cross-competition persists when purified plasma membranes are studied. This effect is completely lost if binding is performed in solution. Purification scheme of the GM-R using DEAE sephacel, wheat germ agglutin-sepharose, Ricin B-sepharose, biotin-GM-CSF-sepharose and HPLC is presented. Binding in solution results in a 10-20 fold reduction in the affinity of the GM-R (10-20pm → 500-1000pm). Chemical crosslinking reveals two cross-linked bands, one of ~ 98kd and another of ~ 200kd. Comparison to the cloned low affinity GM-R reported by Gearing et al. will be presented.

GM-CSF activates neutrophils and enhances the effects of secondary agonists on neutrophil function. GM-CSF directly stimulates neutrophil LTB_4 and PAF synthesis as well as dramatic down-regulation of LTB_4 and interleukin-8 receptors. The down-regulation of these receptors as well as other effects on neutrophil function are dramatically inhibited by two tyrosine kinase inhibitors, erbstatin and genestein. GM-CSF directly induces the tyrosine phosphorylation of at least six substrates in neutrophils within 15 minutes. This effect is inhibited by erbstatin and genestein.

P 413 THE IL-1 RECEPTOR ANTAGONIST BINDS TO THE ~60 kDa IL-1 RECEPTOR
David J. Dripps, Robert C. Thompson, Stephen P. Eisenberg,
Synergen, Inc., 1885 33rd Street, Boulder, CO 80301

The binding of IL-1α and IL-1β to the same receptor on many cell types is an important initial step in the induction of cellular activities involved in the inflammatory response. Recently a third member of the IL-1 family, termed IL-1ra, was isolated and cloned, and found to block the effects of IL-1α and IL-1β by acting as a receptor antagonist. Previous studies have shown that IL-1ra is capable of binding the 80 kDa IL-1 receptor on T cells and fibroblasts with high affinity (K_D=~100-200 pM). We have now investigated the ability of IL-1ra to bind to the second type of IL-1 receptor (~60 kDa) found on two B cell lines.

We show that IL-1ra will compete for the binding of labeled IL-1β to the human B cell line, Raji. IL-1ra binds to these cells with the same affinity as IL-1α (K_D=~2.5nM), but with a ~20-fold lower affinity than IL-1β. With the murine pre B cell line 70Z/3, IL-1ra will bind to the IL-1 receptor but with a much lower affinity (K_D=~340 nM) than to Raji cells. In addition, we show that IL-1ra will prevent the cross-linking of labeled IL-1β to the ~60 kDa receptor on both these B cell lines. These results demonstrate that IL-1ra will specifically block the binding of IL-1β to murine and human B cells. We conclude that IL-1ra will bind to the ~60 kDa IL-1 receptor, and unlike the 80 kDa receptor, this binding is species dependent.

P 414 UP-REGULATION OF P65 IL-1 RECEPTOR (IL-1R) ON BONE MARROW CELLS BY G-CSF AND GLUCOCORTICOIDS. [1]Dubois, C.M., [2]Neta, R., [3]Ruscetti, F.W., [4]Jacobsen, S.E.W., [5]Oppenheim, J.J., and [6]Keller, J.R.[1,6] BCDP-PRI/Dyncorp-,[3,4,5]LMI-BRMP,NCI-FCRF, Frederick, MD. [2] Armed Forces Radiobiology Research Institute, Bethesda, MD.

We have previously shown that injection of IL-1 into mice results in a marked up-regulation of IL-1R on bone marrow (BM) cells. This effect is indirect, presumably mediated in part through the induction of circulating colony stimulating factor (CSF) production by IL-1. To assess this possibility, murine bone marrow were treated with CSFs in vitro and we found that he addition of G-CSF, GM-CSF and IL-3, but not IL-1, to BM cells induced a rapid up-regulation (5-10 fold) of IL-1 binding. Treatment of purified BM progenitor cells with IL-3 resulted in an increase of IL-1R expression suggesting that IL-1R was up-regulated on an early progenitor population. Scatchard analysis revealed that CSFs treatment increases the number of IL-1 R but not receptor affinity. Cross-linking studies indicated that G-CSF augmented the expression of a 65 kD IL-1 binding protein that was cold competed by adding 50 fold excess IL-1 α but was not blocked by anti-type 1 IL-1 receptor antibody (35F5) suggesting that BM cells expressed the type 11 IL-1R. The administration of IL-1 to mice also results in a rapid elevation of glucocorticoids (GCs) which were reported to up-regulate IL-1R expression in vitro. We therefore investigated the contribution of GC in the up-regulation of IL-1R by IL-1. First, we observed that injection of IL-1 into adrenalectomized mice reduced the up-regulation of IL-1R on BM cells as compared with the up-regulation of IL-1R on sham adrenalectomized mice. Second, G-CSF and GCs synergized in vivo to up-regulate IL-1R on BM cells from normal mice. These results suggest that both GC and G-CSF induced by IL-1 are the mediators of the vivo up-regulation of IL-1R.

P 415 INDEPENDENT REGULATION OF 55kDa AND 75kDa TNF RECEPTORS ON HUMAN B LYMPHOCYTES.
Bjørn K. Erikstein, Terje Espevik*, Heidi Kiil Blomhoff, Steinar Funderud, Werner Lesslauer+ and Erlend B. Smeland, Laboratory of Immunology, Institute for Cancer Research, Montebello, N-0310 Oslo 3, Norway; *Institute for Cancer Research, University of Trondheim, N-7006 Trondheim, Norway and +Central Research Units, F. Hoffman-La Roche AG, 4002 Basel, Switzerland.

The expression of 55kDa and 75kDa TNF receptors on resting and activated human peripheral blood B lymphocytes was studied using specific mAbs and flowcytometry. Most resting B cells expressed small amounts of the 75kDa TNF receptor, and this receptor was markedly upregulated upon stimulation with anti-μ or SAC. In contrast, the expression of the 55kDa TNF receptor was low on resting as well as on activated cells. B cell activation was accompanied by an increased binding of biotinylated TNF, and this binding could be blocked by preincubation by utr-1 (anti-75kDa), but not the htr (anti-55kDa) antibodies. TGF-β partly inhibited the anti-μ induced increase in p75 expression. Moreover, TPA led to an early, marked downregulation of p75 expression, followed by a later modest increase after >24 h. Htr mAbs, which may mimic or inhibit TNF effects in other cell systems, had insignificant effects in assays for restimulation of preactivated B cells. In contrast utr-1 markedly inhibited the TNF-β and partly the TNF-α induced proliferation. Taken together, our data suggest that changes in p75 expression are responsible for the increased TNF-receptor expression on activated versus resting peripheral blood B cells and that p75 also may play an important functional role.

P 416 REGULATION OF CELLULAR FUNCTIONS BY MONOCLONAL ANTIBODIES AGAINST TUMOR NECROSIS FACTOR RECEPTORS.
T. Espevik, B. Naume, M.R. Shalaby, M. Brockhaus*, W. Lesslauer* and A. Sundan, Institute of Cancer Research, University of Trondheim, N-7006 Trondheim, Norway; and *F. Hoffmann-La Roche, Basel, Switzerland.

We have used monoclonal antibodies (Mabs) against the 55 kD and 75 kD TNF receptors (TNFRs) and studied their involvement in TNF induced cytotoxicity of U937 cells, activation of human endothelial cells (HECs) and regulation of NK/LAK cell functions. Antibodies against both types of TNFRs inhibited TNF induced cytotoxicity as well as activation of HECs. NK cells were found to express more of the 75 kD than the 55 kD TNFR upon activation with IL-2. Both types of TNFRs were involved in the generation of LAK activity. Proliferative activity of IL-2 stimulated NK-cells was inhibited by neutralizing antibodies against TNF, indicating that endogenously produced TNF is crucial for NK-cell proliferation. Mabs against the two TNFRs also inhibited IL-2 induced proliferation of NK cells. These data indicate that both types of TNFRs are involved in TNF signal transduction in various cell systems.

P 417 ESTABLISHMENT OF MAMMALIAN CELL LINES CONSTITUTIVELY SECRETING THE EXTRACELLULAR DOMAIN OF THE MOUSE INTERLEUKIN 4 RECEPTOR,
Xuedong Fan, Liza Davies, Herinder Lonial, Hanh Nguyen, Nobuyuki Harada*, Maureen Howard*, Satwant Narula, Paul Zavodny, and Daniel Lundell, Department of Biotechnology/Molecular Biology, Schering-Plough Research, Bloomfield, New Jersey, 07003 and *DNAX Research Institute, Palo Alto, California, 94304.
Interleukin 4 (IL4), a T-cell-derived factor, mediates growth and differentiation of various lymphoid and myeloid cells. The biological effects of IL4 are mediated through a specific, high affinity cell surface receptor, which has been recently cloned (Harada, N. et al., 1990. PNAS **87**, 857; Mosley, B. et al., 1989. Cell **59**, 335). The coding region for the extracellular domain of the mouse IL4 receptor was removed from the full-length cDNA clone by use of the polymerase chain reaction (PCR). The PCR primers introduced a SalI restriction site followed by a protein synthesis initiation codon (ATG) in an optimum Kozak context at the 5' end of the cDNA and a termination codon (TAG) followed by a BamHI restriction site at the end of the putative extracellular domain. The PCR-modified coding region for the mouse IL4 receptor extracellular domain was cloned into a pcD-based expression plasmid using an SRalpha promoter (Takebe, Y. et al. 1988. MCB **8**, 466); this plasmid was capable of producing soluble mouse IL4 receptor from Cos cells. The plasmid was modified for stable transformation of mammalian cells by introduction of a mouse IgG heavy chain enhancer and a mycophenolic acid (MPA) resistance marker. After transfection into mouse myeloma cells, >40 MPA resistant clones were isolated. From these isolates, several clones constitutively expressed soluble recombinant mouse IL4 receptor, which was competetive in a cell-based receptor binding assay. Sufficient quantities of soluble mouse IL4 receptor can be prepared from this system to support biochemical and biological analysis.

P 418 A NOVEL LOW AFFINITY HUMAN INTERLEUKIN-7 RECEPTOR. W. C. Fanslow, S. F. Ziegler, L. S. Park and R. J. Armitage, Immunex Corporation, Seattle, WA 98101.

Human recombinant IL-7 was labeled with biotin and used to examine IL-7 receptor expression and regulation on human primary hemopoietic cells and a range of cell lines by flow cytometry. A strong intensity of staining was observed using relatively high (>$1x10^{-7}$M) concentrations of biotinylated IL-7 on the majority of cell types examined. This reactivity, which could be effectively competed with excess unlabeled IL-7, did not correlate with either mRNA levels for the cloned receptor or with estimates of IL-7R expression determined by ^{125}I-IL-7 binding. B-lymphoblastoid cell lines (B-LCL), including RPMI 1788 and Cess, the pre-B line JM1 and the monocytoid line THP1, although staining strongly with biotinylated IL-7, either lacked IL-7R mRNA or had barely detectable levels and only a low number of IL-7 receptors as determined by Scatchard analysis. In contrast, Burkitts' lymphoma lines Daudi and Raji bound biotinylated IL-7 with only low to moderate intensity but expressed strong levels of IL-7R mRNA. Incubation of RPMI 1788 cells with a titration of biotinylated IL-7 revealed binding with a Ka in the range of $1x10^{6}M^{-1}$-$1x10^{7}M^{-1}$ at ligand concentrations >$1x10^{-7}$M. This affinity is 100-1000 times lower than that reported for the cloned IL-7 receptor. Further data suggesting the existence of a distinct low affinity IL-7R was provided by two antibodies specific for the cloned IL-7R. Staining with these mAb correlated with both IL-7R mRNA levels and receptor expression determined by ^{125}I-IL7 binding but was not compatable with the distribution of reactivity seen with biotinylated IL-7. Using tritiated biotin to label IL-7, it was estimated that B-LCL and THP1 expressed a minimum of $1x10^{5}$ binding sites/cell. Pre-B lines and Burkitts' lymphoma lines were found to express total IL-7 binding sites in the range of $3x10^{4}$-$1x10^{5}$ and 1-2.5 x 10^{4} respectively. Proliferation of THP1 cells, expressing only the low affinity form of IL-7R, could be inhibited by the addition of IL-7 in a concentration-dependent fashion indicating that, at least on this cell line, binding of IL-7 with a Ka of $1x10^{6}M^{-1}$- $1x10^{7}M^{-1}$ can transduce a biological signal. Taken together the data presented here demonstrate the existence of a low affinity IL-7R, expressed in high numbers on hemopoietic cells of different lineages, which is the product of a gene distinct from that encoding the cloned IL-7 receptor.

P 419 A MOLECULAR ANALYSIS OF THE STRUCTURE AND FUNCTION OF THE HUMAN IFNγ RECEPTOR, Michael A. Farrar, Jose Fernandez Luna, Jesus Calderon, and Robert D. Schreiber, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

Murine L cells transfected with the full length human IFNγ receptor cDNA respond only to murine IFNγ and not to human IFNγ. In contrast, transfection of the full length human IFNγ receptor cDNA into murine L cells that contain human chromosome 21 (SCC16-5) resulted in the expression of a human IFNγ receptor that displayed the appropriate Ka ($3.1 \pm 1.4 \times 10^{9}$ M^{-1}) and in the generation of a cell that responded to both human and murine IFNγ (induction of MHC class I). SCC16-5 transfected with a truncated form of the human IFNγ receptor lacking 97% of the intracellular domain did not respond to human IFNγ, did not internalize and degrade ligand, and bound human IFNγ with a 30 fold lower affinity ($1.6 \pm 0.1 \times 10^{8}$ M^{-1}) than full length receptors on SCC16-5. Furthermore, through the use of recombinant human IFNγ receptor deletion mutants we have mapped the regions required for functional activity and receptor-mediated ligand internalization. A 48 amino acid region of the intracellular domain proximal to the membrane is required for receptor mediated ligand internalization. In addition, functional activity requires the presence of both the 48 amino acid transmembrane proximal and 39 amino acid carboxy terminal portions of the intracellular domain. These observations are consistent with the concept that functionally active IFNγ receptors require the presence of at least two proteins and additionally indicate that the receptor's intracellular domain is required for the generation of a functionally active receptor.

P 420 BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT EXTRACELLULAR DOMAIN OF THE HUMAN IL-4 RECEPTOR AND GENERATION OF MONOCLONAL ANTIBODIES, Jean-Pierre Galizzi, Odile Djossou, Pierre Garrone, Smina Ait-Yahia and Jacques Banchereau, Schering-Plough, Laboratory for Immunological Research, BP 11, 69571 Dardilly Cedex, France

A cDNA encoding the 130 kDa human IL-4 receptor has recently been cloned. It encodes for an open reading frame of 825 amino acids (AA) containing an extracellular domain of 207 AA with six N-linked potential glycosylation sites, a single transmembrane domain of 24AA and an intracellular domain of 569 AA.Two cDNAs encoding the extracellular domain of the IL-4R were constructed using two different endonuclease restriction sites (*Dra* III and *Aha* II). The proteins secreted by COS7 cells block IL-4 binding to cells. The affinity purified soluble IL-4R (*Dra* III construct) binds IL-4 with high affinity since the Kd value (50 -100 pM) is close to the Kd for IL-4 binding to the membrane receptor. The purified soluble IL-4R secreted in COS7 cell supernatant is a glycoprotein of 45,000 Da including 25,000 Da for the oligosaccharide moiety. Treatment of soluble IL-4 receptor (sIL-4R) with N-glycanase or endoglycosidase F did not affect IL-4 binding to the receptor. sIL-4R is a monomer in solution and binds IL-4 according to a 1:1 stoichiometry. sIL-4R was shown to be a powerful antagonist of *in vitro* IL-4 biological effects, including T and B cell proliferation as well as B cell differentiation. Finally, purified sIL-4R was used to generate monoclonal antibodies against the IL-4R.

P 421 THE IFNγ-RECEPTOR AS TOOL FOR THE DISCOVERY OF NEW IMMUNOMODULATORY DRUGS, Gianni Garotta, Michael Fountoulakis, Zlatko Dembic, Laurence Ozmen and Dietrich Stüber, Central Research Units, F. Hoffmann-La Roche Ltd., 4002 Basel, Switzerland

Agents that are able to neutralize the activity of IFNγ may have a potential application in the control of autoimmune diseases, hypersensitivities and alloreactions. The discovery of such antagonistic drugs needs the engineering of a soluble form of IFNγ-receptor. Based on the mapping of the epitope detected by those anti-receptor antibodies that inhibit the binding of IFNγ, we express the extracellular domain of this receptor in *E.coli*. The soluble protein (P41H6) we obtained, binds IFNγ with an affinity which is 10-fold lower as compared to the native membrane-bound receptor. This soluble IFNγ-receptor was used to develop a cell-free,solid phase receptor-binding assay for high-flux screening of natural or synthetic compounds, which inhibit IFNγ binding.

P 422 CHARACTERIZATION OF IL-1 RECEPTORS ON THE LIVER CELL-LINE HEPG2. J. G. Giri, R. Robb and R. Horuk, Du Pont Co., Glenolden, PA. 19036

IL-1 has been shown to induce acute phase proteins and IL-8 expression in hepatocyte lines. Therefore we examined the type of cell surface IL-1 receptors expressed on HepG2 hepatoma cells. Two different IL-1 binding glycoproteins have been identified to date: type I, of 80 kDa and type II of 60 kDa. Different receptor subtypes were shown to be expressed on various cells, but it was not clear whether both forms of IL-1 receptor could be expressed by the same cell. We found that HepG2 cells bind both IL-1α and ß with high affinity, [^{125}I] IL-1α with a K_D of 136 pM, and [^{125}I] IL-1ß with K_D of 180 pM (16,000 and 8500 binding sites/cell, respectively). A monoclonal antibody to the type I receptor did not significantly inhibit IL-1ß binding and phorbol ester treatment reduced binding by 68%, properties consistent with type II receptors. Cross-linking and ligand blotting with [^{125}I] IL-1α demonstrated the presence of a 60 kDa binding protein and in addition variable expression of the 80 kDa receptor. IL-1 induced complement factor B and IL-8 expression in these cells. The nature of the IL-1 receptor responsible for these responses is under investigation.

P 423 AN EPITOPE OF THE MOUSE IL-1 RECEPTOR INVOLVED IN THE BIOLOGICAL ACTIVITY BUT NOT BINDING OF IL-1, Andrew L. Glasebrook, Susan A. Stamnes, D. Lynn Phillips, Deena A. Hepburn, and William D. Roeder, Lilly Research Laboratories, Indianapolis, IN 46285.

We have made monoclonal antibodies (mAb) to the mouse IL-1 receptor (mIL-1R) as tools for investigating its structure and function. One of these mAb, LA-15.1, identifies an epitope of the mIL-1R which is involved in biological function but not binding of IL-1. A hamster cell line transfected with the full length cDNA of the 80kd (T cell form) mIL-1R and expressing $3x10^5$ receptors/cell was used to immunize rats. Spleen cells were fused to SP2/0 and hybridoma supernatants screened for the ability to inhibit proliferation of an IL-1-dependent cell line, T1165.17. One mAb, LA-15.6, completely inhibited T1165.17 proliferation while a second mAb, LA-15.1, effected partial inhibition (70%). When analyzed for their effects on IL-1 binding, it was observed that LA-15.6 completely inhibited the binding of IL-1 and vice versa. In contrast, LA-15.1 had no effect on the binding of IL-1 and was itself not inhibited from binding by IL-1. The epitopes recognized by LA-15.1 and LA-15.6 were different as evidenced by the simultaneous binding of both mAb to either soluble truncated mIL-1R or cell surface mIL-1R expressed on a variety of cell types. We conclude that the epitope defined by LA-15.6 is contained within the IL-1 binding site while the epitope defined by LA-15.1 involves a region of the mIL-1R that is involved in biological activity (i.e. signal transduction) but not binding of IL-1.

P 424 DEMONSTRATION OF IN VITRO AND IN VIVO EFFICACY OF TWO BIOLOGICALLY ACTIVE HUMAN SOLUBLE TNF RECEPTORS EXPRESSED IN E. COLI, Karin J. Hale, Christopher G. Smith, Rebecca W. Vanderslice, Susan Baker, Deborah A. Russell, Ramon I. Rivera, David Dripps, and Hiko Kohno, Synergen, Inc., 1885 33rd Street, Boulder, CO 80301

We purified, cloned, and characterized two human TNF inhibitors which are soluble fragments of the TNF receptors, Type I and Type II. The recombinant TNF inhibitors or binding proteins (rTBPI and rTPBII) are expressed in E. coli, refolded, and chromatographically purified to homogeneity. The rTBP's are stable and biologically active in several in vitro assays and in vivo models. Both recombinant proteins can inhibit the cytotoxic effect of huTNFα on L929 cells; however, rTBPI is ~5x more active than rTBPII. rTBPI and rTBPII both competitively inhibit ^{125}I-TNFα binding to cell surface receptors on U937 cells. However, recombinant and native TBPI were able to block this binding at a 10-30 fold lower concentration than required for recombinant and native TBPII. Using a murine model of cytokine-induced septic shock, we demonstrated the efficacy of multiple injections (IP) over a 12-24 hour period of either rTBPI or rTBPII in reversing the lethality associated with a single bolus injection (SC) of TNFα and IL-1β. In subsequent experiments, induction of increased IL-6 serum levels by hTNFα was prevented by injection (SC) of either protein 30 minutes before and 30 minutes after injection (IV) of the cytokine.

P 425 IDENTIFICATION OF A ESSENTIAL REGION FOR GROWTH SIGNAL IN HUMAN IL-4 RECEPTOR, Nobuyuki Harada, Gloria Yang, Atsushi Miyajima and Maureen Howard, DNAX Research Institute, Palo Alto, CA, USA

Interleukin-4 (IL-4) is a potent factor for growth and differentiation of various lymphoid and myeloid cells. IL-4 acts on these cells via binding to high affinity receptor. We have isolated cDNA encoding the human interleukin-4 receptor (IL-4R) by crosshybridization using murine IL-4R cDNA as a probe. Cos-7 cells transiently transfected with cDNA express high affinity binding sites for human IL-4. The human IL-4R has a 65% identity with the mouse IL-4R at the nucleic acid level and retains the typical structural motif of cytokine receptor family. Unlike many of the growth factor receptor which contain a tyrosine kinase domain within their cytoplasmic region, IL-4 R as well as other cytokine receptors has no consensus motif for tyrosine kinase domain. When the human IL-4R cDNA was transfected into a murine pro-B cell line, stable transformants were able to respond to human IL-4, indicating that human IL-4R transmit the IL-4 signal from cell membrane to nucleus. Using this expression system, we demonstrate that certain deletion mutants in the cytoplasmic domain which express high affinity IL-4R are not able to transmit growth signal.

P 426 DIFFERENCES IN IL-2 SIGNALS TRANSMITTED THROUGH p70 IL-2R AND p70-p55 IL-2R COMPLEX. Dragana Lj. Jankovic, Angelita Rebollo and Jacques Thèze. Unité d'Immunogénétique Cellulaire. Institut Pasteur, 25, rue du Docteur Roux, 75724 PARIS cedex 15, France.

We have isolated an IL-4-dependent T cell clone (LD8) expressing p70 IL-2R, but not p55 IL-2R, from a murine IL-2-dependent cytotoxic T cell line that expressed p70-p55 IL-2R. This clone has lost the capacity to proliferate in response to IL-2. The IL-2-mediated growth of LD8 cells was completely restored after introduction and expression of human p55 IL-2R cDNA. Clone LD8 and its transfectant expressing human p55 IL-2R are useful models for comparing signals transmitted through p70 IL-2R and p70-p55 IL-2R complex and for evaluating those necessary for cell proliferation. We have analysed the expression of genes coding for c-myc, c-myb, p55 IL-2R and transferrin receptor expression. While c-myc is an immediate T lymphocyte activation marker, c-myb, p55 IL-2R and transferrin receptor are early ones. Following stimulation by IL-2 in a dose range necessary to saturate p70 IL-2R, LD8 cells upregulate mRNA coding for the protooncogenes c-myc and c-myb. As soon as 4hr after IL-2 stimulation, p70 IL-2R mRNA is upregulated, while p55 IL-2R mRNA stays undelectable 16 hr after stimulation. Flow cytometric analysis showed the absence and the presence of transferrin receptor induction on LD8 cells after stimulation with IL-2 and IL-4, respectively. In contrast, the LD8 transfectant expressing human p55 IL-2R and cultured in IL-2, but not in IL-4, expresses and upregulates *de novo* murine endogenous p55 IL-2R. In parallel, induction of transferrin receptor is also demonstrated. These results demonstrate that dissociation between IL-2 signals transmitted through p55-p70 IL-2R complex and p70 IL-2R can be made at the level of expression of early activation markers, such as p55 IL-2R and transferrin receptor. The molecular basis for these different signal-transducing capacities of p70 and p70-p55 IL-2R systems is under the study.

P 427 **A Recombinant Soluble Erythropoietin Receptor: Expression and Initial Biochemical/Biological Characterization.** Simon S. Jones* and Sara Yankelev, Genetics Institute, Inc. 87 CambridgePark Drive, Cambridge, MA 02140.

The erythropoietin receptor appears to be the primary membrane component inducing proliferation and differentiation of erythroid progenitors upon binding of erythropoietin. We have recently isolated the gene for the human EPO receptor from an erythroleukemia cell line and fetal liver cDNA (Blood, 1990, 76:31-35), as well as a genomic clone from a peripheral blood lymphocyte library.

To further understand the physical interaction and mechanism of signal transduction by the EPO receptor complex we have generated, through mutagenesis, a cDNA encoding a soluble version of the EPO receptor. The cDNA has been expressed in COS-7 cells and stable CHO lines have been derived. The soluble EPO receptor is secreted as a single 33kDa product, which can be deglycosylated to yield a 27kDa product. The soluble form of the receptor binds iodinated EPO and generates a single, immunoprecipitable species of 72kDa upon crosslinking with disuccinimidyl suberate. Also conditioned media containing the soluble receptor specifically inhibits the incorporation of ^{3}H-thymidine by an EPO-dependent cell line, FDCPE, cultured in the presence of EPO, lu/ml.

The initial purification of the soluble EPO receptor from CHO conditioned media will also be described.

P 428 STRUCTURE-FUNCTION RELATIONSHIPS OF INTERLEUKIN-3 (IL-3): STUDIES BASED ON THE ANALYSIS OF INTERSPECIES IL-3 CHIMERIC MOLECULES. K. Kaushansky, S.G. Shoemaker, V.C. Broudy and N. Lin. Division of Hematology, University of Washington, Seattle, WA. 98195
IL-3 is an acidic glycoprotein involved in the response to inflammatory stimuli. To better understand the mechanism by which IL-3 exerts its effects, we sought to determine the region(s) of the polypeptide required for receptor (R) binding and biological activity. Primate (pr) and murine (mu) IL-3 fail to cross react in assays of R binding and of proliferative activity. Based on these findings and using a series of interspecies chimeric IL-3 molecules and species-specific IL-3 assays we report that the residues between pr IL-3 Val14 and Ile20 and between Phe107 and Glu119 are essential for activity. In contrast, residues in an amino terminal domain alone (Val14 through Gln45) are sufficient for binding to the IL-3 R. These studies imply that R binding and signal transduction can be dissociated. Next, the binding epitopes of a series of monoclonal antibodies (mcAb) to pr and mu IL-3 were mapped. Four distinct epitopes were defined which bind to R-blocking mcAb and their Fab fragments. Three of these epitopes correspond to the regions required for activity. Furthermore, two of the epitopes widely seperated in the primary strucutre of IL-3, cross-compete by ELISA. These findings provide evidence that two non-linear functional regions are in close juxtaposition in the tertiary structure of the molecule. Together, these data define the domains of IL-3 required for biological activity, provide a framework for the development of specific IL-3 antagonists and lead to testable models of the interaction of IL-3 and its receptor.

P 429 FUNCTIONAL RECONSTITUTION OF THE HUMAN GM-CSF RECEPTOR ON MURINE FACTOR-DEPENDENT CELL LINES, T. Kitamura[1], K. Hayashida[1], K. Sakamaki[1], T. Yotota[2], K. Arai[2] and A. Miyajima[1], [1]DNAX Research Institute, Palo Alto, CA 94304. [2]Institute of Medical Science, University of Tokyo, Tokyo, Japan.
We have cloned a second component (β chain) of human GM-CSF receptor, KH97, which was homologous to the murine low-affinity IL-3 receptor (AIC2A) and AIC2B. AIC2B has a 95 % homology with AIC2A but does not bind murine IL-3. Similarly, KH97 does not bind human IL-3 or GM-CSF by itself, but when it was coexpressed in fibroblasts with the low-affinity receptors for human GM-CSF (α chain), it conferred high-affinity binding for human GM-CSF. To study the signaling pathway of human GM-CSF, we introduced the α and/or β chain(s) of the human GM-CSF receptors into murine IL-3-dependent (Ba/F3) and IL-2-dependent (CTLL2) cell lines (summarized below).

	BaF/α	BaF/β	BaF/αβ	CTLL/α	CTLL/β	CTLL/αβ
binding of hGM-CSF(Kd)	2 nM	-	100 pM	2 nM	-	100 pM
response to hGM-CSF	+	-	+	-	-	+

These data indicate that both α and β chains are required for the signaling of the human GM-CSF receptor and that the α chain of the human GM-CSF receptor may interact with a murine homologue of KH97, AIC2A or AIC2B, and transduce the signal of human GM-CSF in Ba/F3 cells.

P 430 EXPRESSION OF MET IN MYELOID LEUKEMIA CELLS, Thomas E. Kmiecik, and George F. Vande Woude, ABL-Basic Research Program, NCI-Frederick Cancer Research & Development Center, Frederick, MD 21702

met is a member of the tyrosine kinase growth factor receptor family. The primary translation product is a 150 kD protein with typical extracellular, transmembrane, and kinase domains. We and others have previously shown that an immature glycosylated form of the newly synthesized protein, $p170^{met}$, undergoes proteolytic cleavage in the extracellular domain to yield 45 kD and 140 kD subunits, which are covalently associated through disulfide linkage in the mature heteroligomeric form.

We have detected expression of met RNA in several murine myeloid leukemia lines (Iyer et al., Cell Growth & Diff. 1:87-95, 1990). All cell lines express a single 7 kb met mRNA species with a mobility equal to that of met mRNA in nontransformed fibroblasts or epithelial cells. Expression was highest in the NFS-58, NFS-60, and NFS-107 myeloid leukemia lines and equaled levels seen in the fibroblasts or epithelial cells. Lower levels of expression were observed in NFS-61, NFS-78, NFS-124, DA1, DA3, and DA34. Expression was not detected by Northern analysis in WEHI-3 or FDCP1. $p170^{met}$ was identified in those cells expressing RNA by immunoprecipitation analysis using an antiserum directed against the carboxy terminal 21 amino acids and pulse chase analysis showed that the protein was processed to the mature $p140^{met}$ form. Relative synthesis rates of the met protein paralleled mRNA levels. Data will be presented on the phosphotyrosine content of met in these cells. We are currently examining these cells for the possibility of autocrine stimulation. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with ABL.

P 431 **ESTABLISHMENT OF A CELL-FREE, NON-RADIOACTIVE CYTOKINE RECEPTOR BINDING ASSAY**
L. Lauffer*, P. Oquendo*, R. Kurrle+ and G. Zettlmeißl*, Dept. of *Mol. Biology and +Immunology, Behringwerke AG, D-3550 Marburg, FRG

We constructed genetic fusions consisting of cDNA fragments encoding the extracellular portions of cytokine receptors and a genomic DNA segment encoding the Fc part of a human IgG1 molecule. Expression of these constructs in mammalian cells results in the secretion of soluble chimeric proteins that possess cytokine receptor and Fc epitopes. Apart from their potential value for therapeutic purposes, these soluble "receptor globulins" are extremely useful in that they can be used to establish a solid-phase, cell-free and non-radioactive receptor binding assay. Without compromising the receptor binding sites, the fusion proteins are bound to ELISA plates precoated with antibodies directed against their Fc part. Biotinilated ligands bind and can be measured using e.g. Streptavidin-POD conjugates. Any agent interfering with binding of ligand to receptor is identified by its decreasing the measured signal. So far, this binding assay has been established for the human receptors for IL-4, TNFalpha (80 kD form) and GM-CSF (low-affinity receptor). They were shown to be specific for the respective ligands. Only unlabelled IL-4, TNFalpha and GM-CSF displace binding of their biotinilated forms to their respective receptors (half-maximal inhibition at 2 ng/ml, 5 ng/ml and 400 ng/ml, respectively). Using the GM-CSF receptor binding assay, we could identify antibodies directed against binding epitopes on GM-CSF. Since these assays can be easily automated, they will also prove useful in other applications, e.g. for receptor screening programs. We thank D. Urdal, Immunex, Seattle and his colleagues for cDNA and recombinant cytokines and B. Seed, MGH, Boston for vectors.

P 432 BIOACTIVITY OF RECOMBINANT HUMAN TNF RECEPTOR FRAGMENTS
Werner Lesslauer, Hisahiro Tabuchi, Reiner Gentz, Ernst-Juergen Schlaeger, Manfred Brockhaus, Georges Grau*, Pierre Francois Piguet*, Pascal Pointaire*, Pierre Vassalli* and Hansruedi Loetscher, Central Research, F. Hoffmann-LaRoche LTD, 4002 Basel, Switzerland
*Department of Pathology, University of Geneva, 1211 Geneva 4, Switzerland

To investigate biological functions of TNF, recombinant soluble fragments of the two human TNF receptors, TNFRα (75kD) and TNFRβ (55kD), were expressed in baculovirus and CHO or myeloma cell expression systems. The extracellular domain of TNFRβ (rsTNFRβ) and a rsTNFRβ/human Cγ3-fusion protein (rsTNFRβ/hCγ3) were purified to apparent homogeneity by TNF- or protein G-affinity chromatography. The equilibrium TNFα binding properties of the recombinant proteins were similar to those of the native receptors with Kd's of 0.4 nM (rsTNFRβ) and 0.6 nM (rsTNFRβ/hCγ3). In a receptor binding assay rsTNFRβ/hCγ3 inhibited the binding of TNFα to native TNFRα and TNFRβ efficiently (>50% inhibition at 1:1 molar ratio), whereas rsTNFRβ was 10-100 fold less potent. Similarly, in a WEHI 164 assay a >50% inhibition of TNF cytotoxicity at a 1:1 molar ratio was found with rsTNFRβ/hCγ3. The efficacy *in vivo* was tested in a well characterised model of TNF mediated lethality. D-galactosamine sensitised mice injected with a lethal dose of LPS (LD 100) were shown to be fully protected by intravenous application of rsTNFRβ/hCγ3. The properties of the recombinant receptor peptides in other *in vivo* systems are currently being investigated.

P 433 DETECTION OF SOLUBLE TNF RECEPTORS IN URINE AND AMNIOTIC FLUID FROM PREGNANT WOMEN. N.B. Liabakk, R. Austgulen, H. Scott*, M. Brockhaus**, W. Lesslauer** and T. Espevik. Institute of Cancer Research, University of Trondheim, N-7006 Trondheim, Norway, *Laboratory for Immunohistochemistry and Immunopathology, The Norwegian National Hospital, Oslo, Norway, and **F.Hoffmann-La Roche AG, 4002 Basel, Switzerland.

Immunoassays were established for detection of soluble 55 kD and 75 kD TNF receptor (R) fragments in biological fluids. These immunoassays were based on monoclonal antibodies against different epitopes of the TNFR and were specific for unoccupied TNFR. Approximately 50% of the urines collected from pregnant women contained high levels of 55 kD TNFR fragments. The levels of 55 kD TNFR fragments in normal control urines was hardly detectable with this assay. The levels of 75 kD fragments seemed to be lower than 55 kD fragments in urines from pregnant women. In addition, amniotic fluids were found to contain high levels of 55 kD TNFR fragments. Antibodies against the 55 kD TNFR were also used for immunohistochemical staining of freeze sections of placenta tissue which revealed p55 TNFR positive cells. These data indicate that soluble TNFRs may play an important role in pregnancy.

P 434 A POINT MUTATION IN THE EXOPLASMIC DOMAIN OF THE ERYTHROPOIETIN RECEPTOR RESULTS IN GROWTH FACTOR INDEPENDENT ACTIVATION AND TUMORIGENICITY., Greg D. Longmore, Akihiko Yoshimura and Harvey F. Lodish, Whitehead Institute for Biomedical Research, Cambridge, MA., 02142.

A retroviral vector containing the wild type EPOR cDNA was packaged and amplified in a mixture of ecotropic/amphotropic helper cell lines. A murine bone marrow-derived cell line, strictly dependent on IL-3 for growth, was infected and cells expressing the EPOR were selected for growth in the presence of erythropoietin. From such a population we isolated cell lines with mutated EPORs which grow in the absence of any hematopoietic growth factors. The mutant EPOR cDNAs contained a point mutation in the exoplasmic domain, with or without a deletion of the 42 carboxyterminal amino acids. The Arginine-Cysteine mutation at codon 129 itself is sufficient to induce factor-independent growth, is retarded in transport from the endoplasmic reticulum, and its protein half-life is prolonged. These changes of receptor metabolism are reminiscent of those seen in the growth factor independent activation of the EPOR through association with the Spleen Focus Forming Virus env gene product gp55. The carboxyterminal deletion enhances the sensitivity to EPO for growth but does not alter EPOR metabolism. Cells expressing constitutively active EPORs or wild type EPOR plus gp55 were tumorigenic in syngeneic mice. Cells expressing wild type or truncated EPORs are not tumorigenic. Experiments studying the role of constitutively active EPORs in hematopoiesis and erythroleukemia are ongoing.

P 435 COMPARISON OF RECOMBINANT SOLUBLE HUMAN GAMMA INTERFERON RECEPTORS PRODUCED IN E.COLI AND MAMMALIAN CELLS, Daniel Lundell, James Fossetta, Mary Petro, Jacqueline Carter, Gregory Deno, Chuan-Chu Chou, Satwant Narula, Paul Zavodny, and Charles Lunn , Department of Biotechnology/Molecular Biology, Schering-Plough Corporation, Bloomfield, New Jersey, 07003

Human interferon-gamma (IFN-g) is a pleitropic lymphokine which exerts a wide variety of biological effects through a high affinity, cell surface receptor which has been cloned (Aguet, M., et al. 1989. Cell **55**, 273). We obtained the complete human gamma interferon receptor cDNA through PCR amplification of a human placenta lambda gt-11 library. The coding region for the extracellular domain of the human IFN-g receptor was removed from the full-length cDNA clone by use of the polymerase chain reaction (PCR). The PCR modified coding region was introduced into expression plasmids for stable transformation of mammalian cells and E.Coli. The mammalian cell expression plasmid contained the intact natural human IFN-g leader peptide, while the mature coding region was fused to a prokaryotic leader sequence for periplasmic expression in E.Coli. Recombinant soluble human IFN-g receptor was purified (>80%) from both sources using a combination of ion exchange chromatography, gel filtration and/or affinity chromatography using an IFN-g agarose column. Both soluble human IFN-g receptor preparations are equally competetive in a cell-based receptor binding assay, as well as equally active in inhibiting the antiviral activity of human IFN-g on human fibroblasts, despite the lack of post-translational modification of the receptor by E.Coli. Sufficient quantities of recombinant soluble human IFN-g receptor may be prepared from both sources to support biochemical and biological characterization.

P 436 PROTEIN KINASE C BUT NOT TYROSINE KINASE IS INVOLVED IN HUMAN INTERLEUKIN-3 (IL-3) INDUCTION OF C-JUN PROTO-ONCOGENE IN NORMAL HUMAN MONOCYTES, R. Allan Mufson, Julie D. Giles and Janet M. Szabo, American Red Cross Holland Laboratory, Cell Biology Laboratory, Rockville, MD 20855

Our laboratory has previously shown that human IL-3 binds to a specific saturable cell surface receptor of approximately 65 kDa molecular weight (Blood 74:2652, 1989). The events occurring after the interaction of human IL-3 with its receptor are, however, not well characterized. We have used normal human monocytes as a model system to begin elucidating the signal transduction mechanism associated with the IL-3 receptor. Normal human monocytes deprived of human serum and colony stimulating factors become quiescent *in vitro*. Stimulation of these cells with recombinant IL-3 induces expression of the c-jun proto-oncogene, as detected by Northern blotting of total monocyte RNA. Thirty minutes after stimulation expression of the c-jun mRNA was maximal and by 120 min after stimulation the mRNA for this gene is no longer detectable. This protooncogene is also induced in these cells by phorbol ester through direct stimulation of protein kinase C. Concentrations of the protein kinase C inhibitor 1-(5-isoquindinylsulfonyl)-2-methylpiperazine (H-7) between 30 and 100 μM (5-20 x Ki) inhibit this induction by phorbol ester. The same concentration range of H-7 completely inhibited the induction of c-jun by human IL-3. A structural analog of H-7 designated HA1004 preferentially inhibits cyclic nucleotide dependent protein kinase rather than protein kinase C. HA10004 at 5-20 x Ki did not inhibit IL-3-induced c-jun mRNA accumulation. Further 30 μM genistein which is an effective inhibitor of cellular tyrosine kinases did not inhibit IL-3 induced c-jun expression. It is therefore likely that IL-3 interaction with its receptor stimulates a phospholipase which generates diacylglycerol and stimulates a cellular Ca^{++}/phospholipid dependent protein kinase C.

P 437 TYROSINE PHOSPHORYLATION OF THE INTERLEUKIN-3 RECEPTOR SIGNALS ITS OWN CLEAVAGE, Alice L-F Mui, Robert J. Kay, R. Keith Humphries, Gerald Krystal, Terry Fox Laboratory, B.C. Cancer Research Centre, Departments of Pathology and Medicine, University of British Columbia, Vancouver, B.C., Canada.

The murine interleukin-3 receptor (mIL-3R), is a member of a recently identified family of hematopoietic receptors characterized by the absence of a tyrosine kinase domain. Nonetheless, this receptor becomes rapidly tyrosine phosphorylated upon binding mIL-3. In order to investigate the signal transduction pathway utilized by mIL-3, we have purified the mIL-3R from a high mIL-3R expressing cell line, $B6SUtA_1$. Interestingly, studies comparing the stability of the 140 kDa tyrosine phosphorylated form of this receptor with its non-tyrosine phosphorylated form reveal that the former is far less stable and is rapidly cleaved to a 70 kDa fragment. Mixing experiments demonstrate that the differential stability of the tyrosine and non-tyrosine phosphorylated mIL-3R's is not due to different levels of co-purifying proteases or inhibitors in the two receptor preparations. Western analyses, with an anti-mIL-3R antibody, of $B6SUtA_1$ cells show that the 70 kDa fragment is also generated in intact cells that have been stimulated with mIL-3. Furthermore, this cleavage occurs with purified plasma membranes and is not inhibited by methylamine, an inhibitor of lysosomal function. Thus, ligand induced tyrosine phosphorylation of the mIL-3R appears to lead, in vivo, to an increased susceptibility to cleavage of this receptor at the cell surface. This proteolytic process may play an important role in the mechanism of action of mIL-3.

P 438 THE MOUSE SOLUBLE IFNγ-RECEPTOR AS POSSIBLE IMMUNOMODULATORY AGENTS, Laurence Ozmen, Reiner Gentz, Michael Fountoulakis, Zlatko Dembic and Gianni Garotta, Central Research Units, F. Hoffmann-La Roche Ltd., 4002 Basel, Switzerland

Several *in vivo* experiments support the hypothesis that an IFNγ antagonist may have therapeutical application in autoimmune diseases, hypersensitivities and alloreactions. IFNγ exerts its biological activity through the binding to a single chain cell surface receptor. In order to provide some proofs of in vivo therapeutical application of IFNγ antagonists, recombinant baculoviruses were constructed and isolated that express the external domain of the mouse IFNγ-receptor. The soluble receptor secreted by insect cells is N-glycosylated, exhibits similar binding capacity as the cell surface receptor, inhibits the binding of IFNγ to cells and neutralizes the biological activity of IFNγ. To test the feasibility of using the soluble receptor as an antagonist we assessed: i) the immunogenicity of this protein after either injection in Freund's adjuvant or chronic injection for two months: ii) the half-life of iodinated soluble receptor after injection into mice. We are now studying the ability of mouse soluble IFNγ-receptor to modify the onset of acute GvHR (alloreaction), chronic GvHR (lupus-like disease) and Schwartzman's reaction.

P 439 CHIMAERIC TNF-RECEPTOR - IgG MOLECULE ACTS AS SOLUBLE INHIBITOR OF TNF MEDIATED CYTOTOXICITY. Karsten Peppel and Bruce Beutler; Howard Hughes Medical Institute, Dallas, TX 75235

Recently two different molecular weight forms (55 kD and 75 kD) of the human TNF receptor have been isolated and cloned. Based upon the published sequence of the 55 kD form we constructed a chimaeric gene consisting of the extracellular domain of its receptor linked to an IgG (G1) constant region. This construct was inserted into a mammalian expression vector. COS cells transiently transfected with this vector secrete high levels of a TNF inhibitory activity when examined by a standart cytotoxicity assay.

Our goal is to determine if we can reduce or eliminate TNF related pathologies in mice. The high affinity and specificity of this molecule to TNF makes it a suitable inhibitior of this cytokine. Here we report on the results of these experiments.

P 440 THE INTERLEUKIN-1 RECEPTOR OF HUMAN TRANSFORMED B CELLS: REGULATION OF RECEPTOR EXPRESSION AND BINDING OF IL-1ß VERSUS IL-1α, Janet Plate, Richard I Cronkhite and Dana Tarandy, Section of Medical Oncology, Department of Medicine and the Department of Immunology, Rush-Presbyterian-St Luke's Medical Center, Chicago, IL 60612.

Our laboratory recently demonstrated that two human B cell lines exhibit dramatic binding differences for IL-1ß and IL-1α. Each cell line, Raji and RPMI 1788, express 100 and 37 times more receptors per cell, respectively, for IL-1ß than for IL-1α. This difference in IL-1ß versus IL-1α binding was further emphasized in competition experiments for IL-1 receptor binding. IL-1ß readily and effectively competed with both radiolabeled IL-1ß and IL-1α for binding to the IL-1 receptor. IL-1α, on the other hand, only effectively competed with IL-1α whereas the same concentration of IL-1α had *no effect* on IL-1ß binding. Cross-linking studies followed by SDS-PAGE analyses confirmed graphically the receptor number differences described for IL-1α and IL-1ß and they revealed receptor sizes of approximately 60,000 daltons with both IL-1α and IL-1ß as cross-linked ligands. In the studies reported here, we confirm the effective upregulation in the surface expression of IL-1 receptors with dexamethasone and further demonstrate even greater increases in the levels of surface IL-1 receptor expression with modulators of cellular activity in the B cell lines. The kinetics of the decreases in specific cellular activity and the coincident affects on IL-1 receptor expression will be reported. Differential affects on regulation of IL-1α and IL-1ß receptor expression will also be included in this presentation.

P 441 MOLECULAR CLONING OF A SOLUBLE FORM OF THE HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR RECEPTOR (GM-CSF-R), M. Raines, L. Liu, S. Quan, J. DiPersio*, D.W. Golde. Department of Medicine, Division of Hematology-Oncology, University of California, Los Angeles, CA 90024, and *Department of Medicine, Hematology Unit, University of Rochester, Rochester, NY 14642.

In an effort to study the structure and function of the GM-CSF receptor, we have molecularly cloned the low-affinity human GM-CSF-R from a choriocarcinoma cell line. Two sets of overlapping PCR primers corresponding to the published sequence[1] were used to amplify the entire human GM-CSF-R coding sequence from BeWo cDNA. The majority of clones isolated were identical to the previously published sequence.[1] Approximately 30% of the PCR amplified clones contained a 97 nucleotide deletion which removed the entire transmembrane domain. This deletion results in an altered translational reading frame and encodes a truncated version of the GM-CSF-R with 16 new amino acids at its C-terminus. The predicted protein should encode a secreted or soluble form of the GM-CSF-R. Both forms of the GM-CSF-R were transfected into ψ-2 cells. Using a soluble ^{125}I-GM-CSF binding assay we have been able to verify the secreted nature of the latter form. Both GM-CSF-R forms displayed similar affinities with K_ds of approximately 10nM. RNase protection analysis indicates that the secreted form is not an artefact of cloning and constitutes approximately 10 to 20% of the total GM-CSF-R mRNA in BeWo and JAR cells. Thus the GM-CSF-R appears to be another member of the hematopoietin receptor family which may be naturally secreted and may play an important role in immune function.

[1]Gearing, D.P., et al. EMBO J. (1989) 8:3667-3676.

P 442 STRUCTURAL AND FUNCTIONAL STUDIES ON THE HUMAN INTERLEUKIN-6-RECEPTOR: BINDING; CROSSLINKING; INTERNALIZATION AND DEGRADATION OF INTERLEUKIN-6 BY FIBROBLASTS TRANSFECTED WITH HUMAN INTERLEUKIN-6-RECEPTOR cDNA

Stefan Rose-John, Erich Hipp, Dorothee Lenz, Hubert Korr, Toshio Hirano*, Tadamitsu Kishimoto*, and Peter C. Heinrich
Institut für Biochemie der RWTH Aachen, 5100 Aachen, FRG and *Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan

Interleukin-6 (IL-6) is a multifunctional cytokine produced by many different cells. It shows a pleiotropic spectrum of action via specific cell surface receptors on different target cells. Studies on the biochemistry of the IL-6-R have been hampered by the fact, that normal cells express extremely low numbers of IL-6-R on their surface. Therefore we have stably expressed a cDNA coding for the human IL-6-R in murine NIH 3T3 fibroblasts. Transfected cells exhibited a single class of binding sites for IL-6. Affinity crosslinking of iodinated IL-6 to the IL-6-R expressing cells led to the identification of three IL-6 containing protein complexes of molecular masses of 100, 120, and 200 kDa suggesting a complex organization of the IL-6-R in the plasma membrane. IL-6 added to the transfected cells exerted a strong growth inhibition as measured by cell counting or ornithine decarboxylase expression. IL-6 was internalized and partly degraded by NIH 3T3 fibrobalsts overexpressing the human IL-6-R.

P 443 **CHARACTERIZATION OF HUMAN INTERLEUKIN 3 AND GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTORS ON THE HUMAN LEUKEMIA CELL LINE M-07: EVIDENCE FOR DISTINCT AND SHARED MEMBRANE RECEPTORS**, B. Ryffel, G. Zenke, G. Woerly, and U. Strittmacher, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland.

The biologic response of the human leukemia cell line M-07 to granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin 3 (IL-3) and interleukin 4 (IL-4) is mediated by a low number of high affinity receptors (kD ~ 100 pM). Cross-competition studies revealed that IL-3 and GM-CSF partially inhibited specific binding of the heterologous radiolabeled ligand, whereas IL-4 binding was not affected by the other cytokines. The molecular mechanism of these findings was investigated by chemical crosslinking and immunoprecipitation. Crosslinking with radiolabeled ligands gave a trimolecular membrane complex consisting of a major 73 kDa and two minor 120 and 128 kDa membrane proteins for IL-3, and a major 84 kDa and two minor 120 and 130 kDa proteins for GM-CSF. Based on saturation and competition studies it is concluded that the major 73 kDa and 84 kDa protein are involved in low affinity binding for IL-3 and GM-CSF respectively, whereas the minor, higher molecular weight proteins have high affinity binding. Whereas the 73 kDa IL-3 receptor protein and the 84 kDa GM-CSF receptor protein represent distinct and non-linked membrane proteins since only the homologous protein was immunoprecipitated from crosslinked cells, it is likely that the minor proteins at least the 120 kDa species is shared for IL-3 and GM-CSF.

P 444 LOCALIZATION IN HUMAN INTERLEUKIN-2 OF THE BINDING SITE TO THE α CHAIN (p55) OF THE INTERLEUKIN-2 RECEPTOR, K. Sauvé, *M. Nachman, *C. Spence, *P. Bailon, #W.H. Tsien, #J. Kondas, #J. Hakimi, and G. Ju, Molecular Genetics, *Protein Biochemistry, #Immunopharmacolgy, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Human interleukin-2 (IL-2) analogs were used to identify specific residues that interact with the 55-kDa subunit (p55) or α chain of the human interleukin-2 receptor (IL-2R). Analog proteins with specific amino acid substitutions within the B helix of IL-2 were inactive in competitive binding assays for p55 but retained substantial competitive binding to the intermediate-affinity p70 subunit. These analogs varied in their ability to interact with the high affinity p55/p70 receptor. Despite the absence of binding to p55, all the analogs exhibited significant biological activity as assayed on the murine CTLL cell line. The K_d's of some of these analogs for p70 were determined to be consistent with intermediate-affinity binding, and were not significantly affected by the presence of p55. These results confirm the importance of the B helix in IL-2 as the locus for p55 receptor binding, and support a revised model of IL-2/IL-2R interaction.

P 445 MOLECULAR CLONING OF THE INTERLEUKIN-6-RECEPTOR FROM HUMAN HEPATOMA CELLS: SEQUENCE IDENTITY WITH THE INTERLEUKIN-6-RECEPTOR FROM NK-CELLS

Heidi Schooltink, Tanja Stoyan, Dorothee Lenz, Toshio Hirano*, Tadamitsu Kishimoto*, Peter C. Heinrich and Stefan Rose-John
Institut für Biochemie der RWTH Aachen, Pauwelsstrasse, 5100 Aachen, FRG
*Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan

Interleukin-6 (IL-6) acts on cells of the immune system, hematopoetic stem cells and on hepatocytes. Since IL-6 acts via specific cell surface receptors, the various biological responses of these different cells could be due to different IL-6-receptors (IL-6-R) or to different signal transduction pathways. Using the cDNA coding for the IL-6-R from NK-cells, we detected a single cross-hybridizing mRNA species in the human hepatoma cell line HepG2, which was up-regulated after treating the cells with dexamethasone. Poly(A)RNA from dexamethasone-treated HepG2 cells was used for the construction of a cDNA library in λgt 10. Screening of this cDNA library with the IL-6-R cDNA from NK-cells resulted in the isolation of several cDNA clones. DNA-sequencing revealed complete identity between the IL-6-R of hepatocytes and of NK-cells. By use of a recombinant E.coli fusion protein a polyclonal antiserum against the hepatic IL-6-R was raised in rabbits. This antiserum precipitated the IL-6-R of HepG2 cells crosslinked to iodinated IL-6, indicating that the cloned cDNA indeed codes for the hepatic IL-6-R.

P 446 **IL1 UPREGULATES SURFACE IL1 RECEPTORS IN A MURINE THYMOMA CELL LINE.**

R. Solari, S.J. Grenfell & N. Smithers. Dept. Cell. Sci, Glaxo Group Res, Greenford, Middlesex & Yamanouchi Res. Inst., Littlemore Hospital, Oxford, OX4 4XN.

In this study, we have investigated the effect that IL1 has on IL1 receptor expression in the murine thymoma cell line, EL4. EL4 cells express both high (65pM, 986 receptors/cell) and low (14.5nM, 10417 receptors/cell) affinity IL1 receptors. Affinity crosslinking studies performed at high and low ligand concentrations revealed an identical profile of ~200, ~80 and ~23kDa binding proteins. Preincubations with IL1α enhanced ^{125}I-IL1α binding to EL4 cells in a time and energy dependent manner. Increased binding could be due to either enhancing the binding affinity or increasing receptor numbers/cell. Since a short incubation with IL1 failed to increase IL1 receptor numbers, increased binding was unlikely to be due to changes in the binding affinity. Receptor numbers may be increased by i) increased receptor synthesis ii) release from an intracellular pool iii) recruitment of cryptic receptors. *De novo* synthesis could not account for increased binding, since the increases in binding could not be prevented by cycloheximide. Equilibrium binding studies on CHAPS solubilized EL4 cells failed to reveal an intracellular pool of IL1 receptors. Recruitment of cryptic receptors may therefore account for the observed increases in ligand binding.

P 447 INITIAL CHARACTERIZATION OF THE RECEPTOR FOR CYTOTOXIC LYMPHOCYTE MATURATION FACTOR (CLMF) ON PHA-ACTIVATED HUMAN PERIPHERAL BLOOD LYMPHOBLASTS (PHA-PBL),

T. Truitt, B. Desai, F. Podlaski, A. Wolitzky, P. Quinn, A. Stern, M. Gately and R. Chizzonite, Roche Research Center, Hoffmann-LaRoche Inc., Nutley, N.J. 07110

CLMF is a novel cytokine which stimulates proliferation of PHA-PBL and synergizes with interleukin-2 to cause the induction of lymphokine-activated killer cells. CLMF is a 75 kDa heterodimer composed of disulfide bonded 40 kDa and 35 kDa subunits. The binding of ^{125}I-labelled CLMF (7-10 uCi/ug; 50-100% bioactive) to PHA-PBL is saturable and specific. Scatchard analysis of the binding data identifies a single binding site with an apparent dissociation constant of ~50-100 pM and 1000-6000 sites per activiated PBL. The kinetics of ^{125}I-CLMF binding to PHA-PBL was very rapid at both 4°C and 22°C; reaching equilbrium within 60 min. The rate of dissociation of ^{125}I-CLMF was slow, with a T1/2 of 6 hrs at 22°C. Affinity cross-linking of ^{125}I-CLMF to PHA-PBL at 4°C produces a major complex of ~250 kDa and a minor complex of ~170 kDa. These data suggest that the receptor may be composed of a single protein of ~180 kDa or two proteins of ~100 kDa and ~180 kDa. Anti-40 kDa subunit antibodies block ^{125}I-CLMF binding to PHA-PBL and neutralize CLMF bioactivity, suggesting that CLMF bioactivity is mediated by binding to this newly identified CLMF receptor on the PHA-PBL.

P 448 REGULATION OF CD27, A T-CELL-SPECIFIC MEMBER OF A NOVEL FAMILY OF MEMBRANE RECEPTORS, René van Lier[1], Rolien de Jong[1], Wil Loenen[2], Miranda Brouwer[1], Evert de Vries[2] and Jannie Borst[2]. [1]Central Lab. Neth. Red Cross Blood Transf. Serv. & Lab. Exp. Clin. Immunol., Univ. of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands; [2]The Netherlands Cancer Institute, Amsterdam.

CD27 belongs to a newly defined family of transmembrane R, together with the Nerve Growth Factor R, two distinct Tumor Necrosis Factor R and CD40. The function of CD27 is unknown, but on the basis of structural and functional properties, we postulate that it plays a role in the events subsequent to T-cell activation, possibly as a cytokine R. Membrane expression of CD27 strongly increases after T-cell activation via the TCR/CD3 complex or the CD2 molecule. In contrast, direct stimulation of protein kinase C (PKC) by phorbol esters markedly downregulates CD27 surface expression. This downregulation most likely does not result from CD27 phosphorylation, since both anti-CD3 mAb and PMA induce hyperphosphorylation of CD27 on serine residues. Stimulation of T cells with anti-CD3 mAb strongly increases steady state CD27 mRNA levels, while PMA treatment greatly reduces these transcript levels. Dissection of the TCR/CD3-induced signalling pathways showed that cytoplasmic cAMP as well as Ca^{2+} concentrations contribute to the increase of CD27 expression. These data indicate that upon antigen-specific T-cell stimulation, membrane expression of CD27 is regulated at the RNA level through the joint action of distinct TCR/CD3-associated signalling pathways.

P 449 STUDIES OF THE STRUCTURE AND FUNCTION OF IL-2 RECEPTORS INDUCED DURING rIL-2 THERAPY ON POST-THERAPY T AND NK CELLS. Stephan D. Voss#, Richard J. Robb*, Mitsuru Tsudo, Kazuo Sugamura and Paul M. Sondel#. #Dept. of Human Oncology, University of Wisconsin, Madison, WI 53792, *E.I. DuPont de Nemours Inc., Glenolden Laboratory, Glenolden, PA.

Patients receiving rIL-2 for the treatment of cancer show a dramatic expansion of $CD56^+$ peripheral blood NK cells during the course of IL-2 therapy. Upon re-exposure to IL-2 in vitro, post-therapy NK cells respond vigorously in both proliferative and cytotoxic assays, while post therapy T cells are nonresponsive to IL-2 and show decreased responses to specific recall antigens and polyclonal mitogenic stimulation. Our efforts at determining the basis of these functional differences in IL-2 responsiveness have focused in part on studies of the IL-2R induced on T and NK cells during the course of IL-2 therapy. We find increased expression of the IL-2R β chain on post-therapy NK cells relative to pretherapy, with no detectable role for the IL-2R α chain (Tac) in either proliferation or IL-2 binding. T cells, in contrast, express high affinity IL-2R. Experiments are underway to determine if the IL-2R expressed on post-therapy T cells internalize IL-2 and trigger the phosphorylation of protein substrates known to be phosphorylated during stimulation of activated T cells with IL-2 (for example, the IL-2R β chain and $p56^{lck}$ kinase). We have also determined that the majority of the IL-2R β chains found on post therapy NK cells are not IL-2 binding, implying a role for a γ chain in the formation of a functional intermediate affinity IL-2 receptor. Experiments in progress are aimed at characterizing the putative γ chain that should be associated with only a minority of the IL-2R β chains on post therapy NK cells and with the majority of the β chains found on YT cells.

P 450 **EXPRESSION AND REGULATION OF IL-4 RECEPTORS ON HUMAN MONOCYTES AND ACUTE MYELOBLASTIC LEUKEMIC CELLS**

AJ Wagteveld, AK v. Zanten, MT Esselink, MR Halie and E Vellenga. Department of Hematology and Nuclear Medicine, State University of Groningen, The Netherlands.

In this study we present evidence that human monocytes and acute myeloblastic leukemic (AML) cells contain both high- and low-affinity interleukin-4 (IL-4) receptors. On monocytes 183 ± 132 high-affinity binding sites/cell with a Kd of 60 ± 29 pMol/l, and 1,500 ± 600 low-affinity receptors with a Kd of 2.3 ± 0.4 nMol/l, (X ± SD, n=6) could be demonstrated. On AML cells a comparable number and binding affinity of IL-4 receptors were observed (77 ± 36 high-affinity receptors with Kd 72 ± 31 pM and 2,400 ± 1,000 low-affinity receptors with Kd of 2.2 ± 0.7 nM). In addition, no cross-competition was shown between radiolabeled IL-4 and IL-1α, IL-3, IL-6, IL-7, G-CSF, and GM-CSF. Both types of receptors on monocytes as well as on leukemic blasts could be down-modulated in a similar fashion by IL-4, activators of protein kinase C (PKC), but not by the calcium-ionophore A23187. The down-modulation by PKC activators was caused by an increased internalization, degradation and release of radiolabeled IL-4 in the medium. Finally, the functionality of the IL-4 receptor was tested on AML cells with a ^{3}H-thymidine proliferation assay. In 8/11 cases IL-4 affected AML proliferation. These data indicate two types of receptors on normal and leukemic cells which can be modulated by external activation signals in an analogous way.

P 451 TNF RECEPTOR EXPRESSION DURING T CELL ACTIVATION, Carl F. Ware*, Todd L. VanArsdale*, Paul C. Crowe*, Janet L. Andrews*, Marcia H. Grayson*, Rita Jerzy‡, Craig A. Smith‡, Raymond G. Goodwin‡, *Division of Biomedical Sciences, Univ. of California, Riverside, CA 92521 and ‡Immunex Corporation, Seattle, WA 98101

Human $CD8^+$ and $CD4^+$ CTL clones and a $CD4^+$ T cell hybridoma (II-23.D7) were characterized for their expression of TNF-α Receptors (TNFR) at the protein and mRNA levels. Immunoprecipitation of ^{125}I-TNF-receptor complexes revealed that the 80 kDa form was the major receptor expressed in both subpopulations of T cells. A band equivalent to the 55 kDa receptor was present in trace amounts. Activation of resting T cells from PBL with anti-CD3 resulted in the expression of several size species of mRNA for the 80 kDa TNFR and this complex pattern evolved with the kinetics of T cell activation. The II-23.D7 line expressed a 4.5 kb mRNA, but upon activation with phorbol ester, exhibited rapid down regulation of TNFR, and the induction of a 4 kb mRNA. In CTL clones the size pattern of the 80 kDa TNFR mRNA appeared to segregate with the CD4 and CD8 surface markers. Thus two factors, the state of T cell activation and the T cell subpopulation, contribute to the observed mRNA size heterogeneity. These results indicate a high level of complexity controlling the expression of TNFR during T cell activation and suggest that transcriptional control may play an important role in regulating the expression of this receptor family in T cells.

P 452 IDENTIFICATION OF AMINO ACID RESIDUES IMPORTANT FOR THE BIOLOGICAL ACTION OF HUMAN INTERLEUKIN-1α. Stephen Yanofsky and Gerard Zurawski, Department of Molecular Biology, DNAX Research Institute, 901 Calif. Ave, Palo Alto, CA 94304

We have generated a "functional map" of human interleukin-1α (hIL-1α) by determining the side chain requirements at each position of the polypeptide chain. This was accomplished by performing substitution mutagenesis on a synthetic hIL-1α gene in which 2-4 codons at a time were replaced by random sequence. Mutant proteins were expressed in E. coli and analyzed for IL-1 activity using the mouse T-cell (D10) proliferation assay. For each region, 500-1000 mutant proteins were analyzed, the DNA for at least 10 active mutant proteins was sequenced, and extracts were prepared to quantitate IL-1 activity levels. This data permitted assignment of the functionally acceptable side chains at each position in the hIL-1α protein. At most positions, no preference was found for any particular side chain or side chain type. However, at 24 positions, hydrophobic side chains were preferred, while at 16 positions we observed a requirement for polar side chains. In addition, 5 positions exhibited a requirement for a specific side chain and 2 positions required a positively charged side chain. Examination of the location of these residues on the crystal structure of the homologous hIL-1β protein revealed that: 1) the majority of the residues with a strong preference for hydrophobic side chains lie in the interior of the protein; these residues are presumably crucial for the correct folding or stability of the molecule 2) most of the residues with a requirement for polar or specific side chains fall on the surface of the molecule. These residues lie on one face of the molecule and are hence likely candidates for residues important for either receptor binding or IL-1 action. Examination of the specific side chain requirements at these positions and characterization of the appropriate mutant proteins should help to further define the role of these residues in IL-1 binding and action.

P 453 REGULATION OF IL-2R β mRNA EXPRESSION IN BALB/C AND NZB/WF_1 B CELLS. Tomohiro Yoshimoto, Kenji Nakanishi, Satoshi Hirose, and Kazuya Higashino. Third Dept.of Int. Med. Hyogo College of Mrdicine, Nishinomiya, Hyogo 663, Japan.

Resting Balb/c CD5(-) B cells required anti-IgM, IL-4, IL-5 and IL-2 and/or IL-6 for their growth and differentiation, while NZB/WF_1 peritoneal CD5(+) B cells required only IL-5 for their growth and differentiation. Here we examined the mechanism whereby anti-IgM and IL-4 prepared CD5(-) B cells but not CD5(+) B cells responsive to IL-2 stimulation. Additionally we compared the IL-2 responsive properties of Balb/c and NZB/WF_1 CD5(-) B cells. Balb/c and NZB/WF_1 CD5(-) splenic B cells constitutively express mRNAs for both IL-2R α and β chains, while NZB/WF_1 CD5(+) peritoneal B cells constitutively express IL-2R α chain alone. The expression of these messages in both Balb/c and NZB/WF_1 B cells is differentialy regulated by anti-IgM and IL-2. IL-2 induced IL-2R β mRNA and caused their growth via preferential up-regulation of IL-2R β chain. By contrast IL-2 failed to induce the expression of IL-2R β mRNA in NZB/WF_1 CD5(+) B cells and their subsequent proliferation. Costimulation with anti-IgM shifted the growth curve provided by IL-2-stimulated B cells indicating that level of IL-2Rα chain determined sensitivity of the cells to IL-2. About their IL-2 responsiveness, we found that NZB/WF_1 CD5(-) B cells responded more strikingly than Balb/c CD5(-) B cells in term of IL-2 induced up-regulation of IL-2Rb mRNA and IL-2 induced proliferation.

Regulation of Cytokine Gene Expression

P 500 EFFECT OF INTERLEUKIN 7 ON HUMAN PERIPHERAL BLOOD MONOCYTES

Mark Alderson, Teresa Tough, Steve Ziegler, Richard Armitage and Ken Grabstein, Departments of Immunology and Molecular Biology, Immunex Corporation, 51 University St. Seattle WA 98101.

We have studied the effect of IL-7 on human peripheral blood monocytes in terms of cytokine secretion, tumoricidal activity and expression of cell surface antigens. IL-7 was found to be a potent monocyte/macrophage activator, inducing IL-1α, IL-1β, IL-6 and TNFα at both the protein and mRNA levels. However, the concentrations of IL-7 required to induce monocyte cytokine secretion were approximately 100-fold greater than those required to induce T-cell activation. The ability of IL-7 to induce cytokine secretion was markedly inhibited by the presence of IL-4. IL-7 was also found to induce monocyte/macrophage mediated tumoricidal activity, a process possibly related, at least in part, to its ability to induce TNFα and IL-1 secretion. Finally, we investigated the ability of IL-7 to regulate the cell surface expression of HLA-DR and CD23 on monocytes. IL-7 up-regulated the expression of both these molecules, though in this regard it was a weaker stimulus than IL-4, GM-CSF or IFN-γ.

P 501 MOLECULAR CLONING AND FUNCTIONAL CHARACTERISATION OF THE OVINE CYTOKINES IL-1α, IL-1β AND TNFα. Arna Andrews, Garry Barcham, Malcolm Brandon and Andrew Nash.

Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Parkville 3052 Australia.

Synthesis of IL-1 and TNFα by activated macrophages can result in profound effects on the immune response as these cytokines are important mediators of both inflammatory and antigen specific responses. This report describes parameters associated with the production of IL-1 and TNFα by ovine alveolar macrophages stimulated with LPS and molecular cloning of the cDNAs encoding these cytokines.

Northern blot analysis using homologous human cDNA probes indicated that exposure of ovine macrophages to LPS led to increased levels of Il-1α, IL-1β and TNFα specific mRNA. TNFα mRNA was induced to maximal levels within 1 hr, declining thereafter. IL-1α mRNA was detected at 1 hr post stimulation with a maximal level at 5 hrs, but none at 24 hrs. In contrast, IL-1β mRNA was not detected until 5 hrs after stimulation with a low level remaining at 24 hrs. Dose response analysis indicated that LPS concentrations of 100pg/ml induced detectable levels of TNFα mRNA while levels as low as 10pg/ml induced secretion of bioactive IL-1.

Based on these results, we constructed a cDNA library with mRNA prepared from ovine macrophages stimulated with LPS. Using homologous human probes the ovine cDNAs for IL-1α, IL-1β and TNFα have been isolated and their sequences obtained.

P 502 BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF A NUCLEAR PROTEIN INVOLVED IN THE INDUCIBLE EXPRESSION OF THE HUMAN IL-2 GENE

Götz Baumann, Michael Sullivan and Paul Ramage, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland

Transcription of the human IL-2 gene is regulated by a number of different cis-acting elements located in a 500 bp region 5' upstream of the transcriptional start site. One region 50 bp upstream of the TATA box ($^{5'}$ATGTAAAA$^{3'}$) reveals striking homology (6/8) with the so-called octamer motif ($^{5'}$ATGCAAAT$^{3'}$). The octamer motif serves as an enhancer element in a variety of housekeeping genes and also in immunoglobulin heavy and light chain genes. We have identified a nuclear protein in human T cells which specifically binds to this motif in the IL-2 promoter in a constitutive manner. The biological relevance of this sequence could be demonstrated by introducing a defined negative mutation of the DNA binding site in the wild type promoter. This mutation, which abolishes protein binding, results in a drastic decrease of antigen-inducible IL-2 promoter activity in transient expression experiments with the reporter gene CAT. By comparative proteolytic clipping band shift assays, molecular mass determination and the use of OTF-1 directed polyclonal antibodies in gel mobility shift assays we could clearly identify the nuclear protein binding to this region of the IL-2 promoter as the transcription factor OTF-1. This protein is known to be present in all cell types and may exert its activating function only when it can interact with additional adjacent transcription factors. We are currently studying the modification and protein/protein interaction pattern of OTF-1 in resting and antigen-stimulated T cells.

P 503 *Regulation of Murine Lymphokine Expression after Activation of Primary Splenic T-cells*

U. Bommhardt, B. Sadlack and A. Schimpl, Institut für Virologie und Immunbiologie, Versbacher Landstr.7, 8700 Würzburg, Germany

After activation of T-cells by antigens or lectins transcriptional as well as post-transcriptional regulatory events lead to the transient expression of such T-cell specific genes as IL2, IL4 and the IL2 receptor genes. In splenic murine lymphocytes, maxima of RNA expression are reached at different times and are of different duration depending on the gene analyzed, and to some degree, the type of stimulus. Removal of the activating signal induces a rapid loss of the mature lymphokine RNAs (1), while IL2 receptor RNA is less influenced and thus can be detected over a longer period of time. In the case of IL2, mRNA downregulation after signal withdrawal seems mainly to be due to processes preceding mRNA stability, while IL4 mRNA becomes labilized after synchronous removal of Con A from the stimulated cells by α-methylmannoside.
Under certain priming conditions IL2 RNA expression in the mouse shows characteristics analogous to those found for the CD28 activation pathway in human lymphocytes (2), resulting in a stabilization of IL2 mRNA, while the stability of e.g. the c-myc and, as preliminary results indicate, also of the IL4 mRNA are hardly affected.
The data thus show that RNA expression of these three genes is differentially regulated.

(1) R. Swoboda et al. (1987), Immunobiology 175: 273
(2) T. Lir[illegible]n et al. (1989), Science 244: 339

P 504 CYTOKINE MAPPING: OBSERVATION AND QUANTIFICATION OF CYTOKINE mRNAs IN K-562 LEUKEMIA CELLS, C.A. Brenner, S. Kanangat[§], R.A. Peterson, B. Rouse[§], and A.T. Ichiki, Dept. Med. Biol., Univ. Tennessee Medical Center, and Dept. Microbiology, Univ. Tennessee, Knoxville, TN 37920

Using the polymerase chain reaction (PCR), a method has been developed to assay and quantitate the expression of specific cytokine messages by message amplification phenotyping, MAPPing. MAPP analysis utilizes a micro-procedure for isolating mRNA from one to a million cells, reverse transcription of total cellular RNA to cDNA and enzymatic amplification at cytokine-specific DNA fragments by PCR. We have constructed a synthetic RNA containing 5' and 3' primers of 14 cytokine mRNAs as an internal standard, which is coamplified along with the specimen. Measurement of the amount of standard allows determination of the cytokine RNA concentration or copy number of the cytokine RNA in the original sample. We have initiated a study to characterize the cytokine pattern produced by K-562 cells by MAPPing as a means of demonstrating the pluripotential nature of the cells. Cells treated with TPA produced the message of IFNγ and IL1α. IL1β-treated cells produced the messages for IL1α, IL5 and IL9. IL5 promotes generation of eosinophils and limited B lymphocyte proliferation, while IL9 supports erythroid colony formation. Cells treated with IL2 produced mRNA for IL1α, IL6, IL8, IL9, and TNFβ. IL6 acts on a variety of target cells, included T cells, B cells, and myeloid progenitors; while IL8 activates neutrophils. TNF-α-treated cells were positive for IL1α and IL9. We are in the process of obtaining quantitative measurements of cytokine messenger RNAs associated with the different K-562 hematopoietic cell lines.

P 505 CHROMOSOME ASSIGNMENT OF THE HUMAN TNF ALPHA RECEPTOR p55 GENE. E. Brownell, G. Ranges* and R. Bayney, Molecular Therapeutics, Inc. and *Miles Institute of Arthritis and Autoimmunity, West Haven, CT 06516.

TNFα (or cachetin) is a polypeptide of approximately 17 kd existing in multimers of two or three subunits which contain several potential glycosylation sites. This cytokine, which is produced primarily by activated macrophages, mediates a range of effects on several different target cell types including the following: 1. cytolysis or cytostasis of allogenic and syngeneic targets, 2. growth of some cell lines (W138), 3. necrosis of meth-A induced sarcoma cells, 4. induction of the expression of MHC molecules and Ig receptors on secretory epithelial cells, 5. activation of polymorphonuclear leukocytes, 6. osteoclast activation and bone resorption, 7. antiviral activity.

The molecular basis for the broad range of activities is unkown, but receptor diversity may account for some of this phenomenon. For example, two cell surface TNFα-binding proteins (p55 & p75), which can be expressed either alone or in combination, have been detected on human target cells. One of our goals is to learn more about the genetic aspects of these receptor molecules to gain insight into how their expression and regulation is acheived. To this end, we have determined the chromosome assignment of the human p55 TNF receptor gene. Its placement suggests that it has evolved as a component of a conserved Hox locus-containing chromosome segment.

P 506 MOLECULAR CLONING OF THE IL-1β PROCESSING ENZYME, Douglas Pat Cerretti, Bruce Mosely, Carl Kozlosky, Nicole Nelson, Kirk Van Ness, Teresa Greenstreet, David Gearing, Carl March, Shirley R. Kronheim, and Roy A. Black, Immunex Corp., 51 University St., Seattle, WA 98101

Interleukin-1β (IL-1β) is a key hormone of the immune system, with roles in hematopoiesis, inflammation, and wound healing. Mature IL-1β is generated by proteolytic cleavage of an inactive precursor between Asp-116 and Ala-117. We have reported previously that lysates of the human monocytic cell line, THP-1, carry out this cleavage. We report here that the processing enzyme (termed IL-1β protease) has been purified to homogeneity and the amino terminal 23 amino acids determined. Full length IL-1β protease cDNAs were then isolated in a three-stage process. In the first stage, amino acid residues, 1-6 and 11-16, were used to design fully degenerate (128 fold) PCR primers for amplification of IL-1β protease specific sequences from cDNA prepared from THP-1 poly-A^+ mRNA. The amplified cDNA, after subcloning and sequencing, was found to encode amino acids 1-16 of IL-1β protease. In the second stage, nucleotides 1-17 were used in a 3'-anchored PCR amplification to generate a cDNA encoding IL-1β protease from the amino-terminal amino acid to the poly-A tail. Finally, in the last stage, full length cDNAs were isolated from a human neutrophil library using the anchored PCR product as a probe. To demonstrate that these cDNAs encode IL-1β processing activity, DNA encoding IL-1β protease were inserted into the mammalian expression vector, pDC303, and co-transfected into COS-7 cells with a second mammalian expression plasmid encoding precursor IL-1β. Transfected COS-7 cells were metabolically labeled with ^{35}S-Met and ^{35}S-Cys and IL-1β proteins were immunoprecipitated from cell lysates. Analysis of autoradiograms after SDS-PAGE show that precursor IL-1β is specifically processed to mature IL-1β in the presence of IL-1β protease cDNA.

P 507 EXPRESSION OF IL-2 GENE TRANS-ACTING FACTORS IN MOUSE THYMOCYTES, Dan Chen and Ellen Rothenberg, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The IL-2 gene is exclusively expressed in T cells, mostly in mature type I helper T cells, but also in triple negative ($CD4^-/CD8^-/TcR^-$) and single positive mouse thymocytes, upon in vitro stimulation under appropriate conditions. The cell-type specificity and induction dependence of IL-2 expression may be regulated by differences in the distribution, abundance, and ratios of various IL-2 transcriptional regulatory factors and/or by differences in the accessibility of the IL-2 gene 5' control region. We approached this problem by making short oligonucleotide probes from the 5'-flanking sequence of the mouse IL-2 gene and using gel retardation assays to probe the existence and inducibility of the known IL-2 gene regulatory factors in different subpopulations of mouse thymocytes. Our previous studies of the EL-4 cell line indicated that the level of NFκB and the factor binding the proximal AP-1 site are correlated closely with IL-2 induction, and both are superinduced in the presence of IL-1. The proximal AP-1 factor is also superinduced in the presence of forskolin, an inhibitor of IL-2 induction. The NFAT-1 and distal AP-1 factors were constitutively expressed in this cell line. Surprisingly, we found that NFAT-1, NFκB, distal and proximal AP-1 factors are present in both PNA^+ (mostly double positive) and PNA^- (mostly double negative and single positive) thymocytes. However, the PNA^- cells increase their expression of NFAT-1 protein significantly more than PNA^+ ones upon induction. We also studied two other novel protein complexes with target sequences upstream of the NFAT-1 binding sequence, which appear to be present in thymocytes constitutively. Current results disfavor the hypothesis that the presence of particular trans-acting factors is sufficient for IL-2 gene expression, but suggest that the absolute amounts or the balance of a battery of transcriptional factors may contribute to IL-2 gene regulation in thymocyte subsets.

P 508 TUMOR CELLS SYNERGIZE WITH ANTI-CD3, ANTI-CD2 AND ANTI-TCRα,β ANTIBODIES TO STIMULATE IL-2-ACTIVATED T LYMPHOCYTES TO PRODUCE IFN-γ and TNF-α. Anita S.-F. Chong, Lloyd H. Graf, Jr. and Philip Scuderi, Departments of General Surgery and Immunology/ Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Center for Research in Periodontal Diseases and Oral Molecular Biology, University of Illinois at Chicago, Chicago, IL 60612 and Department of Experimental Therapeutics, Miles Inc., Berkeley, CA 94701.

Peripheral blood T-lymphocytes are activated by interleukin-2 (IL-2) to express promiscuous cytolytic (LAK) activity. We have previously reported that the cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) are released by the LAK cells during this effector cell-target cell interaction. We here report that the release of cytokines by LAK cells can be further enhanced by the addition of the monoclonal antibodies, anti-CD3, anti-TCRαβ and anti-CD2 monoclonal antibodies (mAbs). Further, both antibodies and tumor cells act on the IL-2-activated T cells (LATs) to induce IFN-γ and TNF-α production. The ability of tumor cells to stimulate LATs is independent of their expression of HLA-antigens and is partially inhibited by mAbs specific for the intercellular adhesion molecule-1 (ICAM-1). However, ICAM-1 alone cannot fully stimulate the production of IFN-γ and TNF-α in LATs because B16/B78H1 mouse melanoma cells transfected with ICAM-1 gene to express elevated levels of surface ICAM-1 do not stimulate greater amounts of cytokine production in LATs compared to the untransfected cells. The possibility that other cell adhesion molecules on target cells synergize with ICAM-1 moecules to stimulate IFN-γ and TNF-α production in LATs will be discussed.

P 509 CHROMATIN STRUCTURE OF HAEMOPOIETIC GROWTH FACTOR GENES, Peter N. Cockerill and Mathew A Vadas, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, 5000, Australia.

Haemopoietic growth factors (HGFs) are a diverse family of cytokines required for the survival, proliferation, differentiation and function of haemopoietic cells. This study concerns the structure and regulation of the two closely linked HGF genes granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL-3). It is anticipated that the study of chromatin configuration around these genes will give indications of the mechanisms that control their expression. Regulatory regions within active chromatin can often be detected as DNaseI-hypersensitive (DH) sites, and these appear to be nucleosome-free regions of DNA. In preliminary DNaseI-digestion studies of the T cell line Jurkat, we identified one DH site spanning the IL-3 proximal promoter and two DH sites further upstream of IL-3. These sites are absent in the B cell line BALL-1. In both Jurkat cells and BALL-1 cells we identified three DH sites just downstream of IL-3. These sites, which lie between IL-3 and GM-CSF, may therefore be constitutive DH sites, while those upstream of IL-3 may be tissue-specific.

P 510 **CHARACTERIZATION OF fms EXPRESSION IN BOVINE TROPHOBLAST** B. Anne Croy and Janice L. Beauchamp, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ont., Canada N1G 2W1

Expression of fms, the CSF-1 receptor, has been reported in human and murine fetal trophoblast cells. In these species trophoblast cells are highly invasive components of hemochorial placentae. Bovine placenta is epitheliochorial and noninvasive trophoblast cells are found either interdigitating with maternal uterine epithelium in structures known as placentomes or in loose adhesion to maternal epithelium in areas between the placentomes. We asked if noninvasive bovine trophoblast expressed a fms-related protein. Using a murine anti-v-fms monoclonal antibody on tissue sections of intercotyledonary trophoblast, cytoplasmic staining of most trophoblast cells was found at all time points studied between day 40 of gestation and term. Unexpectedly, trophoblast cells in the placentomes showed nuclear staining. Binucleate cells that could be maternal, fetal or chimeric are found in bovine placenta and we frequently observed binucleate cells with only one stained nucleus, suggestive of chimeric fusion. Seven day conceptuses, resulting from in vitro maturation of ova followed by in vitro fertilization, expressed fms. Thus, expression of fms does not correlate with invasion of trophoblast and probably does not require maternal expression of CSF-1.

Supported by the Natural Sciences and Engineering Research Council, and the Ontario Ministry of Agriculture and Food.

P 511 DIFFERENTIATION OF T-CELL LYMPHOKINE GENE EXPRESSION. Stefan Ehlers and Kendall A. Smith, Department of Medicine, Dartmouth Medical School, Hanover NH

A simple *in vitro* experimental system was devised to examine the differentiation of T cell effector function at the molecular genetic level. Highly purified neonatal T cells (derived from umbilical cord blood) and adult T cells were activated with solid-phase anti-CD3 mAb 64.1 and compared with respect to their lymphokine gene expression using PCR-assisted message amplification phenotyping. Neonatal T cells, which represent a population of truly unprimed cells, solely expressed the genes for IL 2 and its receptor, while adult T cells comprising both unprimed and primed cells were capable of *transcribing* readily detectable levels of additional mRNAs for IL-3, IL-4, IL-5, IL-6, IFN-γ and GM-CSF. More importantly, primary activation and culture in IL-2 *in vitro* could induce functional differentiation of neonatal T cells which thereby acquired the capacity to express the extended lymphokine gene repertoire characteristic of adult T cells. This model system reflects the *in vivo* generation of a T cell anamnestic response and allows for the functional definition of memory T cells in terms of their differentiated state of inducible effector-lymphokine gene expression.

P 512 **CHARACTERIZATION OF T CELL-DERIVED SIGNALS INVOLVED IN THE INDUCTION OF MONOCYTE IL-1 mRNA DURING ANTI-CD3 MITOGENESIS**, Thomas M. Ellis, Paul T. McAllister, Richard I. Fisher, and R. Clive Landis, Section of Hematology/Oncology and Department of Microbiology, Loyola University School of Medicine, Maywood, IL 60153

Studies from our laboratory have shown that the secretion of IL-1 beta from monocytes during anti-CD3 mitogenesis involves a cell contact signal, which induces IL-1 mRNA and production of cell associated IL-1, and a soluble signal, which induces secretion of IL-1 beta from monocytes. The development of the T cell derived cell-contact signal requires prior activation of T cells but does not require T cell protein synthesis. Whereas both CD4+ and CD8+ T cell subsets induce monocyte IL-1 mRNA, removal of membrane CD4 or CD8 by modulation does not affect the ability of activated T cells to induce monocyte IL-1 mRNA. In contrast to normal T cells, the Jurkat T cell line induces monocyte IL-1 mRNA without prior activation and in the absence of anti-CD3. Furthermore, a CD3/TCR negative variant of the Jurkat cell exhibited a comparable ability to induce monocyte IL-1 mRNA, indicating that induction of monocyte IL-1 mRNA by Jurkat did not involve allorecognition. These studies demonstrate that the induction of monocyte IL-1 mRNA by T cells during immune activation 1) requires direct contact with activated T cells, 2) requires neither the TCR nor the CD4 or CD8 associative recognition structures, and 3) is a constitutive property of the Jurkat T cell line.

P 513 PRODUCTION OF CYTOKINES IN SARCOID LYMPH NODES: PREFERENTIAL EXPRESSION OF IL-1β AND IFN-γ GENES. D. Emilie, O. Devergne, M. Peuchmaur, M.C. Crevon & P. Galanaud. INSERM U131, Clamart, France

Sarcoidosis is a chronic granulomatous disease which may be considered to be a human model for the Delayed Type Hypersensitivity (DTH) reaction. The expression of cytokine genes in organs displaying sarcoid granulomas was analyzed by *in situ* hybridization with several cytokine probes using biopsies from 11 sarcoid lymph nodes (LN). We detected cells expressing IL-1b, TNF-a, IL-6, IL-2 and IFN-g genes in all LN. The major finding of this study was that cytokine genes are independently expressed. Of the monokine genes, the IL-1b gene was preferentially expressed. The distribution of cells containing IL-1b mRNA was characterized by their amalgamation in clusters inside sarcoid granulomas. Cells expressing the TNF-a gene were exclusively located inside granulomas but were always scattered. Cells expressing the IL-6 gene or the IL-1a gene were found scattered inside granulomas and in the residual lymphoid tissue. The number of cells expressing the IL-1b gene was significantly higher than that of cells expressing TNF-a gene ($p=0.001$), IL-6 gene ($p=0.007$) or IL-1a gene ($p<0.001$). Of the cells expressing lymphokine genes, those expressing the IFN-g gene were 31.9 ($\pm$7.6) times more frequent than those expressing the IL-2 gene ($p<0.001$). Cells containing IFN-g mRNA were mainly detected inside granulomas whereas cells containing IL-2 mRNA were randomly distributed. These results show that each monokine gene or lymphokine gene can be independently expressed *in vivo*. The increased expression level of the IL-1b gene and of the IFN-g gene we detected inside granulomas may be specific to DTH immune reactions.

P 514 CYTOKINE-MEDIATED REGULATION OF CELL PROLIFERATION AND GROWTH FACTOR RECEPTOR EXPRESSION OF HUMAN BREAST CARCINOMA CELLS.

Urs Eppenberger, Petra Loop, Willy Kueng and Heinz Mueller, Department of Research and Gynecology, University Medical School, 4031 Basel/Switzerland.

An important mechanism in tumor development is the inhibitory action of growth modulatory cytokines. We tested several cytokines for their inhibitory potency on MCF-7 cell growth. A potent inhibitor was TNF-α at 10^{-8}M, Interferon α (1000 units) and Interleukin 1 (100 units). Neither Interleukin 2 nor Interferon γ had any effect on the IGF-stimulated growth. A synergistic inhibitory effect was observed using Interferon γ (500 units) together with TNF-α throughout the growth experiment. Inhibition of cell growth in the presence of Interferon γ was effective even with non-inhibiting concentrations of TGF-α.

Modulation on growth by cytokines can be a regulation of the availability of growth hormone receptors on the cell surface. Measuring EGF-receptors by utilizing an anti-EGF receptor antibody demonstrated that growth inhibitors like TGF-α suppressed the upregulation of EGF-receptors on MCF-7 cells during growth stimulation. Resulting in a significant reduction of cell surface receptors of growth factors such as EGF-receptors. Growth inhibition by cytokines is a complex mechanism in which the regulation of surface receptors may play a keyrole (supported by SNF-Grant Nr. 31-26523.89 and the Swiss Cancer League Grant No. FOR 341.89.2).

P 515 ROLE OF CD8+ T CELLS AND INTERFERON-ALPHA THERAPY IN AIDS AND KAPOSI'S SARCOMA, Milan Fiala, Department of Neurology, Wadsworth Veterans Administration Center and UCLA School of Medicine, Los Angeles, CA 90024

Benefits of Interferon-alpha 2b (IFN) may be due to its anti-retroviral or immunomodulatory effects. We have treated 12 AIDS and 8 ARC patients with a low dose of IFN (3 million U. 2-3x/week) with Retrovir for an average of 3.6 and 3.9 months respectively. In the AIDS group 8 patients improved clinically with Kaposi's sarcoma (KS) lesions regressing in 4 of 5 patients and *Mycobacterium avium intracellulare* and cytomegalovirus (CMV) infections stabilizing in 2 patients [in 5 of these CD8+ cells increased (mean 511 cells/μL before IFN, 624 cells/μL after IFN)] and 4 patients deteriorated [in 3 of these CD8+ cells decreased (mean 935 cells/μL before IFN, 652 cells/μL after IFN)]. In the ARC group 6 patients improved (in 5 patients CD8+ cells increased) and 2 patients deteriorated (both lost CD8+ cells). As in other AIDS patients, patients with KS and CMV retinitis lost remaining CD4+ and CD8+ T cells faster compared to KS patients without disseminated CMV infection. Neither disseminated CMV infection nor HIV p24 antigenemia were statistically associated with onset of KS. The onset of KS was preceded by a severe decrease of CD4+ T cells and was followed by a decline of CD8+ T cells. Deficiency of CD8+ T cells is an important defect predisposing to disseminated CMV infection and possibly KS which may be improved by IFN therapy. Secondary opportunistic infections in AIDS patients may show improved responsiveness to appropriate therapy in presence of IFN therapy.

P 516 IMMUNOREGULATORY EFFECTS OF NEUROPEPTIDES: UPREGULATION OF IL-2 PRODUCTION IN EL-4 CELLS BY SUBSTANCE-P, Doina Ganea, Pranela Rameshwar and Pedro Gascon, Department of Biological Sciences, Rutgers University, and Hematology Department, UMDNJ, Newark, NJ, 07102.

T lymphocytes have been previously reported to possess receptors for Substance-P (SP), and to proliferate in response to SP. We investigated the effect of SP on the production of interleukin-2 (IL-2) by the murine T cell line EL-4. In the presence of suboptimal or supraoptimal concentrations of phorbol myristate acetate (PMA), SP stimulates IL-2 production by the murine T cell line EL-4 in a dose-dependent manner. The IL-2 produced by the EL-4 cells stimulated with SP and PMA was shown to be biologically active as determined by its effect on the IL-2 dependent T cell line CTLL-2. The effect of PMA and SP is synergistic rather than additive, with optimal concentrations of Substance-P (10^{-8}M) inducing an approximately hundred fold increase in IL-2 activity above the level of activity obtained with either PMA or SP alone. Preincubation of the EL-4 cells with PMA, followed by the addition of fresh medium containing SP indicated that both PMA and SP are required simultaneously for optimal stimulation. Monoclonal anti-IL 2 antibodies completely neutralized the biological activity present in the supernatants from EL-4 cells stimulated with SP. The effect of SP is specific, its activity being successfully competed by spantide, an SP inhibitor, and by physalaemin, an SP antagonist, in a dose-dependent manner. These experiments indicate that SP specifically enhances the generation of IL-2, and suggest a possible mechanism for the regulation of the immune response by neuropeptides.

P 517 KINETICS AND REGULATION OF COLONY-STIMULATING FACTOR PRODUCTION, Bahram Goliaei, Mariam Taheri,and Azra Rabbani, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, IRAN.

We have studied the simultaneous regulation of colony-stimulating factor(CSF) production and protein synthesis by the lung in-vitro. When fresh lung tissue was cultured in serum free culture medium,the first detectable level of the CSF activity appeared 6 hrs after initiation of the culture,continued to rise until 24 hrs and then leveled off for several days. Under similar conditions protein synthesis did not level off but continued to rise after 24 hrs. When the lung tissues which had been cultured for 6,24,and 48 hrs were recultured in fresh tissue culture medium,de novo synthesis of CSF occured as judged by protein synthesis inhibition and stimulation studies. The rate of new CSF synthesis was decreased as the initial culturing period of lung tissues increased from 6 to 48 hrs. There was also a decrease in the rate of protein synthesis and release in the secondary cultures as a function of the initial culturing period of lung tissues. These data indicated that 1)CSF production is specificly regulated and inhibited after 24 hrs.2)aging is partially responsible for the general decrease in CSF production and protein synthesis. Endotoxin stimulation of 24 or 48 hrs cultured lung tissues resulted in de novo synthesis of CSF by the lung. Also when fresh lung tissues were cultured in 24 or 48 hrs lung conditioned medium no new CSF production occured, while under the same conditions protein synthesis was significant as judged by double labling experiments. On the other hand, the 6 hrs lung conditioned medium could support both CSF production and protein synthesis by the fresh lung tissue. In conclusion,the data suggest that CSF production by the lung is regulated by the level of CSF in the medium via a feed-back regulation mechanism.

P 518 REGULATION OF SCI/MIP1α GENE TRANSCRIPTION, M.Grove and M.Plumb, Beatson Institute for Cancer Research, Garscube Estate, Bearsden, Glasgow, G61 1BD, U.K.

The SCI protein is areversible inhibitor of haematopoietic stem-cell proliferation and an inflammatory mediator. SCI is predominantly produced in the bone-marrow in-vivo by macrophages. Hence we are comparing the transcriptional regulation of the SCI gene in a macrophage cell-line(RAW) which constitutively expresses SCI with that in non-expressing cell-lines[fibroblast(STO) and erythroleukaemia(MEL)].

We have isolated and sequenced a genomic clone which contains the complete murine SCI gene. DNAse1 digestion of intact nuclei indicate that the SCI gene promoter is DNAse1 hypersensitive in RAW but not STO cells, correlating with the absence of detectable SCI mRNA in STO cells. Transfection studies in RAW and MEL cells using serial 5' promoter deletion constructs linked to the human growth hormone(hGH) reporter gene reveal that sequences between +20 and -250bp act as a basal endotoxin-responsive promoter in RAW cells, whereas both tissue-specific and non-tissue-specific enhancer sequences are located between -250 and -1000bp.

In-vitro nuclear protein binding studies of promoter sequences(+20 to -250bp) have revealed several binding sites, three of which are similar to the NF-KB transcription factor consensus binding sequence. We have also identified two putative Pu-boxes. NF-KB- and Ets-family-related protein binding sites are present in the promoter regions of several other cytokine genes. We are using serum-starved(quiescent) RAW cells stably transfected with SCI promoter/hGH gene constructs to identify promoter elements which are responsive to specific factors which may be involved(via eg. NF-KB and Ets) in the in-vivo up-regulation of SCI gene expression during the stimulation of cell-proliferation.

P 519 EXPRESSION OF DOUBLE-STRANDED RNA-DEPENDENT ENZYMES IN THE RESPONSE OF MACROHAGES TO IFNγ, G. Luca Gusella, Michael A.Clayton, Tiziana Musso and Luigi Varesio, Biological Carcinogenesis and Development Program, PRI/DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, Biological Response Modifiers Program, Division of Cancer Treatment, NCI, Frederick, MD 21702-1201

IFNγ elicits pleiotropic responses in macrophages and, in combination with low doses of bacterial lipopolysaccharide (LPS), it activates macrophages to tumoricidal activity. In many cell types IFNs induce the expression of 2'5'oligoadenylate synthetase (OAS) and of a protein kinase (dsRNA-PK) whose activation depends on the presence of double stranded RNA. We have analyzed the expression of the OAS and of the dsRNA-PK in murine macrophages stimulated with IFNγ. Treatment of macropahges with IFNγ induced OAS mRNA expression that became maximal after 18 h. In the same conditions IFNγ induced dsRNA-PK as measured by the synthetic dsRNA-dependent phosphorylation of exogenous histones. Addition of LPS did not modify these responses. We have previously reported that the occurence of cytocidal activity in macrophages is associated with rRNA precursor accumulation. We showed that rRNA precursors generated by in vitro transcription can activate dsRNA-PK in a cell-free system demonstrating the existence of secondary dsRNA structures which activate dsRNA-dependent enzymes. These data demonstrate that IFNγ is a potent inducer of dsRNA-dependent enzymes in macrophages. However, only the concomitant exposure of macrophages to IFNγ plus LPS provides dsRNA structures needed for the enzyme's activation. We speculate that the active dsRNA-dependent enzymes are important for the expression of tumoricidal activity.

P 520 Expression of interleukin 6 (IL-6) in C6 glioma cells stimulated with cytokines and spirochetes. Gail S. Habicht* & Jorge L. Benach *#. *Dept. of Pathology & #NY State Dept. of Health, Stony Brook, NY.

The CNS is capable of mounting specific immune responses as evidenced by intrathecal production of antibodies to antigens introduced into the brain. Cells resident in the CNS may be stimulated to express Ia and to act as APC. Some of these cells have also been shown to express cytokines which amplify immunoresponsiveness. During infection of the brain, antibody production by limited numbers of B cells may persist as shown by the stability of oligoclonal banding patterns in the CSF. IL-6 produced locally in the brain may account for the persistence of these B cells. In the present study we have investigated the capacity of lipopolysaccharides, human recombinant IL-1α and TNFα and a bacterial neuropathogen, *Borrelia burgdorferi*, the causative agent of Lyme disease, to induce IL-6 synthesis in cell lines of neural origin. Preliminary studies showed that *B. burgdorferi* induced IL-6 secretion by both macrophage and fibroblast murine cell lines. C6 rat glioma cells, and PC12 rat and NG-108-15 mouse neuroblastoma cell lines were incubated with various concentrations of spirochetes for 24 hours. Supernatants were assayed for IL-6 biological activity using the IL-6 dependent cell line B9. IL-6 activity was produced by the C6 glioma but not by the neuroblastoma cells lines in a dose dependent manner: very high concentrations of spirochetes (>2.5x10^7/ml) were toxic. C6 glioma cells also produced IL-6 in response to both smooth and rough forms of bacterial lipopolysaccharide (LPS) as well as to a LPS/lipoprotein containing extract of *B. burgdorferi*. Cytokines known to be induced by spirochetes were investigated for their ability to enhance IL-6 production. As little as 1 U/ml of hrIL-1α doubled IL-6 production by C6 cells and acted synergistically with LPS. Interferon γ blocked the effects of IL-1 on these cells. TNF also enhanced IL-6 production. That cells of the brain can express immunoregulatory and inflammatory cytokines in response to bacterial pathogens and their products supports the idea that these molecules may be able to act locally to induce prolonged intrathecal antibody production or to produce tissue damage. That some cytokines enhance while others decrease IL-6 production suggests that a tightly controlled cytokine network is normally operative in the brain. Supported by NIH Grant AR36028.

P 521 CHARACTERIZATION OF THE MOUSE COLONY-STIMULATING FACTOR-1 GENE PROMOTER. M.A. Harrington,* L. Pedigo*, H.J. Edenberg* and S. Saxman*. Walther Oncology Center and Indiana University School of Medicine, Indianapolis, IN.

Colony stimulating factor-1 (CSF-1; M-CSF) is a glycoprotein growth factor required for the growth and differentiation of macrophage progenitors and survival of mature macrophages. Expression of the CSF-1 gene in monocytes and fibroblasts is controlled at both the transcriptional and post-transcriptional level. To study the molecular mechanisms which regulate CSF-1 gene expression the 5' flanking region of the mouse CSF-1 gene was isolated. A high degree of structural and sequence (80-85%) homology between the mouse and human CSF-1 genes was observed. A transcriptional start site was located 181 base pairs upstream from the translational start site. Several sequences homologous to known cis-acting elements, including GC boxes, CK-1 and CK-2 elements and AP-1, NF-kB, and PU.1 binding sites were identified in the 5' flanking region. A construct containing over 700 base pairs of the 5' flanking region was able to direct expression of a linked reporter gene in C3H10T1/2 mouse embryo fibroblasts. Exonuclease III was used to generate deletion constructs which contained progressively less of the most distal 5' region. Deletion of a 150 base pair fragment in the distal portion of the promoter resulted in a significant decrease in activity. Gel-shift assays using nuclear extract isolated from C3H10T1/2 cells and fragments containing different portions of the 5' flanking region revealed the presence of DNA binding material. Ongoing studies using DNase I footprinting are being used to determine if any of the putative cis-acting elements identified by sequence homology are responsible for the DNA binding activity.

P 522 **CYTOKINE GENE EXPRESSION AND CELLULAR DIFFERENTIATION INDUCED BY HIV-1 INFECTION OF HUMAN MYELOMONOBLASTIC PLB-985 CELLS** J. Hiscott, M. D'Addario, A. Roulston, S. Caplan and M.A. Wainberg. Lady Davis Institute and Dept. of Hematology, Jewish General Hospital, Depts. of Microbiology and Medicine, and McGill AIDS Center , McGill University, Montreal CANADA H3T1E2.
The human diploid PLB-985 cell line has been characterized as a bipotential myelomonoblastic cell population, capable of either granulocytic or monocytic differentiation upon induction with different agents. In this study, PLB -985 cells or PLB cells chronicallly infected with HIV-1 strain IIIB (PLB-IIIB) were used to investigate the effects of HIV-1 infection on myeloid specific differentiation and cytokine gene expression. Interestingly, selection of chronically infected PLB-IIIB cells generated a cell population with a more monocytic phenotype as determined by differential staining and by monitoring expression of surface antigens CD13 (MY 7), CD14 (MY 4), CD33 (MY 9) and CD34 (MY10). PLB-985 cells were >90% positive for the myeloid specific markers CD13 and CD33, and negative for the monocyte specific marker CD14, whereas PLB-IIIB cells were 25% positive for CD14. Furthermore, the number of CD14 positive cells increased to >50% following PMA treatment.The production of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) was characterized by quantitative PCR mRNA phenotyping following treatment with phorbol ester or tumor necrosis factor. IL-1α and TNF-α were both strongly inducible by phorbol ester in PLB-985 and PLB-IIIB, whereas IL-6 was not induced in either cell type. In contrast, IL-1β was not induced by phorbol ester in PLB cells, but in PLB-IIIB, a constitutive level of IL-1β RNA was observed and this basal level of IL-1β RNA was highly inducible by phorbol ester treatment. These experiments demonstrate that HIV-1 infection of myelomonoblastic cells may alter expression of certain myeloid specific genes and select for cells with a more differentiated monocytic phenotype.

P 523 INTERLEUKIN-1 (IL-1) RELEASE MAY BE DEPENDANT ON CELLULAR INJURY, Kristin A. Hogquist and David D. Chaplin, Department of Medicine and Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110.

IL-1 α and β are cytokines that are thought to function primarily as intercellular mediators and can be detected in plasma and the supernatants of cultured cells; however, they are not released via the classical pathway of constitutive protein secretion.

We have analyzed IL-1 release from LPS-stimulated cells by immunoprecipitation of pulse/chase labeled cultures. Both full-length and proteolytically processed forms can be detected outside the cell. In macrophages, the IL-1 detected in culture supernatants comprised less than 5% of the total cellular IL-1. Stimulation of the cells with PMA, cycloheximide, ionomycin, or other cytokines all had no effect on the release of IL-1. Similar results were obtained using resting, elicited, and activated murine macrophages, as well as murine monocytes. The only stimulus which resulted in the accumulation of IL-1 in the supernatant was treatment with 5mM ATP for 10 minutes. Non-specific release of intracellular contents was determined by measurement of lactate dehydrogenase (LDH) activity in culture supernatants. IL-1 release paralleled release of LDH in all situations. In LPS-stimulated human monocytes, 19% of the IL-1β synthesized during a 30 minute pulse was recovered from the supernatant after a 20 hour chase. In contrast, monocytes cultured in vitro prior to LPS stimulation showed IL-1β release of less than 5%. LDH release was also higher in freshly isolated monocytes than in cultured monocytes. Our data suggest that IL-1 release from monocytic cells occurs non-specifically, perhaps as a consequence of cellular injury.

P 524 TWO DIFFERENT TRANSCRIPTION FACTORS, NF-κB AND NF-GMa, INTERACT WITH THE SAME SITES IN THE TNF-α PROMOTER C.V. Jongeneel(1), C. Drouet(1), S.A. Nedospasov(2), and A.N. Shakhov(1,2). (1) Ludwig Inst. for Cancer Research, 1066 Epalinges, Switzerland; (2) Engelhardt Inst. of Mol. Biol., Moscow 117984, USSR

The TNF-α promoter is inducible by LPS in macrophages and by engagement of the T cell receptor / CD3 complex in subpopulations of T lymphocytes. We have shown previously that NF-κB, a pleiotropic activator involved in the control of many cytokine genes, plays a central role in this transcriptional activation. The TNF-α promoter contains at least 5 sequence elements that bind the heterotetrameric form of NF-κB (two 50 kD DNA-binding subunits and two 67 kD regulatory subunits), albeit with widely differing affinities. All of these sites also bind a less strongly inducible form of NF-κB, which is probably KBF1, a dimer of the 50 kD DNA-binding subunit. In addition to NF-κB and KBF1, two of the κB sites bind an abundant nuclear protein unrelated to NF-κB, which does not bind the authentic κB enhancer from the Ig κ light chain. By cross-competition studies with the "cytokine-1" (CK-1) site from the G-CSF promoter we have identified this protein as NF-GMa, a factor thought to play an important role in the regulation of many cytokine promoters. The consensus sequence of CK-1 is indistiguishable from that of κB-type enhancers, but individual sites can bind either NF-κB (e.g. site κB3 from the mouse TNF-α promoter), or NF-GMa (the CK-1 site from the G-CSF promoter), or both (the CK-1 site from the GM-CSF promoter, site κB2 from TNF-α). Thus, sequences commonly found in cytokine promoters can bind at least two unrelated nuclear factors, both of which could be involved in the modulation of their transcriptional activity.

Bandshift assays performed with nuclear extracts from primary lymph node cells or conA blasts before of after stimulation by PMA and ionomycin show qualitative as well as quantitative modulation of both factors. Mutation of a site in the TNF-α promoter that binds predominantly NF-κB can influence the formation of a DNA-protein complex which depends on the binding of NF-GMa at a different site. Both types of results argue in favor of an interplay among the two factors in the regulation of cytokine gene expression.

P 525 AGE-RELATED EFFECT OF D-PENICILLAMINE ON INTERLEUKIN-2 PRODUCTION AND PROLIFERATION IN MOUSE LYMPHOCYTES, Ilona Kariv and Frederick G. Ferguson, Department of Veterinary Science, The Pennsylvania State University, University Park, PA 16802

D-penicillamine (D-Pen) is widely used in the clinical practice to suppress rheumatoid inflammation. The mode of D-pen action on the immune system reactivity, however, remains unclear. The goal of this research was to clarify the influence of D-pen on cell cycle progression during lymphocyte activation. This study examined interleukin-2 (IL-2) production, occurring during G_1 phase, and DNA synthesis, occurring during S phase of the cell cycle. It also compared the effect of D-pen on young and old C57BL/6 male mice. There was an age-related decrease in IL-2 production and proliferative response in Concanavalin A (Con A)-activated and Con A and 12-O-tetra decanoylphorbol 13-acetate (TPA)-activated lymphocytes. D-pen augmented IL-2 production in both Con A-activated and Con A/TPA-activated lymphocytes from the young and the old animals. The relative increase in IL-2 production was greater in the old mice than in the young mice. D-pen inhibited the proliferative response in the young animals, but not in the old animals. The results suggest that D-pen acts as an inhibitor of the cell cycle progression in the late G_1 after the activation of IL-2 expression, but before initiation of S phase. The augmented IL-2 production may be a result of this inhibition, i.e. the cells produce more IL-2 possibly in an attempt to overcome the inhibition.

P 526 SIGNAL TRANSDUCTION VIA TRANSFECTED AND ENDOGENOUS GROWTH FACTOR RECEPTORS ENHANCES CYTOKINE PRODUCTION BY MAST CELLS, A.D. Keegan, J.H. Pierce, M. Plaut, and W.E. Paul. LI, NIAID and LCMB, NCI, NIH, Bethesda, MD 20892

IL-3 dependent mast cell lines produce cytokines in response to ionomycin and IgE receptor cross-linkage. In this study we have observed that cells pre-cultured in IL-3 produce 10-100 times more cytokine after receptor cross-linkage in comparison to IL-4 pre-cultured cells. Although the receptors for IL-3 and IL-4 are not tyrosine kinases, their occupancy with ligand causes tyrosine phosphorylation of specific substrates. Therefore the contribution of tyrosine kinase activity to the ability of the IL-3 dependent mast cell line, CFTL-15, to produce cytokines was analyzed. CFTL-15 cells were transfected with growth factor receptors containing intrinsic tyrosine kinase activity (EGFR, PDGFR, and CSF-1R) and with a member of the hematopoietin receptor family that induces tyrosine phosphorylation on cellular substrates (EPOR). All of the transfectants are similar in their ability to produce IL-3 in response to receptor cross-linkage. Stimulation of the EGFR, PDGFR, and EPOR transfectants with their respective ligands alone resulted in the production of IL-3, IL-6, and GM-CSF. Stimulation with CSF-1 alone has failed to induced cytokine production. However, all of the growth factors enhanced the amount of lymphokine produced in response to IgE receptor cross-linkage when added simultaneously. EPO was the least effective at <2-fold. CSF-1 usually enhanced 2-5-fold. EGF and PDGF enhanced the response 10-fold. Using pre-culture assays, IL-3 is far superior to all other factors tested thus far in preparing cells to secrete IL-3. The ability of the above mentioned stimuli to induce tyrosine phosphorylation in the transfectants was analyzed with anti-phosphotyrosine antibodies. At least 3 proteins were phosphorylated in response to IgE receptor cross-linkage, ranging from 68 to 84 kD. The effects of IL-3, IL-4, and the growth factors on the phosphorylation levels and patterns in the transfected cells is currently being investigated. These results suggests that tyrosine phosphorylation of certain substrates contributes to the ability of mast cells to produce cytokines.

P 527 DIFFERENT CYTOKINS ARE INVOLVED IN THE IMMUNOMODULATORY EFFECT OF ß-1,3-D-GLUCAN. Zbigniew Konopski, Lill-Tove Rasmussen, Rolf Seljelid and Trond Eskeland. Institute of Medical Biology,University of Tromsø, P. box 977, 9001 Tromsø, Norway. Animals treated with particulate or soluble ß-1,3-D-glucan develop resistance against bacterial, viral, fungal, and protozoan infections, reject implanted tumors, reject antigenically incompatible grafts more rapidly, produce larger amounts of serum antibodies against specific antigens and survive otherwise lethal irradiation. Soluble, aminated ß-1,3-D-glucan (AG) is a novel form of the immunomodulator. AG causes total regression of Meth A sarcoma in mice. ß-1,3-D-glucan attached to plastic microbeads (GDM) and used intraperitoneally protects against otherwise lethal peritonitis in mice. In vitro GDM are internalized via specific ß-glucan receptor on macrophages giving augmented release of IL-1 and PGE_2. The same responce gives AG when it binds to the ß-glucan receptor. The receptor may account for the immunomodulatory effect of ß-1,3-D-glucan. Activity of ß-glucan receptor is modulated by different cytokines.

P 528 SELECTIVE INDUCTION OF EXPRESSION OF PDGF B CHAIN mRNA IN MURINE MACROPHAGES BY cAMP. Elizabeth J. Kovacs, Michelle Frazier-Jessen and Susan VanStedum. Department of Cell Biology, Neurobiology and Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153. Since evidence suggests that prostaglandin E_2 plays a role in expression of cytokines genes, we chose to examine the role of cAMP in the control of expression of IL-1β, TNFα and PDGF B chain mRNAs in the ANA-1 murine Mϕ cell line. Mϕ were cultured with or without agents which induce an elevation in intracellular cAMP in the presence or absence of LPS. Prior to treatment Mϕ do not express IL-1β, but do express low levels of TNFα and PDGF B chain mRNAs. Following stimulation with forskolin, for example, PDGF B chain mRNA is induced. The level of PDGF B chain mRNA induced by forskolin ranged from 25% to 200% of the level induced in 6 hours by LPS. In contrast, while LPS triggers the expression of IL-1β and TNFα mRNAs, inducers of intracellular cAMP accumulation fail to stimulate them. The combination of forskolin and LPS appears to act additively on the expression of PDGF B chain mRNA, suggesting that the effects of LPS are not mediated by a cAMP dependent pathway. These observations demonstrate that macrophages differentially express cytokine genes in response to treatment with inducers of intracellular cAMP. Hence, there may be two classes of cytokine genes, one which is activated by inducers of cAMP and another which is not. Supported by ONR #N00014-89-J-1130 and the Elsa U. Pardee Foundation.

P 529 COMPLEMENTATION ANALYSIS REVEALS DOMINANT ACTIVATORS OF TNF SYNTHESIS IN NON-MACROPHAGE CELL LINES, Veronique Kruys, Kathleen Kemmer, and Bruce Beutler, Howard Hughes Medical Institute, Dallas, TX 75235

A minigene reporting the biosynthesis of tumor necrosis factor (TNF; cachectin) has been used to permanently transfect mouse macrophages (RAW 264.7 cells), as well as three non-macrophage cell lines (L-929, HeLa, and NIH 3T3). The construct contains a chloramphenicol acetyltransferase (CAT) coding sequence, preceded by the TNF promoter and 5'-untranslated region (UTR), and followed by the TNF 3'-UTR. In RAW 264.7 cells, the construct is normally silent, but high levels of CAT are expressed following induction by endotoxin. In HeLa cells and in NIH 3T3 cells, no CAT biosynthesis may be detected, either in the presence or absence of endotoxin. Cells of the L-929 fibrosarcoma line strongly express the reporter gene in a constitutive fashion. In the present study, we have exploited the exquisite sensitivity of the CAT assay, performing cell fusion experiments in order to determine whether the constitutive expression of CAT activity exhibited by L-929 cells represents a dominant or recessive phenotype, and whether complementation might be identified among those cells that fail to express CAT. We now report that L-929 cells contain a dominant activator of CAT biosynthesis, which is capable of stimulating expression of the reporter gene in any of the cell lines tested. We have further observed that HeLa cells and NIH 3T3 cells fail to complement one another when fused, suggesting that they manifest a shared defect in the sequence of events leading to TNF gene activation (i.e., they belong to the same complementation group). However, the fusion of RAW 264.7 cells with HeLa cells leads to a permissive environment for expression of the reporter gene (i.e., complementation is observed). It would therefore appear that L-929 cells and HeLa cells contain distinct activators of TNF gene expression, which may participate in signal transduction pathways that effect TNF biosynthesis.

P 530 STRUCTURE AND FUNCTION OF INTERLEUKIN 2-RECEPTORS (IL-2R) ON SPINDLE CELLS OF AIDS-ASSOCIATED KAPOSI'S SARCOMA (AIDS-KS). Yanto Lunardi-Iskandar[1], Shuji Nakamura[1], Parkash Gill[2], Shinsaku Sakurada[1], Syed Zaki Salahuddin[2], Robert C. Gallo[1]. [1]Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892 and [2]Norris Hospital, University of Southern California, Los Angeles, CA 90033.
IL-2R (p55, Tac antigen, IL-2R alpha) were detected on AIDS-KS-derived cells, human umbilical vein endothelial cells (HUVEC) and normal bone marrow stromal cells (N1181B) by immunofluorescence, using a monoclonal anti-Tac (CD25) antibody. We analyzed the IL-2R on AIDS-KS cells and related controls (HUVEC, N1181B) by combining Scatchard and cross-linking analysis. Only one type of high-affinity receptors was found on AIDS-KS cells, as well as on HUVEC and N1181B, with a dissociation constant (K_d) ranging from 11 to 85 X 10^{-12} M. The number of binding sites per cell ranged from 800 to 1450. Analysis of IL-2R structure revealed two bands corresponding to the IL-2R p55 alpha and p75 beta chains. We found gene expression for IL-2R alpha chain, but not for IL-2 in HUVEC and AIDS-KS cells. HUVEC and AIDS-KS cells have the ability to internalize IL-2 and AIDS-KS cells proliferate in the presence of 10-1000 pM of rIL-2. The activation of IL-2R in AIDS-KS cells may be one of the factors responsible for the growth and invasiveness of Kaposi's sarcoma in vivo.

P 531 NORMAL HUMAN MESOTHELIAL CELLS GROWN IN CULTURE PRODUCE AND RESPONDE TO HEMATOPOIETIC GROWTH FACTORS,Luisa Lanfrancone,Diana Boraschi,Paolo Ghiara,Brunangelo Falini,Emilio Donti,Fausto Grignani,Giuseppe Peri,Alberto Mantovani and Pier Giuseppe Pelicci.
Istituto di Clinica Medica I, Policlinico Monteluce, 06100 Perugia, Italy.
To study the role of oncogenes in the control of normal proliferation, we established and characterized normal, human, growth factor (GF)-dependent mesothelial cultures. Mesothelial cells were isolated from endocavitarian fluids and established in vitro in presence of supernatants of PHA-stimulated peripheral blood mononuclear cells (PHA-CM).Rapidly proliferating, adherent cells exhibit a fibroblast-like morphology and coexpress vimentin and cytokeratins. Cells possess: 1) normal kariotype; 2) no anchorage-independent growth; 3) definite lifespan; 4) absolute requirement of exogenous GFs.Mesothelial cells are capable of producing several hemopoietic (GM-CSF, G-CSF, M-CSF, IL1, IL6, IL8) and non-hemopoietic (PDGF, EGF) GF. Proliferation assays indicate that serum is necessary, even not sufficient, for cell growth and that IL1- and -ß and/or a- and b-FGF can induce long-term proliferation. Moreover,thymidine incorporation assay shows that GM-CSF,IL6 and gIFN can act as mitogenic stimuli, promoting DNA synthesis but not supporting mesothelial growth. IL1- and -ß receptors are expressed on these cells, suggesting an autocrine pattern of growth for IL1. In fact, addition of IL1 to serum-free medium induces IL1- and -ß expression in the cells. Cultures of normal mesothelial cells can provide a useful tool to study gene regulation and signal transduction.

P 532 MONOCYTES FROM AIDS PATIENTS WHICH HAVE A DEFECT IN TISSUE FACTOR EXPRESSION ALSO PRODUCE A SUPPRESSOR FACTOR, Janet L. Lathey*, Jan M. Agosti**, and Stephen A. Spector*, *Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093, **University of Washington, Seattle, WA 98195
Monocytes/macrophages are important in regulating immune and coagulation systems. Such cells can be infected with human immunodeficiency virus (HIV) and infection of the host may alter the regulation of modulating factors. Thus, tumor necrosis factor (TNFα), interleukin 1-ß (IL-1ß) and tissue factor (TF) were evaluated in monocytes from HIV seropositive patients. RNA was extracted from monocytes with or without LPS stimulation, and hybridized with cDNA probes. Steady state levels of TNFα, IL-1ß, and TF mRNA were low to undetectable in all monocytes before LPS stimulation. In contrast, LPS induced levels of TF mRNA were depressed in patients' monocytes, while TNFα and IL-1ß were "normal." Induced TF mRNA was reduced 66% in monocytes from AIDS patients. Preliminary experiments to determine if a soluble factor was involved in the TF defect have also been performed. Supernate from LPS stimulated monocytes from AIDS patients, but not HIV negative individuals could suppress the level of TF mRNA in LPS stimulated monocytes from HIV negative individuals. The suppression from the "AIDS" supernate was 55% and 65% in two different experiments. Thus, the defect in TF may be related to the production of a suppressive cytokine.

P 533 RELATIONSHIP BETWEEN GLUTATHIONE AND INTERLEUKIN-2 DEPENDENCY OF CYTOTOXIC T CELLS. Chi-Ming Liang and Shu-Mei Liang, Molecular Oncology Inc. Gaithersburg, MD 20878 and CBER, FDA, Bethesda, MD 20892
We have previously shown that glutathione (GSH) upregulates the actions of interleukin-2 (IL-2) on the murine cytotoxic T cell lines such as CTLL-2 and CT-4R (J. Biol. Chem. 264, 13519). Here, we further study the interrelationships of GSH depletion, IL-2 dependency and cytotoxic T cells. Flow cytometry analysis showed that when cultured in medium containing IL-2 (10 units/ml) and GSH (0.5 mg/ml; or 3 x 10-5 M 2-mercaptoethanol), there were at least two populations of CTLL-2 cells - 70% with relatively high level and 30% with low level of IL-2 receptors. If the cells were incubated in the culture medium without GSH for 8-18 h, there was little change of the expression of IL-2 receptors in the cells with high level IL-2 receptors. There was, however, a time-dependent decline (from 30% to 2%) in the population of the cells with low level IL-2 receptors. Incubation of CTLL-2 cells with GSH (up to 1 mg/ml) for 1-9 h immediately after 18 h GSH depletion did not restore the number of cells with low level IL-2 receptors. Since GSH upregulates the actions of IL-2, these results suggest that IL-2 dependent cells with low level IL-2 receptors are more sensitive than those with high level IL-2 receptors to GSH depletion and may perish after depletion of GSH for a period of time ($\geq$ 8 h).

P 534 LYMPHOKINE EXPRESSION IN IMMUNODEFICIENT WASTED MICE. C.R. Libertin, J. Chung, M. Padilla, G.E. Wolocschak. Loyola University Chicago, Maywood, IL 60153 and Biological and Medical Research Div., Argonne National Laboratory, Argonne, IL 60439.
Wasted mice bear the autosomal recessive gene wst which is expressed as a combined neurologic and immunologic abnormality. Previous work from our groups have detected T- and B-cell abnormalities in these mice. To determine the potential role for lymphokine abnormalities in the pathology of the disease of wasted mice, we examined expression of lymphokines in thymus and spleens of wst/wst mice relative to age-matched controls. Relative accumulation of mRNA specific for IL1, IL2R, IL2, IL5, gamma-IFN, and TGF-beta were determined by dot blot hybridization and microdensitometry. IL2 and gamma-IFN in thymocyte derived RNA from wasted mice were elevated compared to controls. All other cytokines were expressed at control levels except IL5 in thymus, which showed a five-fold reduction in wasted mice. These results may be explained by low percentages of Th2-cells or a generalized cytokine dysregulation in lymphocytes from wasted mice. The results also support a potential role for IL5 deficiency (and perhaps a compensatory increase in TGF-beta expression) in the secretory immune deficiency. (Supported in part by OHER, USDOE, Contract No.W-31-109-ENG-38).

P 535 DOMINANT POSITIVE AND NEGATIVE SELECTION USING A HYGROMYCIN PHOSPHOTRANSFERASE-THYMIDINE KINASE FUSION GENE. Stephen D. Lupton, Laurie L. Brunton, Victoria A. Kalberg, and Robert W. Overell. Department of Molecular Biology, Immunex Corporation, 51 University Street, Seattle, WA 98101-2977. The bacterial hygromycin phosphotransferase (*hph*) gene was fused in-frame with the *Herpes simplex* virus type I thymidine kinase (HSV-I TK) gene. The resulting selectable fusion gene (termed HyTK) encodes a bifunctional fusion protein that confers hygromycin B resistance (Hm^r) for dominant positive selection and ganciclovir sensitivity (GCV^s) for negative selection, and provides a means by which these selectable phenotypes may be expressed and regulated as a single genetic entity. Plasmid vectors containing the HyTK selectable fusion gene were constructed and used to demonstrate the efficacy of the gene for positive and negative selection in NIH/3T3 and Rat-2 cells. The HyTK selectable fusion gene was slightly more effective than the *hph* gene at conferring Hm^r in both cell types. When compared with the HSV-I TK gene in Rat-2 cells, the HyTK selectable fusion gene was slightly less effective at conferring the ability to grow in HAT medium (HAT^r), but was markedly more effective at conferring GCV^s. Retroviral vectors were constructed containing the HyTK selectable fusion gene. High titer virus stocks were generated, which conferred both Hm^r and HAT^r on infected cells. Moreover, cells infected with the retroviruses contained unrearranged proviruses, and were killed (>99.9%) by GCV. The HyTK selectable fusion gene is therefore a useful addition to the existing repertoire of selectable genes, because it allows positive and negative selection in wild-type cells. In particular, the HyTK selectable fusion gene should be of significant value as a 'suicide gene' in gene therapy applications, permitting graft ablation with GCV.

P 536 GENE EXPRESSION IN LYMPHOID CELLS FOLLOWING INFECTION WITH MURINE CYTOMEGALOVIRUS, C. L. Martens, R. Lee, N. Street, and L. J. Murray, Dept. of Immunology, DNAX Research Institute, Palo Alto, CA 94304.

Infection of mice with cytomegalovirus results in strong induction of cytotoxic T cells and suppression of hemopoiesis, both of which may result from changes in cytokine production. Two strains of mice, genetically resistant (C3H) or susceptible (BALB/c) to MCMV infection, were compared at various times after sublethal infection with MCMV. C3H mice had a consistently higher ratio of $CD8^+$ to $CD4^+$ T cells throughout the infection than did BALB/c. Short-term $CD8^+$ T cell clones generated from infected animals were assessed for T-cell receptor $V\beta$ gene usage. Six of 14 short-term $CD8^+$ T cell clones grown from infected C3H mice transcribed the $V\beta8.2$ gene, while only one utilized $V\beta8.1$; of four $CD8^+$ T cell clones grown from MCMV-infected BALB/c mice, one utilized $V\beta8.1$, and none transcribed $V\beta8.2$. FACS staining of fresh spleen cells for T-cell receptor $V\beta$ regions suggested that $V\beta8.2$ was expressed at a much lower level in the BALB/c mice than in the C3H mice, both before and during infection. Thus induction of a larger population of $CD8^+$ T cells and/or a $V\beta8.2^+$ subset of T cells may be involved in the greater resistance of C3H mice to MCMV.

Cytokine gene expression was evaluated in T cell subsets freshly isolated from infected mice without *in vitro* culture. In both BALB/c and C3H mice, IL-2 mRNA levels were suppressed from day 2 to 5 after infection. Interferon-γ transcript levels increased through day 7 post-infection in $CD8^+$ T cells, and serum IFN-γ levels increased on days 5 and 7 after infection. Short-term $CD8^+$ T cell clones from BALB/c mice all transcribed GM-CSF, while only half of the C3H clones expressed GM-CSF. All $CD8^+$ T cell clones from both strains transcribed TNF-α, TNF-β, and IFN-γ, but not IL-2, IL-4, or IL-10. The differences in cytokine expression may be related to differences in T cell subsets which develop after infection with MCMV.

P 537 INDUCTION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN THE IMMEDIATE EARLY RESPONSE OF MACROPHAGES TO COLONY-STIMULATING FACTOR 1, Hitoshi Matsushime,[1] Martine F. Roussel,[1] Kouji Matsushima,[2] and Charles J. Sherr,[1,3] Department of Tumor Cell Biology[1] and Howard Hughes Medical Institute,[3] St. Jude Children's Research Hospital, Memphis, TN 38105, and Laboratory of Molecular Immunoregulation,[2] National Cancer Institute, Frederick, MD 21701

Apart from its role in hematopoiesis, colony-stimulating factor 1 (CSF-1) acts on mature macrophages to modulate their production of inflammatory mediators. Using a library prepared from CSF-1 stimulated macrophages, a cDNA encoding the murine interleukin-1 (IL-1) receptor antagonist (IL-1Ra) was cloned, sequenced, expressed in mammalian and bacterial cells, and shown to compete with IL-1α in binding to type I IL-1 receptors. Mouse IL-1Ra is a 22 kDa glycoprotein that is 76% related to its human counterpart but shows considerably less homology to the α and β forms of IL-1. CSF-1 treatment of macrophages leads to a rapid and sustained increase in IL-1Ra mRNA in the G1 phase of the cell cycle that was not inhibited by cycloheximide. Induction of IL-1α mRNA occurred somewhat more slowly and was abrogated by protein synthesis inhibitors. No induction of IL-1 or IL-1Ra was observed in CSF-1 responsive NIH/3T3 cells engineered to express the human CSF-1 receptor, demonstrating that regulation of both genes by CSF-1 depends on cell context and can be dissociated from the proliferative response. In agreement, bacterial lipopolysaccharide, a macrophage activator, induced both IL-1 and IL-1Ra mRNA in macrophages. The kinetics of its induction and presence of a signal peptide suggest that IL-1Ra may be secreted more rapidly than IL-1. The data suggest that macrophages can both positively and negatively regulate the IL-1 response of T cells and fibroblasts.

P 538 INDUCTION OF IL-1ß PRODUCTION IN HUMAN DERMAL FIBROBLASTS BY IL-1α AND TNF-α. Existence of protein kinase C-dependent and adenylate cyclase-dependent pathways.

Alain Mauviel, Nathalie Temime*, Dominique Charron*, Gérard Loyau and Jean-Pierre Pujol. Laboratoire de Biochimie du Tissu Conjonctif, CHU Côte de Nacre, F-14040 Caen Cédex, * : Laboratoire d'Immunogénétique Moléculaire, Paris, France.

We have investigated the mechanisms involved in the regulation of IL-1ß production by cultured human dermal fibroblasts. We show that IL-1ß is constitutively expressed as a cell-associated form, no soluble form being observed in control as well as in stimulated cell supernatants. IL-1α and Tumor Necrosis Factor-α (TNF-α) exerted a dose-dependent stimulation on the production of this cell-associated IL-1ß, as estimated with a specific enzyme linked immunosorbent assay. As expected, this effect was associated with a huge release of prostaglandin E_2 (PGE_2) and by a transient rise in intracellular cyclic AMP. IL-1ß production was also increased, but to a lesser extent, by addition of increasing concentrations of the protein kinase C activator phorbol myristate acetate or with low concentrations (0.001 and 0.01 µg/ml) of PGE_2. In contrast, higher concentrations (0.1 and 1 µg/ml) of PGE_2 were clearly inhibitory, as well as exogenously added dibutyryl-cyclic AMP. H7, an inhibitor of protein kinase C, reduced the stimulatory effect of IL-1α and TNF-α, suggesting the implication of protein kinase C in the upregulation of IL-1ß expression in fibroblasts. As a consequence of indomethacin addition, not only prostaglandin synthesis was inhibited but also cyclic AMP formation was dramatically reduced, probably because secondary stimulation of adenylate cyclase by PGE_2 was lacking. This resulted in a strong potentiation of the stimulatory effect of the two cytokines studied, supporting the role of both cyclooxygenase and adenylate cyclase pathways in the endogenous downregulation of IL-1ß induction by IL-1α and TNF-α.

P 539 RADIATION-INDUCED TNF PRODUCTION, William H. McBride, Sian Comora, Chi-Shiun Chiang, Kristina Rhoades and James Economou, Departments of Radiation Oncology and Surgical Oncology, and Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA 90024
Clinically relevant low dose (2 Gy) in vitro irradiation of macrophages, astrocytes, and microglial cells primes these cells for TNF production as evidenced by their ability to respond to endotoxin. The kinetics of the TNF response are essentially unaltered, only the magnitude is changed. The primed state is associated with the appearance of TNF mRNA. In contrast, doses of radiation greater than 4 Gy decrease TNF production. Local abdominal irradiation of mice also primes peritoneal macrophages for TNF production with dose-response characteristics similar to those seen with in vitro irradiation. Transient transfection systems using the TNF promoter ligated to the luciferase gene are being used in attempts to define the cis-acting response elements within the promoter region. TNF production may be responsible for some of the early responses to radiation. The dose response characteristics suggest that cellular responses at clinical doses are different from those at higher doses, and this may have significance in helping to define the best therapeutic radiation dose.
(Supported in part by PHS grant number CA-31612 awarded by the NCI, DHHS.)

P 540 DIFFERENTIAL cDNA SCREENING OF ORGANISED cDNA LIBRARIES AS A MEANS FOR ISOLATING NEW CYTOKINES, A. Minty[1], P. Chalon[1], J.C. Guillemot[2], C. Labit[3], P. Leplatois[4], P. Liauzun[1], G. Loison[4], J. Lupker[3], M. Kaghad[1], M. Magazin[2], B. Miloux[3], C. Minty[1], P. Ramond[5], D. Shire[6], N. Vita[2], D. Caput[1] and P. Ferrara[2], Depts. of Molecular Biology (1), Protein Biochemistry (2), Recombinant Animal Cells (3), Recombinant Microorganisms (4), Computer Analysis (5) and Organic Chemistry (6), Sanofi Elf Bio Recherches, Labège 31328 FRANCE
We have constructed and organised cDNA libraries from activated human peripheral blood cells and bone marrow stromal cells. These libraries have been screened with cDNA probes from (1) peripheral blood lymphocytes treated with different activators (PMA, PHA and anti-CD 28) or inhibitors (cyclosporin A), (2) a macrophage cell line (U937) stimulated with PMA with and without cycloheximide, and (3) astrocytes and bone marrow stromal cells with and without IL1 stimulation.
Amongst a number of new inducible mRNAs discovered in this way, we have found members of the IL8-related "small induced secreted" (SIS) cytokine family (hSISI,II,III). hSISI and hSISII correspond to the groβ and groγ genes recently cloned by Haskill et al Proc. Natl. Acad. Sci. USA 87, 7732-7736, and Tekamp-Olsen et al J. Exp. Med. 172, 911-919. These mRNAs are expressed in the U937 monocyte and U373 astrocyte cell lines. Differential expression of SIS and other cytokines in these cells will be discussed. We are also investigating the possible identity of receptors for IL8 with those for other hSIS proteins. Finally, some biological activities of recombinant hSIS cytokines will be discussed.

P 541 CHARACTERIZATION OF A NOVEL FAMILY OF TRANSCRIPTIONAL FACTORS REQUIRED FOR IL-1β EXPRESSION. Maria A Monroy, Brian G. Monks, Jon Buras, Gary W. Hunninghake, and Matthew J. Fenton, Departments of Pathology and Medicine, Boston University Medical School, Boston MA 02118, and University of Iowa Hospital and Clinic, Iowa City IA 52242.
Interleukin 1 (IL-1) α and β are pluripotent proinflammatory cytokines that play a critical role in the pathogenesis of rheumatoid arthritis. We have identified a novel nuclear protein, termed NFIL-1βA, that binds to a highly conserved sequence upstream of the TATA box of the human and murine IL-1β promoters. NFIL-1βA is present in virtually all cells which are capable of expressing IL-1. Some cell types express multiple forms of NFIL-1βA, and it is possible that these isoforms represent either distinct DNA-binding proteins, heterodimers that share a common DNA-binding moiety, or post-translationally modified forms of the same protein. Distinct isoforms can be distinguished by their elution profile from DEAE columns and sensitivity to brief heat treatment. UV-crosslinking studies have been performed to determine the molecular sizes of these isoforms. Studies of the functional role of NFIL-1βA suggest that this factor is required for basal promoter function, as well as for the ability of the promoter to be trans-activated by CMV immediate early gene products. These findings are consistent with the role of NFIL-1βA as a "bridging" factor that may receive or channel stimulatory signals from inducible upstream enhancer elements.

P 542 IL-2 INDUCES IL-6 EXPRESSION IN HUMAN MONOCYTES,
Tiziana Musso[1], Igor Espinoza-DelGado[2], G. Luca Gusella[1], Kari Pulkki[2] and Luigi Varesio[2], [1] Biological Carcinogenesis and Development Program, PRI/DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, [2] Biological Response Modifiers Program, Division of Cancer Treatment, NCI, Frederick, MD 21702-1201.
Monocytes are the main producers of IL-6 in the blood. We investigated the effect of IL-2 on IL-6 production in human monocytes. We found that IL-2 (100, 500, 1000 U/ml) induces IL-6 mRNA expression and IL-6 secretion in the supernatant, in a dose dependent manner. Since IL-2 treatment induces IL-1β expression in human monocytes, we examined whether the IL-2 induction of IL-6 is mediated by IL-1β. Monoclonal antibodies against IL-1β were able to block the IL-1 induced IL-6 at the level of mRNA and secretion in the supernatant but failed to block the IL-2 induced IL-6 demonstrating that the effect is not mediated by IL-1β. Because IL-6 is a potent effector molecule in inflammatory processes, it is concivable that its production is controlled by stimulatory and inhibitory signals. We investigated the effect of TGFβ1 on IL-6 production in human monocytes. TGFβ (0,1, 1, 10 ng/ml) inhibited, in a dose dependent manner, the induction of IL-6 by IL-1 and IL-2 at the level of messanger RNA and bioactivity. The inhibitory effects of TGFβ on IL-6 production may be an important mechanism in the control of IL-6 levels.

P 543 REGULATION OF FRESHLY-ISOLATED IL-4-PRODUCING T CELLS BY IL-2 AND IFN-γ,
Marisa F. Naujokas and Alison Finnegan, Deptartments of Immunology/ Microbiology and Medicine, Section of Rheumatology, Rush Medical Center, Chicago, IL, 60612.
It has been reported that IL-4-producing Th2 clones are regulated by IL-2 and IFN-γ. The present studies investigate whether freshly-isolated IL-4-producing T cells (IL-4p) were similarly regulated by IL-2 and IFN-γ. Limiting dilution analysis (LDA) was used to quantitate IL-4p responding to anti-CD3. This system detects IL-4p after 3 days in culture, and requires proliferation of responding cells. Addition of exogenous IL-2 increased the frequency of IL-4p 5-20 fold in a manner that was not completely dependent on proliferation. IL-4p were not detected when endogenous IL-2 activity was blocked by anti-IL-2 antibodies. These data suggest that IL-4p are dependent on IL-2 for detection. The ability of IFN-γ to inhibit freshly-isolated IL-4p is controversial in the literature. In this LDA system, addition of exogenous IFN-γ did not significantly inhibit the frequency of IL-4p detected. However, partially blocking endogenous IFN-γ activity resulted in a 2-3 fold increase in the detected frequency of IL-4p, suggesting that endogenous IFN-γ may be inhibiting induction of some IL-4p in the cultures. These data suggest that the ability to detect freshly-isolated IL-4p is dependent on the amount of endogenous IFN-γ present in the cultures. Evaluating effects of IFN-γ in antigen-specific responses may provide more information on this question. Furthermore, studies to determine whether functionally distinct cells may be differentially sensitive to inhibition by IFN-γ are in progress. In conclusion, freshly-isolated IL-4p appear to be regulated positively by IL-2 and negatively by IFN-γ, similarly to Th2 clones.

P 544 REGULATION OF CYTOKINE GENE EXPRESSION THROUGH NF-κB: ROLE OF SEQUENCE CONTEXT AND DNA BENDING IN NF-κB INTERACTION WITH ITS TARGET SITE,
S.A.Nedospasov, A.N.Shakhov R.L.Turetskaya and D.V.Kuprash, Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Moscow 117984 USSR.
NF-κB system is involved in transcriptional regulation of the genes encoding many cytokines and their receptors. Role of the upstream κB sites in the activation of the tumor necrosis factor (TNF) gene was previously demonstrated. Similarly, transfections of promoter deletion/CAT constructs of the human lymphotoxin (LT) gene into lymphoid cells suggested a role for a conserved κB site 100 bp upstream to promoter. In view of NF-κB induction by TNF and LT these sequences might be involved in positive feedback loop regulation. Since TNF loci contain a number of presumed κB sites, located in homologous and non-homologous positions both upstream and downstream to the two genes, we wished to compare their role in gene regulation. Synthetic oligonucleotides corresponding to various sites showed different affinity to NF-κB in vitro. In particular, conserved κB site in the promoter region of LT and non-conserved site #3 from the mouse TNF gene, both with their natural flanks, were active in transfection/CAT assay and showed strongest binding in vitro. Site #3 had sequence features which implied that DNA conformation and/or bending properties might be important for binding. We then addressed more general question: how sequences which flank the consensus "core" of κB target site influenced affinity to NF-κB from nuclear extracts prepared from macrophages and from lymphoid cell lines. Our data indicate that substitution of the natural flanks could change affinity of a particular site. To directly analyse bending properties of various κB-related sequences, synthetic duplexes were cloned into pBend2 vector and short labeled DNA fragments with different locations of the target site were prepared using PCR. Band shift data indicate that κB sequences, which have no intrinsic bend, are significantly bent once bound to NF-κB. Estimation of the bending angle suggests that DNA is bent differently in low and high molecular weight complexes from lymphoid cells.

P 545 TNFα AS A MEDIATOR OF FLUID EXSORPTION AND TISSUE DAMAGE IN SALMONELLA-INDUCED INTESTINAL DISEASES, David W. Niesel, Judy W. Arnold, Johnny W. Peterson and Gary R. Klimpel, Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550

Tumor necrosis factor (TNF) production in the intestinal tract following *Salmonella typhimurium* infection was investigated using a mouse ligated ileal loop model. Intestinal segments infected with *S. typhimurium* had high levels of fluid secretion as early as 6 hrs post-bacterial infection. At this time, low levels of TNF activity were also present in the fluid obtained from infected segments. At 20 hrs post-infection, high levels of TNF activity were present in fluid obtained from infected intestinal segments and was characterized as TNFα by neutralization experiments using rabbit antisera to TNFα. TNFα production was further verified by Northern blot analysis using RNA obtained from cells eluted from the infected intestinal segments. No TNF activity was found in fluid obtained from intestinal segments challenged with cholera toxin. To investigate the role TNFα plays in *Salmonella*-induced inflammation and/or fluid secretion, intestinal segments were injected with recombinant mouse TNFα (rTNFα). Intestinal segments injected with rTNFα had high levels of fluid secretion at 12-24 hrs post-rTNFα challenge. Further, the histological profile of intestinal segments injected with rTNFα appeared identical to segments infected with *S. typhimurium*. These results document the production of TNFα in the intestinal tract following *S. typhimurium* infection and suggest that TNFα may be involved in the fluid secretion and/or pathology associated with *Salmonella* infections of the intestinal tract.

P 546 **ALTERATIONS IN T-CELL EXPRESSION OF GM-CSF AND IL-3 BY HTLV-INFECTION.** Stephen D. Nimer, Maurice J. Wolin, Roy Lau, Soon Ki Shin, Ming Peng, Karen Kwan, and Masayo Kornuc. Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024.

GM-CSF (granulocyte-macrophage colony-stimulating factor) and IL-3 (interleukin-3) are early acting hematopoietic growth factors expressed by activated but not resting T lymphocytes. All HTLV-infected T-cell lines tested to-date constitutively express GM-CSF and can be stimulated to increase GM-CSF expression by stimulating with PHA and TPA. In contrast IL-3 mRNA is neither constitutively expressed nor inducible in these cell lines. We and others have previously demonstrated that the Tax proteins of HTLV-I and -II can transactivate GM-CSF promoter CAT constructs transiently transfected into HTLV-uninfected T cells. We now find that Tax can also activate IL-3 promoter-CAT constructs in these HTLV-uninfected T-cell lines, which is surprising given the lack of IL-3 expression by HTLV-infected T-cell lines. We have prepared nuclear extracts from HTLV-infected and uninfected T-cell lines and have characterized DNA-protein interactions within the GM-CSF and IL-3 upstream flanking sequences. We are utilizing this strategy to determine the cellular basis for the discordant expression of GM-CSF and IL-3 in these cells.

P 547 **GM-CSF REGULATES A NOVEL MEMBER OF THE MIP-1 CYTOKINE FAMILY,** Amos Orlofsky, Mark S. Berger and Michael B. Prystowsky, University of Pennsylvania, Philadelphia, PA 19104

We have used a differentially screened cDNA library prepared from GM-CSF-stimulated murine bone marrow cultures to isolate six novel hematopoietic-specific cDNA clones. Two of these clones (A1 and C10) are induced >10-fold within one day of culture in GM-CSF. The sequence of C10 predicts a secreted protein of 95 aa after signal peptide cleavage. This amino acid sequence has 32% average identity to other murine members of the family of host defense-related cytokines of which the prototype is macrophage inflammatory protein-1 (MIP-1). Unlike the other members of this family, however, C10 mRNA is only very weakly stimulated by activating agents in macrophages and is strongly reduced, rather than stimulated, in activated T-lymphocytes. Conversely, MIP-1 is not rapidly stimulated in GM-CSF-treated bone marrow. C10 mRNA is also stimulated during G-CSF-induced granulocytic differentiation of the myeloid precusor cell line, 32D cl3. The C10 clone may encode a cytokine with an unusual pattern of regulation in hematopoietic cells.

P 548 EFFECTS OF DIFFERENT POLYSACCHARIDES ON TNF-α PRODUCTION FROM HUMAN MONOCYTES. Marit Otterlei, [1]Gudmund Sjåk-Bræk, Liv Ryan, [1]Olav Smidsrød and Terje Espevik, Institute of Cancer Research and [1]Institute of Biotechnology University of Trondheim, N-7006 Trondheim, Norway.

Polysaccharides other than LPS are reported to have immunostimulating activity, but little is reported about the effects of different polysaccaridens on cytokine production from human monocytes. In this study we show that several well-defined polysaccharides including polymers of different size of β1-4 linked D-mannuronic acid (poly-M and M-blocks), β1-4 linked partly N-deacetylated chitin (chitosan), β1-4 linked D-glucoronic acid (C60XY) and β1-3 linked D-glucose (SG) induces human monocytes to produce TNF-α. Poly-M was the most potent polysaccharide tested, approximately equal to LPS from E. coli. TNF-α production was shown to depend strongly on the m.w. of poly-M and chitosan, with a maximal TNF-α production for m.w. above 50,000 and 20,000, respectively. These data indicate that β1-4 linked polysaccharides are potent stimulators of TNF-α production.

P 549 MURINE MEGAKARYOCYTOPOIESIS DEPENDS ON BUTYRYLCHOLINESTERASE GENE EXPRESSION D. Patinkin*, S. Seidman*, F. Eckstein**, F. Benseler**, H. Zakut*** and H. Soreq*. *Dept. Biol. Chem., HUJ, Jerusalem 91904, Israel. **Abteil. Chem., Max Planck Inst., Gottingen, FRG; ***Dept. Obstet. & Gynecol., Wolfson Med. Center, Holon, Israel.
We have investigated the relationship between butyrylcholinesterase (BCHE) gene expression and megakaryocytopoiesis (MGPS) by three different methods, using a serum-free methyl cellulose culture system with interleukin 3(IL-3) as inducer. (1) Megakaryocytes (MGs) stained with indolyl acetate, an esterase stain, in the presence of specific inhibitors to each of the cholinesterases (CHEs), expressed both acetylcholinesterase and BCHE. The expression was evidenced in the cytoplasm and in cells of all stages of development. (2) The incorporation of 15-mer antisense oligonucleotides (AS-CHE), flanking the initiator AUG region of BCHEmRNA, to murine bone marrow (BM) cultures at a concentration of 5 μM, reduced the BM colonies by 50%. A sharp drop in MG and a significant rise in macrophage populations was noted, demonstrating a shift in stem cell differentiation. Equivalent concentration of the corresponding sense oligo had no effect. Pulse-labeling of the oligo cultures with ^{35}S-Methionine indicated that total protein synthesis remained similar to controls and MG reduction was due to specific gene product suppression rather than to toxicity. (3) Growth of murine BM cells with the incubation medium of Xenopus laevis oocytes expressing recombinant BCHE enhanced colony counts by 80% after 4 days in culture as compared to IL-3 controls while medium from oocytes sham-injected with frog saline exhibited a non-significant rise in colonies. These observations implicate BCHE, a humoral component, specifically in MGPS and suggest application of oligos in the modulation of BM development.

P 550 DIFFERENTIAL PRODUCTION OF IL-1 alpha AND IL-1 RECEPTORS BY THY-1$^+$ AND THY-1$^-$ MURINE LUNG FIBROBLAST SUBSETS, Richard P. Phipps, Maria Silvera, Stephen Derdak, David Penney, Peter Keng and Deborah Brown. Cancer Center and Dept. of Microbiology and Immunology, University of Rochester School of Medicine, Rochester, NY 14642.
The purpose of this study was to determine whether subsets of normal murine lung fibroblasts produced IL-1, possessed IL-1 receptors, and if so, whether IL-1 affected their ability to synthesize collagen. We previously identified two major subsets of murine lung fibroblasts based on the expression of Thy-1. Thy-1$^+$ and Thy-1$^-$ fibroblasts synthesize fibronectin and type I and III collagen. These cells lack the characteristics of epithelial, endothelial and hematopoietic cells. Initial studies using a bioassay demonstrated that small quantities of IL-1 were synthesized only by the Thy-1$^-$ subset. IL-1 synthesis was greatly up-regulated by TNF alpha in Thy-1$^-$, but not Thy-1$^+$ fibroblasts. A neutralizing anti-IL-1 alpha monoclonal antibody (161.1) completely inhibited all IL-1 activity from the Thy-1$^-$ fibroblasts. Studies using PCR and Northern blot analysis showed mRNA for IL-1 alpha, but not the beta form. Interestingly, flow cytometric analysis using a monoclonal anti-IL-1 receptor antibody (M5), indicated that the Thy-1$^+$ fibroblast lines and clones displayed relatively high levels of IL-1R in contrast to the low level on Thy-1$^-$ fibroblasts. However, both subsets responded to exogenous IL-1 by increasing production of collagen 2 to 3 fold. These findings support the existence of functional fibroblast subsets and demonstrate that IL-1, which is often elevated in lavage fluid from patients with fibrotic lung disease, may play a key role in promoting aberrant collagen production in pulmonary fibrotic disease. Supported by HL-39949, CA-42739, CA-11198 and 57RR05403-27.

P 551 IL-6 PRODUCTION BY PSORIATIC FIBROBLASTS INDEPENDENT OF SERUM AND GROWTH FACTOR(S). Errol P. Prens, Reno Debets, Klazina 't Hooft-Benne, Theodoor Van Joost and Robbert Benner, Departments of Dermatology and Immunology, University Hospital Rotterdam-Dijkzigt and Erasmus University, Rotterdam, The Netherlands.
We have recently shown that IL-6 levels are elevated in suction blister fluid from lesional psoriatic skin, probably derived from dermal fibroblasts. Since IL-6 was recently reported to induce keratinocyte proliferation, it's role in psoriasis needed further clarification. In the present study, IL-6 production was measured in the supernatants (SN) of cultured fibroblasts from psoriatic lesions and uninvolved skin, and compared to control SN from healthy individuals. The synthesis of IL-6 in keratinocytes was shown to be highly serum dependent. To determine a possible constitutive IL-6 production capacity of fibroblasts, these cells were cultured to sub-confluency, replated and switched to four different culture media. The supernatants were collected on 5 consecutive days, and stored in aliquots at -80 °C. The level of IL-6 was determined using the B9 bio-assay. Samples were always compared to a standard curve, where one unit/ml of IL-6 corresponded to half-maximal [3H]TdR incorporation. Fibroblasts from 90% of psoriasis patients produced low levels of IL-6 (1 to 15 U/ml) under serum-free and growth factor(s) free conditions, while only 15% of the healthy controls produced comparable levels of IL-6 under the latter conditions. This difference in IL-6 production between psoriatic and control fibroblasts was completely restored in the presence of serum or growth factor(s). These differences were found to correlate to IL-6 mRNA levels. The results indicate that psoriatic fibroblasts are consitutively able to produce IL-6 under unstimulated conditions.

P 552 TUMOR NECROSIS FACTOR SECRETION REFLECTS INTRACELLULAR THIOL CONCENTRATION OF HUMAN PERIPHERAL BLOOD MONOCYTES *IN VITRO*
Ronald L. Rabin, Frank J.T. Staal, Marcia M. Bieber, Leonore A. Herzenberg, and Nelson N.H. Teng, Departments of Gynecology and Obstetrics, and of Genetics, Stanford University School of Medicine, Stanford CA 94305
Glutathione, a thiol which detoxifies reactive oxidative intermediates, may regulate TNF secretion via regulation of NF-κB, a transcription factor which regulates the mRNA synthesis of TNF. When intracellular glutathione concentrations (GSHi) fall, NF-κB activity increases, and vice-versa. This relationship between GSHi and the regulatory pathway of TNF secretion led us to ask whether TNF secretion by stimulated peripheral blood monocytes (PBM) is preceded by a decrease in intracellular thiols.
We purified PBMs, stimulated them with LPS, and stained them with monochlorobimane (a fluorochrome specific for GSHi). We report that mean GSHi concentrations fall rapidly (10 minutes), reach their nadir 3 hours post-stimulation, and then recover, (though not completely) at 18 hours. TNF secretion by the stimulated PBMs, as determined by the murine fibroblast cytotoxicity assay, reflect the fall in GSH, peaking 4 hours after LPS stimulation, and fall to near zero 18 hours after stimulation.

P 553 POST TRANSCRIPTIONAL STABILIZATION OF GM-CSF mRNA BY A LABILE PROTEIN IN HUMAN T CELLS. D. Razanajaona, C. Lavezzi, M. Lopez, M. Mouren P. Mannoni.and J. Gabert. Department of Molecular and cellular Biology-U119 Centre Anticancéreux, 232 Bd. Ste Marguerite 13009 MARSEILLE - FRANCE
GM-CSF mRNA regulation was studied in Jurkat,a human T cell line, known to synthesize GM-CSF upon activation. Many structural studies on the regulatory regions (promotor and 3' untranslated region) of the GM-CSF gene have been carried out on human T cells but to our knowledge no dynamic study on GM-CSF mRNA expression has yet been reported in these cells.The analysis included Northern Blot analysis, Run On experiments and half life measurement after Actinomycine D chase. These experiments were performed on cells activated by TPA or/and PHA with or without Cycloheximide added at different culture times.We thus observed that TPA induced a peak of GM-CSF mRNA at 6 h, but after a decrease at 12 h, a second peak arose at 24 h. It was shown that this pattern of GM-CSF mRNA expression corresponded to transcriptional and post-transcriptional mechanisms of regulation. The balanced state seen on Northern analysis at 24h of TPA activation was linked mainly to an increase in the half-life (5h) of GM-CSF mRNA. In contrast to the expected cycloheximide effect , well known to stabilize the unstable transcripts like the GM-CSF mRNA, after 24 h TPA induction the cycloheximide decreased this half-life (45').Similar results were found with the PHA and PHA+TPA activations. Our data suggests that besides the classical regulation already described by the structural studies , stabilizing labile proteins are involved in GM-CSF mRNA regulation in human T cells, underlying the complexity of the regulation of this cytokine.

P 554 ALTERATIONS IN C/EBP-LIKE PROTEINS DURING THE ACUTE-PHASE RESPONSE, David Ron, Allan R. Brasier and Joel F. Habener, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02114

Transcriptional activation of the rat angiotensinogen gene during the acute-phase response is mediated by a cis-acting DNA element that binds an IL-1 and TNF-inducible NFkB-like protein as well as a family of C/EBP-like proteins. By a mechanism of mutually-exclusive binding, the C/EBP-like proteins attenuate the activation of the angiotensinogen promoter by NFkB. We now demonstrate that in addition to nuclear translocation of NFkB, injection of rats with lipopolysaccharide leads to an alteration in the profile of C/EBP-like DNA-binding proteins in liver nuclei. Western blot analysis reveals that induction of the acute-phase response is followed within 4-6 hours by a diminution in C /EBP immunoreactivity detected in nuclear extracts. This diminution is correlated with loss of a predominant 43 Kd DNA-binding protein in Southwestern blots probed with labeled C/EBP recognition site oligonucleotide. Similar results were obtained in 3T3-L1 adipocytes that had been treated with monocyte-conditioned medium. Evidence that these changes in C/EBP level are functionally significant is provided by the demonstration that transcriptional activity of reporter genes that are dependent on C/EBP-activation falls on treatment of hepatoma cells with monocyte conditioned medium. In adipocytes the monocyte conditioned medium-induced changes in C/EBP level correlates with a fall in the mRNA of several adipocyte specific genes known to be transcriptionally regulated by C/EBP. Pulse-chase labeling of 3T3 adipocytes followed by immunoprecipitation of C/EBP demonstrates that the monocyte conditioned medium-induced fall in C/EBP levels is effected, in part, by an increase in its rate of degradation. The functional consequences of the inflammation-mediated changes in liver and adipose tissue complement of C/EBP-like proteins are discussed.

P 555 AN EXAMINATION OF CYTOKINE INHIBITION BY PENTAMIDINE AT THE TRANSCRIPTIONAL, TRANSLATIONAL, and POST-TRANSLATIONAL LEVEL, Gary J. Rosenthal, Emanuela Corsini, and William Craig, Immunotoxicology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Regulation of cytokine release by cells at varying levels of protein processing can have substantial therapeutic value in terms of specificity and toxicity. We have recently demonstrated the ability of the *Pneumocystis carinii* therapeutic, pentamidine isethionate (PT), to inhibit the elaboration of interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor (TNF) from isolated rat and human macrophages at therapeutically relevant concentrations. That this inhibition occurs in vivo is demonstrated by the reversal of both mortality and hypothermia induced by high doses of endotoxin. Macrophages treated in vitro with lipopolysaccharide or in vivo with endotoxin were examined for gene expression of IL-1, IL-6 and TNF. In rat macrophages, the subcellular level of cytokine inhibition by PT appears to be quite distinct from corticosteroid induced inhibition. Specifically, PT modulation of transcription is minimally affected while dexamethasone completely inhibits cytokine mRNA. While extracellular release of cytokines are blocked > 80% by PT concentrations >10-6M, cytosolic levels of bioactive cytokine appear normal suggesting that translation of mRNA was taking place. Cell associated 31 kDa precursor IL-1 exists in substantial quantities as detected by Western blot and was specifically associated with the membrane as determined by bioassay. These studies demonstrate that unlike classical cytokine inhibitors such as corticosteroids which block at the level of mRNA, PT modulates cytokines at a post-translational stage of processing and may represent a valuable anti-inflammatory agent as well as an important tool in understanding intracellular protein processing.

P 556 GM-CSF-RESPONSIVE SEQUENCES UPSTREAM OF THE PRIMARY RESPONSE GENE, EGR-1/TIS8, IN FACTOR-DEPENDENT HUMAN MYELOID LEUKEMIA CELL LINES, Kathleen M. Sakamoto and Judith C. Gasson†, Division of Hematology-Oncology, Children's Hospital of Los Angeles 90054-0700; †Departments of Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles CA 90024.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein that promotes proliferation and differentiation of myeloid progenitors and enhances the function of neutrophils, monocytes and eosinophils. The biochemical pathways mediating GM-CSF action are unknown. However, we previously demonstrated that the effects of GM-CSF on cell growth and function involve rapid and transient induction of specific primary response genes, including EGR-1/TIS8 in murine myeloid leukemia cells (32DCl3) and human neutrophils (Varnum et al., *Mol Cell Biol* 9:3580-3583, 1989). In order to identify sequences mediating GM-CSF-induced gene expression, we isolated and mapped the human EGR-1/TIS8 gene. We sequenced approximately 700 base pairs (bp) of the 5′ upstream region and found a number of putative regulatory elements, including two Spl binding sites, a TPA-like responsive element, five serum response-like elements, an EGR-1 binding site, and two cAMP-like elements. We subcloned 600 bp of the human EGR-1/TIS8 promoter region into a vector containing the chloramphenicol acetyltransferase (CAT) reporter gene. This construct was transiently transfected into factor-dependent human myeloid leukemia cell lines that were stimulated with GM-CSF or TPA. Both GM-CSF and TPA induced significant increases in CAT activity over that found in unstimulated cells. We can therefore use early nuclear events as an endpoint, and by working backwards, elucidate the mechanisms by which GM-CSF transmits the biological signals required for myeloid cell proliferation and maturation.

P 557 **ISOLATION AND CHARACTERIZATION OF FcεR1 POSITIVE CELLS IN BOTH NORMAL AND ANTI-IgD STIMULATED MICE WHICH PRODUCE IL-4 UPON STIMULATION**, Robert A. Seder, Marshall Plaut, Fred D. Finkelman, Steven J. Galli, Ann M. Dvorak, Anne Kagey-Soboyka and William E. Paul. LI, NIAID, NIH, Bethesda, MD 20892, The Johns Hopkins Asthma and Allergy Center, Baltimore, MD, 21224 USUHS, Bethesda, MD, 20814 and Beth Israel Hospital, Boston, MA 02215

We have previously demonstrated the existence of a population of cells in both spleen and bone marrow that can be induced to secrete IL-4 upon crosslinkage of FcεRI. We now report purification of FcεR positive cells from both spleen and bone marrow from normal and anti-IgD stimulated mice. This population represents 0.1% of spleen cells in both naive and stimulated mice, 1% of bone marrow cells from naive donors, and 3-5% from anti-IgD stimulated mice. These FcεR positive cells can be induced to produce IL-4 by crosslinkage of FcεRI, FcγRII or ionomycin while no IL-4 could be detected from FcεR negative cells to the same stimuli. The frequency of FcεR positive cells capable of producing IL-4 upon stimulation is approximately 1/5 from stimulated mice and 1/25 from naive donors. These cells are enriched in Alcian blue positive cells and histamine-containing cells. They also show enrichment of mast cell precursors which form colonies when stimulated in liquid or semi-solid media. By electron microscopy, more than 90% of granulated cells appear to be mature or immature basophils. However, this FcεR positive population also contained non-granulated monocytoid cells. Currently, efforts are underway to determine which cell population is the principal IL-4 producer. These results demonstrate the purification of mouse basophils and show that all the capacity of splenic and bone marrow non-B, non-T cells to produce IL-4 in response to FcεR crosslinkage or ionomycin is found in the FcεR positive cells.

P 558 MODULATION OF HUMAN IL-6-RECEPTORS, Luc Snyers and Jean Content, Department of Virology, Institut Pasteur du Brabant, B-1180 Bruxelles (Belgium).

Modulation of hormone and cytokine receptors is thought to play an important role in the responsiveness of cells to these factors. Particularly, synergy between two or more molecules in the induction of a biological effect can sometimes be accompanied (and maybe partly explained) by mutual actions on surface specific receptors.
We have initially observed that human IL-6 receptor was up-regulated by the glucorticoids hormone analog dexamethasone on epithelial cells and found that this effect was observable on two hepatoma cell lines (Proc. Natl. Acad. Sci. USA, 87, 2838-2842, 1990), which was consistent with the well known synergy between glucorticoids and IL-6 in the induction of acute phase proteins. Here we demonstrate that in the hepatoma cell line Hep3B, IL-6R is positively regulated by IL-1β and by IL-6, and negatively by TNF. The results have been obtained by Northern blot analysis but curiously, we failed to demonstrate an effect of IL-1 on IL-6 binding capacity of the cells. We also show that IL-6R mRNA is super-induced by cycloheximide and are currently investigating if this occurs by a transcriptional mechanism or rather by an effect of stabilisation of the mRNA or both.

P 559 MOLECULAR CHARACTERIZATION OF THE HUMAN MONOCYTE INTERLEUKIN-1 (IL-1) RECEPTOR Melanie K. Spriggs*, Pamela J. Lioubin*, Richard Armitage*, Kenneth Grabstein*, David Cosman*, John Sims*, and Joachim Bauer# * Immunex R&D Corp, 51 University St. Seattle, WA, #Medizinische Universitatsklinik Freiburg, Federal Republic of Germany

Primary human monocytes, neutrophils, and monocytic cell lines increase their expression of IL-1R ~10 fold through treatment with PGE2 and steriods. Northern blot analysis of both cell types reveal abundant mRNAs homologous to both the type I IL-1R and the type II IL-1R induced simultaneously in response to steroid or PGE2 and steroid treatment. However, cross-linking of induced monocytes and neutrophils using radiolabeled IL-1β shows a single IL-1R on the cell surface with an apparent molecular mass of ~66kD. Full length cDNAs representing type I and type II mRNAs have been isolated from induced monocytic cells and sequenced. Peptide maps and serologic cross-reactivity are compared between the type I, type II and monocyte IL-1R.

P 560 CONTRASTING EFFECTS OF IL-4 AND TGF-β1 IN REGULATING THE DEVELOPMENT OF $CD4^+$ PRECURSORS INTO HELPER EFFECTORS AND MEMORY CELLS. Susan L. Swain, Andrew Weinberg, Michael Croft, Gus Atkins, Linda Bradley and Gail Huston, Department of Biology, UCSD, La Jolla, CA 92093

We have developed an in vitro model in which mature, but naive $CD4^+$ T cells proliferate and differentiate to generate a population of T cells with helper effector function. We have evaluated the effects of different lymphokines on the phenotype, lymphokine secretion pattern and helper function of the effectors which are generated. The most interesting effects were mediated by IL-4, by IFNγ and by TGFβ1. Addition of these three lymphokines at the initiation of cultures driven by mitogen or antigen led to the development of distinct populations. IL-4 promoted development of short lived effector cells secreting IL-4, IL-5, IL-3 and GMCSF in large amounts (Th2 like), whereas IFNγ promoted development of cells which preferentially secreted IFNγ with IL-3 and GMCSF. Effectors generated in either IL-4 or IFNγ were activated and CD45RB high. In contrast TGFβ1 favored development of a longer lived population which secreted IL-2 and IFNγ (Th1 like) and which has low expression of CD45RB. This later population had several phenotypic properties shared with memory T cells.

P 561 TNF MEDIATED REGULATION OF FERRITIN HEAVY CHAIN GENE EXPRESSION: A ROLE FOR CYTOKINES IN IRON HOMEOSTASIS. Langdon Miller, *Alan Taylor, Steven Miller, Suzy Torti, Yoshiaki Tsuji, and Frank M. Torti. Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305 and the Veterans Administration Medical Center, Palo Alto, CA 94304.
Tumor necrosis factor (TNF), a 17,000 Dalton protein synthesized by stimulated macrophages, has been shown to have not only tumoricidal activities, but also pleiotrophic effects *in vitro* and *in vivo* that are consistent with its role as one of the principal mediators of both the physiologic and pathologic effects of inflammation and septic shock. We recently observed that TNF alpha induces ferritin heavy chain mRNA levels both in mouse TA1 adipocytes and human muscle cells. Ferritin is the major cellular iron storage protein. To explore the relationship between TNF and iron in regulating ferritin, we have examined this regulation in primary human myoblasts. Iron increases the synthesis of ferritin largely by promoting translation of pre-existing mRNA's for both the heavy and light ferritin isoforms. Evaluation of mRNA showed that TNF regulated ferritin heavy chain specifically, provoking no change in steady state levels of ferritin light chain mRNA; iron, in contrast, increased the mRNA of both isoforms. Actinomycin-D blocked the TNF-induced changes in ferritin heavy chain but did not inhibit the translation induction of ferritin seen with iron treatment. Equal TNF induction of ferritin heavy chain mRNA was observed in iron-loaded cells and in cells depleted of iron by an intracellular chelator, 2,2'-dipyridyl. The ferritin heavy chain induction by TNF and iron was almost exactly additive over the entire range of iron concentration. However, the role of iron in translational regulation of ferritin was retained in TNF-treated cells. We conclude that TNF acts independently of iron in its induction of ferritin heavy chain mRNA but requires the presence of iron for this effect to be fully expressed at the protein level.

P 562 **THE INFLUENCE OF RACE, GENDER AND CIGARETTE SMOKING ON SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR LEVELS.** **David J. Tollerud, Carol C. Kurman, Linda Morris-Brown, David L. Nelson, William A. Blattner. University of Pittsburgh, Pittsburgh PA 15261 and the Metabolism Branch & Epidemiology and Biostatistics Program, NCI, Bethesda MD 20892.**

Soluble interleukin-2 receptor (sIL-2r) is a modified form of the IL-2 cell surface receptor which is released from the cell membrane after T-cell activation. sIL-2r can be detected in the blood of normal subjects, and concentrations are increased in patients with a variety of immunologically mediated conditions. Studies in children have shown that concentrations are increased in those < 6 years of age. The influence of age, race and gender have not been well defined in adults. We analyzed serum samples from a population-based survey of 455 healthy adults, ages 20 to 69, including 282 whites and 173 blacks. In univariate analyses (t test), white race, male gender and current smoking were each associated with higher serum sIL-2r concentrations (geometric mean U/ml): RACE (Whites 498 vs Blacks 399; $p \leq 0.0001$); GENDER (Men 477 vs Women 424; $p \leq 0.02$); SMOKING (Smokers 507 vs Nonsmokers 418; $p \leq 0.0002$). Multivariate analyses showed the race and smoking effects to be independently significant ($p \leq 0.0001$). Levels were highest in white smokers (550 U/ml), intermediate in white nonsmokers and black smokers (454 and 452 U/ml), and lowest in black nonsmokers (365 U/ml). These results will be integrated with data on T-cell subsets and serum immunoglobulin levels in this population, to define the interrelationships of these immunologic parameters in a healthy adult population. Normative data such as these will be important for the appropriate interpretation of clinical and epidemiological investigations applying these biologic markers.

P 563 INTERLEUKIN-4 PREVENTS THE INDUCTION OF G-CSF AND IL-6 mRNA IN HUMAN ADHERENT MONOCYTES IN RESPONSE TO ENDOTOXIN AND IL-1 STIMULATION. Edo Vellenga, Joost Th.M. de Wolf*, and M. Ruud Halie*. Department of Hematology, University of Groningen, P.O.Box 30001, 9700 RB GRONINGEN, The Netherlands.

Human recombinant interleukin-4 (IL-4) was studied for its effects on the expression of G-CSF and IL-6 mRNA in human adherent monocytes in the absence and presence of endotoxin and IL-1-α. IL-4 (15 ng/ml) did not induce G-CSF and IL-6 transcripts in monocytes nor did IL-4 prevent the induction of the cytokines in response to endotoxin and IL-1 (50 U/ml) stimulation when added simultaneously. However, monocytes pre-exposed to IL-4 during 3-8 hrs, did not express G-CSF and IL-6 mRNA in response to endotoxin and IL-1 stimulation. This effect was independent of the presence of fetal bovine serum but dependent of the IL-4 dose. In addition it was shown that the induction of G-CSF and IL-6 mRNA by the calcium-ionophore A23187 or by c-AMP elevating agents could be blocked by IL-4. These suppressive effects of IL-4 were not related to changes in the half-life of G-CSF mRNA. In addition the effects were independent of protein synthesis since IL-4 suppressed the G-CSF and IL-6 mRNA induction in cycloheximide treated monocytes. Finally it was demonstrated that IL-4 had comparable effects on the G-CSF secretion of endotoxin and IL-1 stimulated human monocytes tested in a murine bone marrow assay.
These results indicate that IL-4 down regulates the expression of G-CSF and IL-6 gene and secretion of proteins in human activated monocytes.

P 564 **INVERSE COORDINATED GENE REGULATION OF TWO ESSENTIAL ENDOTHELIAL BOUND COFACTORS BY TUMOR NECORISIS FACTOR,** Dietmar von der Ahe, Bertfried Jost, Harald Oderwald and Bernd Pötzsch, Max-Planck-Society,Kerckhoff Clinic, Unit of Haemostasis Research, 6350 Bad Nauheim, F.R.G.

Cytokines are both regulated by transcription factors and responsible for the induction of specific nuclear factors, which in turn influence the transcription of genes in response to enviromental stimuli. Tumor Necrosis Factor alpha (TNF alpha) is considered as important mediator of inflammation, and has many actions on a wide variety of cell types. One major target of TNF is the endothelium, a cell of many talents: e.g. maintaning blood fluidity and important functions in hemostasis, inflammation and wound healing. To study signaltransduction by cytokines we have cloned two endothelial cell cofactor genes including the 5'-flanking regions: (i) the Tissue Factor (TF) gene, an essential cofactor of the extrinsic pathway of coagulation, is positively regulated by TNF alpha (ii) the Thrombomodulin (TM) gene, an essential cofactor of the anticoagulant pathway is negatively regulated by TNF alpha. Both, TF activation and TM suppression by TNF alpha in endothelial cells is a primary transcriptional event (data shown). We used this system as a model to study the TNF signal transduction pathway. For this purpose, the promoter region of both genes have been fused to the Chloramphenicolacetyltransferase (CAT) reporter gene and tested by transfection into primary endothelial cells and cell lines. In both genes basal and TNF dependent regulatory elements are located within 400 bp upstream of the transcription start site. DNase I protection assay revealed several protein binding sites within the TF and TM promoter, among them an AP-2 binding site (TF) and an unusual polypurin/polypyrimidin Box (TM promoter). Currently we are studying these putative promoter elements and how they are involved in the TNF dependent signal transduction pathway.

P 565 ELEMENTS REGULATING INTERFERON-γ (IFN-γ) GENE EXPRESSION. Christopher B. Wilson and Laurie Penix. Depts of Pediatrics and Immunology, University of Washington, Seattle, WA 98195

Initial studies suggested that IFN-γ and IL-2 were co-ordinately regulated, but recent data indicate that this is not the case - naive T cells (defined as CD45RA+ cells in the human), produce IL-2 in amounts similar to memory T cells but produce much less IFN-γ. To address mechanisms mediating these differences, we are studying regulatory elements in the IFN-γ gene. IFN-γ promoter sequences (-41 to +63) and varying amounts of 5' flank were linked to a lacZ reporter and transiently transfected into Jurkat T cells. Minimal activity was observed with any construct in the absence of activation. IFN-γ constructs containing 540 bp of 5' flank gave maximal activity (9.8-fold induction), compared to constructs containing up to 2.7 kb or as little as 110 bp of 5' flank (70% and 30% as active); the promoter alone had minimal activity. Activity of the -540 IFN-γ construct was similar to that of a construct in which lacZ was driven by the IL-2 enhancer/promoter (15-fold induction). Optimal induction required calcium ionophore and PMA and little activity was seen in a human B cell line. These results suggest that critical regulatory elements of the IFN-γ gene are contained in the near 5' flank, wherein are contained several sequences with partial homology to known protein binding sites. Studies to define the role of individual elements are in progress.

Late Abstracts

MUTANT PROTEINS OF INTERLEUKIN 2 AS PROLIFERATION FACTORS FOR PRIMARY HUMAN T-CELLS.

Albert Duschl, Ulrich Weigel, Marita Meyer and Walter Sebald, Physiologisch-Chemisches Institut, Biozentrum der Universität, D-8700 Würzburg, FRG.

Mutant proteins (muteins) of interleukin 2 were generated by oligonucleotide-directed mutagenesis *in vitro* and expressed in *E.coli.* All charged residues as well a four lipophilic amino acids contained within four hydrophobic segments were exchanged. With two exceptions (F44K and E110G), all mutant proteins produced could be renatured, purified and tested as proliferation factors. ConA-prestimulated blast cells from healthy donors as well as IL-2 dependent mouse CTLL cells were used to study the biological activity of the muteins. Among the most interesting findings were:

- a mutein (D20N) with a 20-fold reduced proliferative activity and high-affinity receptor binding, but unchanged affinity to the TAC-antigen
- a mutein (R38Q) with unchanged proliferative activity, but 20-fold reduced binding affinity both to the high- and the low- affinity receptor
- a mutein (L94K) with a more then tenfold improved renaturation yield as compared to the wild type.

INTERLEUKIN-7 INDUCES WEAK LAK ACTIVITY FROM MURINE SPLENOCYTES LATE AFTER CULTURE WHEN COMPARED TO INTERLEUKIN-2 Howard D. J. Edington, M.D. and Michael T. Lotze, M.D., Departments of Surgery, Molecular Genetics and Biochemistry. University of Pittsburgh, Pittsburgh, PA 15261

Interleukin-7 (IL-7) is a novel cytokine elaborated by bone marrow and thymic stromal cells initially described as a B-cell growth factor and subsequently found to act as a T cell growth factor. IL-7 enhances the allogeneic response in mixed lymphocyte cultures and generates LAK activity from human peripheral blood mononuclear cells.[1,2] Incubation of murine splenocytes in IL-7 (3500u/cc) generates LAK activity that is weak and delayed, peaking after 6 days culture relative to LAK activity generated by IL-2 (1000u/cc) which peaks after 3 days of culture. Lytic activity tested against the syngenic methlcholanthrene induced sarcomas - MCA 102 and 105, NK cell sensitive lymphoma - YAC and a spontaneously arising murine squamous cell carcinoma - SCC. These results are in accord with those of Lynch and Miller[3]. Studies evaluating the effect of IL-7 on expansion and activation of murine tumor infiltrating lymphocytes (TIL) are ongoing. Recombinant human IL-7 was kindly provided by Sterling Pharmaceutical.

Targets:	YAC			MCA-102			MCA-105			SCC		
LU/10^6	Med.	IL-2	IL-7	Med.	IL-2	IL-7	Med.	IL-2	IL-7	Med.	IL-2	IL-7
Day 3	<1	59	<1	<1	37	<1	1	56	1	<1	25	<1
Day 6	<1	10	1	1	36	1	1	160	3	<1	4	<1

1. Lotze MT, et al. T cell growth factors and the expansion of Lymphoid cells with antitumor activity in vitro and in vivo. In: Lotze MT and Finn O (eds) Cellular Immunity and the Immunotherapy of Cancer. Wiley-Liss, Inc pp. 91-104, 1990.
2. Stotter H, et al. Interleukin-7 induces human lymphokine activated (LAK) cells and interleukin-4 activity. J. Immunol 146: 150-155, 1991
3. Lynch DH and Miller RE. Induction of murine lymphokine-activated killer cells by recombinant IL-7. J. Immunol., 145: 1983-1990, 1990.

INTERLEUKIN-6, A MYOCARDIAL DEPRESSANT FACTOR AND IT'S POSSIBLE ROLE IN MYOCARDIAL REPERFUSION INJURY IN MAN. Brack G. Hattler, M.D., Ph.D., and Mitchell S. Finkel, M.D. Departments of Surgery, Medicine and Pharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Cardiopulmonary injury is the primary cause of death following cardiac surgery. Mechanisms of injury are complex but include an inflammatory component in which both cellular and humoral mechanisms are involved. Pro-inflammatory cytokines are produced not only by immune cells, but also endothelial cells, fibroblasts, and other epithelial and mesenchymal tissues. We now have evidence that one of the cytokines, Interleukin-6 (IL-6), can be detected in the circulation of patients immediately following cardiopulmonary bypass. Our laboratory has demonstrated that IL-6 is a negative inotrope in hamster papillary muscle and in human atrial muscle and that this effect is dose-dependent and reversible. Samples were taken from the coronary sinus, pulmonary veins, and bronchoalveolar lavage in patients immediately before and following cardiopulmonary bypass. During surgery, there is a slight rise in IL-6 that is more prominent in the pulmonary venous samples (IL-6 100-200u/ml). Immediately after surgery, levels increase dramatically in both the coronary sinus and pulmonary veins (IL-6 1000-1500u/ml). This increase in IL-6 levels is selective and not shared by IL-1 or TNF. The highest IL-6 values immediately after surgery were noted in the bronchoalveolar lavage (IL-6 1800-4000u/ml).

ABNORMAL RESPONSE TO IL-5 IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS, Teresa G. Hayes, Annemarie B. Moseley, David P. Huston, Baylor College of Medicine, Houston, TX 77030

In B cell chronic lymphocytic leukemia (B-CLL), neoplastic lymphocytes are arrested at discrete stages of differentiation. One hypothesis for the failure of B-CLL cells to complete development is an abnormal response to physiologic lymphokine signals. B lymphocytes from two B-CLL patients were treated *in vitro* with the T-cell dependent mitogens *Staphylococcus aureus* Cowan I (SAC) and *Branhamella (Moraxella) catarrhalis* (BCAT) in combination with recombinant human interleukins. The results demonstrate a selective deficiency in response to IL-5 in cells from the B-CLL patients compared to healthy controls. As in healthy B lymphocytes, SAC or BCAT-activated B-CLL cells proliferated and secreted Ig in response to IL-2, and proliferation and differentiation of activated B-CLL cells were inhibited by IL-4. However, B cells from both CLL patients failed to secrete Ig after stimulation by BCAT plus IL-5. Lack of response to IL-5 could potentially explain the arrested development in the B lymphocytes of some B-CLL patients.

IDENTIFICATION OF A POSSIBLE REPRESSOR OF IL-2 GENE TRANSCRIPTION, Sang-Mo Kang*#, Mariagrazia Grilli*, Annie-Chen Tran*, and Michael J. Lenardo*. *LI, NIAID, NIH, Bethesda, MD 20892. #HHMI-NIH Research Scholar.

Nearly all detailed studies of gene regulation have utilized transformed cell lines as their model system. Although transformed cells have clearly been useful and informative, they have important limitations. Most significantly, they are aberrant: besides dividing indefinitely without a stimulus, many in-vivo phenomena often cannot be reproduced. We have recently developed methods for studying gene regulation in non-transformed murine T-helper lymphocyte clones.

An important aspect of T-cell physiology is the exquisitely tight control of IL-2 gene transcription found in normal cells. Unlike tumor lines, significant transcription of the IL-2 gene appears to occur only in the presence of T-cell receptor signalling coupled with an as yet ill defined "costimulatory signal" present on the surface of most antigen presenting cells. In addition, the kinetics of IL-2 mRNA accumulation display a sharp "on-off" behavior. We are currently studying the various enhancer elements thought to activate IL-2 gene transcription in order to define the basis of this tight regulation.

Using murine CD4+ TH_1 cells, we have identified a novel nuclear factor which binds to a site in the IL-2 enhancer shown previously to interact with the well-characterized nuclear factor NF-kB. This factor, which we call NF-Cyt1, appears to bind with approximately six-fold higher affinity for the IL-2 site than NF-kB. Interestingly, the regulation of NF-Cyt1 would suggest that it behaves as a repressor of IL-2 gene transcription: the binding activity of NF-Cyt1 declines dramatically with activation of TH1 clones. The regulation of NF-Cyt1 binding activity and the functional role of NF-Cyt1 in IL-2 gene transcription are currently under study.

GENERATION AND CHARACTERIZATION OF A SOLUBLE ERYTHROPOIETIN-BINDING PROTEIN

Linda S. Mulcahy, Manisha R. Agarwal, Sally S. Varga, J. Lee Pellegrino-Gensey, Timothy E. Tracy, and Linda K. Jolliffe, Department of Molecular and Cellular Biology, R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08869

Erythropoietin (EPO), the primary regulator of human erythropoiesis, stimulates erythroid progenitor cells via a specific cell surface receptor. The receptor consists of large extracellular and intracellular domains, separated by a short transmembrane domain. We were interested in expressing the extracellular domain as a "soluble receptor" to facilitate structure-function studies on the ligand binding domain.

The mouse EPO receptor cDNA was truncated 5' to the transmembrane domain, inserted into a mammalian expression vector, and expressed in COS cells. The presence of EPO binding activity in the culture medium was confirmed by a competitive radioligand binding assay. The soluble receptor was able to block the ability of EPO to induce proliferation of EPO dependent cells. Anti-EPO receptor peptide antisera was generated and used to characterize the secreted protein. Both immunoprecipitation and Western blot analysis of the soluble receptor reveal a 26-28 kD band that is not detected in control cell culture medium or with normal rabbit serum. This is consistent with the predicted MW for this truncated protein.

THE PRODUCTIVITY AND LONGEVITY OF HUMAN BONE MARROW CULTURES IS SIGNIFICANTLY ENHANCED BY THE MEDIUM PERFUSION RATE AND ADDED HEMATOPOIETIC GROWTH FACTORS,
Bernhard O. Palsson, Richard M. Schwartz and Stephen G. Emerson*, Department of Chemical Engineering and Department of Internal Medicine*, The University of Michigan, Ann Arbor, MI 48109
Hematopoiesis, the process of blood cell differentiation, involves complex interactions between hematopoietic and stromal cells, hematopoietic growth factors, and the hematopoietic microenvironment. Successful reconstitution of functional bone marrow *ex vivo* involves understanding the relationship between these factors in an attempt to mimic the hematopoietic environment *in vivo*. We have examined the effect of the medium perfusion rate, the innoculum density, and several of the hematopoietic growth factors on hematopoietic differentiation and proliferation in long-term bone marrow cultures. The medium perfusion rate of 3.5 medium volumes per week produced stable levels of non-adherent progenitor cells for 18 weeks and a higher total number of non-adherent cells than did long-term bone marrow cultures perfused at 1 or 7 medium volumes per week. Addition of the hematopoietic growth factors IL-3, GM-CSF, and EPO, individually or in combinations, to the culture medium resulted in increased hematopoietic progenitor cell and non-adherent cell production over controls in long-term bone marrow cultures. IL-3 and EPO display a synergistic effect on the production of progenitor cells CFU-GM and BFU-E and thus may both play a role in early hematopoiesis.

THE ANALYSIS OF IMMUNE EFFECTOR MECHANISMS USING RECOMBINANT VACCINIA VIRUSES THAT EXPRESS THEIR OWN CYTOKINES
Ramshaw I.A. Karupiah G. Sambhi S. Ruby J. Ramsay A. and Kohonen-Corish M. Viral Engineering Group, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia 2601.
We have constructed recombinant vaccinia viruses (vv) that express IL-2, TNF-α or IFN-γ. Although the growth of these viruses in vitro is equivalent to similarly constructed control viruses, their growth in vivo is markedly attenuated. Indeed, athymic nude mice which lack competent T cells are able to survive infection with virus expressing IL-2, TNF-α or IFN-γ, but die from infection with control virus. The virus-encoded IL-2 mediated protection could be reversed by treating athymic nude mice with monoclonal antibodies (Mab) against IFN-γ, suggesting that the production of host derived cytokines is an important mechanism of virus clearance. These findings have led us to question the role of cytokines in the normal physiological process of virus clearance involving CD8+ cutotoxic T lymphocytes. It was found that in an adoptive T cell transfer model the anti-viral effector function of T cells could be inhibited with monoclonal antibodies (Mab's) against IFN-γ. We suggest that the principal role of T cells, therefore, may be to focus cytokines at sites of virus replication, rather than, as previously thought, the lysis of virus infected cells.

TRANSCRIPTIONAL REGULATION OF THE MELANOMA GROWTH STIMULATORY ACTIVITY ($MGSA_1/Gro_\alpha$) GENE,
Rebecca L. Shattuck[1], Lauren Wood[1], Nancy E. Baker[1], and Ann Richmond[1,2] (1) Department of Cell Biology, Vanderbilt University School of Medicine, and (2) Veterans Affairs Medical Center, Nashville, TN 37232-2175
Two biological activities, neutrophil chemotaxis and melanocyte growth simulation, have been associated with the cytokine, melanoma growth stimulatory activity (MGSA). The nucleotide sequence of MGSA is identical to that of human Gro. Since the initial isolation of $MGSA_1/Gro_\alpha$, two additional genes have been isolated which encode $MGSA_2/Gro_\beta/Mip2\alpha$ and $MGSA_3/Gro/Mip2\beta$. A genomic clone encoding $MGSA_1/Gro_\alpha$ has been isolated, and the structural composition determined to consist of four exons, interrupted by three introns (Baker, N.E., Kucera, G.B., & Richmond, A. (1990) Nucleic Acids Research, 18, 6453). The mRNA for MGSA/Gro is barely detectable in normal human melanocytes, but both MGSA mRNA and protein are strongly expressed in benign nevi and metastatic melanomas, as well as in normal epidermal keratinocytes. The various MGSA/Gro/Mip2 mRNA levels in human melanoma and keratinocytes are regulated by serum, interleukin-1, lipopolysaccharide, TGFβ and MGSA itself. Approximately 3.0Kb of the 5' regulatory region of the $MGSA_1/Gro_\alpha$ gene has been cloned, partially sequenced and the transcriptional regulation of the MGSA expression has been investigated using a CAT reporter system. The transcriptional regulation of MGSA by various agents and the 5' untranslated regions involved in this regulation will be presented in detail.

AN EOSINOPHIL-DEPENDENT MECHANISM FOR THE ANTITUMOR ACTIVITY OF IL-4,

Robert I. Tepper*, Robert Coffman# and Philip Leder*, *Department of Genetics, Harvard Medical School, Boston, MA 02115 and #Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304

Murine IL-4 exhibits potent antitumor activity when present at sufficiently high concentrations at the site of tumor-cell challenge. This effect is observed against a wide array of tumor cell types in vivo but not in vitro. A consistent finding at the site of tumor cell inoculation is an inflammatory infiltrate comprised predominantly of eosinophils and macrophages with a notable absence of lymphocytes. To assess the role of eosinophils in mediating IL-4-induced tumor cytotoxicity, we treated animals with both TRFK-5, an anti-IL-5 antibody, and RB6-8C5, which is cytotoxic for terminally-differentiated granulocytes, including neutrophils and eosinophils, but not monocytes/macrophages. Tumor formation by murine J558L plasmacytoma cells, which is completely inhibited by IL-4 present locally, was restored in 100% (5/5) of syngeneic Balb/c mice treated with the combined antibody treatment. Histologic analysis demonstrated a marked reduction in the number of tumor-infiltrating eosinophils in comparison with PBS-treated controls. Eosinophil-mediated cytotoxicity therefore appears to be an important mechanism of action for the antitumor activity of IL-4. These results have implications for designing therapeutic strategies that simultaneously exploit both lymphoid and non-lymphoid-mediated cytokine-induced tumor killing.

Differential Cytokine Expression by IgD$^+$ and IgD$^-$ B Cells.

H. Martinez-Valdez, C. Guret, T. Defrance, F. Briere, B. Vanbervliet, N. Burdin, F. Rousset, and J. Banchereau. **Laboratory for Immunology Research, Schering-Plough, 27 Chemin des Peupliers B.P. 11, 69571 Dardilly, Cedex FRANCE**

We have isolated two distinct tonsillar B cell populations, defined by the presence or absence of IgD on their surface. Flow cytometry confirmed the selective purity of both IgD$^+$ and IgD$^-$, which was always above 95%. A finer phenotype analysis of the two populations revealed surface markers consistent with their identity as mantle zone and germinal center cells respectively. In particular CD23 was only expressed by IgD$^+$ cells. Using, the polymerase chain reaction and northern blot analysis, we have examined the constitutive levels of cytokines in these cells and have found that both populations expressed a broad cytokine repertoire. We observed however, distinct patterns of cytokine expression in these two subpopulations, namely IgD$^+$ cells but not IgD$^-$ cells expressed IL-6. In contrast, only IgD$^-$ cells selectively expressed IL-1a and IL-8. T cell specific cytokines such as IL-2 and IL-4 were not detected, even after stimulation with polyclonal mitogens, which ruled out the T cells as a source of the examined cytokines. The possibility of monocyte or macrophage contribution was also ruled out by flow cytometric analysis. Therefore, the differential expression of cytokines by IgD$^+$ and IgD$^-$ cells, further defines the dichotomy between mantle zone and germinal center B cells.

EFFECT OF CYTOKINES ON THE LYMPHOID DIFFERENTIATION "IN VITRO" OF EMBRYONIC STEM CELLS Jose C. Gutierrez-Ramos and Ronald Palacios. Basel Institute for Immunology. Basel .Switzerland.

With their recent and more thorough characterization, Embryonic Stem cells (ES) are becoming an incresingly useful complement to direct studies on early embryos and have the potential at least to yield greater insight into the functional aspects of growth factors in the early differentiation and commitment to the lymphoid lineage. Following this approach, we have defined culture conditions that support in vitro the differentiation of the embryonic stem cell line D3 into T-cell progenitors, B-cell progenitors and myeloid cells as assesed, at various times of culture, by FACS with a panel of monoclonal antibodies against several surface markers (Thy1.2, Pgp1, B220, Joro37.5, IgM, F4/80 and Mac-1). We found that different bone marrow stromal and fetal liver stromal cell lines have distinct capacities of inducing differentiation of D3 stem cells into T- and B-cell progenitors (Thy1$^+$Pgp1$^+$Joro37.5$^+$ or B220$^+$IgM$^-$) and myeloid cells (F4/80$^+$ Mac1$^+$). Such functional properties of the different stromal cell lines can be distinctly modified by recombinant interleukins (IL3,IL4, IL5,IL6 and IL7) when added at different times of culture. Our results show that, although the presence of IL6 is esential at early stages of the "in vitro" differentiation , if the differentiation of ES cells is performed in the exclusive presence of IL6, mainly fibroblast-type cells are obseryed in the cultures. However, when IL6 is provided in conjunction with IL3 and IL7, lymphoid progenitors, that have rearanged IgH or TcRγ loci, are generated and expanded. Thus, we have been able to define a precise sequence of signals required to induce in vitro embryonic stem cells into T- and B-cell progenitors or myeloid cells; if this sequence of signals provided (by stromal cell lines and interleukins) is altered, the final products as well as the efficiency of the system dramatically change.

FREQUENCY OF CYTOKINE SECRETING CELLS IN THE CD45RA AND CD45R0 LYMPHOCYTE SUBSETS IN RESPONSE TO VARIOUS ACTIVATION STIMULI.
Colin A. Michie, Peter C. L. Beverley, Human Tumour Immunology Group, Imperial Cancer Research Fund, University College and Middlesex Schools of Medicine, London, England.

A single cell assay technique has been developed based on complement-mediated haemolysis of sheep red blood cells coated with mAb to the required cytokine. The method estimates the frequency of cells secreting cytokine, and measurement of haemolytic plaque areas gives an estimate of the quantity of cytokine produced. Negatively selected populations of human CD45RA and CD45R0 lymphocytes were stimulated with mitogen, and via CD2 and CD3. It is found that the CD45RA population have a lower frequency of cells producing IFNγ, and no cells producing IL4 or IL6 in response to these stimuli. Frequency of IL2 secretion is similar to the CD45R0 population. There is no response to stimulation via CD2 in this subset. A higher frequency of CD45R0 cells produce IFNγ, in larger amounts, and secretion of IL4 and IL6 was observed after 48hrs, particularly following activation via CD2.

THE EFFECT OF ANTI-INTERLEUKIN-4 TREATMENT ON THE DEVELOPMENT OF MURINE THYMOCYTES, David B. Agus, Cynthia Watson, and William E. Paul, Laboratory of Immunology, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Interleukin-4 (IL-4) is known to have potent regulatory effects on the growth and differentiation of T lymphocytes and has been reported to affect the development of thymocytes *in vitro*. Recently, transgenic mice over-expressing IL-4 have been shown to have striking abnormalities in T cell development within the thymus and in the peripheral T lymphocyte compartment. We have studied the role IL-4 may play physiologically in T cell development by treating mice through their late gestational (day 12 to birth) and early post-natal period with a neutralizing monoclonal anti-IL-4 antibody (11B11). Neonates examined at birth, after treatment of pregnant mothers with 11B11, showed ~2.6-fold increase in thymus cell number, although no marked abnormalities in the phenotypic distribution was observed. Studies of observed abnormalities of thymic structure in the neonates and of T cell development and function in the early post-natal period are now in progress. Results from these studies will be presented in the context of a model for the role of IL-4 in thymocyte development.

LECTIN-CARBOHYDRATE INTERACTIONS IN MYELOPOIESIS. Othmar Förster and Walter Krugluger. Inst. of Exptl. Pathology, Univ.of Vienna, Austria.
Rat macrophages derived from bone marrow culture in the presence of M-CSF or GM-CSF (BMDMΦ) bind strongly soy bean agglutinin (SBA). Only about 20% of freshly isolated bone marrow cells (BMC) show such strong binding, whereas about half of these cells seem to bind SBA to a very low degree as demonstrated by FACS analysis. Sorting of BMC by FACS into SBA negative, SBA weakly positive and SBA strongly positive populations reveiled macrophages and eosinophils in the strongly positive and the majority of blast like cells in the weakly positive fraction, whereas neutrophils were SBA negative. SDS-PAGE analysis of radioiodinated, solubilized, SBA-agarose bound membrane glycoproteins suggested that the strong SBA binding of BMDMΦ is due to the expression of a 160 kDa glycoprotein not present on fresh BMC, whereas the weak SBA binding by blast-like BMC is to a 26 kDa structure not found on BMDMΦ. Addition of SBA to BMC cultures greatly enhanced the proliferation inducing effect of CSF and increased the NBT reduction by the resulting BMDMΦ. SBA alone had no effect. When SBA was added to BMDMΦ after 1 week culture in CSF these cells seemed to undergo final differentiation as indicated by a change in several surface antigens, and showed intensive pseudopodia formation on the bottom of the culture plate. We conclude that carbohydrate-lectin interactions may provide additional (microenvironmental?) signals at various stages during myelopoiesis.

THE EFFECT OF ANTI-INTERLEUKIN-4 TREATMENT ON THE DEVELOPMENT OF MURINE THYMOCYTES, David B. Agus, Cynthia Watson, and William E. Paul, Laboratory of Immunology, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Interleukin-4 (IL-4) is known to have potent regulatory effects on the growth and differentiation of T lymphocytes and has been reported to affect the development of thymocytes *in vitro*. Recently, transgenic mice over-expressing IL-4 have been shown to have striking abnormalities in T cell development within the thymus and in the peripheral T lymphocyte compartment. We have studied the role IL-4 may play physiologically in T cell development by treating mice through their late gestational (day 12 to birth) and early post-natal period with a neutralizing monoclonal anti-IL-4 antibody (11B11). Neonates examined at birth, after treatment of pregnant mothers with 11B11, showed ~2.6-fold increase in thymus cell number, although no marked abnormalities in the phenotypic distribution was observed. Studies of observed abnormalities of thymic structure in the neonates and of T cell development and function in the early post-natal period are now in progress. Results from these studies will be presented in the context of a model for the role of IL-4 in thymocyte development.