CYTOKINES AND CYTOKINE RECEPTORS: FROM CLONING TO THE CLINIC

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Poster Sessions

Cytokines that Influence Hematopoietic Stem Cells-I

E 001 DO MOUSE HEMATOPOIETIC STEM CELLS SELF-RENEW? Gerald J. Spangrude, Laboratory of Persistent Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Rocky Mountain Labs, Hamilton, MT.

The most primitive of hematopoietic 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. However, the self-renewal aspect may not be apparent at a single cell level but may be a result of gradual differentiation with each cycle of division. Methods for the isolation of populations of mouse hematopoietic stem cells have allowed a degree of insight into this organizational aspect of the hematopoietic compartment. For example, the multipotent characteristic of hematopoietic stem cells can be separated from the self-renewal characteristic by cell sorting based on retention of the vital dye rhodamine-123. In order to better understand the complexity of the hematopoietic stem cell compartment in mice, the expression of various cell surface markers on primitive progenitors has been characterized among a variety of mouse strains. The results indicate a wide variability in hematopoietic stem cell phenotypes, in contrast to the expected result that the expression of cell surface markers would be an invariant characteristic of these cells. For example, the Thy-1 antigen is expressed at low levels by virtually all hematopoietic stem cells in Thy-1.1 haplotype animals, but in Thy-1.2 haplotype mice only about 20% of marrow repopulating activity can be recovered in the Thy-1^{low} population of cells. Further, the Ly6A/E antigen is a useful marker for hematopoietic stem cells only in $Ly6^b$ haplotype mice, where 99% of the marrow-repopulating activity of normal bone marrow is included among cells expressing the antigen compared to less than 20% in strains of the $Ly6^a$ haplotype. Therefore, in certain strains of mice the Thy-1 and Ly6A/E antigens are expressed by virtually every hematopoietic stem cell, while in other mouse strains these antigens are present on only a subset of stem cells. It is not clear whether this subset is functionally distinct from the remaining stem cells that do not express the markers. If so, this observation may provide a means by which the hierarchy of the hematopoietic stem cell compartment may be further characterized. Isolation of various populations of hematopoietic stem cells in this laboratory continues to result in co-localization of radioprotective activity and long-term repopulating activity, in contrast to the reports of others. Whether this discrepancy is due to differences in isolation methods which may alter the functional characteristics of the cells, strain or age differences in bone marrow donors, or other factors is as yet unknown.

Cytokine Discovery-An Update (Session Sponsored by Genetics Institute, Inc.)

E 002 CD23 - A RECEPTOR OR A CYTOKINE, M. Sarfati, and G. Delespesse, Notre-Dame Hospital Research Center, Montreal, Canada H2L 4M1.

The CD23 antigen is a member of a recently described superfamily of type II integral membrane proteins displaying a C-type lectin motif. CD23 is spontaneously cleaved from the cell surface into soluble fragments (sCD23) containing the lectin domain and differing by their stability and their molecular weight. CD23 may be expressed on a large variety of hematopoietic cells and on some epithelial cells. The CD23 gene is a single copy gene which codes for two productive transcripts (named A and B) differing only in their short intracytoplasmic region. B cells may express the two isoforms whereas all the other types capable of expressing CD23 display only the isoform B. Historically, the first function ascribed to CD23 and its soluble fragments is their ability of binding to IgE, explaining why these molecules are also known respectively as low affinity receptor for IgE (FcεRII) or IgE-binding factors (IgE-BF). Until very recently, all the functions described for surface CD23 were IgE-dependent whereas most of the activities of sCD23 were clearly IgE-independent, implicating that CD23 is capable to functionally interact with other ligands than IgE. CD23 should be viewed not only as a receptor expressed on the surface of several cell types but also as the membrane precursor of a soluble cytokine with multiple activities. Hence there is compelling evidence that CD23 is a functional IgE receptor. Binding of IgE to cell surface CD23 reduces the cleavage into sCD23, signals the cells and changes their biological activities. Both the IgE-dependent signaling and biological activities of surface CD23 depend upon the cell type on which it is expressed. On inflammatory cells, IgE-dependent functions of CD23 (type B) include: phagocytosis, cellular cytotoxicity and the release of inflammatory mediators. On B cells, type A CD23 is involved in IgE-dependent presentation of Ag to T cells. Our recent studies, using anti-sense oligonucleotides specifically directed to CD23 mRNA type A or type B, formally demonstrate that CD23 plays a role in B cell activation. This function is IgE-independent, and it is most likely mediated by the CD23 type B. The group of J.-Y. Bonnefoy reported that CD21 is a ligand for CD23 as evidenced by rosette formation between CD21 bearing cells and either liposomes containing full length CD23 or CD23 bearing cells. The data suggest that CD23 may play the role of an adhesion molecule involved in contact-dependent interactions between several types of cell, however it is still unknown whether the interaction between these two complementary molecules signals the CD23 bearing cells. The same group also provides suggestive evidence that CD21 may be a functional receptor for sCD23 that is involved in the potentiating effect of sCD23 on the in vitro synthesis of IgE. Further studies are required to examine whether CD21 is also involved in the other IgE-independent activities of sCD23 including on the differentiation of early thymocytes, the proliferation of myeloid precursors, the rescue of germinal center B cells from apoptosis or the proliferation of CD4 T cells. The possibility exists that, like TGF-ß, CD23 has several ligands that may mediate distinct CD23 biological activities. Structure-function studies revealed that the lectin motif is involved in the IgE-binding and cytokine activities of CD23/sCD23; the role of the lectin motif is also underlined by the suppressive effect of fucose-1-phosphate on the binding of IgE and CD21 to CD23.

E 003 ANTIBODIES TO MURINE CD40 STIMULATE NORMAL B CELLS BUT INHIBIT PROLIFERATION OF B LYMPHOMA CELLS, Maureen Howard, Nobuyuki Harada, Leopoldo Santos-Argumedo, Raul Torres†, Edward Clark† and Andrew Heath, DNAX Research Institute, Palo Alto, CA 94304 and †University of Washington, Seattle, WA 98195

A rat anti-mouse CD40 antiserum has been prepared by hyperimmunization of Lewis rats with a highly purified preparation of the recombinant extracellular domain of murine CD40. This antiserum specifically binds CD40-expressing L cell transfectants, but not untransfected L cells, and induces vigorous proliferation of highly purified small dense B cells obtained from the spleens of unstimulated mice. Anti-CD40-induced B cell proliferation can be augmented by the addition of IL-4, and isi nhibited by highly purified recombinant soluble mouse CD40. Interestingly the same anti-CD40 antiserum specifically inhibits the *in vitro* growth of A.20 murine B lymphoma cells in a cell density dependent manner. The specificity of this inhibition can be demonstrated by reversing the effect with highly purified recombinant soluble mouse CD40. These data implicate CD40 as a possible target for therapeutic intervention in the treatment of B lymphomas.

E 004 THE TNF/NGF SUPERFAMILY OF CYTOKINES AND RECEPTORS, Craig A. Smith, Douglas E. Williams, Richard J. Armitage, Brian Gliniak, Kenneth Grabstein, William Fanslow, Terry Farrah, Ben Falk, Wenie S. Din, Terri Davis and Raymond G. Goodwin, Immunex Research and Development Corp., Seattle, WA. 98101.

Receptors for TNF and NGF are members of a superfamily of at least nine transmembrane proteins that includes CD40, CD30, FAS, CD27, OX40, and a pox-viral gene product encoding a soluble form of the cellular TNF receptor. CD40, a ligand for which has recently been cloned, is a largely B cell-specific antigen; CD27 and OX40 are T cell activation antigens, and CD30 is a marker antigen for tumor cells of Hodgkin's disease. Using a soluble, recombinant form of CD30 as a probe (consisting of the extracellular region only), we have identified a corresponding ligand (CD30L) on the surface of the induced T cell, 7B9. We then isolated a 1.6 kb cDNA clone encoding this protein by a direct expression approach. The sequence revealed the ligand to be a 239 amino acid type II membrane protein, whose C-terminal domain shows significant sequence homology to TNFα, TNFβ and the CD40 ligand. The receptor binding site is presumably localized to this region, and CD30L can be modeled in 3 dimensions after the crystal structures of human TNFα and β. These results suggest the emergence of a ligand superfamily parallel to the receptor superfamily with which they interact. Expression studies of the recombinant ligand establish the cytokine, like its receptor, to be glycosylated in the extracellular region. A 1.7 Kb human CD30L cDNA clone was isolated by cross hybridization from an induced peripheral blood T cell lambda library. The encoded protein is also predicted to be a type II membrane protein and is ~75% identical to its murine homologue at the amino acid level. Biological activities of the recombinant ligands and their possible relevance to the pathogenesis of Hodgkin's lymphoma are under investigation.

E 005 **BIOLOGICAL SIGNIFICANCE OF CELL SURFACE APOPTOSIS ANTIGEN FAS ;** **Shin Yonehara[1], Ai Ishii[2], Yoshiko Nishimura[1], Minako Yonehara[2], Yuko Kobayashi[1], Yoshinori Yamazaki[1], and Shuji Kishi[1], [1]Pharmaceutical Basic Research Laboratories, JT Inc., Yokohama 236, Japan and [2]The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan.**

It is well known that normal cell death, termed apoptosis or programmed cell death, plays an important role for embryonic development, cell turnover in healthy tissues, metamorphosis, and atrophy. Apoptosis can also be seen in tumor regression. In the immune system, autoreactive thymocytes and mature T cells can lead to apoptotic cell death by the stimulation through antigen receptor. Fas antigen is a cell surface protein which mediates apoptosis. We have prepared anti-Fas mAb with associated apoptosis-inducing activity. Cloning of Fas antigen cDNA indicates that Fas antigen is a member of the cell surface receptor family of TNF and NGF receptors. Transfection of human Fas cDNA renders mouse cells to be sensitive to the apoptosis-inducing activity of anti-human Fas mAb, indicating that Fas antigen directly mediates apoptosis. Recently, mice carrying the lymphoproliferation (*lpr*) mutation were reported to have defects in the Fas antigen gene. *lpr* mice are well known to suffer from a systemic lupus erythematosus-like autoimmune disease. These data indicate an important role of Fas antigen in the negative selection of autoreactive T cells.We analyzed the expression and function of Fas antigen on T cells from thymus and periphery. After more than 5 days stimulation of peripheral T cell with IL-2, anti-CD3 mAb or superantigen SEB, expression of Fas antigen was strongly induced and the stimulated T cell can be killed by the treatment with anti-Fas mAb. These data suggest that Fas is involved in clonal depletion of chronically activated or autoreactive peripheral T cells. In case of human thymocytes, significant amounts of Fas antigen is expressed on both $CD4^-CD8^-$ and $CD4^+CD8^+$ undifferentiated thymocytes, and most of differentiated $CD4^+$ or $CD8^+$ thymocytes express undetectable level of Fas antigen. After 5h incubation of human thymocytes with anti-Fas mAb, chromosomal DNA fragmentation can be observed, indicating that Fas antigen on thymocytes is functional. Thus, undifferentiated thymocytes and chronically stimulated peripheral T cells express functional Fas antigen. It is well known that negative selection of autoreactive T cell with apoptotic cell death is occurred on undifferentiated $CD4^+CD8^+$ thymocytes and chronically stimulated peripheral T cell. All the data indicate that Fas antigen may be involved in the elimination of autoreactive T cell in either thymus or periphery.

Cytokine Receptor Function and Signal Transduction-I

E 006 **NMR STRUCTURE OF CYTOKINES**, Angela M. Gronenborn[1], P.T. Wingfield[2], E. Appella[3], K. Matsushima[3], R. Powers[1], D.S. Garrett[1], C.J. March[4], A. Frieden[4] and G.M. Clore[1], [1]NIDDK, NIH, Bethesda; [2]OD, NIH, Bethesda; [3]NCI, NIH, Bethesda; [4]Immunex Co., Seattle.

Understanding the relationship between macromolecular structure and function together with the potential to predict and redesign such structures will permit the unraveling of complex cellular processes at a hitherto unprecedented level of detail. The cytokines constitute an important class of proteins in the signalling pathways in the immune and inflammatory response exhibiting considerable pleiotropy in their actions. Three members of the interleukin family have been studied in structural terms by NMR and for all three proteins high resolution three dimensional solution structures were determined within a remarkably short time after these proteins were discovered and purified. Structural findings for each of the three cytokines, Il-8, Il-1β and Il-4, will be presented and mutational data will be discussed in the light of these structures. In addition, the NMR solution structures will be compared to single crystal X-ray structures and differences will be discussed.

E 007 HEMOPOIETIC GROWTH FACTOR (IL-3, GM-CSF AND IL-5) RECEPTORS AND SIGNAL TRANSDUCTION, Atsushi Miyajima, DNAX Research Institute, Palo Alto, CA 94304.

Pleiotropic hemopoietic growth factors, IL-3, GM-CSF, and IL-5 display different spectram of target cells. However, they exhibit similar function on some common target cells, such as eosinophils. High affinity receptors for these cytokines are composed of α and β subunits. The α subunits are specific for each cytokine and bind their respective cytokine with low affinity by themselves. Only one type of β subunit (common β subunit, β_c) is present in the human receptors for the three cytokines. The human β_c does not bind any cytokines by itself but forms high affinity receptors for all the three cytokines with cytokine specific α subunits. In contrast to the human, the mouse has two distinct β subunits that are 91% identical: one (AIC2A, or β_{IL3}) is specific for the IL-3 receptor and the other one (AIC2B, or β_c) is common among the three receptors. The β subunits are not only required for high affinity binding, but are also essential for signal transduction. The common β subunit with signalling function provides a molecular basis for common biological activities of IL-3, GM-CSF and IL-5 and the expression of the α subunits is responsible for specificity to cytokines. Although all these cytokines induce protein tyrosine phosphorylation and activation of PI-3 kinase, ras, raf, and MAP kinase, neither the α nor β subunit has an intrinsic tyrosine kinase, suggesting that the receptors activate a tyrosine kinase(s) directly or indirectly. Deletion analysis identified the critical cytoplasmic domain of β_c for proliferation. Interestingly, the β_c deletion mutant incapable of inducing major tyrosine phosphorylation still transmitted herbimycin A sensitive growth signal, suggesting that the major tyrosine phosphorylation is not required for proliferation and that multiple tyrosine kinases may be invoved in the signalling pathway. Antibodies against the receptor subunits were prepared to identify proteins that interact with the receptor. Two distinct proteins were found to be coimmunoprecipitated with the IL-3 receptor and characterization of these proteins is in progress.

Cytokine Receptor Function and Signal Transduction-II

E 008 INTERLEUKIN-1 SIGNAL TRANSDUCTION, Timothy Bird[1], Duke Virca[2], Jennifer Slack[1], Margit Gayle[3] Judith Giri[1], Heather Schule[1], Paul deRoos[2], John Sims[3], and Steven Dower[1], Departments of [1]Biochemistry, [2]Protein Chemistry, and [3]Molecular Biology, Immunex Corporation, Seattle.

Two types of interleukin-1 receptor (IL-1RI and IL-1RII) have been identified by molecular cloning studies. Both receptors are capable of binding the two agonist forms of IL-1 (IL-1α and β) and the IL-1 receptor antagonist, though there are differences in the relative affinities of the two receptors for the three types of ligand. Both receptors have similar extracellular regions consisting of three immunoglobulin-like domains. The main difference between them is that the cytoplasmic domain of IL-1RII is much shorter than that of IL-1R1 (29 versus 200 amino acid residues, respectively). IL-1RII is widely distributed; on some B cell lines and neutrophils it is expressed as the predominant receptor, while it is co-expressed with IL-1RI on other cells such as HepG2 hepatoma. Most connective tissue cells express IL-1RI exclusively. Many other cytokine receptors consist of heteromeric complexes between signal-transducing and smaller, purely ligand-binding subunits. An extensive series of experiments was carried out to determine whether a similar situation pertains in the IL-1R system. Cross-competition binding studies, and other experiments using natural, full-length recombinant, and truncated 'soluble' forms of both receptors revealed that they bind IL-1 independently and do not interact to form a heterodimeric IL-1-binding complex. Furthermore, using a specific neutralizing monoclonal antibody raised against IL-1RII, it has not been possible to antagonise a wide variety of IL-1 mediated responses in IL-1RII-bearing cells. In all cases we have studied (induction of surface immunoglobulin in pre-B cells, acute phase protein induction and NF-κB activation in hepatocytes, IL-8 induction in a macrophage line) a small number of co-expressed type I receptors appears to mediate IL-1 signalling. In fact, the role of IL-1RII may be to act as an inhibitor (or carrier) of circulating IL-1. Signalling via IL-1RI involves changes in the activity of serine/threonine kinases. We identified one of these kinases as MAP (mitogen -activated protein kinase, $p44^{MAPK}$) kinase. In common with many other extracellular stimuli, IL-1 increases the activity of MAP-kinase by activating an upstream regulator, MAP kinase-kinase. Unlike many of these other activators of MAP kinase, IL-1 does not activate $p21^{ras}$. By using selective inhibitors of MAP kinase activation, we were able to show that it is a necessary but not sufficient step leading to the induction of PGE_2 and IL-6 release by KB epidermoid cells. Activation of the transcription factor NF-κB, on the other hand, is independent of MAP kinase. A second IL-1-activated protein kinase (p50 hsp kinase) phosphorylates the small heat shock protein hsp27. Hsp kinase also lies downstream of MAP-kinase, which is capable of directly activating it *in vitro*.

E 009 IL-4 AND INSULIN INDUCE OVERLAPPING SIGNALING PATHWAYS IN FACTOR-DEPENDENT MYELOID CELLS Ling Mei Wang[1], Achsah Keegan, Weigun Li[1], Silvio Gutkind[1], Morris White[2], Stuart A. Aaronson[1], William Paul[1] and Jacalyn H. Pierce[1], [1]National Institutes of Health, Bethesda, MD 20892 and [2]Harvard Medical School, Boston, MA 02215.

Insulin, insulin-like growth factor-1 (IGF-1), and IL-4 induced potent DNA synthesis in the IL-3-dependent hematopoietic cell lines, FDC-P1 and FDC-P2. While none of these factors alone could sustain long-term proliferation of these lines, a combination of IL-4 with insulin or IGF-1 did support continuous growth. When the IL-4 receptor was overexpressed in FDC-P2 cells, this transfectant could then be propagated indefinitely in IL-4. Similar results have been obtained after transfection of the IGF-1 receptor into FDC-P1 cells[1]. IL-4, insulin, and IGF-1 each induced pronounced tyrosine phosphorylation of a band migrating at 170 kD in both lines. This substrate strongly associated with the 85 kD subunit of PI 3-kinase after stimulation with each factor. V8 digestion indicated that the 170 kD proteins phosphorylated by the three factors were identical to each other. Interestingly, the half-maximal dose required for proliferation correlated precisely with that needed to observe tyrosine phosphorylation and PI 3-kinase association with the 170 kD substrate. The major tyrosine-phosphorylated substrate induced by insulin in fibroblasts, termed IRS-1, has a molecular size of 160-185 kD and also tightly associates with PI 3-kinase[2]. An anti-rat IRS-1 serum weakly detected the tyrosine-phosphorylated 170 kD protein induced by IL-4 or insulin in FDC cells, suggesting that the hematopoietic cell substrate is either related or identical to IRS-1. When an expression vector containing rat IRS-1 was transfected into FDC-P1, tyrosine-phosphorylated IRS-1 was readily detected in the lysates from factor-stimulated cells, providing evidence that IL-4, as well as insulin and IGF-1, can induce phosphorylation of the actual IRS-1 protein in a hematopoietic cell background. Taken together, our results indicate that IL-4, insulin, and IGF-1 utilize at least one overlapping signaling cascade in IL-3-dependent myeloid cells and that the degree of activation of this pathway correlates with the growth potential of these factors. We are currently introducing expression vectors containing IRS-1 and/or receptors for IL-4 or insulin into IL-3-dependent cells that do not respond to IL-4 or insulin in an attempt to more clearly define the importance of IRS-1 in signal transduction.

References

1. McCubrey, J.A., Steelman, L.S. *et al.* Blood 78, 921-929, 1991.
2. Backer, J.M., Myers Jr., M.G. *et al.* EMBO 11, 3469-3479, 1992.

E 010 REQUIREMENT OF NUCLEAR PROLACTIN FOR T LYMPHOCYTE PROLIFERATION. Michael B. Prystowsky and Charles V. Clevenger, University of Pennsylvania, Philadelphia.

The clonal expansion of T lymphocytes is often a necessary component of an effective immune response. Proliferation is a highly regulated process which requires the sequential stimulation of cell surface receptors. For at least some T lymphocytes, antigenic stimulation causes the production of lymphokines including interleukin 2 (IL2) and the expression of IL2 receptor. IL2 induces *de novo* gene expression and G1 progression, but the neuroendocrine hormone prolactin (PRL) is required for entry into S phase. During G1 progression PRL binds to its cell surface receptor and is translocated to the nucleus. PRL is required for expression of Histone H3 and cyclin B, and cell cycle progression

E 011 IL-2 RECEPTOR AND ITS TARGET GENES IN THE HEMATOPOIETIC CELL CYCLE. Tadatsugu Taniguchi[1], Yasuhiro Minami[1], Hiroshi Shibuya[1], Takeshi Kono[1], Naoki Kobayashi[1], Mitsutoshi Yoneyama[1], Atsuo Kawahara[1], Kyoko Yamada[1], Seijiro Minamoto[1], Masanori Hatakeyama[1], Roger M. Perlmutter[2], [1]Institute for Molecular and Cellular Biology, Osaka University, JAPAN, [2]Howard Hughes Medical Institute, University of Washington, School of Medicine, USA.

Interleukin-2 (IL-2) has been known as a critical regulator of lymphocyte proliferation. The IL-2 receptor (IL-2R) comprises at least three distinct IL-2 binding components, the IL-2Rα chain (p55, Tac-antigen), the IL-2Rβ chain (p70-75) and the IL-2Rγ chain (p64). Structural and functional analyses of these components revealed that (i) IL-2Rα and IL-2Rβ bind IL-2 with "low" and "intermediate" affinities (Kd:~10nM and 1nM), respectively, and the three chains together constitute the "high-affinity" IL-2 receptor (Kd: ~10pM); (ii)IL-2Rβ but not IL-2Rα is responsible for the intracellular signal transduction, (iii)Although the IL-2Rγ is required for IL-2 internalization, the role of IL-2Rγ in signal transduction is still unclear, (iv)IL-2Rβ and IL-2Rγ belong to a new superfamily of cytokines receptors. Detailed analysis of structural domains of the human IL-2Rβ chain has revealed a critical cytoplasmic region rich in serine residued ("serine-rich" region) proximal to the cell membrance, important for ligand-mediated growth signal in an IL-3-dependent cell line, BAF-BO3. Adjacent to this region, there is a region rich in acidic amino acids("acidic" region). Priviously, we provided evidence for the formation of a stable complex of IL-2-2Rβ with the lymphocyte specific protein tyrosine kinase (PTK) $p56^{lck}$.The primary interaction sites appear to be the acidic region of the receptor and the N-terminal half of the kinase domain that is well conserved among the *src*-family kinases. As a result of this interaction, IL-2Rβ becomes phosphorylated in vitro by $p56^{lck}$. Furthermore, this interaction appears to be critical for the PTK activation by IL-2. These data suggest the participation of $p56^{lck}$ as a critical signaling molecule downstream of IL-2R via the novel interaction. These findings are also suggestive of the involvement of *src*-kinases other than $p56^{lck}$ in the IL-2 mediated signals. In fact, more recently, we have shown the interaction of IL-2Rβ with $p59^{fyn}$ in BAF-BO3. Recent study indicates that the IL-2 receptor β chain (IL-2Rβ) is linked to at least two intracellular signalling pathways. One pathway may involve a protein tyrosine kinase of the src family, which leads to the induction of the *c-jun* and *c-fos* genes, among others. A second pathway, involving an as yet unknown mechanism, leads to *c-myc* gene induction. We provided evidence that the *c-myc* induction by the second pathway is critical for the BAF-BO3 cell cycle progression.

New Murine Models for Cytokine Discovery

E 012 T-B COGNATE INTERACTION--THE ROLE OF CD40 AND ITS LIGAND. William C. Fanslow, Richard J. Armitage, Mark R. Alderson, Charles Maliszewski, Tim Sato, Kenneth H. Grabstein, Fred Ramsdell, Michael B. Widmer, Ky Clifford, Brian MacDuff, Laura Strockbine, Melanie K. Spriggs. Immunex Research and Development Corporation, Seattle.

CD40 is a 50 kDa surface glycoprotein expressed on B-lineage cells, epithelial cells, dendritic cells and some carcinoma cell lines. CD40 is a member of the recently described TNF receptor family of molecules which includes receptors for nerve growth factor and tumor necrosis factor. Monoclonal antibodies to CD40 mediate several effects on B cells including induction of proliferation and IgE secretion in the presence of IL-4 , suggesting that a ligand for CD40 (CD40-L) would be important for B cell development and function. We have isolated cDNAs which encode both murine and human CD40-L. The predicted amino acid sequence of the murine and human CD40-L indicates that they are both type II integral membrane proteins and that they share regions of sequence similarity with TNFα and TNF β. The expression of CD40-L is induced on murine and human T cells following activation. Recombinant membrane-bound CD40-L induces proliferation of both murine and human B cells in the absence of a costimulus. CD40-L mediates the secretion of multiple immunoglobulin isotypes, including IgE , by activated murine and human B cells. The activity of recombinant soluble CD40-L and its comparison to membrane-bound CD40-L will be discussed as will the effects of CD40-L in other relevant biological systems.

E 013 IMMUNODEFICIENCY CAUSED BY A TARGETED DISRUPTION OF INTERLEUKIN - 2 GENE IN MICE, Benjamin Sadlack[1], Hubert Schorle[1], Anneliese Schimpl[1], Hartmut Merz[2], Alfred C. Feller[2] and Ivan Horak[1] [1]Institute of Virology and Immunobiology and [2]Institute for Pathology, University of Würzburg, 8700 Würzburg, Germany.

Mice deficient for interleukin-2 (IL-2) were generated by gene targeting to analyze the function of this pleiotropic lymphokine in vivo[1]. Mice homozygous for the IL-2 gene mutation analyzed at 4 weeks of age, were normal with regard to thymus development and thymocyte and peripheral T-cell subset composition. However, their B - lymphocyte differentiation is severly affected as indicated by changes in the isotypes levels of immunoglobulins and by the presence of immature B-cells in peripheral lymphoid organs. We report that the IL-2 deficient mice develop normaly during the first 3 to 4 weeks of age, but they soon become severely compromised and about 50% die within the first 9 weeks of age. The histological and immunological analyse of the complex pathological changes will be presented. The fact that no evidence for a primary involvement of a contagious pathogen was found and the same pathological findings were observed in the progeny of two independent IL-2-/- lines, which originated from two different ES- cell clones, provides a strong argument for a causal linkage between the IL-2 defect and disease symptoms

1) Schorle, H., Holtschke, T., Hünig, T., Schimpl, A., and Horak, I. Nature 352 (1991) 621.

E 014 A NOVEL IMMUNOSUPPRESSIVE CYTOKINE DERIVED FROM $CD4^-CD8^-\alpha\beta^+$ T CELLS, S. Strober, T. Niki, and P. Van Vlasselaer. Stanford University, Stanford, CA 94305.

A panel of $CD^-4^-CD8^-\alpha\beta^+$ T cell clones was derived from the spleens of BALB/C mice after culture in IL-2 enriched medium and cloning by limiting dilution. The cloned cells suppress the mixed leukocyte reaction (MLR), and acute lethal graft versus host disease. Stimulation of the cloned cell line, TLI-2.C7, in vitro with calcium ionophore and PMA elicits the secretion of a cytokine which suppresses ^3H-thymidine incorporation and IL-2 secretion in the MLR. The cytokine was purified biochemically, and is a 20 kd protein with a unique N-terminal amino acid sequence. The partially purified protein inhibits the function of antigen presenting cells (APC) such as cloned thymic macrophages and B cells. APC's incubated with the cytokine have a reduced ability to present antigen to T cell hybridomas. Polyclonal rabbit antibodies directed against a synthetic peptide based on the N-terminal amino acid sequence are able to remove bioactivity from supernatents of the TLI-2.C7 cell line after solid phase immunoabsorption.

Inhibition of IL-1 Action (Joint)

E 015 IL-1 RECEPTOR ANTAGONIST: PRODUCTION AND ROLE IN BIOLOGY, William P. Arend, Robert W. Janson, Jr., Mark Malyak, Charlotte Kutsch, John K. Jenkins, Lorise C. Gahring, and Michael F. Smith, Jr., University of Colorado School of Medicine, Denver, CO 80207.

IL-1ra is the first described naturally-occurring human molecule that functions as a specific receptor antagonist of a cytokine. Two forms of IL-1ra have been described: secretory or sIL-1ra is produced by monocytes and macrophages whereas intracellular or icIL-1ra is a major product of keratinocytes and other epithelial cells. IL-1ra and IL-1β production by human monocytes appear to be regulated differently, and possibly in a reciprocal fashion. LPS induces both proteins in this cell whereas adherent IgG induces only IL-1ra. Other cytokines also influence IL-1ra production by monocytes. GM-CSF modestly induces IL-1ra production by fresh monocytes but is more important as a differentiating agent to enhance IL-1ra production by macrophages. LPS-induced monocytes exposed to IL-4 exhibit an inhibition in IL-1β production and an enhancement in IL-1ra production. IL-10 is also a potent inhibitor of LPS-induced IL-1β production by monocytes but only weakly enhances IL-1ra production. Two other cytokines, IL-1α and IL-3, are weak inducers of IL-1ra production by monocytes but do not change the effects of LPS. Thus, different cytokines may influence IL-1ra and IL-1β production by monocytes through different mechanisms.

Monocyte differentiation into macrophages leads to the acquisition of new patterns of induction of IL-1β and IL-1ra. Both *in vitro*-derived and alveolar macrophages spontaneously produce IL-1ra in culture; this production is not dependent upon adherence or the presence of serum. GM-CSF enhances IL-1ra production by macrophages but in contrast to monocytes, neither LPS nor adherent IgG have a positive effect. Another important cell as a source of IL-1ra is the human neutrophil. Both freshly-isolated peripheral blood and synovial fluid neutrophils contain a small amount of IL-1ra protein (but not IL-1β) without any detectable mRNA. This observation suggests that these cells were stimulated earlier in their life span to produce IL-1ra. Cultured neutrophils exhibit a modest induction of IL-1ra transcription and translation in the presence of LPS, IL-4, TNFα or GM-CSF. Although this IL-1ra is the glycosylated secretory variant, stimulated neutrophils secret little IL-1ra. These cells produce less than 1% the amount of IL-1ra per cell as do monocytes or macrophages. However, dying neutrophils present in large numbers in inflammatory exudates may be an important source of extracellular IL-1ra.

Human keratinocytes produce large amounts of icIL-1ra spontaneously in culture. This structural variant lacks a leader sequence and remains intracellular. Production of icIL-1ra by keratinocytes is enhanced by cell differentiation or by exposure to TNFα; other cytokines have no effect. The ratio of IL-1ra to IL-1 in normal human skin is ≈100:1, suggesting that IL-1ra may exert potent anti-inflammatory effects in the skin when released from dying keratinocytes in the stratum corneum. Synovial fibroblasts and alveolar macrophages can simultaneously produce both forms of IL-1ra during *in vitro* culture. The icIL-1ra to sIL-1ra ratio in cultured synovial fibroblasts is ≈20:1. Small amounts of icIL-1ra mRNA are produced by alveolar macrophages in response to culture with CMV.

Studies on the transcriptional regulation of sIL-1ra indicate the presence of cis-acting DNA elements in the most proximal 300-bp of the promoter that are important in both constitutive and LPS-induced IL-1ra production by macrophage cell lines. The icIL-1ra molecule is encoded for by a different first exon acting through an alternate splice receptor site in the mRNA. The icIL-1ra first exon and its promoter are located at least 5-kb upstream from the sIL-1ra genomic DNA. The two forms of IL-1ra appear to be under different transcriptional control.

Extracellular IL-1ra may be important as a competitive inhibitor of IL-1 in the cell microenvironment. However, intracellular IL-1ra may play additional roles in regulation of cell function that have yet to be defined.

E 016 CLINICAL USE OF HUMAN RECOMBINANT IL-1 RECEPTOR ANTAGONIST, Michael A. Catalano, Synergen, Inc., Boulder, CO 80301-2546.

Interleukin-1 (IL-1) is a pro-inflammatory cytokine implicated in a wide range of human diseases. There exists a naturally-occurring inhibitor of IL-1 which antagonizes its actions at the receptor level. A recombinant form of the IL-1 receptor antagonist (IL-1ra) has been effective in animal models of rheumatoid arthritis, sepsis, inflammatory bowel disease, graft-versus-host disease, and asthma. It is now being studied in clinical trials of these diseases. Some of the results will be described.

E 017 MODIFICATION OF ALLERGIC LATE-PHASE RESPONSE BY SOLUBLE HUMAN IL-1 RECEPTOR (RHU IL-1R). Michael F.Mullarkey[1], Abbe Sue Rubin[2], Eileen R. Roux[2], Roberta K. Hanna[2], Cindy A. Jacobs[2],[1]Swedish Hospital Medical Center, Seattle,[2]Immunex Corporation, Seattle.

Allergic respiratory disease affects an estimated 10% of the American population. When allergens are injected under the skin of atopic patients a characteristic wheal-and-erythema reaction is noted within 20 minutes. Two to 8 hours after this immediate reaction patients experience a second reaction without additional exposure to antigen. Late-phase reactions result in sustained inflammation in the skin, nose and lungs of allergic patients. The time course, pathology and etiology of late-phase allergic responses are distinct from classic delayed hypersensitivity reactions. An initial clinical trial in humans was designed to investigate whether rhu IL-1R might inhibit late-phase response to allergen in patients with allergic rhinitis. Fifteen volunteers were skin tested with clinically relevant allergens at concentrations necessary to elicit late-phase responses. Patients were then injected S.C. with rhu IL-1R and sera collected 14 days later to determine antibody status. All patients were antibody negative. Skin tests were then repeated in duplicate on each forearm with 2x the concentration required for late-phase responses and additional control sites were placed on patients' backs. Antigen injection on the forearms was followed by the immediate S.C. injection of rhu IL-1R or placebo at allergen sites. Cohorts of three patients received 1, 10, 25, 50, and 100 ug/site. Sites were monitored for erythema, induration and itching or discomfort. Drug and placebo were administered according to a prospective, double-blind design. Induration in the tested arm versus the control arm was reduced at 1 ug/site during multiple observations obtained between 2 and 8 hours ($p<0.005$). At 10 ug/site induration was reduced in the experimental arm between 2 and 8 hours ($p<0.05$), and systemic effects were seen causing a reduction of induration at control sites on the back. At higher doses, late-phase was suppressed at both control and experimental sites in a dose-dependent fashion. All patients remained antibody negative when tested 14 days after the second exposure to rhu IL-1R. No toxicities were noted throughout the study. Rhu IL-1R is a remarkably potent inhibitor of allergen induced late-phase inflammation in the skin, with a high safety profile. It may be of value as a therapeutic agent in allergic diseases.

Inhibition of TNF Action (Joint)

E 018 NATURALLY-OCCURRING ANTIBODIES TO IL-1, IL-6 AND OTHER CYTOKINES, Klaus Bendtzen, Morten Svenson, Morten B. Hansen, and Marcus Diamant. Laboratory of Medical Immunology TTA 7544, Rigshospitalet University Hospital, DK-2200 Copenhagen N, Denmark.

A very specific means of modifying the function of cytokines has recently been demonstrated by the presence in sera of specific, high-affinity autoantibodies (aAb) to IL-1α and IL-6. These aAb are found in at least 15 - 75% of normal individuals and, at variable concentrations, in patients with immunoinflammatory disorders. The Fab fragments of the respective aAb bind in a saturable manner to IL-1α and IL-6 with exquisite specificity and with remarkably high affinities (Table). The aAb to IL-1α and IL-6 are the single most important binding proteins of these cytokines in normal human sera.

'Autoantibodies' to other cytokines, e.g. IL-2, TNFα, and the interferons, have been reported in normal individuals. However, naturally occurring antibodies to cytokines, apart from those against IL-1α and IL-6, not always bind the cytokines in a specific manner or, indeed, with any appreciable affinity. Western blotting techniques, for example, may show some degree of specificity even though the binding between antibody and ligand is weak and topographically unassociated with the specific binding sites of the antibodies. Even though Western blotting, RIA and ELISA techniques may be used for screening purposes, demonstration of ligand binding to the Fab fragments of the immunoglobulins, combined with saturation binding analysis, is necessary to confirm the presence of specific aAb to a given cytokine.

The biological role of aAb to IL-1α and IL-6 is not yet understood, although their binding forces suggest that they interfere with immune and inflammatory processes, for example by blocking or destroying antigen-presenting cells carrying membrane-bound cytokine and/or by scavenging bioactive IL-1α and IL-6 released by cells at inflammatory sites. Indeed, the aAb inhibit receptor binding and thus the bioactivities of the cytokines. They are polyclonally derived, because they are recognized by antibodies to both immunoglobulin light chains. They are almost exclusively of the IgG class, IgG_4 constituting about 50% of the aAb to IL-1α (IgG_4 normally amounts to 1% of total IgG). IgG_4 does not activate complement, and lattice formation is limited. Hence, precipitation of IL-1α/anti-IL-1α IgG_4 is unlikely to occur to any significant degree *in vivo*. IgG_4 aAb to IL-1α may therefore function as specific carriers, and thus systemic regulators, of circulating IL-1α. The clinical significance of these aAb is obvious, because:

1: aAb in serum interfere with biochemical and biological assays for IL-1α and IL-6,
2: formation of aAb to cytokines in general is important when considering therapeutic use of cytokines as bioresponse modifiers,
3: inappropriate production/function of aAb to inflammatory cytokines may be pathogenetically involved in immunoinflammatory diseases, including systemic complications to infections and trauma,
4: the therapeutic use of naturally occurring aAb to cytokines is of potential interest. Thus, the presence of blocking aAb to IL-1α and IL-6 in pharmaceutically prepared normal human IgG might perhaps explain why high-dose IgG therapy is beneficial in a number of pathogenetically obscure immunoinflammatory conditions.

Table. Autoantibodies to IL-1α and IL-6 in healthy adults.

	anti-IL-1α Ab	anti-IL-6 Ab
Frequency	30-75%	15%
Increased with age and in males	yes	no
Block CK rec.-binding and bioactivity	yes	yes
Recognize native cytokines	yes	yes
Immunoglobulin classes	$IgG_{4,2,1}$	IgG_1
In pharmaceutical IgG preparations	yes	yes
Ligand binding:		
K_d	$< 10^{-11}$ M	$< 10^{-10}$ M
maximum binding	30 ng/ml serum	300 ng/ml serum
bind with Fab fragments	yes	yes

References: Svenson M et al. Scand J Immunol *1989*; **29**: 489-492.
Bendtzen K et al. Immunol Today *1990*; **11**: 167-169.
Hansen MB et al. Scand J Immunol *1991*; **33**: 777-781.

E 019 TNF-RECEPTORS KINETICS IN MICE, W.A. Buurman, Dept. of Surgery, University of Limburg, Fac. II, P.O. Box 616, NL-6200 MD Maastricht, The Netherlands.

TNF-Receptors are considered to be more than cell signalling proteins. They are claimed to be responsible for inactivation of TNF spilling into the circulation during inflammation. The kinetics of TNF-R is studied by ELISA in mice treated with LPS. Antibodies to TNF are used to investigate the mechanism responsible for TNF-R plasma level kinetics. Furthermore the role of the kidney in the clearance of TNF-R and TNF-R-TNF complex was studied in nephrectomized and normal mice. The data indicate that TNF appears very early after TNF induction by LPS and preceding the increase in plasma TNF-levels. TNF plays a role in the regulation of TNF-R receptor levels, since anti-TNF antibodies reduced the increase in TNF-R levels. The kidney is found to be responsible for clearance of both TNF-R and TNF-R-TNF complexes.

In conclusion LPS leads to increased systemic TNF levels that are preceded by an increase in circulating TNF-R levels, a process in which TNF is a responsible factor. Both TNF-R and the complexes are primarily cleared by the kidney.

E 020 COUNTERACTING THE EFFECTS OF TNF WITH SOLUBLE TNF RECEPTORS, Kendall M. Mohler, Dauphine S. Torrance, Craig A. Smith, Raymond G. Goodwin and Michael B. Widmer. Immunex Corporation, Seattle, WA, 98101.

Two forms of the extracellular, ligand-binding portion of the human p80 cell surface receptor for tumor necrosis factor, a monomer (sTNFR) or a dimer linked to the Fc portion of human IgG1 (sTNFR:Fc), were used to antagonize the biological activities of TNF. As predicted from the multivalent interaction of TNF with its cell surface receptor(s), the divalent sTNFR:Fc was a more potent inhibitor of TNF binding (50-100X) and TNF bioactivity (500-1000X) *in vitro* than the monomeric sTNFR. In addition, monomeric and dimeric forms of the sTNFR were also utilized *in vivo* in two mouse models of inflammation; LPS-induced septic shock and hypersensitivity pneumonitis induced with *M. faeni*. In LPS-induced septic shock, administration of dimeric sTNFR:Fc to mice at doses ranging from 10-100 ug saved them from an otherwise lethal injection of LPS, whereas sTNFR at doses up to 260 ug did not. Surprisingly, the dimeric sTNFR:Fc construct demonstrated a beneficial effect even when administered 3h after a lethal LPS injection (i.e., after serum TNF levels had peaked and receded). To study the mechanism by which the soluble TNF receptor functions, serum TNF levels were examined in mice given LPS in the presence or absence of soluble receptor. Administration of a mortality-reducing dose of sTNFR:Fc ablated the rise in serum TNF bioactivity that normally occurs in response to LPS, as measured by an *in vitro* L929 cytolysis assay. However, TNF bioactivity was revealed in these "TNF-negative" samples when a monoclonal antibody which blocks the binding of murine TNF to the human soluble TNF receptor was included in the L929 bioassay. These results indicate that (1) the absence of direct cytolytic activity in the L929 assay was due to neutralization of TNF, rather than to an absence of TNF in the serum and (2) TNF readily dissociates from the sTNFR:Fc *in vitro*. Utilizing the L929 bioassay in conjunction with the blocking mAb to the sTNFR:Fc molecule, we have demonstrated that the sTNFR:Fc molecule can prolong the presence of serum TNF *in vivo* following administration of LPS. Moreover, administration of either monomeric sTNFR or low doses of dimeric sTNFR:Fc actually resulted in increased serum TNF levels compared to mice given LPS but no soluble receptor. However, these "agonistic" doses of receptor did not lead to increased mortality when an LD60 dose of LPS was given. In an *in vivo* model of local inflammation in the lung (hypersensitivity pneumonitis), intranasal (i.n.) installation of *M. faeni* antigen alone induced a marked influx of granulocytes and monocytes into the lung on d4. Simultaneous instillation (i.n.) of sTNFR:Fc (100 ug), but not human IgG1, decreased the inflammatory response. Instillation of monomeric sTNFR at doses up to 40 ug did not reduce the lung inflammatory response. Collectively, these results demonstrate that (1) recombinant soluble TNFR is a potent *in vitro* and *in vivo* antagonist of TNF bioactivity, especially in dimeric form and (2) soluble TNF receptors may prevent toxic effects of TNF *in vivo* by serving as biological buffers for TNF. Under some circumstances the soluble receptors function simultaneously as both TNF "carriers" and antagonists of TNF biological activity.

Clinical and Pre-Clinical Investigation of Cytokines

E 021 **MODULATION OF INTERLEUKIN-6 IS ASSOCIATED WITH CHANGES AND PROLIFERATION OF CULTURED AIDS-KS DERIVED CELLS.** S.A. Miles*, D. Gearing**, P. Linsley***, T. Maione****, T. Kishimoto*****, A. Rezai*, L. Magpantay*, O. Martínez-Maza*. *UCLA CARE Center and Dept. of Ob/Gyn, UCLA School of Medicine, Los Angeles, CA, **Immunex Corporation, Seattle, WA, ***Bristol-Myers Squibb Research Institute, Seattle, WA, ****Repligen Corp., Cambridge, MA, and *****Osaka Univ, Osaka, Japan.

Interleukin-6 (IL-6) and the IL-6 receptor is produced in large amounts in cultured AIDS-Kaposi's sarcoma derived cells and in KS lesions *in vivo*. Direct modulation of the IL-6 pathway using oligonucleotides targeted to sequences within the IL-6 gene, IL-6 receptor gene (80 kD subunit), or the NF-IL-6 binding site of the IL-6 promotor decrease proliferation of AIDS-KS derived cells. Multiple cytokines (such as IL-1ß, TNF-α, high dose gamma IFN, low dose TGF-ß and Oncostatin-M) and the transactivating protein of HIV, *tat*, increase interleukin-6 (see figure). In each case, these increases are associated with increases in proliferation of AIDS-KS derived cells in culture. Moreover, the effects of these proteins can be blocked by IL-6 antisense but not sense oligonucleotides. Cytokines which decrease IL-6 production (such as PF4, IL-4 and high dose TGF-ß) decrease the proliferation of AIDS-KS cells in culture. This data suggests that IL-6 is an important factor in the growth of KS cells *in vitro*, that modulation of IL-6 expression or response in KS cells may be a common mode of action for multiple growth regulators and that interruption of the IL-6 pathway *in vivo* may have therapeutic potential. One proliferative cytokine, Oncostatin-M, can utilize both the LIF and IL-6 receptors for function. In comparison to endothelial cells and smooth muscle cells, OncoM, but not LIF or IL-6 have mitogenic effects. This activity can be blocked by a mixture of antibodies to the gp130 subunit of the IL-6 receptor. On both smooth muscle cells and endothelial cells, LIF and OncoM have activity. The activity of LIF can be blocked by soluble hu-LIF receptors and the activity of both LIF and OncoM can be blocked by antibodies to the gp130 sub-unit of the IL-6 receptor. The activity of all three of these cytokines correlates with specific differences in post-receptor signal pathways involving *ras*, c-*raf*, PI3K and MAP kinase. These studies provide support for the concept that AIDS-KS derived cells are functionally different from their mesenchymal counterparts.

Cytokine Control of Lymphopoiesis

E 022 **CYTOKINE CONTROL OF HUMAN B LYMPHOCYTE DIFFERENTIATION**, Jacques Banchereau, Dominique Blanchard, Francine Brière, Serge Lebecque and Françoise Rousset. Schering-Plough, Laboratory for Immunological Research, Dardilly, France.

INTRODUCTION : Antigens breaking the cutaneous or mucosal barrier are rapidly taken up by Dendritic/Langerhans cells which migrate to the draining lymph nodes where they initiate the development of T and B cell reactions. Antigen specific B cells proliferate and differentiate into plasma cells. Some B cells migrate within the follicles to generate germinal centers where they expand, undergo somatic mutations, isotype switching, antigen selection and differentiation into either memory cells or plasmablasts secreting antibodies of higher affinity and specificity. In order to understand the mechanisms controlling these steps, we have studied the role of cytokines in the growth and differentiation of B cells activated in two different systems : the CD40 system and the CD3 system.

THE CD40 SYSTEM: Resting B cells proliferate when cultured in the presence of anti-CD40 and a fibroblastic cell line (L cells) expressing an IgG Fc receptor ($Fc\gamma R_{II}$/CDw32). Additional triggering of the sIg results in T cell-independent differentiation of B cells. Naive $sIgD^+$ B cells produce mostly IgM and isotype committed $sIgD^-$ B cells produce mostly IgG and IgA. IL-4 strongly stimulates the proliferation of B cells cultured in the CD40 system and factor dependent B cell lines could be maintained for up to 10 weeks. Single B cells can give rise to colonies of 50-500 cells within two weeks. IL-10 enhances short term B cell proliferation and combinations of IL-4 and IL-10 result in strong multiplication of B cells. Naive B cells undergo isotype switching towards IgE in response to IL-4. This is demonstrated by using the clonal growth of G8 idiotype positive single B cells and by sequencing of the $VDJC_H$ products of single daughter B cells. B cells secrete large amounts of IgM, IgG and IgA in response to IL-10 following differentiation into plasma cells. Naive $sIgD^+$ $sIgM^+$ B cells, which produce essentially IgM in response to IL-10, are induced to secrete both IgA_1 and IgA_2 following addition of TGFß. In contrast, TGFß suppressed the IL-10 mediated IgG, IgM and IgA secretion by $sIgD^-$ B cells. The CD40 system displays many of the features of B-cell immunopoiesis, including intense proliferation, isotype switching and plasma cell differentiation, but does not to induce somatic mutations.

THE CD3 SYSTEM: Some $CD4^+$ clones, activated with immobilized anti-CD3, induce growth and differentiation of resting B cells. The proliferation is limited because cells readily differentiate into non proliferating plasma cells. These clones also induced isotype switching in naive B cells. Addition of IL-4 antagonists to cultures result in inhibition of IgE production while the secretion of other isotypes and the proliferation is poorly affected. Addition of IL-2 antagonists inhibits B cell proliferation and production of IgG, A, M without inhibiting that of IgE. T cell clones activated for 24-28 h and washed free of cytokines fail to induce B cell proliferation and differentiation. Addition of IL-2 (but neither of IL-4 nor of IL-10) to such cultures results in strong B cell growth and Ig secretion and IL-10 further enhances these effects.

PERSPECTIVES : Identification of culture conditions yielding somatic mutations will represent a major step towards the "*in vitro* immunization" of naive B cells. This would ultimately permit us to generate human monoclonal antibodies of desired specificity and unavailable in the human repertoire.

E 023 THE PHENOTYPE DYNAMICS OF T CELLS ARE CONTROLLED BY STEROID AND POLYPEPTIDE HORMONES, Raymond A. Daynes, and Barbara A. Araneo, Department of Pathology, University of Utah Medical School, Salt Lake City, UT 84132.

The capacity for rapid adaption to new and/or changing environmental conditions represents an essential component of the mammalian immune system. This functional necessity emanates from the nomadic lifestyle which characterizes this organ system's major cellular elements, and the flexibility in effector mechanism selection that is required of the system for successful function in host protection. The dynamic nature of lymphocyte recirculation, a process that associates with a constant movement of cells into and out of unique tissue microenvironments, must be paralleled by equally dramatic changes in the potential of these cells to elicit particular types of responses. The effector mechanisms that are mobilized following an encounter with an infectious agent, must also be plastic. Route of infection, the presence of antigen-associated cell stimulatory factors (eg. LPS), the extent of tissue injury at infection sites and the physiological status of the host at time of exposure, all contribute to the complex process of response selection.

Our research has been focused on understanding the biochemical and cellular basis of the information transfer systems, which function to fulfill the 3-dimensional requirements of the *in vivo* immune system. We have been able to elucidate some novel roles played by two classes of endogenous substances; the steroid hormones and the polypeptide growth factors (eg. PDGF and TGFβ). Most important are those steroid hormones which undergo tissue-specific end-organ metabolism from inactive precursors to active species. Included in this group are dehydroepiandrosterone (DHEA) sulfate, testosterone, and 25 hydroxyvitamin D_3. These molecules are enzymatically converted to the active species, DHEA, dihydrotesterone and 1,25 dihydroxyvitamin D_3 respectively at local tissue sites. The active hormones, through classical biochemical pathways, alter the potential of lymphoid cells to produce lymphokines and cytokines following activation. The cells resident in local microenvironments, rich or deficient in a particular steroid hormone, would be influenced accordingly. The polypeptide growth factors, released from preformed platelet stores at tissue injury sites, or produced *de novo* by local or infiltrating cell types, also can function to redirect the biosynthesis of individual lymphokine species by activated lymphoid cells. We believe that it is the collective influences of these afferent acting processes, and not the predetermination of genetic potential, which provides the mammalian immune system with its high degree of flexibility. In support of this argument, the data to be presented will demonstrate that; 1) lymphocyte behavior is microenvironmentally controlled by the actions of end-organ metabolized steroid hormones and growth factors, 2) aging and severe trauma cause immune dysfunction due to definable alterations in information transfer, and not because of intrinsic defects, 3) immunosenescence and trauma-associated immunosuppression can be reversed/prevented by appropriate therapeutic intervention, and 4) there are many non-immunologic conditions which are normally being controlled through mechanisms that are identical to those that provide afferent regulation to the mammalian immune system.

E 024 CYTOKINE CONTROL OF EFFECTOR T CELL GROWTH AND DIFFERENTIATION, M.B. Widmer, K.H. Grabstein, W.C. Fanslow, R.J. Armitage, M.K. Spriggs and M.R. Alderson, Immunex Corporation, Seattle, WA.

Cytokines play an integral role along with antigen in the growth and differentiation of antigen-specific cytolytic T lymphocytes (CTL) from their quiescent precursors. One system which has proven to be particularly useful for the analysis of the immunoregulatory effects of individual cytokines is the mixed leukocyte culture (MLC), in which the generation of alloantigen-specific CTL can be readily quantified. We have employed two investigative approaches to the study of cytokine effects on CTL generation in both human and murine MLC: 1) exogenous cytokines were added to MLC to determine their potential for regulating the response 2) cytokine antagonists were used to determine the role of the corresponding endogenous cytokines in the response. In addition to the activity demonstrated by conventional, T cell-active cytokines in these systems, results of recent experiments indicate that CTL generation can be influenced in a positive manner by cell-bound ligands which interact with certain members of the TNF receptor family. As some of these molecules are normally not considered to affect T cell growth and differentiation directly, additional studies will focus on determining which of the multiple cell types present in the culture system is the target for the activity of these molecules and whether other known cytokine intermediaries are involved.

Cytokine Control of the Immune Response to Infection

E 025 COLONY STIMULATING FACTORS FOR THE TREATMENT AND PREVENTION OF INFECTIOUS DISEASES, David C. Dale, University of Washington School of Medicine, Seattle, Washington.

Numerous preclinical and clinical trials have now demonstrated the effectiveness of the colony stimulating factors to elevate blood neutrophil counts. This occurs *in vivo* because of stimulation of neutrophil production in the bone marrow. Both granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) cause increased proliferation of myelocytes and earlier neutrophil precursors. They also appear to increase blood counts by accelerating the post mitotic maturation and entry of neutrophils into the circulation.

The quality of the neutrophils produced in response to G-CSF and GM-CSF has been investigated in a variety of ways. Morphologically the cells released into the blood within the first days of treatment appear larger than normal. They also have prominently staining primary granules, so-called "toxic" granulation. Their functional capacity, as reflected by *in vitro* measurements of oxygen consumption, glucose utilization, the generation of hydrogen peroxide and chemiluminescense is normal or may even be enhanced. Studies of the killing of bacteria or fungi by neutrophils exposed to the CSF *in vitro* or produced in subjects treated with either G- or GM-CSF indicate that these functions are intact or possibly increased.

A critical neutrophil function for host defenses is the capacity to circulate freely and migrate to a site of inflammation. *In vitro* studies suggest that the CSFs may be weak chemoattractants. *In vivo*, however, it appears that the increases in cell numbers in the blood do not necessarily result in increased migration of cells to a tissue site of inflammation. Using a skin chamber technique reduced migration has been observed with both. These findings may be attributable to alterations of adhesins on the surface of neutrophils or their interactions with endothelial cells, but the full biological basis for these findings are not yet known.

Several studies now show that treatment with the CSFs can accelerate marrow recovery following chemotherapy or bone marrow transplantation and reduce the susceptibility to infection associated with acute neutropenia in these settings. In animal studies it can be demonstrated that pretreatment with G-CSF to increase the neutrophil supply reduces the occurrence and severity of infections in models of burns, neonatal sepsis and alcohol induced marrow suppression. Clinical trials are under way to determine if the CSFs can be used to enhance host defense mechanisms with common infections in non-neutropenic subjects.

E 026 THE ROLE OF CYTOKINES IN THE REGULATION OF HIV EXPRESSION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Following initial infection with HIV, infected individuals experience a prolonged asymptomatic period. Although HIV replication occurs throughout all stages of HIV infection, it is clear that during this time, the HIV genome that is present in certain cells in the body may remain in a latent state or direct the cell to produce low-level amounts of virus. In an effort to understand the mechanisms involved in the conversion of a latent, or low-level HIV infection into active disease, we have focussed on the identification of factors that can activate virus expression. We have found that cytokines can alter HIV expression in chronically infected cells. We have demonstrated the induction of HIV expression by tumor necrosis factor (TNF)-α, interleukin (IL)-6 and granulocyte-macrophage colony stimulating factor (GMCSF). We have shown that TNF-α-induced upregulation of HIV expression involves a transactivating mechanism in which a DNA-binding protein binds to the NFkB sites on the HIV LTR. We have extended these observations to the regulation of HIV expression by transforming growth factor (TGF)-β. We have shown that TGF-β exhibits a bifunctional effect on HIV replication. Although initially thought to suppress HIV infection, we have recently observed that interferon-gamma actually induces the induction of HIV replication within intracellular vesicles. We have investigated the role of pharmacologic agents such as retinoic acid (RA) and anti-oxidants in the suppression of HIV infection. We have shown that retinoic acid closely resembles TGF-β in its pattern of suppression of HIV expression. At the molecular level, TGF-β and RA appear to involve multiple levels of HIV expression. We have also shown that the anti-oxidants, glutathione (GSH) and n-acetyl cysteine (NAC), suppress the induction of HIV expression by PMA, TNF-α, and IL-6. On a molecular level, we have demonstrated that NAC, but not GSH, suppresses transcription of HIV mRNA. We have also investigated the potential role of a pharmacological antagonist of PAF, RP 55778, on HIV replication. It is hoped that an understanding of the role of cytokines and pharmacologic agents in the regulation of HIV expression may lead to the development of therapeutic strategies that can forestall progression of HIV disease.

E 027 CYTOKINES OF TH2 CELLS, Tim Mosmann, Jean-Marie Heslan and Larry Guilbert, Department of Immunology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The T helper subsets TH1 and TH2 differ markedly in the patterns of cytokines that they secrete after antigen stimulation, and these patterns are responsible for very different functional properties of the two subtypes. TH1 cells induce an inflammatory response, including activation of granulocytes and macrophages. In contrast, TH2 cells are excellent helpers for B cell antibody production, and in the absence of a significant Interferon γ response, TH2 cells induce a strong allergic response due to secretion of IL4, IL5 and IL10. IL10 is also able to inhibit TH1 responses at the level of macrophage activation and indirectly, by inhibiting TH1 cytokine production. Another cytokine, P600, was initially characterized as an induction-specific cDNA clone isolated from TH2 but not TH1 cells. We have now expressed recombinant P600 protein and characterized the functions of this cytokine. P600 does not have activity in many of the assays in which TH2-specific cytokines are active. However, P600 induces proliferation and/or activation of an adherent cell population from a bone marrow precursor. The resulting cells have high expression of the macrophage markers MAC1 and F4/80. Although these cells are not phagocytic for antibody-coated erythrocytes, they are effective antigen-presenting cells for T cell clones specific for particulate, soluble or alloantigens.

Workshop

E 028 NEW T-CELL GROWTH FACTORS IN THE TREATMENT OF CANCER Michael T. Lotze, Joshua T. Rubin, Walter J. Storkus, Steven Hurd, Chet L. Nastala, Howard E. Edington, Thomas G. McKinney, Barbara Pippin, Kiyoshi Nishihara, Hideaki Tahara, Elaine Elder, Theresa Whiteside, Robert Zimmerman and Arthur Louie. Section of Surgical Oncology, Department of Surgery, Department of Molecular Genetics and Biochemistry, Division of Biologic Therapy, Pittsburgh Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, PA 15261 and Chiron, Inc., Emeryville CA 94608.

There are now at least 5 described T-Cell Growth Factors. These include IL-2, IL-4, IL-7, IL-10 and IL-12. IL-2 is the prototypic T-Cell Growth Factor. We have administered it in a recently completed study to 14 patients with a variety of malignancies to demonstrate that IL-2 can be given safely as a bolus over 1-2 minutes. The two doses employed were 360,000 or 540,000 IU/kg administered thrice daily. Of the 5 patients with melanoma, one had a near complete response, and two have had partial responses. The half-life was similar to that observed with IL-2 administered conventionally, with a T 1/2 alpha of .064 hours and a T 1/2 beta of 0.59. There were no deaths observed in this series of patients. Of five patients with renal cell carcinoma, one had stable disease and one has had a partial response. We are currently evaluating the use of PEG-IL-2 as an adjuvant for vaccines and similarly are about ready to begin a trial of high dose systemic IL-2 with or without pentoxifylline in the treatment of patients with disseminated cancer. This is done to decrease the toxicity associated with TNF release in the context of IL-2 treatments. Such treatments have been shown not to adversely impact on the response to therapy in murine models.

Interleukin 4 is a TH2 cytokine which we have administered in phase I and Phase II testing alone or in conjunction with IL-2. More recently we have treated two patients with gene marked TILS expanded in IL-2 and IL-4, at up to 2×10^{11} cells. Rapid expansion of specific TILS was noted in combinations of IL-2 and IL-4. The transfer of such cells was well tolerated when given with concurrent IL-2 and IL-4 treatment in vivo. We are now modifying our protocol to allow administration of TILS grown in IL-2 and IL-4 with systemic IL-2 alone. In addition we have recently been approved by the Recombinant DNA Advisory Committee to administer IL-4 as part of a gene therapy vaccine. IL-4 transfected fibroblasts will be mixed with autologous tumor and sequential biopsies obtained from injected sites. This protocol was based on previous regimens identified as being effective in murine models.

Interleukin 7 is a stromal derived cytokine which promotes B-Cell maturation and T-Cell growth. We have previously demonstrated that IL-7 leads to preferential outgrowth of $CD4^+$ tumor infiltrating lymphocytes and can be used to expand such cells in culture for long periods especially in combination with IL-2. We are considering initiating a trial of IL-7 expanded $CD4^+$ cells in the treatment of patients with AIDS or cancer.

Interleukin 10 is a cytokine produced primarily by T-cells acting as a cytokine synthesis inhibitory factor through its effects on macrophages. We have created retroviral vectors expressing the viral IL-10 homologue for potential use in the gene therapy of transplantation. Similarly we have recently confirmed results from colleagues at Schering that IL-10 indeed induces lymphokine activated killing activity. Thus, we consider all of these cytokines as being cytotoxic inducers of NK cells.

IL-12 is a novel heterodimeric cytokine consisting of p35 and p40 chains. We have created retroviral vectors expressing both chains and have been able to successfully transfect both murine fibroblasts and human melanomas. In murine studies we have been able to show that not only does IL-12 induce LAK activity but that it also enhances the response in allostimulation although not nearly as great as that observed with IL-2. IL-12 delivery at the site of a tumor delays the growth of such a tumor as well as being of benefit in the setting of vaccination allowing the growth of subsequent challenge of tumor. The future development of each of these individual cytokines and their combinations will require considerable ingenuity and time in the future.

Cytokines and Hematopoiesis

E 100 EFFECTS OF STEM CELL FACTOR ON RADIATION-INDUCED MYELOSUPPRESSION: KINETICS OF EARLY AND LATE PROGENITORS RELEASE FROM BONE MARROW, Camille N. Abboud*, Maureen C. Kempski*, Philip K. Gregory+, Kris M. Zsebo#, Departments of Medicine* and Radiation Oncology+, University of Rochester School of Medicine, Rochester, NY 14642, and AMGEN Inc# CA 91320

In order to study the *in vivo* action of Stem Cell Factor (SCF) on the kinetics of early and late progenitors we elected to study mice subjected to 400 cGY radiation. Prior studies have indicated that at a dose of 200 cGY one can suppress marrow CFU-S and CFU-GM content for 7 days. B6D2F1 mice were treated with a single dose of 400 rads after which they received a sham vehicle injection or recombinant rat SCF 100μg/kg daily for a period of 21 days. Marrow and spleen cellularity and progenitor content were analyzed at weekly intervals. Myeloid (CFU-GM) and erythroid (BFU-E) progenitors were quantified as well as more primitive high-proliferative potential colony forming cells (HPP-CFC), and Day 14 splenic colony-forming units (CFU-S). In all categories of early and late progenitors the marrow at Day 7 displayed similar or slightly decreased content of progenitors in the SCF treated group. By contrast, splenic size and content of progenitors especially Day 14 HPP-CFC and CFU-S was markedly increased in the SCF treated group at 7, 14 and 21 days. The early increase in splenic CFU-S and HPP-CFC at 7 days, preceded the actual recovery of marrow CFU-S and HPP-CFC in the SCF treated animals at 2 weeks, suggesting a role for stem cell factor mediated egress of early progenitors from bone marrow to splenic tissue. These studies underscore the utility of SCF in stem cell mobilization and recovery after radiation-induced myelosuppression, and can be used as a model to design similar experiments in patients undergoing hemibody irradiation for the therapy of cancer.

E 101 PROLIFERATIVE AND DIFFERENTIATIVE EFFECTS OF GROWTH FACTORS, SERUM AND STEROIDS ON HIGHLY ENRICHED HEMATOPOIETIC LONG TERM REPOPULATING CELLS (LTRC) CULTURED AS SINGLE CELLS. Stephen H.Bartelmez, Ewa Sitnicka, Gregory V.Priestley, Norman S.Wolf Department of Pathology, University of Washington, Seattle, WA98195.

Detailed analysis of hematopoietic stem cell populations requires the in vitro isolation of this rare (~1 stem cell per 50,000 marrow cells) subpopulation. Our recent murine studies have focused on a new cell fractionation approach based largely on a sequential Hoechst 33342-Rhodamine 123 selection in the final stages of the fractionation. This procedure results in an extremely enriched primitive cell fraction, 20 of which cells will completely long term repopulate a lethally irradiated recipient in all hematopoietic lineages. These primitive cells are essentially devoid of CFU-S 8 day and CFU-S 12 day spleen colony forming cells, however, ~8 cells per 10 cells plated in vitro will form high proliferative potential colonies (>50,000 cells) if exposed to c-kit ligand (KL), IL-6, IL-3, IL-1 and CSF-1. To elicidate growth requirements for these primitive cells during the first several cell divisions , single cell cultures were established. The isolated cells either lysed, remained as single cells for >10 days, or proliferated depending on the specific growth factors, serum or hydrocortisone (H.C.) present. Without added cytokines, the primitive cells invariably lysed within 2-3 days. In contrast, if either KL, IL-6, IL-3, IL-1, IL-11 or G-CSF were present alone up to 50% of single sorted cells were still present after 10 days. However, only KL or IL-3 alone were mitogenic. While KL stimulated ~15% of primitive cells to form small clones (range 2->128 cells), IL-3 stimulated ~30% of cells to form both small and large (>5000cells) clones. Maximal clonal efficiency (80-90%) was attained when KL+IL-6 were present from day 0. Delayed addition of IL-3 to KL+ IL-6 indicated that IL-3 increased clone size. Growth factors+FCS (25%) produced the highest cloning efficiency and growth rates but smallest clone size, whereas horse serum (25%) produced the largest clone size, coupled with slow growth rate. The addition of H.C. to either serum slowed proliferation and reduced clonal formation.

E 102 MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) PRODUCTION BY A NEONATAL SPLEEN:MYELOMA HYBRIDOMA LINE (NBXFO): ROLE IN NEONATAL SUPPRESSION, Beverly E. Barton, H. Terry Wepsic and Martin R. Jadus, Department of Immunology, Schering-Plough Research Institute, Bloomfield, NJ 07003 and Department of Laboratory Service, Veterans Affairs Medical Center, Long Beach, CA 90822

The hybridoma, NBXFO, was created by fusing neonatal DBA/2 splenocytes with FO myeloma cells. The supernate derived from this cell line is capable of supporting hematopoietic progenitor activity from the bone marrow of adult mice and rats. We have identified the cytokine responsible for this action to be macrophage colony stimulating factor (M-CSF) also known as colony stimulatory factor-1 (CSF-1). The bone marrow progenitor cells which grow out of the NBXFO supernates are non-specific esterase positive monocyte/macrophages. Neutralizing antibody to M-CSF, but not antibody to IL3, IL7, GM-CSF or stem cell factor could inhibit the growth of the progenitor cells. NBXFO cells also possess cell surface M-CSF as determined by flow cytometry as well as in a functional analysis, since formalin-fixed NBXFO cells could support hematopoietic growth. NBXFO cells also possess 2 types of M-CSF transcripts: 1.6 and 4 kb mRNAs. Eight percent of neonatal splenocytes possessed cell surface M-CSF and the conditioned media from neonatal splenocytes could sustain the growth of macrophage colonies. From this work we believe that the fetal cell which contributed the other half of the hybridoma was probably derived from a cell which produced M-CSF. Finally, we discuss the possible relevance of M-CSF in the origin and activation of neonatal natural suppressor cells.

E 103 DIFFERENTIAL EFFECT OF RECOMBINANT CYTOKINES ON FDCP-MIX 1.8 CELL PROLIFERATION AND MAINTENANCE OF IL-3 RESPONSIVENESS, William R. Benjamin, Sharon Bowen and Nadine S. Tare, Department of Inflammation and Autoimmune Diseases, Hoffmann-La Roche Inc. Nutley, NJ 07110

FDCP-mix is a murine IL-3-dependent cell line which possesses the capacity to differentiate into multiple hematopoietic lineages. When cultured in the absence of cytokines, FDCP-mix cells rapidly (24-36 hr) differentiate into granulocytes which subsequently undergo apoptosis. The immature phenotype of FDCP-mix cells and the ability of various cytokines to direct their differentiation into multiple lineages suggests that these cells may provide a system for further defining the effects of cytokines on hematopoietic cell development. A subline of FDCP-mix cells (original line kindly provided by D. Williams), designated FDCP-mix 1.8, was established in our lab based on its ability to retain an undifferentiated phenotype when cultured with irradiated Swiss 3T3 fibroblasts. The ability of various cytokines and hematopoietic growth factors to replace the function of 3T3 cells with regard to stimulating FDCP-mix 1.8 cell proliferation and maintaining IL-3 responsiveness (prevention of differentiation and apoptosis) was determined. As expected, IL-3 was active in both assays. However, FDCP-mix 1.8 cells also proliferated in response to GM-CSF and marginally to KL, IL-5 and G-CSF. In contrast to control cultures, FDCP-mix 1.8 cells cultured for 48-72 hr with each of the four cytokines retained significant responsiveness to IL-3 (KL > IL-5 > GM-CSF > G-CSF). No other cytokine tested exhibited activity in either assay. Thus, FDCP-mix 1.8 cells proliferate and maintain IL-3 responsiveness in response to GM-CSF but are selectively prevented from differentiating by KL, G-CSF and IL-5. The range of cytokines to which FDCP cells respond may partially explain their multilineage capability and suggests that these cells may be useful for the identification of additional cytokines which modulate hematopoietic cell development.

E 104 LONG TERM RECONSTITUTION OF MICE WITH CULTURED BONE MARROW CELLS: DIFFERENTIAL RESPONSES OF BONE MARROW AND ENRICHED STEM CELLS TO IL-3. Amy E. Berson, Maureen A. McNally, Karla M. Knobel, Diane Rood, Lydia Kilinski, Thomas B. Okarma, and Jane S. Lebkowski. Applied Immune Sciences, Inc., 200 Constitution Drive, Menlo Park, CA 94025.

The ability to culture bone marrow cells while still maintaining their ability for long term rescue and engraftment of lethally irradiated recipients will have important implications for gene therapy and bone marrow transplantation (BMT).

We have cultured nonadherent bone marrow mononuclear cell (NBMMC) and stem cell enriched ($Thy1.2^+Lin^-$) populations with IL-3 for up to 6 days. By six days NBMMCs maintained in culture decreased to 6% of input cell number whereas $Thy1.2^+Lin^-$ cells increased to 2310%. Doses of 95,000; 100,000; 50,000; 250,000 NBMMCs maintained in culture for 0, 1, 2, and 6 days respectively, rescued 50% of lethally irradiated mice. Likewise, doses of 8,000 and 21,000 $Thy1.2^+Lin^-$ cells cultured 0 and 1 day respectively, rescued 50% of the irradiated mice. The same $Thy1.2^+Lin^-$ cells cultured 6 days failed to rescue recipients. When donor cell engraftment was evaluated, all animals maintained a high level of donor reconstitution regardless of cell dosage, cell type (NBMMC or $Thy1.2^+Lin^-$), time of assay, and number of days in culture. Moreover, the percent T-cell engraftment did not significantly change between 2 and 17 months post BMT. These data indicate that the ultimate stem cell function of rescue and engraftment can be maintained in NBMMCs and $Thy1.2^+Lin^-$ cells cultured with IL-3 for up to six days and one day.

To further understand the requirements for extended stem cell function in culture, future work will investigate other accessory cells and cytokines that may provide the growth factors and support.

E 105 TGF-ß2 DECREASES CHEMOTHERAPY-INDUCED MORTALITY IN MICE. Joseph A. Carlino and Richard W. Gregory. Department of Immunology and Cell Biology, Celtrix Pharmaceuticals, Santa Clara, CA 95054. Transforming Growth Factor-Beta 2 (TGF-ß2) has been shown to suppress the proliferation of normal murine hematopoietic progenitors following in vitro and in vivo treatment. However, initial efforts by our laboratory and by other groups to utilize this suppressive activity to protect bone marrow and reduce chemotherapy-related mortality has instead resulted in chemosensitization. We now show that a chemoprotective effect is elicited by TGF-ß2 when given at doses below those that induce maximal bone marrow suppression. This protection is dependent upon the chemotherapeutic drug administered, as well as the timing of TGF-ß2 administration. Initial studies in normal male C3H mice demonstrated that intraperitoneal or intravenous administration of 0.1µg/day of TGF-ß2 for 5 days induced little or no shift of bone marrow cells into G0/G1. However, doses ≥1µg/day, which induced maximal bone marrow suppression, also induced a significant weight loss and decrease in bone marrow cellularity. When 0.1µg/day of TGF-ß2 was given either before a lethal dose of Adriamycin (ADR) or after a lethal dose of Cyclophosphamide (CPA), there was a significant reduction in the mortality induced by the chemotherapeutic drugs. In contrast, doses of TGF-ß2 which induced maximal bone marrow suppression in normal mice significantly increased mortality in ADR or CPA treated mice. The schedule-dependent effect of TGF-ß2 was confirmed in experiments where a dose administered prior to ADR which afforded chemoprotection acted as a chemosensitizing dose when administered after the ADR. In vitro and in vivo studies designed to address the mechanism of action of TGF-ß2 in this system indicate that the chemoprotective effects of TGF-ß2 are dependent upon a balance of its pleiotropic activities as an inhibitor of bone marrow and intestinal epithelial proliferation, induction of CFU-GM in the marrow, and its effects upon lymphoid tissue cellularity.

E 106 MURINE STROMAL CELLS SUPPORT EXPANSION OF EARLY HUMAN HEMATOPOIETIC STEM CELLS AND GROWTH FACTOR-DEPENDENT CELL LINES.
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The goal of this study was to design in vitro assays to identify and expand very primitive multipotent human stem cells and subsequently analyse molecular mechanisms associated with their commitment and differentiation. To that purpose, we have investigated the ability of marrow-derived murine and human stromal cells to support the development of very early $CD34^{++}/CD38^-$ hematopoietic progenitor cells. In colony-assays, $CD34^{++}/CD38^-$ require at least a three growth factors combination (IL-3, Steel factor (SF-AMGEN), and Epo) to form colonies. When added to these assays MS-5 cells will synergize with any growth factor combination, to (i) increase cloning efficiency and colony size by a factor 2-5; (ii) trigger the development of late-appearing multipotent blast progenitors (CFU-Bl) with a very high recloning potential. Murine cells could not be replaced by human stromal cells. MS-5 cells, but not human stromal cells, also supported a 2-10 fold expansion of primitive hematopoietic progenitor cells in long-term cultures (LTC) established by coculturing $CD34^{++}/CD38^-$ cells 6-8 weeks on murine feeders. There was no need for exogenously supplied human growth factors in these LTC. Interestingly, only early progenitors were amplified, and MS-5 did not support differentiation of these cells into mature clonogenic progenitors and terminally differentiated cells. Limiting dilution experiments revealed that 2-5% of initial $CD34^{++}/CD38^-$ cells were capable of clonal expansion in these conditions. In parallel experiments, we also showed that MS-5 cells stimulated the proliferation of the GM-CSF/IL-3/Epo dependent leukemic cell line UT-7 in clonogenic assays as well as in LTC, in the absence of the human growth factors normally required. An intimate contact between UT-7 and MS-5 was required, and soluble SF did not replace MS-5 cells suggesting the existence of novel stromal-derived species cross-reactive activities which may act as membrane-bound forms or closely associated with extracellular matrix components.

E 107 BFU-E, CFU-MEG, CFU-EO AND LONG-TERM-CULTURE INITIATING CELLS (LTC-IC) IN MINIATURE SWINE BONE MARROW. David A. Crouse[1], Jim Rogers[2], J.Graham Sharp[1] and John D. Jackson[3], Depts. of Cell Biology & Anatomy[1] and Pathology/Microbiology[3], Univ. Neb. Med. Ctr., Omaha, NE 68198 and Albert Einstein College of Medicine[2], Bronx, NY 10461

Therapy for a variety of cancers now involves the collection and cryopreservation of hematopoietic stem cells prior to the administration of high dose therapy and subsequent rescue with the cryopreserved material. In order to better evaluate the wide variety of potential clinical protocols used in such therapy, it is necessary to develop a pre-clinical animal model which accurately reflects the human situation. We have previously reported the development of GM-CFC, HPP-CFC and long-term cultures in the miniature swine. The studies reported here demonstrate BFU-e, CFU-meg and CFU-eo as well as LTC-IC from both fresh and frozen swine BM cells. These assays were modelled after the protocols for human cells and employ recombinant human cytokines and/or swine spleen lymphocyte conditioned media (LCM). BFU-e ($27\pm4/10^5$ cells) were observed only when swine LCM plus EPO were employed. Pure, Luxol-blue-staining CFU-eo colonies ($4\pm1/10^5$) were observed when recombinant human IL-5 was employed as a stimulus. Recombinant human IL-3 plus GM-CSF led to the production of mixed eosinophil colonies. CFU-meg ($2\pm1/10^5$) positive for the human platelet glycoprotein IIIa antigen (Dakopatts) were stimulated by rhIL-1 plus rhIL-3 plus rhGM-CSF plus rhG-CSF. LTC-IC were assayed in a limiting dilution analysis using a pre-established murine stromal cell population as the supporting adherent layer. In this assay, the frequency of morphologically detectable LTC-IC (cobblestone areas) was found to be 1 in 15 to 30,000 cells pre-processing and 1 in 3 to 8,000 cells post-processing. Unfortunately, attempts to identify primitive swine marrow cells bearing CD34 (My 10) or Sca-1 were not successful. Nonetheless, all of the above data are similar to data from human materials and support the continued development of the miniature swine as an appropriate model for pre-clinical studies with human cytokines.

E 108 THE STIMULATION OF CANINE NEUTROPHILS (PMN) BY rhIL-8 OR PHORBOL-12-MYRISTATE,13-ACETATE (PMA) FOLLOWING THE PROPHYLACTIC ADMINISTRATION OF rcG-CSF BEFORE AND AFTER THE INDUCTION OF GRAM NEGATIVE SEPSIS (GNS). Ann M. Farese, Eloise McLaughlin, Peter Eichacker, Charles Natanson and Thomas J. MacVittie. EXH, AFRRI and CCMD-NIH, Bethesda, MD.

GNS is a primary cause of morbidity and mortality after irradiation and/or trauma. Even though the PMN is the host's key cellular defense against invading pathogens, it may be detrimental to the host due to the release of toxic oxygen radicals. G-CSF stimulates the production of PMNs and primes them for increased functional activity. We compared the PMA- or IL-8-stimulated respiratory burst activity (RBA) of PMNs, following the prophylatic administration of rcG-CSF before and after the induction of GNS. Awake beagles were divided into 3 protocols. At 9 days (d) before and daily until 3d after bacterial clot placement (BCP) (*E. coli* 0111, 15 x 10^9 organisms/kg) groups (grp) of dogs received: 1. high dose (HD, n=17) rcG-CSF, 5 μg/kg 2. low dose (LD, n=17) rcG-CSF, 0.1 μg/kg and 4.9 μg/kg human serum albumin (HSA) or heat-inactivated canine serum (CS), or 3. HSA or CS (C, n=20) 5 μg/kg. Complete blood counts (CBC) and PMN RBA were evaluated. RBA was assessed flow cytometrically using the dye 2, 7, dichlorofluorescin, at 15 min post IL-8, PMA or 37°C stimulation. At d0, canines were treated with cephtriaxone (100 mg/kg, 1 x/d for 5d) and Ringer's lactate solution (60 ml/kg, iv) at 2, 6, 24, and 48h after BCP. **CBC.** Prophylaxis increased systemic PMNs to 800% ($p<.01$) and 160% (p=ns) of baseline (BL) in HD and LD respectively by d9, the time of BCP. The C grp remained at BL. BCP induced a marked depletion of systemic PMNs within hrs. C and LD PMNs decreased to 30-40% of BL but, the HD PMNs only decreased to 300% of BL. PMNs in all grps then increased through 48 hr post BCP (C, LD 240%, HD 825% of BL). **RBA.** PMA Prior to BCP, HD rcG-CSF administration depressed the PMN's PMA-stimulated RBA (46-71%). LD and C PMNs remained at BL values. After BCP, the PMA-stimulated RBA of HD PMNs remained depressed (46%). Depressed PMN PMA-stimulated RBA did not occur in LD and C grps until d2-4 post BCP (69-30% respectively). Following the cessation of cytokine therapy and the resolution of GNS, the PMNs from all grps exhibited an increased RBA. IL-8 Prior to BCP, HD rcG-CSF administration increased IL-8-stimulated RBA (206%) by PMNs. The PMNs from both the C and LD exhibited increased RBA to IL-8 stimulation (112, 141% respectively) at d3 of therapy but returned to BL values prior to BCP. After BCP, PMNs from all 3 grps produced an elevated IL-8-stimulated RBA. After the cessation of cytokine therapy and the resolution of GNS, there was a depressed IL-8-stimulated RBA in the PMNs from the HD (d10-21 BCP, 68-85%), and LD (d14-21 BCP, 67-75%), whereas, the C PMNs remained ≥ BL values. **Survival.** 9 of 20 C, 7 of 17 LD, and 2 of 17 HD rcG-CSF treated animals died. A trend toward increased survival was seen among the 3 grps (p=.052). Pretreatment with rcG-CSF was associated with improved survival in this canine model of GNS.

E 109 CHARACTERIZATION OF EARLY HEMATOPOIETIC CELLS IN ES DIFFERENTIATION, Meri T. Firpo, Sabine Tiefenthaler and Gordon Keller, Department of Basic Immunology, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

The capacity of murine embryonic stem (ES) cells to generate hematopoietic cells in culture provides a unique model system for studying the earliest stages of hematopoietic commitment. When cultured in methylcellulose, ES cells form colonies called embryoid bodies, which contain differentiated cells of many types, including hematopoietic cells. Committed erythroid progenitors can be detected in embryoid bodies between days 4 and 5 of differentiation. Myeloid progenitors can be detected by day 7. Cells with lymphoid characteristics can be found after day 14. The existence of erythroid restricted progenitors as early as day 4 in culture suggests that a more primitive multipotent hematopoietic progenitor is present in earlier embryoid bodies.

We used monoclonal antibodies ECMA-7 and Fall-3 as well as monoclonal antibodies specific for CD44, CD45 and heat stable antigen (HSA) to detect cell surface markers identifying hematopoietic progenitors in ES cultures. Using FACS analysis, we have determined that the ECMA-7 determinant, a marker of undifferentiated ES cells is expressed on most undifferentiated ES cells, but is gradually lost over six days in culture. Preliminary experiments indicate that HSA and the Fall-3 determinant, markers of early hematopoietic progenitors, are not expressed on undifferentiated ES cells. However, cells expressing these markers can be detected in embryoid bodies by day 3, supporting the idea that primitive hematopoietic cells are present at this stage of differentiation. CD44 and CD45, markers of hematopoietic progenitors and lymphoid cells are not expressed until 10 to 11 days of differentiation.

Current experiments are aimed at isolating these early hematopoietic cells from embryoid bodies and defining their potential *in vitro* and *in vivo*.

E 110 OXIDATIVE ACTIVITY OF REGENERATING BONE MARROW, SPLENIC, AND PERIPHERAL BLOOD MYELOID CELLS IN IRRADIATED MICE TREATED WITH GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF). **Roxanne Fischer and Myra L. Patchen, Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5603.**

G-CSF has been shown to be an effective therapy for the treatment of radiation-induced hemopoietic aplasia via its ability to enhance granulocyte progenitor cell proliferation and differentiation, hence, accelerating the production of peripheral blood neutrophils. In this study, the functional activity of myeloid cells generated in G-CSF-treated irradiated mice was assayed based on myeloid cell oxidative burst activity. Female B6D2F1 mice were exposed to a high-sublethal 7.75 Gy dose of cobalt-60 radiation administered at 0.4 Gy/min. Saline or G-CSF (rhG-CSF, 100μg/kg, s.c.; Amgen) administration was initiated on day one postirradiation and continued daily for 17 days. On days 14, 17, and 22 postirradiation, the oxidative burst activity of bone marrow (BM), splenic (SPL), and peripheral blood (PB) myeloid cells was assayed in unprimed cells and in cells primed with C5a, using opsonized zymosan as a stimulus. Results were compared to responses obtained from both saline-treated irradiated mice and from unirradiated (normal control) mice. At day 14 the oxidative responses of unprimed BM and SPL cells from G-CSF-treated mice were 678% and 450% of the responses in saline-treated mice and 122% and 129% of the responses in normal mice. Oxidative responses in primed BM and SPL cells at this same time were 381% and 243% of the responses in saline-treated mice and 188% and 155% of the responses in normal mice. Significant elevations in the oxidative activity of BM and SPL cells obtained from G-CSF-treated mice continued to be observed through day 22 postirradiation. At day 14 postirradiation, PB cells from saline-treated mice exhibited no significant oxidative activity, while the oxidative activities of both unprimed and primed cells from G-CSF-treated mice exhibited greater than normal activities (483% and 220%, respectively); these cells continued to exhibit greater than normal oxidative activities through day 22 postirradiation (153% and 113%, respectively). These studies illustrate that BM, SPL, and PB myeloid cells produced following G-CSF therapy of radiation-induced hemopoietic aplasia exhibit not only greater oxidative activity than cells obtained from irradiated saline-treated mice, but also greater oxidative activity than cells obtained from normal (nonirradiated) mice.

E 111 HIGHLY ENRICHED AND INFECTABLE HEMATOPOIETIC STEM CELLS FROM ADULT MOUSE BONE MARROW

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In order to find a population highly enriched for hematopoietic stem cells, we are fractionating adult mouse bone marrow on the basis of a variety of cell surface markers using both magnetic and flow cytometric cell sorting. Examining all possible fractions, and using competitive repopulation in a long term (12-16 weeks) transplantation assay, we have shown that all assayable stem cells are c-kit$^+$, sca$^+$, AA4$^-$, lin (CD4, CD8, CD5, Mac1, Gr1, B220)^{low}, CD45$^+$, CD43$^+$, M169$^+$ and Thy-1.2^{low}.

Furthermore in an attempt to separate stem cells which are able to be infected by retroviruses (i.e. cycling cells) from quiescent, un-infectable cells, we have used the vital cell-cycle dye Hoechst 33342. This has allowed us to identify a population which represents a small proportion of the sca$^+$ lin^{low} cells from normal bone marrow and appears to be highly enriched for hematopoietic stem cell activity from both normal and 5FU-treated mouse bone marrow.

We are currently investigating the extent of purity of this population, the true cell cycle status, and the infectability of these cells when purified from normal bone marrrow. We are also beginning to scale up these efforts in order to assay these cells for a variety of activities including spleen colony formation, *in vitro* colony formation, and their response to numerous growth factors.

E 112 RHIL7 INDUCES EXTRAMEDULLARY HEMATOPOIESIS IN MICE THROUGH THE EXPORTATION OF MYELOID PROGENITOR CELLS FROM THE BONE MARROW TO PERIPHERAL SITES. Grzegorzewski, K., Komschlies, K., Kaneda,● K., Faltynek,† C., Ruscetti,¶ F.W., Wiltrout,★ R.H., BCDP, PRI/*DynCorp*, ★LEI,¶LMI, BRMP, DCT, National Cancer Institute-FCRDC, Frederick, MD 21702-1201 and ‡Sterling Winthrop Pharmaceuticals Research Division, Malvern, PA 19355

RhIL7 has been shown to affect the growth and/or activity of B and T lymphocytes. Recently, our laboratory (Damia et al., *Blood* **79**: 1121, 1992) has shown that the administration of rhIL7 to mice causes a decrease of 60-80% in the total number of myeloid progenitors (CFU-c) in the bone marrow (BM) and a simultaneous 5- to 8-fold increase in the spleen. The data presented herein suggests that one possible mechanism for these apparently divergent effects of rhIL7 is the exportation of progenitor cells from the BM to peripheral organs. In mice treated daily with rhIL7 ip for 7 days, we observed an increase in the total number of leukocytes and CFU-c in the blood. Also, there was a 6- to 7-fold increase in nonparenchymal cells (NPC) and a 15- to 20-fold increase in the total number of myeloid progenitors obtained by enzymatic digestion of the liver. Similar results were obtained when cells were isolated by liver lavage. These data suggest an increase in leukocyte trafficking in response to rhIL7. To determine the magnitude of this phenomenon, mice were splenectomized and treated for 7 days with rhIL7 or diluent. Untreated splenectomized mice, express a compensatory increase in both; NPC and CFU-c. In splenectomized mice, that were treated with rhIL7, we observed a 3-fold increase in both the number of NPC and the total number of myeloid progenitors as compared to nonsplenectomized mice. Phenotypic analysis of NPC obtained from the livers of rhIL7-treated mice showed an 6- to 7-fold increase in the total number of myelomonocytic ($MAC\text{-}1^+$) and a 8- to 9-fold increase in granulocytic ($8C5^+$) cells, further confirming changes in myelopoiesis. The rhIL7-related decrease in myeloid progenitors in the BM was not affected by splenectomy. Thus, these data support the hypothesis that the *in vivo* administration of rhIL7 to mice stimulates the trafficing of myeloid progenitors from the BM and their subsequent localization in the spleen and liver.

E 113 HEMATOPOIETIC AND SYSTEMIC EFFECTS OF DYSREGULATED INTERLEUKIN-11 EXPRESSION. R.G. Hawley, A.Z.C. Fong, S.C. Clark,* B. Ngan and T.S. Hawley, Division of Cancer Research, Sunnybrook Health Science Centre, Toronto, Ontario M4N 3M5, Canada, and *Genetics Institute, Cambridge, MA 02140

Interleukin (IL)-11 was originally isolated based on its ability to stimulate proliferation of an IL-6-dependent mouse plasmacytoma cell line. Subsequent studies have demonstrated that IL-11 can functionally substitute for IL-6 in several contexts. In particular, like IL-6, IL-11 acts on early hematopoietic cells. We recently reported that lethally irradiated mice whose hematopoietic systems were reconstituted with bone marrow cells infected with an IL-6 retrovirus developed a myeloproliferative disease characterized by excessive neutrophil production (1). Despite chronic stimulation of neutrophil excess, IL-6-producing bone marrow progenitors demonstrated considerable proliferative longevity (the longest documented clone enduring for a total of 72 wk in multiple secondary, tertiary and quaternary recipients). To likewise examine the hematologic consequences of long-term exposure to IL-11, mice have been engrafted with bone marrow cells infected with an IL-11 retrovirus. Analyses of proviral integration patterns in hematopoietic tissues of secondary recipients three months after transplantation demonstrated productive infection of multipotential stem cells. Further serial transfers are planned to ascertain the life span of IL-11-producing stem cells.

With two notable exceptions, high circulating IL-11 levels resulted in minimal alterations in peripheral leukocyte counts. The exceptions, two secondary recipients of bone marrow from the same donor, both developed myeloid leukemia. Current investigation is directed toward establishing a causal role of IL-11 in the leukemogenic process. Otherwise, the predominant finding in the IL-11 mice was increased splenic megakaryocytopoiesis with concomitantly higher circulating platelet numbers (160% of controls). The most notable nonhematologic changes were loss of body fat and frequent hyperactivity. Curiously, several mice had grossly distended stomachs or bowels. These results highlight differences between IL-11 and IL-6 bioactivities but reveal functional similarities with other members of the IL-6 cytokine family (e.g., leukemia inhibitory factor). (1) R.G. Hawley *et al.* (1992). J. Exp. Med., in press.

E 114 IN VITRO STUDIES OF EARLY EMBRYONIC HEMATOPOIETIC STEM CELLS OF THE MOUSE: ISOLATION AND RESPONSE OF YOLK SAC CELLS TO STIMULATORY AND INHIBITORY GROWTH AND DIFFERENTIATION FACTORS, Hua Huang, Du Miao, Jane Bielich, and Robert Auerbach, Center for Developmental Biology, University of Wisconsin, Madison, WI 53706

The yolk sac is the first site of hematopoiesis in the mammalian embryo. However, little is known about the initial stem cells that give rise to the early nucleated erythrocytes, nor has it been determined whether stem cells of the yolk sac form a separate lineage with finite life span or whether, in fact, they include the cells that ultimately give rise to the various lineages of the hematopoietic system. In the latter case, hematopoietic yolk sac stem cells would migrate into organs such as the liver, thymus, spleen and bone marrow as these structures in turn become active sites of hematopoiesis during embryogenesis.

We have developed a procedure to isolate yolk sac hematopoietic stem cells using combinations of non-adherence to plastic, density gradient centrifugation, immunocytoadherence and cell sorting. Isolated non-adherent, density <1.077, AA4.1 positive and WGA bright cells give rise to multiple lineages including T cells, B cells and various myeloid cells in fetal thymus organ culture, on S17 stromal feeder layers or in methylcellulose, respectively. Stem cell frequencies as well as cell surface phenotypes for these stem cells have been determined.

The yolk sac hematopoietic stem cells can directly respond to IL-3 and stem cell factor (SCF), but not to leukemia inhibitory factor. They have high proliferating potential, forming large colonies consisting (>90%) primarily of undifferentiated blast cells when grown in presence of IL-3, GM-CSF and SCF. Stem cell inhibitory factor (SCI) has no effect on yolk sac stem cells, while TGFb inhibits 70-80 % of colonies that respond to combinations of IL-3 and GM-CSF. A high percentage of TGFβ resistant colonies give rise to secondary colonies.

Our experiments demonstrate that the mouse yolk sac contains the most primitive pluripotential hematopoietic stem cells identified to date, and that the regulatory mechanisms for these primitive stem cells are different from those found in fetal liver or bone marrow.

E 115 PHOTOFRIN (PII) STIMULATES MYELOPOIESIS IN NORMAL MICE AND MICE TREATED WITH 5-FLUOROURACIL (5-FU) OR τ-RADIATION, David W.C Hunt, Robert A. Sorrenti* and Julia G. Levy, Department of Micobiology, University of British Columbia, and *Quadra Logic Technologies, Vancouver, B.C., Canada V5Z 4H5.

Certain porphyrin molecules possess tumour-localizing and strong light absorbance properties. These characteristics have been utilized to elicit dissolution of tumours through an oxygen-dependent mechanism when the tissue is exposed to visible light. Evidence from clinical trials and animal studies has indicated that the administration of porphyrin compounds may also influence the immuno-haematopoietic system. To follow up on these observations, we have administered PII (dihaematoporphyrin ether) and benzoporphyrin derivative (BPD) to normal male DBA/2 mice and monitored the haematpoietic response, in the absence of activating light. A single dose (10 or 25 mg/kg) of PII caused a 1.6X increase in relative spleen weight, spleen cell numbers and a 1.3X increase in circulating leukocytes within 7 days, compared to the solvent-injected controls. Significant increases in CFU-GM were noted in the bone marrow within 1-2 days and in the spleen from 3-7 days post-injection. In contrast, administration of BPD (10 mg/kg) did not alter any of these parameters. When mice were pre-treated with a myeloablative dose of 5-FU (150 mg/kg), PII accelerated the recovery of blood leukocyte concentration (1.4X) and spleen cell numbers (1.6X) relative to control mice 8 days later. When mice were given a sub-lethal dose of τ-radiation (400 Rads), PII enhanced recovery of blood leukocyte numbers (1.4X) and spleen cell numbers (2.4X) relative to the controls 15 days later. Haematological rescue following 5-FU and τ-irradiation was associated with large increases (2-6X) in CFU-GM levels in the bone marrow and the spleen. The cellular mechanisms by which PII stimulates myelopoiesis are not understood but this response to PII is not associated with significant changes in serum levels of IL-1α, IL-6 or GM-CSF or the activation of splenic T lymphocytes. In vitro assays demonstrated that PII is neither mitogenic for mouse spleen cells nor possesses intrinsic colony-stimulating activity. Thus, PII is a novel therapeutic agent which possesses both photosensitizing and haematopoietic-stimulating activity.

E 116 THROMBOPOIETIC ACTION OF NATURAL HUMAN INTERLEUKIN-6 IN RABBITS. Nobutaka Ida, Emi Yoshida, Yu-ichirou Satoh, Ken-ichi Yoshizawa, Kiyoshi Okano, Tasuku Okamoto and Masanobu Naruto, Basic Research Lab., Toray Ind. Inc., Tebiro, Kamakura 248, Japan.
Thrombopoietic action of interleukin-6 has been expected for the pharmaceuitical use against thrombocytopenia. We have examined the thrombopoietic activity of highly purified natural human IL-6(nHuIL-6) and found that rabbits were the most sensitive for the response. In both iv and sc route, single injection of nHuIL-6 (100 μg/kg) to New Zealand White rabbits caused 50% increase of platelet (PLT) after 4 days while the PLT increase was marginal in rats and mice by the same treatment. Furthermore, even for the first 24h-iv-drip infusion alone, 100 μg/kg nHuIL-6 resulted in 92% increase of PLT in rabbits after 6 days. Repeating injection (14 consecutive days) of nHuIL-6 at doses of 0.3-30 μg/kg/day exhibited the superior efficacy of sc route(130% increase)to the iv route(50% increase). The greatest PLT increase in rabbits was achieved by administration of nHuIL-6 using Alzet osmotic pump inplanted subcutaneously. The constant release of the cytokine from the pump (10 μg/kg/day, for7 days) permitted almost 300% increase of PLT number on day 8 and more than 50% increase still remained even on day 15. Collagen induced aggregating potential and electron microscopic examination of the PLT showed no significant difference in saline treated rabbits and in rabbits that received nHuIL-6 by the Alzet pump method. These results indicate that continuous low dose administration is the most beneficial modality for thrombocytopenia therapy by nHuIL-6 and rabbits are useful animal to examine the mechanism of megakaryocyte differentiation and PLT formation by nHuIL-6.

E 117 CNTF AND LIF ACT ON NEURONAL AND HEMATOPOIETIC CELLS VIA SHARED SIGNAL TRANSDUCING COMPONENTS GP130 AND LIFRβ. N. Y. Ip, S. Davis, T. Aldrich, T. G. Boulton, N. Stahl and G. D. Yancopoulos. Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591.
Recent analyses suggest that ciliary neurotrophic factor (CNTF) is a distant structural relative of a number of hematopoietic cytokines including interleukin-6 (IL-6), leukemia inhibitory factor (LIF) and oncostatin M. However, unlike its related cytokines that have a broad spectrum of activities, CNTF appears to have actions restricted to the nervous system. Utilizing neuronal cell lines that are responsive to CNTF as well as LIF, we have demonstrated that the tyrosine phosphorylations and gene activations induced by these two cytokines are indistinguishable and are very similar to signalling events which characterize LIF and IL-6 responses in hematopoietic cells. We further demonstrated that both CNTF and LIF utilized the IL-6 signal transducing receptor component gp130, as well as a protein first identified based on its ability to bind LIF (hereon LIFRβ). We now show that gp130 and LIFRβ together comprise a functional LIFRβ receptor complex, but that CNTF requires a third receptor component, CNTFRα, to convert this receptor complex into a functional CNTF receptor. In contrast to the widespread distributions of gp130 and LIFRβ which allow for broad actions of LIF, CNTFRα expression is mostly restricted to the nervous system thereby generally limiting the actions of CNTF to neurons. Our studies on the *in vivo* localization of CNTFRα reveal high levels of CNTFRα expression in neuronal precursors during embryonic development. We provide evidence that CNTF may act in concert with other neurotrophic factors to effect the growth and differentiation of neuronal precursors during neuropoiesis in a way analogous to that exhibited by the cytokines during hematopoiesis.

E 118 *EX VIVO* TREATMENT OF SWINE BONE MARROW WITH HUMAN CYTOKINES INCREASES PROGENITOR CELLS, J. D. Jackson, B. J. O'Kane-Murphy, S. R. Clausen and J. G. Sharp, Departments of Pathology and Microbiology and Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198
The period of absolute neutropenia following high dose therapy and transplantation is a critical time for the patient largely due to risk of infections. *In vivo* cytokine therapy can shorten post-transplant aplasia by about 7 days; however, this treatment accentuates the steepness of the recovery curve for neutrophils but does not decrease the initial period of total aplasia following high dose therapy. One possible approach to decreasing the initial period of aplasia is to manipulate the bone marrow using cytokines *ex vivo* prior to transplantation. In these studies we used a Yucatan miniature swine model to examine the *ex vivo* treatment of swine bone marrow cells with human recombinant cytokines in an attempt to increase the number of progenitor cells. Swine bone marrow cells were incubated for one hour with single or combinations of human recombinant cytokines. Following the incubation of the swine bone marrow cells with the cytokines, the cells were washed to remove any residual cytokine and the cells plated in an *in vitro* agar colony assay. The data show that an exposure of one hour to IL-3, GM-CSF, IL-3+GM-CSF, increased the number of progenitor cells. In addition, the second generation hybrid IL-3-GM-CSF molecule (PIXY321) also increased the number of progenitor cells. These data suggest that short-term incubation of swine bone marrow cells with recombinant human cytokines can enhance *in vitro* hematopoiesis by increasing the number of colony forming cells. Whether this translates into a shortening of aplasia *in vivo* remains to be defined. However, because swine bone marrow cells respond to human cytokines, the Yucatan miniature swine offers an excellent transplant model for examining the ability of *ex vivo* cytokine treated bone marrow to reduce the initial period of aplasia and also maintain a long-term durable graft.

E 119 TNF-α IS A POTENT INHIBITOR OF MURINE HPP-CFC STIMULATED BY SCF AND OTHER HEMATOPOIETIC GROWTH FACTORS. Frede W. Jacobsen[1], Erlend B. Smeland[2] and Sten E. W. Jacobsen[2]. [1]Laboratory of Clinical Pharmacology and [2]Department of Immunology, The Norwegian Radiumhospital, Montebello, 0310 Oslo, Norway.

Tumor necrosis factor α (TNF-α) has been demonstrated to mediate both stimulatory and inhibitory effects on hematopoietic progenitor cell growth. However, previous studies have not investigated the effects of TNF-α on the most immature hematopoietic progenitor cells. Therefore the effects of TNF-α were studied on murine high proliferative potential colony forming cells (HPP-CFCs), the most primitive progenitor cells measurable in vitro. As previously shown, HPP-CFCs enriched by 5-Fluorouracil treatment and harvested 2 days post-treatment, only formed HPP-colonies in response to multiple cytokines. The formation of these colonies was inhibited 50-70% by TNF-α, regardless of which cytokines were used to stimulate growth. In particular, and previously not shown, stem cell factor (SCF)-supported growth of HPP-CFC was inhibited >70% in response to TNF-α. These effects were observed with murine as well as human rTNF-α, suggesting that the inhibitory effects of TNF-α on hematopoietic progenitor cells are mediated, at least in part, through the type I TNF-α receptor. In conclusion, TNF-α inhibits the growth of immature murine hematopoietic progenitor cells regardless of the cytokines inducing their growth.

E 120 DIRECT EFFECTS OF SYNERGISTIC HEMATOPOIETIC GROWTH FACTORS ON MURINE BONE MARROW PROGENITOR CELLS. Sten E. W. Jacobsen, Erlend B. Smeland, Eli Lien, Francis W. Ruscetti, Maria Ortiz, and Jonathan R. Keller, Department of Immunology, The Norwegian Radium Hospital, Oslo, Norway, and Laboratory of Molecular Immunoregulation, BCDP-PRI/DYNCORP, FCRDC-NCI, Frederick, MD 21702.

A number of hematopoietic growth factors have been demonstrated to have potent synergistic effects on colony stimulating factor (CSF)-induced in vitro growth of hematopoietic progenitor cells, while they have no colony stimulating activity as single factors. However, little is known about the relative role of direct effects (on the progenitor cells), and indirect effects (through induction of other cytokines) of these synergistic hematopoietic growth factors (HGFs) on bone marrow progenitor cells. Therefore, the in vitro effects of some synergistic factors were studied on unfractionated murine bone marrow cells and highly enriched progenitor cells. While IL-1 synergized with IL-3 to stimulate colony formation of both unfractionated bone marrow cells and Lin- progenitors in soft agar, this effect was not observed when cells were plated individually. In addition, no synergistic effects of IL-1 was observed on IL-3-induced proliferation of the multi-potent Lin-Thy-1+ progenitors. In contrast, IL-1 was found to synergize with GM-CSF and CSF-1 also at the single cell level on all investigated progenitor cell populations, while no synergistic activity (direct or indirect) was observed on G-CSF-induced proliferation. In contrast to IL-1, the synergistic effects of IL-4, IL-6, IL-11, and Steel Factor (SF) on G-CSF-, CSF-1-, GM-CSF-, as well as IL-3-induced colony formation were all directly mediated. Thus, IL-1 exerts potent direct as well as indirect synergistic effects on committed and multi-potent hematopoietic progenitor cells, while the synergistic effects of IL-4, IL-6, IL-11, and SF are directly mediated.

E 121 MAST CELL GROWTH FACTOR PROMOTES THE SURVIVAL OF PROGENITOR CELLS IN THE ABSENCE OF CELL DIVISION. J.R. Keller, S.E.W. Jacobsen, M. Ortiz, J. Gooya, F.W. Ruscetti, BCDP-PRI/Dyncorp, LMI-BRMP, FCRDC-NCI, Frederick, MD 21702 and Department of Immunology, Norwegian Radium Hospital, Oslo, Norway.

MGF is a member of a group of synergistic factors (SFs) which stimulate the growth of primitive hematopoietic progenitor\stem cells in the presence of other hematopoietic growth factors (HGFs). While it has been shown that HGFs alone or in combination promote the survival of progenitor\stem cells through proliferation and differentiation, little is known how HGFs regulate stem cell survival. Since SFs alone have little or no effect on the growth of purified stem cells, we examined whether MGF in comparison with other early acting growth factors could promote the survival of stem cells in the absence of proliferation. Purified bone marrow cells were plated in soft agar in MGF at time 0, prior to the addition of the combination of MGF plus interleukin-3 (IL-3) . Greater than 95% of the progenitor cells that give rise to colonies in soft agar in response to the combination of MGF/IL-3 at the initiation of the culture survived for 24 and 48 hours with a gradual decline by 96 hrs. In comparison, other early acting factors including interleukin-6, granulocyte-colony stimulating factor, leukemia inhibitory factor and interleukin-1 showed no effect on survival even after 24 hrs and were comparable to medium controls. Progenitors responsive to MGF/IL-3 were recovered from liquid cultures grown in the presence of MGF plus nocodozale, a mitotic inhibitor, after 24 and 48 hrs comparable to cultures containing MGF alone . Thus, unique among the early acting factors tested, MGF can directly promote the survival of progenitor cells in the absence of cell division.

E 122 LEUKEMIC CELLS OBTAINED FROM PATIENTS WITH AML AND CML ENGRAFT AND PROLIFERATE IN SCID MICE IN RESPONSE TO CYTOKINES. T. Lapidot*, C. Sirard*, J. Vormoor*, A. Keating~, M. Minden#, T. Hoang† B. Patterson# and J. E. Dick*. Dept of Genetics, Hosp for Sick Children; and Dept of Molecular and Medical Genetics, Univ. of Toronto, Toronto, Ont Canada*; Div of Hematology, Toronto Hosp~; Dept of Medicine, Ontario Cancer Inst#; Clinical Research Inst of Montreal†.

The ability to transplant both normal and leukemic human hematopoietic cells into immune-deficient mice provides a powerful in vivo model to characterize the human hematopoietic system. Although in vivo models have been developed for some human leukemias, progress to establish a model of chronic and acute myeloid leukemia has been limited We report the growth of human CML and AML cells obtained from patients at diagnosis in SCID mice. Cells obtained from CML patients in chronic phase were transplanted into SCID mice using a similar protocol to the one we developed for normal human bone marrow. Human growth factors were administered on a regular basis to transplanted mice to stimulate the leukemic stem cells to proliferate in vivo and to engraft durably. CML cells proliferate in SCID mice over a two month period. Human CFU-GM, BFU-E and CFU-GEMM progenitors that express the bcr/abl oncogene were present in the marrow. This novel in vivo model for human CML greatly advances our capability to investigate the molecular and cellular mechanisms that underlie this stem cell leukemia. Cells from >20 patients, obtained at diagnosis or at relapse, with AML of the FAB subtypes M1 or M4, homed to the mouse bone marrow and proliferated extensively at this site in 3 to 4 weeks of growth factor stimulation. Although both FAB phenotypes disseminated to other organs, M4 cells showed more rapid and extensive dissemination to lungs, spleen, liver and kidney; consistent with the clinical disease in M4 patients. The growth of M4 cells resulted in morbidity and mortality of the animals by two weeks post transplant while M1 cells took >8 weeks. Some of the leukemic cells differentiated in vivo resulting in the appearance of abnormal eosinophils. Significant numbers of leukemic blast progenitors were detected in the bone marrow using in vitro assays suggesting that self-renewing leukemic blast cells durably engrafted the mouse marrow. Limiting dilution analysis revealed that as few as 10^5 M1 AML cells can engraft the mice. The development of an vivo assay for human leukemia blast cells provides a powerful approach to characterize molecular alterations involved in leukemia transformation and progression. Furthermore, this model provides a valuable new tool to evaluate, in vivo, new therapeutic strategies.

E 123 RECOMBINANT HUMAN INTERLEUKIN-11 (rhIL-11) STIMULATES PROGENITOR CELL PRODUCTION AND ACCELERATES PERIPHERAL PLATELET AND HEMATOCRIT RECOVERY IN MYELOSUPPRESSED MICE. Leonard JP, Neben TY, Quinto C and Goldman SJ. Genetics Institute, 87 Cambridge Park Drive, Cambridge, MA.

Interleukin-11 (IL-11) is a multifunctional hematopoietic cytokine which has been shown to stimulate megakaryocyte maturation and increase peripheral platelet number in vivo. We have studied the effects of rhIL-11 on peripheral platelet recovery following combined sublethal irradiation and Carboplatin treatment. Female C57BL/6 mice received 500 cGy total body irradiation from a ^{137}Cs source followed by a single i.p injection of the chemotherapeutic drug Carboplatin (1.2mg/mouse). rhIl-11 (250μg/Kg/day) was administered by subcutaneous injection B.I.D beginning either 1 day or 7 days after irradiation and continuing until day 20. Peripheral blood counts were compared to saline treated mice at various time points post irradiation. The combined modality regimen produced severe thrombocytopenia with the platelet nadir ($95x10^6$ cells/ml) occurring on day 12 after treatment and platelet counts remaining substantially reduced (<20% untreated control value) for a further 6-8 days. In the mice treated with rhIL-11 starting from day 1, the nadir in platelet counts was reached earlier (day 10) and was less severe ($246x10^6$ cells/ml) than the saline controls. rhIL-11 administration also reduced the duration and extent of the thrombocytopenia with platelet counts returning to normal by day 20 ($1013x10^6$ cells/ml) compared to day 25 ($912x10^6$ cells/ml) in the saline controls. Mice receiving rhIL-11 starting on day 7 after treatment had comparable platelet counts to the saline treated controls indicating that the ability of rhIL-11 to reduce thrombocytopenia is lost if administration is delayed for 7 days. A steady decline in the hematocrit was seen in both the saline treated and rhIL-11 day 7 treated mice, reaching a nadir on day 17 (17% and 18% respectively) and returning to near normal by days 28 and 25 respectively. Administration of rhIL-11 day 1, reduced the nadir in hematocrit (27%, day 14) and accelerated the recovery (37%, day 17 and 46% day 20). Changes in BFU-E in the rhIL-11 day 1 treated mice sacrificed on day 14 were consistent with the changes seen in hematocrit in these mice. The number of BFU-E in the bone marrow and spleen were significantly increased compared to both the saline treated controls (2.5 fold and 100 fold respectively) and rhIL-11 day 7 treated mice (6 fold and 16 fold respectively). There was also a modest increase in the number of BFU-E in the spleen in the rhIL-11 day 7 treated mice compared to the saline controls, which was reflected by the slightly accelerated hematocrit recovery in these mice. The results indicate that administration rhIL-11 commencing one day, but not 7 days after the combined modality regimen can reduce the extent and duration of thrombocytopenia and accelerate the hematocrit recovery. This latter effect appears to be mediated, at least in part, by the stimulation of erythroid progenitors in the bone marrow and spleen.

E 124 MYELOID PROGENITOR RESCUE BY STEM CELL FACTOR IN 4-HC TREATED MARROW, Jane L. Liesveld, Abigail W. Harbol, and Camille N. Abboud, Department of Medicine, University of Rochester School of Medicine, Rochester, NY 14642

Ficoll-Hypaque separated light density marrow cells, when treated with 4-hydroperoxycyclophosphamide (4-HC) have <1% of starting CFU-GM and fail to form adherent stromal layers when plated under Dexter-type long term culture conditions. When light-density marrow cells treated with 60 μg/ml 4-HC were plated over irradiated marrow stromal layers, the addition of stem cell factor (SCF), the ligand of the *c-kit* proto-oncogene resulted in detection of CFU-GM at one week vs. 4 weeks in control cultures. This effect of SCF could be prevented by simultaneous addition of SR-1, a neutralizing anti-*c-kit* monoclonal antibody, to the culture flasks at a dilution of 1:2,000. The presence of SCF during 4-HC treatment was not protective of pre-existing CFU-GMs, however. The earlier generation of CFU-GM from 4-HC treated marrow in the presence of SCF confirms its action at a pre-CFU level. The action of SCF at a pre-CFU level is also demonstrated by its ability to increase CFU-GM output from $CD34^+$ progenitor cells, an effect that is additive to that of a pre-existing stromal layer. The factors present in such stromal layers which enhance the effects of SCF on $CD34^+$ proliferation and CFU-generating ability remain incompletely defined. In the culture system utilized here, the addition of IL-11 did not result in more CFU-GM output than in the presence of SCF alone. The role of SCF in promotion of pre-CFU proliferation and maturation does not appear to be promotion of adhesion to stromal elements as normal $CD34^+$ progenitors, KG1a cells, and Mo7e cells demonstrate no adherence to CHO-derived SCF-coated tissue culture plates. Further experiments with blocking antibodies in LTMBC cultures are ongoing to delineate the roles of these early acting cytokines. The effect of SCF on 4-HC treated marrow suggests action at a pre-CFU level and might indicate potential for this cytokine to enhance CFU-GM recovery in purged marrow in *in vivo* transplantation settings or *in vitro* in culture systems which are stroma-based or which contain an appropriate combination of cytokine growth factors to substitute for stromal layer-derived trophic effects.

E 125 A1, A NOVEL HEMATOPOIETIC-SPECIFIC EARLY RESPONSE GENE, Elaine Y. Lin, Amos Orlofsky, and Michael Prystowsky, Dept. of Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

As part of an investigation of GM-CSF regulated gene expression, a novel murine gene, **A1**, was identified. Comparison of A1 nucleotide and deduced protein sequences to several data banks indicated no homology to any known sequence. Northern analysis demonstrated that expression of the A1 gene was limited to hematopoietic lineages, including T-helper lymphocytes, macrophages, and granulocytes. In bone marrow derived macrophages, A1 mRNA accumulation was rapidly and stably induced by GM-CSF. The elevation of A1 mRNA appeared within 4 hours of GM-CSF treatment and reached a plateau at 16 hours (>10-fold induction). Cycloheximide did not effect the induction of A1 mRNA accumulation by GM-CSF, suggesting that A1 is an early response gene of GM-CSF. A1 mRNA accumulation was also stably induced in bone marrow derived macrophages by IL-3 and IL-4. In addition, a strong but transient induction was produced by treatment with LPS. These results suggest that A1 is a hematopoietic-specific early response gene whose expression is regulated by a variety of cell differentiation and activation factors.

E 126 ALTERATIONS OF MAST CELL GROWTH FACTOR (MGF) METABOLISM IN HUMAN MASTOCYTOSIS. Jack Longley[a], Lynda Tyrrell[a], Dirk Anderson[b], Douglas Williams[b], and Ruth Halaban[a], Yale University[a], New Haven, CT; Immunex Corporation[b], Seattle, WA.

Lesions of cutaneous mastocytosis are characterized by infiltrates of dermal mast cells and may show hyperpigmentation because of increased epidermal melanin. MGF, the ligand for the c-Kit proto-oncogene product, stimulates the proliferation of mast cells and increases melanin production by melanocytes. To investigate the potential role of MGF in cutaneous mastocytosis, we used immuno-peroxidase techniques to identify MGF protein in skin sections and RNA-PCR to identify MGF-mRNA in skin from patients with cutaneous mastocytosis and from normal controls. Tissue staining of skin from normal individuals showed a pattern consistent with cell bound MGF expressed by keratinocytes and dermal fibroblasts. In contrast, in normal and lesional skin of mastocytosis patients, MGF was present in the intercellular spaces between keratinocytes and free in the dermis, consistent with the presence of the soluble form of this ligand. Messenger RNA containing exon 6, capable of producing soluble MGF, was detected in epidermis and dermis of patients and control subjects as well as in cultured keratinocytes and fibroblasts. Sequence analysis did not reveal abnormalities in patients' MGF mRNA. We conclude that there is abnormal production of a soluble as opposed to the cell-bound form of MGF in the skin of patients with cutaneous mastocytosis. This phenomenon is probably due to abnormal proteolytic processing since differences in the splicing or sequence MGF mRNA could not be detected between mastocytosis patients and normal controls. Soluble MGF may thus be the cause for the characteristic accumulation of mast cells and hyperpigmentation of skin in lesions of cutaneous mastocytosis. These findings suggest that some forms of mastocytosis represent reactive hyperplasia rather than mast cell neoplasia.

E 127 EX VIVO EXPANSION OF G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS WITH PIXY 321, J. Mason, M. Mullen, T. Shea, University of California San Diego Medical Center, Division of Hematology/Oncology, San Diego, CA 92103-8421

The duration of myelosuppression in patients receiving high dose chemotherapy with autologous progenitor cell rescue is inversely proportional to the number of reinfused progenitor cells. We have previously reported a greater than 2 fold ex vivo expansion of G-CSF mobilized peripheral blood progenitor cells during a 48 hour incubation with Il-3. Ongoing clinical trials utilizing G-CSF mobilized blood progenitor cells have enabled us to compare the ex vivo expansion mediated by Il-3 compared with PIXY 321, the Il-3/GM-CSF fusion protein (Immunex). Peripheral blood mononuclear cells were collected by phlebotomy from patients receiving G-CSF (5ug/kg/day sq) as priming prior to cytopheresis. Following density sedimentation (Ficoll) the mononuclear cells were incubated in short term liquid culture with RPMI 1640 + 10% autologous plasma with either Il-3 (5ng/ml), PIXY 321 (25u/ml) PIXY + stem cell factor (10ng/ml) or Il-3 + SCF. Control cultures were incubated in identical media lacking supplemental cytokine. Progenitor cell number was determined by semi-solid media culture with colonies of CFU-GM, BFU-E and CFU-GEMM scored at 14 days. Following Il-3 incubation CFU-GM showed a 2.49 fold increase from baseline, BFU-E showed a 2.30 fold increase and CFU-GEMM showed a 2.33 fold increase ($n=7$). Progenitor cells incubated with PIXY showed a 1.99 fold increase in CFU-GM, a 2.01 fold increase in BFU-E and a 4.27 fold increase in CFU-GEMM ($n=4$). The addition of stem cell factor to Il-3 and PIXY 321 failed to show evidence of synergy. There was no statistical difference between the groups but there was a trend for greater CFU-GEMM expansion in PIXY stimulated cells. Maximal expansion in all groups occurred at 48 hours, and cells incubated with no added cytokine showed a progressive decline from baseline. These results indicate that in a short term culture system PIXY 321 mediates at least an equivalent and possibly greater (CFU-GEMM) expansion compared with Il-3.

E 128 DIFFERENTIAL INDUCTION OF EITHER MYELOPOIESIS OR ERYTHROPOIESIS FROM PURIFIED CD34$^+$ CD45RA^{lo} CD71^{lo} HUMAN CORD BLOOD CELLS USING DIFFERENT CYTOKINE COMBINATIONS. **Hector Mayani, Wieslawa Dragowska & Peter M Lansdorp. Terry Fox Laboratory, BC Cancer Centre, Vancouver, Canada.**

Blood cell production is the result of proliferation and differentiation of hemopoietic stem/progenitor cells (HSC) and is regulated by a group of glycoproteins known as hemopoietic cytokines. Despite an increased understanding of the biochemistry and molecular biology of these cytokines, their roles in the biology of primitive HSC are not fully understood. In order to directly study the effects of various cytokines on purified HSC, we have isolated a population of primitive hemopoietic cells (CD34$^+$CD45RA^{lo}CD71^{lo}) from human umbilical cord blood and analyzed their patterns of proliferation and differentiation in serum-free cultures in the absence or in the presence of different cytokine combinations. When these cells were cultured in the absence of any cytokine, the total cell number after 8 - 10 days decreased to 20 [7-30]% (mean and range of 7 separate experiments) of the input levels. Addition of MGF and IL-6, resulted in a 16 [10-21]-fold increase in the total cell number, and a 3 [1-5]-fold increase in the number of CD34$^+$ cells. In the presence of MGF, IL-6, IL-3 and Epo, there was a 812 [448-1300]-fold increase in the total cell number and a 28 [6-32]-fold increase in CD34$^+$ cells. In the presence of MGF, IL-6, GM-CSF/IL-3 fusion protein, M-CSF and G-CSF, a 662 [284-1500]-fold increase in the total cell number was observed, and the number of CD34$^+$ cells increased 42 [20-48]-fold. When all the above cytokines were added together, the total cell number increased 1292 [648-2370]-fold, and CD34$^+$ cells increased 53 [14-123]-fold. Maximum numbers of erythroid progenitor (CD34$^+$CD45RA^{lo}CD71$^+$) and mature (CD34$^-$ CD45RA^{lo}CD71$^+$) cells were observed in the presence of MGF, IL-6, IL-3 and Epo. In contrast, maximum levels of myeloid progenitor (CD34$^+$CD45RA$^+$CD71$^+$) and mature cells (CD34$^-$CD45RA$^+$CD71$^+$) were found in cultures supplemented with MGF, IL-6, GM-CSF/IL-3 fusion protein, M-CSF and G-CSF. Simultaneous addition of all the above cytokines resulted in maximum levels of both erythroid and myeloid cells. Thus, our results indicate that a significant and differential expansion of erythroid or myeloid progenitor cells can be achieved *in vitro* by using different cytokine combinations. This observation suggests that either (i) some hemopoietic cytokines can act on very primitive HSC and influence their committment towards the erythroid or the myeloid lineages, or (ii) such cytokines act on already committed progenitor cells inducing their expansion. Current studies using clonal analysis of purified progenitors are aimed to discriminate between these two possibilities.

E 129 THE ROLE OF IL-3R EXPRESSION IN HEMATOPOIETIC CELL LINEAGE COMMITMENT DURING *IN VITRO* DEVELOPMENT OF EMBRYOID BODIES. Terrill McClanahan, Takahiko Hara, Atsushi Miyajima and Frank Lee. DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304.

To identify early steps of lineage commitment in the hematopoietic cell lineage during mouse embryogenesis, we have studied the *in vitro* development of blastocyst-derived embryonic stem cells into embryoid bodies. Events *in vitro* closely mimic the morphology of early egg-cylinder stage embryos, with formation of yolk sac-like cysts containing blood islands with nucleated erythrocytes and macrophages. We have identified reproducible patterns of gene expression for hematopoietic growth factor receptor genes which occur as *in vitro* development proceeds. Two of the earliest genes to undergo a dramatic induction are the AIC2A gene, encoding a β subunit unique to the high affinity receptor for IL-3, and the AIC2B gene, encoding the common β subunit of the high affinity receptors for IL-3, GM-CSF and IL-5. The induction of these two genes early during *in vitro* development suggests that they are early events in hematopoietic lineage commitment. Using a specific monoclonal antibody, we have demonstrated that the AIC2A molecule is detectable on developing embryoid body cells starting at day 9 of development. We have also shown that cells of day 9 embryoid bodies respond to IL-3, leading to the induction of c-*fos* mRNA. Stable transfectants of ES cells over-expressing high affinity IL-3R bind IL-3 and transduce biochemical signals including phosphorylation of MAP kinase. Although induction of the β subunits is an early event of hematopoietic development, functional expression of IL-3R alone appears to be insufficient for lineage commitment, since ES cells which over-express IL-3R do not form solely hematopoietic cells in the presence of IL-3. The relevance of IL-3R expression in developing embryoid bodies will be discussed.

E 130 INHIBITION OF NORMAL HEMATOPOIESIS AND GROWTH FACTOR-DEPENDENT CELL LINES BY C-RAF ANTISENSE OLIGONUCLEOTIDES. K.W. Muszynski, U.R. Rapp, F.W. Ruscetti and J.R. Keller, BCDP-PRI/Dyncorp, LMI-BRMP, LVC, FCRDC-NCI, Frederick, MD. 21701.

A number of studies have shown that the c-raf gene may be required for the proliferation and differentiation of hemopoietic cells. In particular, in vitro immune complex kinase assays have shown that a variety of hemopoietic growth factors, including IL-2, IL-3, GM-CSF, CSF-1 and EPO activate Raf-1 kinase activity. In addition, studies using antisense oligonucleotides to block c-raf gene expression have established a requirement for Raf-1 kinase activity in IL-3 and EPO induced proliferation of FDCP-1 cells. To further characterize the role of c-raf gene expression in hemopoietic cell growth we examined the effects of antisense raf oligonucleotides on 1) colony stimulating factor (CSF)-induced colony formation of normal bone marrow cells in vitro and 2) the proliferation of several different growth-factor dependent cell lines in response to hemopoietic growth factors. C-raf antisense oligonucleotide treatment of murine bone marrow cells inhibited IL-3, CSF-1 and GM-CSF-induced colony formation by 70% to 98% in comparison to cells grown in the presence of sense oligonucleotides or growth factor alone. In addition, c-raf antisense completely inhibits factor-induced proliferation of cell lines in response to IL-4, CSF-1, G-CSF, and SCF. Thus, c-raf expression is required for the CSF-induced proliferation of normal hemopoietic cells and is required for the proliferation of progenitor cell lines stimulated with several cytokines.

E 131 COMPARISON OF c-kit LIGAND AND PRO-INFLAMMATORY CYTOKINES IN RADIOPROTECTION OF MICE. Ruth Neta, James E. Liedman, James Mitchell and Douglas Williams. AFRRI, NCI, Bethesda, MD. and IMMUNEX, Seattle, WA.

Our previous work has established that interaction of IL-1, TNF and IL-6 is necessary for innate as well as LPS-, IL-1-, and TNF-induced radioprotection (J.Exp.Med. 173, 1177, 1991 and 175, 689, 1992) We now show that in IL-1- and LPS-induced radioprotection, participation of c-kit ligand is also required. This is based on the finding that anti-c-kit ligand antibodies abrogate radioprotection by both, IL-1 and LPS . Administration of c-kit ligand by itself enhances survival of lethally irradiated mice. However, this radioprotection was not diminished with doses of anti-IL-1, anti-TNF or anti-IL-6 antibody which blocked IL-1- or LPS-induced radioprotection. Comparison of the in vivo effects of IL-1 and c-kit ligand showed several differences. IL-1 induces an increase in circulating IL-6 and CSF's, which participate in radioprotection. In contrast, the radioprotective regimen of c-kit ligand does not induce the presence of CSF's in the sera of mice. Conversely, treatment with anti c-kit ligand antibodies of IL-1- or LPS-treated mice did not reduce the titers of serum CSF, thus suggesting that an increase in these hematopoietic growth factors may not be required for c-kit ligand radioprotection. c-kit ligand unlike IL-1, IL-6, LPS or TNF did not induce an increase in acute phase proteins, again indicating that radioprotection with c-kit ligand and pro-inflammatory cytokines operate via different mechanisms.

E 132 IFNγ-IFNγR MEDIATED SIGNALS CONTROL GROWTH AND APOPTOSIS OF HUMAN LEUKEMIC T CELLS, *Francesco Novelli°, Francesco Di Pierro°, Paola Francia di Celle°, Gianni Garotta^, and Guido Forni°, °Immunogenetics and Histocompatibility Center, CNR, 10126 Turin , Italy and ^Hoffmann-La Roche Ltd., Basel, Switzerland.*

The consequences of the interaction between IFNγ and IFNγR were investigated on 3 human T cell leukemia lines maintained in different culture conditions. Flow-cytometric analysis of T cell lines cultured in the presence of fetal calf serum shows that 10-20% of the cells express IFNγR (fluorescence mean from 10^1 to 10^2) as detected by a mAb to human IFNγR (γR99). The addition to the culture medium of 1000 U/ml of IFNγ increases their proliferation and down-modulates IFNγR expression. On the contrary, when T cell lines are cultured for 48 h in serum-free medium, the expression of IFNγR dramatically increases (80-90% of the cells are stained, fluorescence mean from 10^2 to 10^3). Chromatin from T cell lines overexpressing IFNγR was cleaved into fragments that are multiple of 200 bp, producing a characteristic "ladder" pattern on DNA elettrophoresis that is known to be the hallmark of apoptosis. Morever, the addition of IFNγ to 48h FCS-deprivated cultures increases the apoptosis of the T cell lines and further up-modulates IFNγR expression on their surface (fluorescence mean from 10^3 to 10^4). Take as whole, these data indicate that IFNγ-IFNγR interaction has dichotomic effect on T-cell lines depending on their culture condition. They further suggest that IFNγR is a marker of apoptosis.

E 133 **HEMOPOIETIC EFFECTS OF STEM CELL FACTOR (SCF) AND GRANULOCYTE COLONY-STIMULATING FACTOR, ALONE AND IN COMBINATION, IN APLASTIC IRRADIATED MICE.** Myra L. Patchen, Roxanne Fischer, and Thomas J. MacVittie, Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5603.

In vitro, SCF (c-kit ligand) has been shown to synergistically enhance the proliferation of various progenitor cells when used in combination with specific CSFs; in vivo effects of SCF-containing cytokine combinations, however, have not been extensively evaluated. In vivo, G-CSF has been shown to significantly enhance myeloid regeneration following myelosuppressive chemotherapy or radiation exposure. Based on this, we evaluated whether simultaneously administered SCF and G-CSF could enhance G-CSF-induced hemopoietic regeneration in irradiated mice. Female B6D2F1 mice were exposed to an aplasia-inducing high-sublethal dose of cobalt-60 radiation (7.75 Gy @ 0.4 Gy/min). Daily, on days 1-16 postirradiation, mice were subcutaneously administered either rrSCF (100 μg/kg; Amgen), rhG-CSF (100 μg/kg; Amgen), or both cytokines. Bone marrow (BM) and splenic (SPL) CFU-s and GM-CFC recoveries were analyzed on days 14 and 17 postirradiation. As expected, G-CSF therapy progressively enhanced CFU-s and GM-CFC regeneration. Already by day 14, CFU-s recoveries in BM and SPL of G-CSF-treated mice were 153% and 795% of those in saline-treated mice and GM-CFC recoveries were 199% and 1097% of those in saline-treated mice. Surprisingly, SCF therapy alone delayed hemopoietic regeneration, especially bone marrow regeneration. On day 14, CFU-s recoveries in BM and SPL of SCF-treated mice were only 19% and 88% of those in saline-treated mice and GM-CFC recoveries only 12% and 95% of those in saline-treated mice. By day 17, SPL CFU-s and GM-CFC recoveries had surpassed those in saline-treated mice (157% and 241%), however, BM recoveries remained less than in saline-treated mice. Furthermore, CFU-s and GM-CFC recoveries in mice administered both SCF and G-CSF were consistently less than those observed in mice treated with only G-CSF. These results illustrate, that following severe radiation-induced hemopoietic aplasia, (1) G-CSF enhances CFU-s and GM-CFC recovery, (2) SCF delays CFU-s and GM-CFC recovery, and (3) SCF used in combination with G-CSF dampens the G-CSF-induced recovery.

E 134 A CYTOKINE RESPONSIVE PRIMITIVE HEMATOPOIETIC STEM CELL IS ASSOCIATED WITH THE STROMAL LAYER OF MOUSE LONG-TERM BONE MARROW CULTURES, Jim A. Rogers and Joan W. Berman, Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Mycophenolic acid treatment of long-term bone marrow cultures depletes the cultures of all assayable or observable hematopoietic precursors. The residual stromal cells are functional and support hematopoiesis if new progenitors are supplied. However, when treated with tumor necrosis factor (20-200 Units/ml), the apparently pure stromal cultures undergo an intense burst of hematopoietic activity. The magnitude of this activity is such that after 4 days the cultures contain approximately $2x10^6$ hematopoietic cells, and, by one week, are indistinguishable from control long-term cultures which have not been treated with mycophenolic acid. Even assuming a doubling time of 8-12 hours, the speed with which the cultures are reconstituted indicates a hematopoietic stem cell is present in the MPA stromal layers at a relatively high frequency (approximately 1,000-10,000/culture). This equates to approximately 0.01-0.10% of normal whole bone marrow. Although the MPA resistant, stromally associated stem cells are capable of complete long-term culture reconstitution (complete hematopoiesis, including cobblestone areas and large numbers of cells in the non-adherent layer, maintained >3 months), they are unable to form spleen colonies or myeloid colonies in semi-solid medium, indicating that they are a population of very primitive stem cells. This stem cell is somewhat responsive to other cytokines. CSF-1, IL-1 and IL-3 also induce hematopoiesis from the MPA stromal layers, but none do so with the speed and intensity of TNF. These data demonstrate that, in addition to the non-adherent, single-cell elements usually isolated for stem cell studies, the adherent population of bone marrow also contains hematopoietic stem cells with very primitive characteristics. We hypothesize that the proliferation and differentiation of this stem cell is accomplished by a regulated cascade of cytokine activities which can be initiated by TNF-alpha.

E 135 CHARACTERIZATION OF SEVERAL SEQUENCES FROM A FACTOR INDEPENDENT FDC-P1 HEMATOPOIETIC PROGENITOR CELL LINE ISOLATED BY SUBTRACTIVE HYBRIDIZATION. Elizabeth A. Romanik, Jean Leif and Joel S. Greenberger, Department of Radiation Oncology, University of Massachusetts Medical Center, Worcester, MA 01655.

The cocultivation of an FDC-P1 hematopoietic progenitor clonal cell line, FDC-P1JL26, with gamma irradiated D2XRII stromal cells results in the evolution of factor independent cell lines (Greenberger et al. Exp. Hematol. 20:92-102, 1992). One subclonal cell line FDC-P1 cl 2 demonstrated a phenotype of growth factor independent proliferation, rapid tumorigenicity and increased radioresistance. We have used subtractive hybridization technology to examine the genetic differences between the parent FDC-P1JL26 and factor independent FDC-P1 cl 2 (FI) cell lines. Sequences from the parent cell line were subtracted from the FI cell line. After two rounds of subtraction, the remaining FI sequences (10% of the original) were used as a probe library for screening a cDNA library constructed from the FI lines. Currently, we have isolated 5 clones ranging in size from 0.6 - 3.6 kb. The results from initial sequencing analyses show that two of the clones appear to have homology to RNA binding proteins, another has homology to a B2 repetitive element and one has homology to sequences found in serine proteinases. We have been unable to match the last cDNA to any sequences deposited in the GenBank/EMBL database. We are pursuing the analysis of the clones and believe that this study will describe sequences critical for understanding the proteins involved in and the mechanisms of uncontrolled hematopoietic cell proliferation.

E 136 FUNCTIONAL DIFFERENCES BETWEEN DR- AND CD38- SUBFRACTIONS OF CD34+ HUMAN HEMATOPOIETIC PROGENITOR CELLS, Leiv Rusten, Sten Eirik W. Jacobsen, Steinar Funderud and Erlend B. Smeland. Department of Immunology, The Norwegian Radium Hospital, Montebello N-0310, Oslo, Norway.

Both DR- and CD38- subfractions of human CD34+ bone marrow cells are highly enriched for immature progenitor cells. The present studies compared the ability of CD34+CD38- and CD34+DR- progenitors to form colonies in vitro. Both populations were isolated using a combination of positive and negative selection with monoclonal antibodies and immunomagnetic beads, or by cell sorting. The average recovery was 4.6% and 3.0% of total CD34+ cells for CD38- and DR- cells respectively. In agreement with previous studies, both the CD34+CD38- and CD34+DR- subfractions contained fewer in vitro colony forming cells (CFC) than the unfractionated CD34+ population. Furthermore, the difference in colony forming ability was more pronounced with optimal concentrations of single hematopoietic growth factors (IL-3 or G-CSF), than when stimulated with an optimal combination of cytokines (G-CSF, SCF and PIXY 321), supporting the conclusion that both CD34+DR- and CD34+CD38- cells contain relatively more primitive progenitors than the unfractionated CD34+ cells. Significant differences were also observed between these two subpopulations, in that the CD34+CD38- progenitors contained less than 60% of the CFC obtained by CD34+DR- progenitors, regardless of whether the cells were stimulated by single or multiple cytokines. In particular, the CD38- cells contained only 17% of the BFU-E's contained in the DR- population. Thus, while both the CD34+CD38- and the CD34+DR- subpopulations are enriched for primitive progenitors, they differ in their in vitro erythroid and myeloid colony forming ability.

E 137 MULTIPLE MECHANISMS UNDERLYING SYNERGY BETWEEN IL-3 AND SCF, Connie S. Tettenborn, and Jeffrey N. Siegel, Naval Med. Res. Inst., ICBP, Bethesda MD 20889-5055

IL-3 and SCF act synergistically on purified bone marrow progenitor cells to promote colony formation, and on cell lines *in vitro* to promote proliferation. We studied this synergy by comparing the effects of IL-3 and SCF alone or in combination on M07e cells, a human megakaryoblastic IL-3 dependent cell line. After a period of growth factor deprivation, cells exposed to SCF did not increase their cell number over a 5 day period of observation. Substituting IL-3 for SCF did result in increased cell numbers, and treatment with both SCF and IL-3 had a synergistic effect on cell number. Surprisingly, although SCF-treated cells did not increase in cell number, several indicators of cell cycle progression were elevated to a comparable degree by SCF as by IL-3, including the proportion of cells passing from G_1 into $S+G_2+M$, the level of ^{3}H-TdR uptake, and the expression of immediate early genes as assessed by reverse transcriptase-PCR. In contrast, ^{3}H-TdR uptake was synergistically increased with SCF and IL-3 together. We demonstrated using morphologic and flow cytometric studies a consistent excess in apoptotic cell death with SCF compared to IL-3. The excess cell death was not due to variable expression of the SCF receptor (c-kit) as these cells were uniformly positive (>90%). Interestingly, IL-3 upregulated SCF receptor expression. These data suggest that several mechanisms may together account for the synergy between IL-3 and SCF: (1) IL-3 action may complement an inherently diminished ability of SCF to prevent apoptosis and (2) IL-3 action may potentiate SCF effects on cell division by upregulating expression of its cell surface receptor.

E 138 LIPIDS DEVELOP DURING THE ROUTINE STORAGE OF BLOOD THAT PRIME NEUTROPHILS THROUGH THE PLATELET ACTIVATING FACTOR (PAF) RECEPTOR AND ARE STRUCTURALLY DISTINCT FROM PAF. C. Silliman, C. Johnson, K. Clay, G. Thurman, D. Ambruso. Dept of Ped, Univ of CO Sch of Med and Natl Jewish Hospital, and Bonfils Memorial Blood Center, Denver, CO.

Lipids developed during the routine storage of whole blood (WB) and packed red blood cells (PRBCs) that significantly primed the PMN NADPH-oxidase. This priming activity became maximal at product outdate. Fresh or stored plasma did not prime the oxidase. Significant PAF immunoreactivity by radioimmunoassay was documented in both PRBCs and WB after two and four weeks of storage, respectively, and at outdate was 10 ng/ml for PRBCs and 2 ng/ml for WB. Levels of the PAF acetylhydrolase enzyme in these components was 80-120% of normal plasma levels. Gas chromatography/mass spectroscopy showed less than 0.1 ng/ml of PAF in all PRBC or WB samples. The lipids were separated by HPLC, resolubilized in albumin and the priming capacity assayed. Priming was not found at the retention time of PAF or phosphatidylcholine (PC) but was found at the retention time of phosphatidylethanolamine in WB and following the elution of PC in both PRBCs and WB. Priming was also present prior to the retention time of PE in PRBCs. Priming was inhibited by WEB 2170, implying that the process required the PAF receptor. Extraction and HPLC separation of fresh plasma demonstrated no priming activity. We conclude: 1) lipids are produced during storage of cellular, blood components that prime PMNs through the PAF receptor, 2) these lipids have PAF immunoreactivity, 3) they are structurally distinct from PAF, and 4) they are produced in the presence of PAF acetylhydrolase suggesting an insensitivity to this enzyme. Such priming agents may play a role in severe transfusion reactions such as acute lung injury.

E 139 TGF-B1 HAS PLEIOTROPHIC EFFECTS ON HEMATOPOIETIC LONG TERM REPOPULATING CELLS INCLUDING ANTI-DIFFERENTIATION .Ewa Sitnicka, Norman S. Wolf, Gregory V. Priestley, Stephen H. Bartelmez Department of Pathology, U.W, Seatte WA 98195

Primitive hematopoietic cells have previously been shown to be markedly growth inhibited in the presence of TGF-B1, however, the effect on the differentiative state of these cells has not been reported. In this study we used single and multiple cell cultures of highly enriched, murine long term repopulating cells, 20 cells of which will completely tri-lineage repopulate an irradiated recipient. Eight of 10 cells from this primitive stem cell population were high proliferative potential colony forming cells (HPP-CFC). As these primitive cells differentiate, HPP-CFC numbers decline generating abundant CFU-GM. The highest cloning efficiency in single cell cultures occurred in the combined presence of c-kit ligand (KL), IL-6 and IL-3. Under these conditions, the primitive cells differentiated very rapidly, i.e.after 5 days of culture the number of HPP-CFC declined to 0 compared to 60 +/- 9 at day 0. Addition of increasing amounts of TFG-B1 to both single and multiple cell cultures in the presence of KL, IL-6 and IL-3 progresively inhibited the proliferation of these primitive cells. In cultures with no or very low levels of TGF-B1, no HPP-CFC could be detected after 8 days. Surprisingly, in cultures with 1-10 ng/ml TGF-B1 we observed an increased number of HPP-CFC. Addition of TGF-B1 to single cell cultures between day 0-3 resulted in profound growth inhibition, e.g. the number of small clones (2-8 cells) was increased in cultures with TGF-B1 (30%) compared to control (4%). Day 0 addition of TGF-B1 was highly effective at inhibiting the first cell division and the number of non-dividing cells was higher in these cultures (40%) than in cultures with no TGF-B1 (25%). When cells were exposed to TGF-B1 for varying periods then allowed to proliferate by neutralizing TGF-B1 with specific anti-TGF-B1 moAb, no alteration of growth kinetics was observed up to 8 days in culture. After that time their ability to proliferate was progressively, irreversibly inhibited.

E 140 EFFECTIVE MOBILIZATION OF BLOOD STEM CELLS FOR TRANSPLANTATION USING ERYTHROPOIETIN. D.M. Smith*, S. Clausen†, and J.G. Sharp†.
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High dose therapy for cancer requires post-therapy infusion of a source of hematopoietic stem cells in order to overcome the cytopenias induced by therapy. Blood stem cells, harvested by apheresis, are an effective alternative to bone marrow. This study employed young adult (25-35 kg) Yucatan miniature swine to evaluate the effectiveness of erythropoietin (EPO) (donated by Ortho Biotech) as an agent capable of mobilizing stem cells into blood for collection. In swine 4 non-mobilized apheresis gave hematopoietic recovery equivalent to bone marrow (2×10^8 cells/kg). Neutrophils recovered to 500 µl by days 16-17 and platelets to 20×10^3 µl by days 18-22. Four aphereses, mobilized using 500 units EPO/kg, provided recovery of neutrophils by days 13-14 and platelets by days 14-15. Furthermore, two unmobilized apheresis, a suboptimal dose, resulted in recovery of 500 neutrophils/µl by days 16-42 (mean 28) and platelets to 20×10^3/µl by days 31-41 (mean 33) while 2 apheresis, mobilized with EPO, resulted in recovery of 500 neutrophils/µl by days 11-13 (mean 13) and 20×10^3 platelets by days 14-16 (mean 15). Therefore, EPO mobilization accelerated hematopoietic recovery following transplantation of an optimal number of collections of blood stem cells and produced rapid recovery following transplantation of a very sub-optimal number of harvested cells.

E 141 MOLECULAR, PHENOTYPIC AND FUNCTIONAL ANALYSIS OF T-CELLS IN THE THYMUS BONE MARROW, SPLEEN AND BLOOD OF MICE RECEIVING SPLIT DOSE MULTI-DRUG CHEMOTHERAPY J. E. Talmadge, J. D. Jackson, and G. A. Perry Dept. of Path. Micro. and Cell Bio. and Anat., U. of Neb. Med. Ctr., Omaha, NE. 68198.
We examined the effect of a maximum tolerated, split dose chemotherapy protocol of cyclophosphamide, cisplatin, and BCNU on neutrophil (PMN) and lymphocyte sub populations in the blood, thymus, bone marrow (BM) and spleen. This chemotherapy protocol was modeled after that used with autologous BM transplantation (AuBMT) for breast cancer. We have obtained molecular, phenotypic and functional data on the reconstituting cells in chemotherapy treated mice with or without BMT and/or CSF-G treatment. These studies demonstrate a rapid reconstitution of PMN (by 15 to 18 days) with a slower lymphocyte reconstitution which varies by site (blood, spleen, BM and thymus) with the normalization of all phenotypes by day 60. In contrast to the recovery of the CD-3, CD-4 and CD-8 cells on both a percentage and absolute cell number basis, the ability of the splenic lymphocytes to respond to Con-A remained depressed. The lymphocytic dysfunction was not associated with any specific phenotype including $V\beta\text{-}5^+$; $V\beta\text{-}8^+$; γ/δ^+ or CD-4$^-$, CD-8$^-$ α/β^+ cells. Furthermore, no thymic or splenic natural suppressor (NS) cell activity was observed (BM NS activity was found but did not correlate with loss of splenic mitogenesis). These studies also revealed, on a time dependent basis, a marked thymic response to Con-A and helper cell activity. Balb/c mice have high levels of splenic IL-10 (RT-PCR) and we are examining the potential regulatory role of IL-10 and IL-4 in this model of immune dysfunction. Therapeutic studies in mice bearing a large metastatic tumor burden (mammary cancer) using this chemotherapy protocol have revealed that BMT and CSF-G prevent infectious disease associated mortality but provide no additional therapeutic efficancy. Supported in part by the Nebraska Cancer and Smoking Disease Research. Program.

E 142 THROMBOPOIETIC ACTIVITY OF RECOMBINANT HEMOPOIETIC FACTORS, Tsuyoshi Tange and Hiroshi Miyazaki, Department of Pathology, the University of Tokyo, Tokyo, and Pharmaceutical Laboratory, Kirin Brewery Co., Ltd., Maebashi, Japan
Although many recombinant hemopoietic factors have been developed, thrombopoietin alone has not yet been purified.
We performed the in vitro proplatelet formation (PPF) assay to examine the thrombopoietic activity of newly discovered recombinant human hemopoietic factors controlling megakaryopoiesis. These include erythropoietin(rEPo), interleukin 6(rIL-6), interleukin 11(rIL-11), leukemia inhibitory factor(rLIF), activin or erythroid differentiation factor(rEDF), stem cell factor(rSCF) and other recombinant colony stimulating factors.
For the PPF assay, the megakaryocytes were isolated from the rat bone marrow with the immunomagnetic beads method using anti-rat platelet GP IIb/IIIa monoclonal antibody(P55). About 5x 10 megakaryocytes were cultured in 24 well or 96 well plates containing IMDM with or without 2 to 10% FCS and factors. The coculture with stromal cells was also performed. The number of proplatelet clusters was counted daily on an inverted phase contrast microscope(Nikon). Proplatelets were yielded well by rIL-6(0.1-100 ng/ml) and rEPo(0.01-2U/ml) with a maximum in number on Day 2 to 3. Both factors were synergistic. IL-11, LIF, EDF and SCF alone seemed to be ineffective but could be effective sinergistically to rIL-6 or rEPo at an optimal dose. The coculture of megakaryocytes with stromal cells greatly enhanced PPF under rEPo or rIL-6. From these results, it is suggested that many cytokines and stromal cell contact might synergistaically stimulate thrombopoiesis.

E 143 **EFFECTS OF STEM CELL FACTOR ON HUMAN THYMOCYTES, Christel H. Uittenbogaart, Ingrid Schmid, Sylvia Kiertscher, Hungyi Shau, Deborah J. Anisman, Loran T. Clement and Krisztina M. Zsebo. UCLA School of Medicine, Los Angeles, CA 90024 and Amgen Inc., Thousand Oaks, CA 91320**
Recent isolation and cloning of the gene for human stem cell factor (SCF) as well as development of an antibody (SR-1) to the SCF receptor have enabled us to study the effect of recombinant SCF on the survival, maturation, and receptor expression of human thymocytes. SCF increased the viability and size of a subset of thymocytes in-vitro, but did not independently induce phenotypic changes on thymocytes indicative of T cell differentiation. The SCF-responsive thymocytes had characteristics of large granular lymphocytes that did not express either T or B cell antigens or the CD56 or CD16 antigens. In combination with IL-2, however, SCF induced the appearance of large granular cells that expressed the CD56 antigen. The synergistic action of SCF and IL-2 was also seen in the induction of cytotoxic effector cells from thymocyte precursors that were able to lyse both NK-sensitive and NK-resistant cell lines. Studies of SCF receptor expression using the SR-1 antibody showed that SCF receptors are expressed on thymocyte subsets which include mature "bright" $CD3^+$ cells, immature "dim" $CD3^+$ cells as well as $CD3^-$ cells. Cytokines such as IL-4, IL-7 and especially IL-2, increased the percentage of SCF receptor-positive cells in cultured thymocytes. Therefore, SCF appears to be a survival/growth factor for immature T cells and NK/LAK precursors and may be useful for T cell regeneration and improvement of NK/LAK activity in patients with cytopenias.

E 144 CONSTANT SUBCUTANEOUS INFUSION OF RECOMBINANT HUMAN INTERLEUKIN-11 INDUCES STEM CELL MOBILIZATION AND CAUSES PROLONGED ELEVATION OF PERIPHERAL PLATELETS, A. Witsell, P. Mauch, T.Y. Neben*, C. Quinto*, J.P. Leonard*, C. Lamont and S.J. Goldman*, Genetics Inststute, 87 Cambridge Park Drive, Cambridge and Harvard Medical School, Boston, MA

Previous studies have shown that subcutaneous (SQ) bolus administration of rhIL-11 stimulates megakaryocytopoiesis and increases peripheral platelet counts in naive mice. This study was designed to compare SQ bolus administration of rhIL-11 to constant SQ infusion by Alzet pump. In addition to monitoring peripheral hematology, the effect of rhIL-11 on the stem cell compartment was determined by quantitating CFU-S from bone marrow, spleen and peripheral blood. Female C57BL mice were implanted subcutaneously with an Alzet mini-osmotic pump containing either rhIL-11 with 0.5% homologous mouse serum (delivery rate of 250 ug/kg/day for 7 days) or vehicle alone. Continuous infusion of rhIL-11 resulted in a significant and sustained increase in spleen weight and cellularity. The number of CFU-S/spleen was elevated on days 3, 7 and 10 (33 and 25 fold increases on days 7 and 10 respectively). Increases in CFU-S in the peripheral blood were seen on days 3, 7 and 10 (15 and 6 fold respectively). Animals treated with rhIL-11 administered by SQ bolus injection also showed increased CFU-S in the spleen and peripheral blood, however, the magnitude of the increase was less than in animals receiving rhIL-11 by continuous SQ infusion. Peripheral blood counts were also measured. Administration of rhIL-11 by Alzet pump stimulated increases in peripheral platelet counts that were evident from day 3 and reached the maximum observed value on day 10 (240% of saline control). In the bone marrow, a shift to higher-ploidy megakaryocytes (increase in 32N) was seen three days after IL-11 pump implantation and was still present on day 10. In mice receiving rhIL-11 by SQ bolus injection, increased platelet counts and a shift to higher ploidy megakaryocytes was seen on days 3 and 7 but did not persist on day 10. Mice implanted with a vehicle pump showed a transient shift to higher ploidy on day 3 which was not observed on day 7. This shift in ploidy was not accompanied by increased platelet counts. These results indicate that constant infusion of rhIL-11 induces a profound mobilization of stem cells from the bone marrow to the periphery (spleen and blood), a shift to higher ploidy megakaryocytes and a prolonged increase in peripheral platelet counts. Although similar effects were seen following rhIL-11 administration by SQ bolus injection, in all cases the magnitude of the responses was reduced.

E 145 EXPRESSION OF STEM CELL FACTOR (c-*kit* LIGAND) IN A HUMAN BONE MARROW DISORDER, APLASTIC ANEMIA, A. Wodnar-Filipowicz, M. Slanicka & C. Nissen, Research Department, University Hospital Basel, Switzerland

Aplastic anemia (AA) is characterized by a failure of proliferation of hematopoietic stem cells. We have recently demonstrated that stem cell factor (SCF) improves the *in vitro* growth of AA bone marrow cells (BLOOD 79, 3196,1992). We have now measured concentration of SCF in serum of 32 patients with severe AA. The pre-treatment values varied between 0.33 and 6.1 ng/ml and no correlation between hematopoietic function and SCF serum levels was noted. Therapy with antilymphocyte globulin or bone marrow transplantation did not result in any recognizable pattern of changes in SCF serum levels. However, the concentration of SCF in serum of many AA patients was at a low range of levels observed in sera from healthy blood donors; in only 21 serum samples out of 128 tested prior and post therapy, a value of 3.3 ng/ml, the mean estimated for 167 control sera has been found. The results suggest, that defficiency of SCF may be one of the underlying causes of the disease. Therefore, we are currently analysing expression of SCF mRNAs encoding two SCF forms: soluble and membrane-bound, in stroma cultures established *in vitro* from the bone marrows of AA patients.

E 146 THE RELATIONSHIP BETWEEN BIOLOGICAL ACTIVITY, RECEPTOR BINDING, AND POLYETHYLENE GLYCOL MODIFICATION OF MACROPHAGE COLONY STIMULATING FACTOR, Ralph Yamamoto[1], Robert Bauer[1], Gavin Dollinger[4], Danute Nitecki[2], Ross Cox[3], Robert Milley[3], John Young[1] and Jolanda Schreurs[1]. Departments of Pharmacology[1], Analytical Chemistry[2], Purification Process Development[3], and Chemical Therapeutics[4], Chiron Corporation, Emeryville, CA 94608.

Abstract. The effects of modification of recombinant human macrophage colony stimulation factor (MCSF) by monomethoxy polyethylene glycol (PEG) were examined for changes in plasma pharmacokinetics, biological specific activity in vitro and receptor binding. The plasma pharmacokinetics of both M-CSF and PEG-MCSF were described by bi-exponential curves; the area under the curve for PEG-MCSF was about 7-fold greater than for unmodified M-CSF, signifying that PEG-MCSF has a substantially increased plasma half-life. As assessed by measurements in vitro, PEG modification of MCSF reduced the biological specific activity and increased the equilibrium dissociation constant and rate of association for the MCSF receptor. Both the number of modifying PEG moieties, as well as the modifier chain length, govern the absolute magnitude of the changes observed in the measured interactions between ligand and receptor. The results support a model whereby PEG attachment to MCSF sterically hinders the interaction of MCSF with the receptor, leading to a decrease in the rate of association, but not the maximum amount of binding. We suggest that the controlled modification of M-CSF with PEG at amino acids distant from the receptor binding site will lead to further improvements in both the in vitro properties and in vivo efficacy of M-CSF.

E 147 RADIOPROTECTION BY IL-1 RESULTS FROM PROTECTION OF HEMATOPOIETIC STEM CELLS? James Zucali, Yvonne Zhang, Jennifer Alderman, Wendy Gibbons, and Jan Moreb, Department of Medicine, University of Florida, Gainesville, FL. 32610

Administration of Interleukin-1 (IL-1) has been shown to protect mice from an irradiation insult. Whether this protection results from protecting hematopoietic stem cells or other organs such as gut or lung remains speculative. Using both an in vitro and in vivo approach, we investigated whether IL-1 has a direct protective effect on hematopoietic reconstituting cells. Bone marrow cells were collected from male B6D2F1 mice and were incubated for 20 hours in the presence and absence of 100 ng/ml IL-1. The cells were then irradiated at 8.0 Gy and injected intravenously into irradiated (9.5 Gy) female B6D2F1 mice. Survival was recorded and at two week intervals, DNA was extracted from bone marrow, spleen and thymus of the recipient female mice. The presence of donor male cells was determined using a specific Y chromosome probe and southern analysis. Transplantation with IL-1 treated cells results in increased survival (60%) of irradiated recipients when compared to mice receiving media treated cells (16%). Male cells could be detected in the spleens of animals receiving IL-1 or media treated cells at two weeks following transplantation. At 4 weeks post transplant, male cells could only be detected in the bone marrow, spleen and thymus of mice receiving IL-1 treated cells; whereas, no male cells were present in mice receiving media treated cells. These results indicate that IL-1 treatment protects short-term repopulating hematopoietic cells from irradiation. By 8 weeks post transplant, male cells could be detected in the bone marrow, spleen and thymus of mice receiving both IL-1 and media treated cells. However, by 12 weeks post transplant, male cells could only be detected in the tissues of mice receiving IL-1 treated cells. Serial transplantion studies were performed. Bone marrow cells were obtained from female recipient mice which had received both IL-1 and media treated male cells 8 weeks previously and were injected into lethally irradiated 2° female recipients. Survival results and the presence of primary donor male cells in the bone marrow, spleen and thymus of these secondary and tertiary recipients will be reported.

Cytokine Receptor Characterization

E 200 VACCINIA VIRUS ENCODES A SECRETED RECEPTOR FOR INTERLEUKIN-1 THAT MODULATES THE HOST RESPONSE TO INFECTION. Antonio Alcamí and Geoffrey L. Smith. Sir William Dunn School of Pathology. University of Oxford. Oxford OX1 3RE, U.K.

Vaccinia virus is the representative member of the poxvirus family of cytoplasmic DNA viruses. We have identified and characterized a soluble IL-1 receptor secreted from vaccinia virus-infected cells. The binding activity is encoded by B15R open reading frame, which shows homology to the extracellular domain of the IL-1 receptor. The vaccinia IL-1 receptor, in contrast with the cellular counterparts, binds IL-1β but does not bind IL-1α or the natural competitor IL-1 receptor antagonist and therefore constitutes a novel IL-1 receptor . The protein was secreted in an active form from insect cells when expressed in a baculovirus vector and competed the binding of IL-1β to the natural receptor on T cells. Deletion of B15R from vaccinia virus accelerated the onset of symptoms of illness in intranasally infected mice, suggesting that the blockade of IL-1β by vaccinia virus can diminish the systemic acute phase response to infection. The IL-1β binding activity has been found in other orthopoxviruses.

The protein encoded by vaccinia virus B18R, a structurally related protein with homology to the IL-1 receptor, has been identified in the supernatant of infected cells but no binding to IL-1 was detected.

E 201 ANALYSIS OF RESIDUES CONTRIBUTING TO HIGH AFFINITY BINDING AND BIOACTIVITY IN MURINE GM-CSF BY SUBSTITUTION MUTAGENESIS. Scott W. Altmann, Armen B. Shanafelt and Robert A. Kastelein, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a four alpha-helical bundle protein with distinct topology as determined by X-ray crystallography. Previous investigations have identified regions in human and murine GM-CSF that are important for bioactivity. Alanine scanning of the first alpha-helix in mGM-CSF has identified several residues involved in high affinity receptor binding and activation. In the present study, a panel of multiple amino acid substitutions were made at residues Arg11, Lys14, His15, Lys20 and Glu21. The impact of the mutations on bioactivity as well as receptor binding were measured as a means of characterizing the specific role each residue plays in mGM-CSF ligand-receptor recognition. Sensitivity to sidechain substitutions varied for each amino acid position suggesting the relative contribution the residue has to the function of the molecule. These results also indicate that the chemical nature as well as the bulkiness of the amino acid sidechains are critical to optimal protein-protein interactions. Structural similarity as well as conservation of several amino acid residues in the first alpha-helix among GM-CSF and a variety of other cytokines suggests that a common ligand-receptor recognition motif is utilized by this family of molecules.

E 202 IDENTIFICATION AND CHARACTERIZATION OF RECEPTORS FOR THE HEMATOPOIETIC STEM CELL INHIBITOR MACROPHAGE INFLAMMATORY PROTEIN - 1α. Belinda R. Avalos, Kevin Bartynski,Mark Kotur and Neil M. Wilkie, Divisions of Bone Marrow Transplantation and Hematology/Oncology, The Ohio State University, Columbus, OH. 43210.

Macrophage inflammatory protein 1-α (MIP-1α) is a pleiotropic cytokine originally characterized as a potent mediator of inflammation and more recently identified as an important inhibitor of stem cell proliferation. To further investigate the mechanism of action of MIP-1α on hematopoietic progenitor cells, binding of ^{125}I-labeled recombinant murine MIP-1α was examined on a variety of cell types. A single class of high affinity receptors was identified on the human acute myelogenous leukemia cell lines, HL-60 and THP-1, as well as on primary cells from a patient with stem cell leukemia (Kd=245-565 pM, 1220-5250 sites/cell). Affinity cross-linking and immunoprecipitation of ^{125}I-MIP-1α produced a similar profile of labeled bands on these cells. The most prominent bands competed by excess unlabeled MIP-1α were apparent at 100,000 and 60,000 daltons. Studies are currently in progress to further define the subunit structure and distribution of MIP-1α receptors in progenitor and mature hematopoietic cell compartments. These studies are critical to understanding the mechanism of action of cytokines with negative regulatory effects on stem cells which may prove to be clinically useful.

E 203 RANDOM MUTAGENESIS OF THE EXTRACELLULAR DOMAIN OF THE HUMAN ERYTHROPOIETIN RECEPTOR, Francis P. Barbone, Linda K. Jolliffe and Linda S. Mulcahy. The R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J. 08869

To further understand the interaction of human erythropoietin(EPO) with its receptor, the cloned cDNA of the extracellular domain of the human EPO receptor was subjected to random mutagenesis. This 225 amino acid region of the receptor has been expressed in *E.coli*, shown to bind EPO, and therefore has been termed the human EPO binding protein, or hEBP. Two methods were employed to generate random mutants of the hEBP. The first method targets conserved amino acid motifs by using an overlap PCR protocol and degenerate oligonucleotide primers spanning short(60-70bp) regions of the hEBP. The second mutagenesis method uses altered PCR conditions to favor incorrect base incorporation by *Taq* polymerase thus allowing for potential mutations at any position of the cloned gene. Libraries of hEBP mutants were cloned into a phagemid vector creating a fusion protein of the hEBP with the C-terminal half of the geneIII protein of bacteriophage M13. Bacterial colonies were tested for the ability to bind ^{125}I-EPO using a colony ligand assay, and were evaluated for expression of the hEBP fusion protein by western blotting. Specific mutations of individual clones were identified by automated DNA sequence analysis. Using the above methods, regions and individual amino acid residues have been identified which may be critical for ligand binding.

E 204 CLONING AND EXPRESSION OF MOUSE OX-40, THE HOMOLOGUE OF A RAT CELL SURFACE PROTEIN RESTRICTED TO $CD4^+$ T BLASTS

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The MRC OX-40 antigen is a 40 kD cell surface glycoprotein found only on activated $CD4^+$ T cells. Molecular cloning of the OX-40 cDNA in the rat showed that the extracellular domain of this protein is related to the nerve growth factor receptor, both receptors for TNF, the antigens recognized by anti-CD27 and anti-CD30 monclonal antibodies and to the B cell antigen CD40. In order to further study the function of this protein, the murine homologue was cloned using the polymerase chain reaction. Substrate cDNA was synthesized from ConA activated spleen and lymph node RNA. Oligonucleotides complementary to the 5' and 3' ends of the rat sequence were used to amplify the entire mouse coding region. Mouse OX-40 cDNA sequence is highly homologous to the the rat, with greater than 90% identity at the amino acid level. We have recently isolated a partial genomic clone for the mouse OX-40 gene and we will use this to map of the intron/exon borders of the coding sequence. In addition, we have generated transfected eukaryotic cell lines which express mouse OX-40 on the surface, and lines which secrete the extracellular domain of the protein. This recombinant protein will be used both as an immunogen for the generation of monoclonal antibodies, and as a probe for possible cell surface or soluble ligands of the OX-40 protein.

E 205 BIOCHEMICAL CHARACTERIZATION OF THE EPITHELIN RECEPTOR , Gary W. Carlton, Jean-Michel Culouscou and Mohammed Shoyab, Growth Regulators Department, Bristol-Myers/Squibb Pharmaceutical Research Institute, Seattle WA 98121

Members of the epithelin family of cysteine rich molecules were originally isolated from rat kidney. Identical proteins called granulins have been isolated from human leukocytes. DNA encoding the epithelin precursor is a 2152 bp domain that includes 7 highly conserved tandem regions encoding all known members of the epithelin family. Epithelins exhibit similarity in size (approx. 6 kDa) and cysteine content (20%). Epithelin 1 possesses growth modulatory activity on cells of epithelial origin that is antagonized by epithelin 2 . Here we report the characterization of a receptor for epithelins on MDA-MB-468 human breast carcinoma cells. Chemical cross-linking of ^{125}I-epithelin 1 to MDA-MB-468 cells revealed an approximately 140 kDa receptor molecule. Cross-linking was inhibited not only by unlabeled epithelin 1 but also by unlabeled epithelin 2 and epithelin 3. Saturation binding and Scatchard analysis demonstrated the existence of high affinity (K_{d1}=330 pM; 110 sites/cell) and low affinity (K_{d2}=62 nM; 32000 sites/cell) binding sites for epithelins at the surface of MDA-MB-468 cells. Epithelin 2 and epithelin 3 compete with ^{125}I-epithelin 1 for binding to MDA-MB-468 cells with equal efficiency. Taken together these data suggest that epithelins may utilize the same receptor. Competition of ^{125}I-epithelin 1 binding to MDA-MB- 468 cells with other known growth modulators and cytokines showed no binding inhibition. We have demonstrated the existence of a receptor for epithelins that is unique and not used by other known factors. It remains to be tested if the rest of the epithelin family members also utilize this receptor.

E 206 ANALYSIS OF THE EXPRESSION OF THE DIA/LIF RECEPTOR, Ian Chambers, Morag Robertson, Meng Li and Austin Smith, Centre for Genome Research, King's Buildings, University of Edinburgh, West Mains Rd., Edinburgh EH9 3JQ Scotland

The components of the DIA/LIF receptor complex present on ES cells have been analysed. To do this a fragment of the murine DIA/LIF receptor (DIA-R) cDNA corresponding to sequences encoding amino acids 196-515 (as described by Gearing et al. EMBO J. 10, 2839-2848) was cloned by reverse transcription-PCR of adult liver RNA. Northern blot analysis demonstrated that ES cells express DIA-R mRNAs of 10kb, 5kb and 3kb. Induction of differentiation of ES cell cultures with either retinoic acid or 3-methoxybenzamide, which cause differentiation into fibroblastic or epithelial cell types does not affect the steady state levels of these DIA-R mRNAs, indicating that a decrease in expression of DIA-R mRNAs is not required for differentiation to proceed. ES cells also express gp130. Analysis of RNA from a variety of tissues revealed the presence of DIA-R transcripts of approximate size 10kb, 5kb, 3kb and 2kb, with the levels of the transcripts varying independently in different tissues. Thus lung expresses higher levels of the 10kb transcript than any other tissues tested, while the highest levels of the 3kb transcript are found in liver. Interestingly, liver lacks both the 5kb and the 10kb trasncripts, suggesting that the presence of the protein encoded by the 3kb transcript is sufficient for signal transduction in liver cells. The structure of the various transcripts and their coding potential is being assessed by analysis of cDNAs isolated from an ES cell cDNA library. Antibodies being raised against portions of DIA-R will be used to examine expression of DIA-R proteins in ES cells.

E 207 INITIAL CHARACTERIZATION OF THE IL-12 RECEPTOR (IL-12R) ON CONCANAVALIN-A ACTIVATED MOUSE SPLENOCYTES, R. Chizzonite, T. Truitt, M. Griffin, E. Nickbarg*, B. Hubbard*, B. Desai, A. Stern, U. Gubler and M. Gately, Roche Research Center, Hoffmann-LaRoche Inc., Nutley, N.J. 07110 and *Genetics Institute, Inc., Cambridge, MA 02140.

Mouse IL-12 (msIL-12) is a ~75 kDa heterodimeric cytokine that stimulates proliferation of both Con-A activated mouse splenocytes (Con-A splenocytes) and PHA-activated human lymphoblasts (PHA-PBL). The binding of [125]-I-labelled msIL-12 (20-25 uCi/ug) to Con-A splenocytes was saturable and specific. At the time of peak IL-12R expression (3 days post Con-A activation), Scatchard analysis of the binding data identified a single binding site with an apparent dissociation constant of ~20-70 pM and 700-1000 sites per activated splenocyte. The kinetics of [125]-I-msIL-12 binding to Con-A splenocytes was very rapid at both 4°C and 22°C; reaching equilbrium within 15 min. The rate of dissociation of [125]-I-msIL-12 was slow, with a T1/2 of 6 hrs at 22°C. Neither resting mouse splenocytes, P388D1 macrophage cells, EL-4 thymoma cells, SP2/0 myeloma cells nor 70Z/3 pre-B cells express detectable IL-12R. Affinity cross-linking of [125]-I-msIL-12 to Con-A splenocytes produces a major complex of ~210-250 kDa. The binding of [125]-I-msIL-12 to the human IL-12R on PHA-PBL occurs with the same affinity and kinetics as binding to the msIL-12R. These data demonstrate that the msIL-12R, like the huIL-12R, may be composed of a single high affinity binding subunit of ~140-180 kDa that is usually maximally expressed at 2-4 days after T-cell activation.

E 208 IDENTIFICATION OF A VARIANT FORM OF THE INTERLEUKIN 6 RECEPTOR IN CELL LINES OF DIFFERENT ORIGIN, Francesco D'Alessandro, Oscar Colamonici *, Michael Loeloff and Richard P. Nordan. Clinical Pharmacology Branch, National Cancer Institute, NIH, Bethesda, MD 20892, and *Department of Medicine, University of Chicago, Chicago, IL 60637

Interleukin 6 mediates its effects by interacting with two transmembrane glycoproteins, gp80 and gp130, with molecular masses of 80 kDa and 130 kDa. The gp80 receptor molecule directly binds IL-6 with low affinity and has been shown to associate with gp130 in the presence of IL-6. Although the gp130 molecule has not been reported to directly bind IL-6, studies with IL-6 plus soluble forms of recombinant gp80 implicate gp130 as a signal transducer in the generation of IL-6 responses.

Affinity crosslinking studies, in our laboratory, using myeloma cell lines and PBLs revealed a 3-band pattern of IL-6-containing crosslinked complexes with molecular masses of 100, 120 and 150 kDa (1). Our analysis indicated that the 150 kDa crosslinked complex consisted of IL-6 associated with a 130 kDa protein, presumably gp130, and that the 100 and 120 kDa crosslinked complexes consist of an 80 kDa protein, presumably gp80, associated with one and two molecules of IL-6 respectively.

Extending our crosslinking studies to hepatomas, melanomas and prostate carcinomas yielded the 100 and 150 kDa crosslinked complexes but not the 120 kDa crosslinked complex, suggesting that variant forms of the IL-6 receptor complex are present on cell lines different origin. This hypothesis is supported by studies with a monoclonal antibody developed in our laboratory that recognizes gp80 on myeloma cells and PBLs but fails to detect gp80 on other cell types.

1. F. D'Alessandro, O.R. Colamonici and R.P. Nordan. Direct association of interleukin 6 with a 130 kda component of the interleukin 6 receptor system. J.B.C. (in press).

E 209 CHARACTERIZATION OF LIGAND-INDUCED CHANGES IN TGF-β RECEPTOR BINDING BY FLOW CYTOMETRY, James R. Dasch and Patricia R. Segarini, Department of Immunology and Cell Biology, Celtrix Pharmaceuticals, Inc., Santa Clara, CA 95054-1815

Three major cell surface binding proteins for TGF-β have been described. The type II and type III receptors have been cloned, however the primary sequence of the type I receptor is unknown. Recently, we described apparent positive cooperativity between the type I and type II receptors on the rat L6 myoblast cell line, Segarini, et al., J. Biol.Chem. 267:1048. In receptor cross-linking studies, excess non-labeled TGF-β2 was found to enhance the binding of iodinated TGF-β1 to the type II receptor at concentrations that caused the type I receptor to be saturated by TGF-β2. We have now observed a similar phenomenon on the L6 myoblasts when the cells are observed by flow cytometry. TGF-β receptor binding is followed by incubation of the cells with biotinylated TGF-β1 or TGF-β2 and then phycoerythrin-labeled streptavidin. In these experiments, when excess non-biotinylated TGF-β is added to the cells, rather than competing the biotinylated TGF-β, we see an increase in the fluorescent signal. Control experiments with a TGF-β neutralizing monoclonal antibody (1D11.16) completely block the binding of biotinylated TGF-β to cells, demonstrating the specificity of the binding. When cells are pre-incubated with excess non-labeled TGF-β, washed, and then incubated with biotinylated-TGF-β, the enhanced fluorescence is still observed. This ruled out the possibility of micro-aggregation of high concentrations of TGF-β and showed the effect to be at the cell surface. Studies are underway to assess whether the positive cooperativity is a result of an allosteric change in the type II receptor or due to a change in receptor numbers.

E 210 CHARACTERIZATION OF THE SOLUBLE TNF RECEPTORS sp55 AND sp75, Harald Dinter, Helmut Harms, Wolf-Dieter Schleuning and Peter Scholz, Research Laboratories of Schering AG, 1000 Berlin 65, Germany.

The DNA fragments coding for the extracellular domains of both TNF receptors were inserted in the eukaryotic expression vector pMPSV/CMV and transfected into CHO and/or BHK cells. Stably transformed cell clones secreted the soluble receptor into the tissue culture medium. The soluble receptors were purified from the medium in a three step procedure. Both soluble receptors started with the predicted amino-terminus indicating correct processing of the signal peptide . The receptors were glycosylated with sialic acid carbohydrates. However, glycosylation is not necessary for TNF binding.

The affinity of the soluble receptors to TNF-α and TNF-β was determined: sp55 had a similar affinity to TNF-α and TNF-β. sp75 showed a fourfold higher affinity to TNF-β. The TNF neutralizing activity of sp55 was thoroughly analyzed in a cytotoxicity assay using a human cervical carcinoma cell line.

In addition, receptor deletion mutants were created and cloned in an eukaryotic expression vector. After transfection into BHK and/or CHO cells the mutants of the soluble receptor were analyzed for their TNF neutralizing activity. The results suggest that both receptors contain multiple binding sites for TNF.

E 211 MUTATIONAL ANALYSIS OF STRUCTURE-FUNCTION RELATIONSHIP OF THE HUMAN GM-CSF RECEPTOR.
Parul D. Doshi, Maureen C. Kempski and John F. DiPersio.
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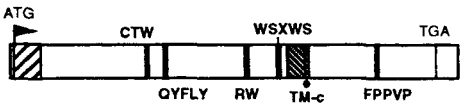
The α subunit of the human granulocyte-macrophage colony stimulating factor (GM-CSF) receptor (GM-R) constitutes a low affinity receptor and shares some highly conserved structural features with other members of the hematopoietin receptor superfamily. We are studying the regions of GM-R(α) involved in its biological function by systematically altering conserved sequence motifs or structural domains crucial for receptor functions such as ligand binding and transmembrane signaling. We have altered the following conserved sequence motifs by oligonucleotide-directed *in vitro* mutagenesis: (1) CTW (nt # 577, trp to leu) (2) RW (nt # 860, trp to cys) (3) Substitution of each amino acid of the WSXWS motif (nt # 1068-1083) (4) Cys in TM domain (nt # 1160, cys to ser).



We have found that mutations in the CTW, the RW or the WSXWS sequence motifs results in the loss of binding of ^{125}I-GM-CSF to COS cells transfected with the mutant cDNAs suggesting that these sequence motifs are important for binding of GM-CSF to its receptor. On the other hand, mutation of the cys residue in the transmembrane domain does alter binding of ^{125}I-GM-CSF to its receptor. We have also generated various truncations in the cytoplasmic domain which do not alter factor binding. Two specific amino acid sequence motifs, QYFLY (nt # 590) and FPPVP (nt # 1218) were deleted by standard molecular manipulations to determine if these regions are involved in ligand binding and/or signal transduction. As expected, the QYFLY motif in the extracellular region eliminates binding of the ^{125}I-GM-CSF to transfectants. The FPPVP motif is present in the cytoplasmic domain and does affect factor binding. We have used FACS analysis to determine the intracellular fate of these receptors. We are now in the process of studying the effects of each of these mutations on growth and signal transduction in factor dependent and independent cell lines.

E 212 INTERACTIONS BETWEEN HUMAN IL-3 AND ITS RECEPTOR, G.J. Goodall, C.J. Bagley, R.J. D'Andrea, M.A. Vadas and A.F. Lopez, Hanson Centre for Cancer Research, Institute of Medical & Veterinary Science, Frome Road, Adelaide, South Australia 5000 Australia

We have proposed (1) that the structures of human IL-3 and each of its receptor subunits are sufficiently similar to the known structures of GM-CSF and the growth hormone/GH receptor complex to allow prediction of regions in IL-3 and its receptor that contact each other. On this basis we predict that the receptor α chain contacts IL-3 on helix D, the C-terminal half of helix A, and the loop between helices A and B, while the β chain contacts helix C and the N-terminal half of helix A. Regions of the receptor α chain predicted to contact IL-3 include the loops between β strands A and B and E and F as well as strand G of the N-terminal domain, and the loops between strands B and C and F and G of the C-terminal domain. We are testing the binding and function of mutant forms of IL-3 and IL-3 receptor to test these predictions.

An IL-3 helix D mutant, Lys116Val, exhibits enhanced binding to COS cells transfected with IL-3R α chain, supporting the involvement of helix D in interactions with the α chain. The binding properties of mutations within IL-3 helices A and C, and in the receptor α chain, will be presented.

(1) G.J. Goodall, C.J. Bagley, M.A. Vadas and A.F. Lopez, Growth Factors, in press.

E 213 MONOCLONAL ANTIBODIES SPECIFIC FOR THE α SUBUNIT OF THE MOUSE IL-3 RECEPTOR, Daniel M. Gorman, Takahiko Hara, and Atsushi Miyajima, DNAX Research Institute, Palo Alto, CA 94304

A high affinity murine IL-3 receptor is composed of two subunits, α and β. The α subunit binds IL-3 with low affinity and forms high affinity receptors with either of the two homologous β subunits that were initially designated as AIC2A and AIC2B. AIC2A is the specific β subunit for the IL-3 receptor and AIC2B is the common β subunit shared by the IL-3, IL-5 and GM-CSF receptors. Using a mouse T-cell line CTLL void of endogenous expression of these IL-3 receptor components we established stable transfectants expressing one or more of these subunits. CTLL cells expressing the α subunit were used to immunize rats in order to isolate monoclonal antibodies against the α subunit. Hybridomas secreting mAB's were screened by differential FACS staining profiles generated against CTLL transfectants and parental CTLL cells. Two rat IgG monoclonal antibodies were positive in this screening and were further analyzed. A panel of cells including bone marrow cells were stained with these antibodies and the staining profiles correlated with the expression of the mouse IL-3 receptor. Using these antibodies, an IL-3 nonresponsive variant cell line derived from an IL-3 dependent cell line was found to possess a dominant mechanism to shut off the expression of the IL-3 receptor α subunit.

E 214 THE ROLE OF THE TWO INTERLEUKIN-6-RECEPTOR SUBUNITS IN INTERNALIZATION OF INTERLEUKIN-6, Lutz Graeve, Elke Dittrich, Claudia Gerhartz, Dietlind Zohlnhöfer, Stefan Rose-John, Heidi Schooltink and Peter C. Heinrich, Institute of Biochemistry, RWTH Klinikum, Aachen, Germany

Interleukin-6 (IL-6) exerts its pleiotropic actions via cell surface receptor complexes that consist of an IL-6 binding protein (gp80) and a signal transducing subunit (gp 130) capable of binding the IL6/gp80 complex. Our goal is to understand the role played by the two IL-6R subunits in binding and internalization of IL-6 and in ligand induced down-regulation of the IL-6R.

Human hepatoma cells (HepG2) expressing both, gp80 and gp130, display about 2.000 high-affinity receptors per cell (kd = 60pM). Upon overexpression of gp80 from a stably transfected expression vector that contains an inducible mouse metallothionein promoter, additional 10,000 low-affinity binding sites (kd = 500 pM) are detected. HepG2 cells rapidly internalize bound 125I-IL-6. Concomitantly cell surface gp80 is down-regulated. Internalized IL-6 is degraded. The reappearance of IL-6 binding sites after down-regulation takes at least 8h and is inhibited by cycloheximide.

gp80 and gp130 cDNAs were transfected singly or together into IL-6R negative Madin-Darby canine kidney (MDCK) cells. Cells expressing gp 80 display only low-affinity binding sites. A clone expressing both subunits displays high- and low-affinity receptors. At 1nM IL-6 both transfectants internalize IL-6 with similar efficiencies, while at 50 pM IL-6 the double transfectant is two to threefold more efficient in internalization. These results indicate that gp80 alone can internalize but that the internalization of a high-affinity complex (IL-6/gp80/gp130) is more efficient. Pulse-chase experiments indicate that the half-life of gp80 in MDCK cells is short (about 2h) and not significantly influenced by IL-6. The receptor is mostly degraded intracellularly, only a small percentage is shed into the culture medium.

E 215 BOTH TNF RECEPTORS, TR60 AND TR80, CAN MEDIATE CYTOLYSIS INDEPENDENTLY AND IN AN ADDITIVE MANNER, Matthias Grell, Peter Scheurich, Gudrun Zimmermann and Klaus Pfizenmaier, Institute for Cell Biology and Immunology, University of Stuttgart, Pfaffenwaldring 57, W-7000 Stuttgart 80, Germany

The human rhabdomyosarcoma cell line KYM-1 expresses high numbers of both tumor necrosis factor (TNF) receptors, TR60 and TR80, and is highly sensitive to TNF-induced cytotoxicity even in the absence of metabolic inhibitors. We show here for the first time that in this cell line each of the constitutively expressed TNF receptors can mediate cytotoxicity independently upon selective stimulation. To perform this we used receptor specific antibodies and sera, able to compete with ligand binding to the respective receptor molecules. It was found, that the anti TR60 (60 kD TNF receptor, type I) monoclonal antibody H398 was strongly agonistic, i.e. cytotoxic, in the presence of a polyclonal anti-murine immunoglobulin (IgG) which could crosslink H398 bound to TR60. Fab fragments of H398, still efficient competitors for TNF binding, did not show any cytotoxic effects.

Purified Ig fractions of polyclonal antisera to TR80 as well as a monoclonal antibody directed against TR80 were also able to induce strong cytotoxicity in KYM-1 cells. Interestingly, when both receptor subsets were stimulated in parallel by limited crosslinking via the respective antibodies additive effects were observed. Blocking of single TNF receptor subsets using the respective antibody Fab fragments indicated that the main cytotoxic effect of the natural ligand TNF is mediated via TR60 receptors (< 10% of total receptor number), but that in KYM-1 cells also TR80 plays a role in mediating TNF-induced cytotoxicity.

The importance of TR80 for TNF induced cytotoxicity is stressed by the fact that several TNF resistant KYM-1 clones obtained by prolonged TNF treatment showed a near to total loss of membrane expression of this receptor subset. Desensitation of TR60 on the other hand had occured at the post receptor level

E 216 RECOMBINANT SOLUBLE HUMAN INTERLEUKIN-5 (hIL-5) RECEPTOR MOLECULES : CROSSLINKING AND STOICHIOMETRY OF BINDING TO hIL-5

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Recombinant soluble human Interleukin-5 receptor alfa (shIL-5Rα) has been expressed in Cos-1 cells and in Baculovirus-infected insect cells (yield : 1-2 μg/ml). The protein was purified from the supernatant by chromatography on ConA-Sepharose, Mono Q and gel filtration on TSK-G 2000 SWG. To analyze whether this shIL-5Rα might be useful as an hIL-5 antagonist, we have studied its binding characteristics. SDS-PAGE of shIL-5Rα crosslinked to radiolabeled hIL-5, suggested that one receptor molecule stoichiometrically binds to one hIL-5 homodimer. Gel filtration studies of the receptor-ligand complex pointed towards the same 1:1 molar ratio. shIL-5Rα was found to be able to neutralize the IL-5 dependent proliferation of mouse CTLL-CA1 cells, transfected with hIL-5Rα cDNA).
We also constructed a chimeric receptor protein (hIL-5Rα-hγ3) by fusion of the cDNA corresponding to shIL-5Rα to the cDNA coding for the Fc-part of the human IgGCγ3 chain. Baculovirus-infected Sf9 cells secreted the chimeric fusion as a disulfide-linked homodimer (0.5-1 μg/ml). The protein was purified by Protein G affinity chromatography.
In a solid phase ligand binding assay, the shIL-5Rα and the bivalent hIL-5Rα-hγ3 were found to bind IL-5 with a similar affinity, corresponding to the membrane-bound, low affinity hIL-5Rα expressed by Cos-1 cells transfected with hIL-5Rα cDNA.

E 217 **EXPRESSION OF A SOLUBLE GM-CSF RECEPTOR,**

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Granulocyte-macrophage colony stimulating factor (GM-CSF) plays an important role in hematopoiesis and host defense by interacting with specific cell surface receptors in target tissues. The effects of GM-CSF are mediated by interaction with a specific receptor (GMR) which consists of a unique alpha subunit (GMRα) which has low binding affinity for GM-CSF, and a beta subunit (GMRβ) which is common to GM-CSF, IL-3, and IL-5. GMRβ is required for high affinity GM-CSF binding, but does not appear to have intrinsic GM-CSF binding activity. This laboratory identified a naturally-occurring soluble isoform of the low affinity GM-CSF receptor (sGMRα) by cDNA cloning. The soluble isoform lacks 84 C-terminal amino acids including the transmembrane region, and contains 16 different amino acids at its C-terminus. Soluble isoforms of several other hematopoietin receptors have been identified and may be a general feature of this receptor family. We have now isolated the coding sequences that flank the transmembrane region from a genomic phage library. Sequence analysis of intron-exon junctions reveals that the sGMRα cDNA is exactly predicted by alternative splicing with loss of the transmembrane exon. This result implies that sGMRα expression can be regulated at the level of RNA processing. We have developed a reverse transcription-PCR method which provides a rapid, sensitive measure of sGMRα and GMRα expression in order to explore differential regulation. The expression of sGMRα in hematopoietic cell lines, tumor cell lines and normal and neoplastic tissues will be presented.

E 218 REGULATION AND FUNCTION OF MEMBRANE-BOUND AND SOLUBLE HUMAN INTERLEUKIN-6 RECEPTOR,

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Interleukin-6 (IL-6) has been identified as the major inducer of acute-phase protein synthesis in rat and human hepatocytes in primary culture as well as in the rat in vivo. IL-6 exerts its action via a specific hepatic cell surface receptor composed of an 80 kD IL-6-binding protein and a 130 kD signal transducing subunit. Both subunits are differently regulated. In human hepatoma cells (HepG2) the 80 kD IL-6-receptor (IL-6-R) mRNA as well as the functional receptor protein is up-regulated by glucocorticoids, but not by IL-6. In contrast, expression of mRNA for the signal transducing subunit gp130 is stimulated by IL-6, particularly in combination with dexamethasone (dex). Under conditions of IL-6-R up-regulation by dex in HepG2 cells acute-phase protein mRNA induction by IL-6 is stronger and occurs earlier than without dex.
We also show that IL-6 down-regulates its surface receptor via endocytosis. The reappearance of IL-6-binding sites at the cell surface required >8h and was sensitive to cycloheximide suggesting that gp80 is not recycled after internalization.
In pulse-chase experiments with COS-7- and NIH/3T3-cells transfected with a cDNA coding for the gp80 subunit of the human IL-6-R, we observed the formation of a soluble form of the IL-6-R from the membrane protein by limited proteolysis ("shedding"). The shed IL-6-R as well as a genetically engineered soluble receptor bind IL-6 ($K_d \approx 6$ nM). Unlike most soluble cytokine receptors the soluble IL-6-R in combination with its ligand acts agonistically on HepG2 cells with down-regulated gp80-receptors via the signal transducing subunit leading to the induction of acute-phase proteins.
Finally, we report on the construction, bacterial expression, refolding and purification of a series of chimeric human/mouse IL-6 proteins. From binding and activity studies on human and murine cells of these chimeras we were able to deduce two receptor recognition domains.

E 219 LIGAND BINDING AND SIGNALLING OF THE HUMAN INTERFERON α/ß RECEPTOR

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The interferons (IFNs) α and ß are a family of homologous cytokines with species-specific antiviral, immunoregulatory and antiproliferative properties. Many IFNα subtypes and IFNß compete for receptor binding and were therefore considered to share a common receptor component. However, a cloned human IFNα receptor (IFNαR) when expressed in murine cells, conferred an antiviral response to IFNα8 but not IFNα2 nor IFNß; indicating that a cofactor was required either for complete binding and/or signalling.

In this study, we have examined ligand binding and signalling of the cloned huIFNαR in heterologous cells (CHO, COS). In our experiments, the IFNαR does bind ^{125}I-labelled IFNα2 and IFNß. Since the human IFNαR gene is located on chromosome 21, we also investigated IFN responses using a series of CHO hybrid cell lines which contain parts of human chromosome 21. The cell line containing all of chromosome 21 binds ^{125}I-huIFNα2 with Kd of 511±37pM, and 1255±54 sites per cell; cf. virtually undetectable binding in the parent CHO cell line. In the former cells, 2'-5' oligoadenylate synthetase (OAS), an IFN-induced enzyme, is induced and an antiviral response is developed. However in another cell line missing part of human chromosome 21, ^{125}I-IFNα2 binds with Kd of 557±115pM, and 1086±142 sites per cell but 2'-5' OAS is not induced, nor does an antiviral response develop. Studies with IFNß in these cell lines showed similar trends. Northern blot analysis shows an IFNαR transcript in both hu chr21- containing hybrid cell lines. These studies show that the cloned hu IFNαR interacts with a broader range of hu IFNs than originally reported and suggests that another component is necessary for response to all IFN subtypes. Furthermore, there appears to be a factor encoded on chromosome 21 which is involved in signal transduction, but probably not the receptor binding, of IFNs α and ß. This may be of significance in Down Syndrome, if at least 2 genes involved in response to IFNs α and ß are overexpressed.

E 220 ROLE OF THE TYPE I AND TYPE II INTERLEUKIN-1 RECEPTORS (IL-1R) ON HEMATOPOIETIC PROGENITOR GROWTH IN VITRO. K. Hestdal, F.W. Ruscetti, R. Chizzonite, M. Ortiz, J. Gooya, D.L. Longo, and J.R. Keller, BCDP, PRI/DynCorp Inc., LMI-BRMP, NCI-FCRDC, Frederick, MD, and Hoffman-LaRoche, Nutley, NJ.

Although interleukin-1 (IL-1) alone cannot promote the proliferation and differentiation of hematopoietic progenitor cells in vitro, it has been shown to act synergistically with the colony-stimulating factors (CSFs) on purified bone marrow cells (BMCs). Two types of IL-1R, p80 type I (IL-1R I) and p65 type II (IL-1R II), have been identified. We have previously shown that neither receptors can be detected on progenitors using ^{125}I-IL-1. To define the role of the two types of IL-1R on progenitor cells, we examined the synergistic effect of IL-1 on GM-CSF-, CSF-1- and IL-3-induced progenitor cell growth (both CFU-c and single cell assays) in the presence or absence of the monoclonal antibodies 35F5 and 4E2, which block the IL-1 binding to the IL-1R I or IL-1R II respectively. The synergistic effect of IL-1 on IL-3-induced progenitor growth was shown to be indirect through the IL-1R I. The direct synergistic effect of IL-1 on CSF-1- induced proliferation of Thy-1^{negative} cells was through IL-1R I. In contrast, the direct synergistic effect of IL-1 on both GM-CSF and CSF-1-induced progenitor growth of Thy-1^{positive} cells was both through IL-1R I and IL-1R II. Thus, depending on the CSFs and target cells used, IL-1 can indirectly and directly synergize through the type I IL-1R or directly through the type II IL-1R.

E 221 REGULATION OF CD27 EXPRESSION DURING DIFFERENT STAGES OF T-CELL ONTOGENY

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CD27, a transmembrane molecule that together with the TNF-R's, CD40 and FAS/APO-1 belongs to the NGF-R family, is expressed on both CD4 and CD8 positive T lymphocytes and medullary thymocytes. TCR/CD3 triggering induces high CD27 expression with a concomitant proteolytic cleavage of a soluble 32 kD CD27 form (Hintzen et al. J. Immunol. 1991, 147:29). Paradoxically, after prolonged activation CD27 expression is gradually switched off. T-cell clones for example neither express, nor release CD27. Within the CD4+ subset of fresh PBL, memory (CD45RA-) T cells can be divided in CD27$^+$ and CD27$^-$ fractions, whereas naive cells are all CD27$^+$. Comparison of CD27-expression and regulation with the related FAS/APO-1 molecule indicated largely opposite requirements. Studies with purified T-cell subsets revealed that after TCR/CD3 stimulation of PBL, naive T cells show a strikingly predominant upregulation of CD27, whereas, relatively, memory T cells seem to have lost the capacity to upregulate CD27. Moreover, it was found that when, next to TCR/CD3 triggering, a second signal is provided (IL-2, αCD5 or αCD28) CD27 upregulation is inhibited. T-cells of the memory phenotype that have no CD27 expression, cannot be induced to reexpress the molecule at both the protein and mRNA level.

From analysis of both the CD27$^+$ and CD27$^-$ memory subsets in immunized and allergic individuals, it was concluded that antigen specific T cells with differentiated Th1 or Th2-like phenotypes reside within the CD27$^-$ fraction (R. de Jong et al., Eur. J. Immunol.,1992, 22:993 and J. Immunol. in press). These data together indicate that CD27$^-$ cells define a separate subset with irreversibly differentiated phenotype, arising after prolonged antigenic stimulation.

Functional probing with large panels of CD27 mAb's has not yielded a clear clue for the physiological role of this molecule, therefore identification of its ligand will be required for elucidation of this matter.

E 222 FUNCTIONAL DIFFERENCES BETWEEN α SUBUNITS OF THE HUMAN IL-3 AND GM-CSF RECEPTORS

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The high-affinity receptors for human IL-3, IL-5, and GM-CSF consist of a cytokine specific α subunit and a common β subunit. Each α subunit has a primary role in cytokine binding and the β subunit is essential for signal transduction. The three cytokines exhibit some overlapping biological activities as well as biological activities unique to each cytokine. The common biological activities can be explained by the shared β subunit. Although different expression patterns of the α subunits partly explain the unique function associated with each cytokine, each α subunit may have different function in signal transduction. To address this question, we have examined fucntional difference between the α subunits of the human IL-3 and GM-CSF receptors using stable transfectants of a mIL-2 dependent T cell line CTLL-2 and a mIL-3 dependent FDC-Pmix cell line which is known to differentiate in response to mGM-CSF. First, the requirement of the cytoplasmic domain of the hIL-3Rα for signal transcuction was much more strict than that of the hGM-CSFRα in CTLL-2 cells. Second, in the presence of hIL-3, CTLL-2 transfectant expressing only hIL-3Rα gave rise to hIL-3 dependent sublines which were found to express the endogenous mouse β subunit, AIC2B. Expression of AIC2B and adaptation to hGM-CSF were never found in CTLL-2 transfectant expressing hGM-CSFRα. Third, whereas hIL-3 stimulated the proliferation of FDC-Pmix expressing hIL-3Rα alone, hGM-CSF did not stimulate the proliferation and differentiation of the transfectants expressing hGM-CSFRα. Unexpectedly, the FDC-Pmix transfectants expressing the α and β subunits of hGM-CSF proliferated in response to hGM-CSF. These results may suggest differential roles of IL-3 and GM-CSF in hematopoiesis.

E 223 TNFα BINDS TO BACTERIA: EVIDENCE FOR A HIGH AFFINITY RECEPTOR AND ALTERATION OF BACTERIAL VIRULENCE PROPERTIES, Gary R. Klimpel, Radwan A. Shaban, Luo Guoyang, Elizabeth A. Grim, and David W. Niesel, Department of Microbiology, University of Texas Medical Branch, and Department of Immunology Shriners Burns Institute, Galveston, Texas 77555

Human and murine receptor(s) for tumor necrosis factor α (TNFα) are present on most somatic cells and have been characterized and cloned. In contrast, very little is currently known about whether TNFα can bind to pathogens and whether such binding results in important biological consequences for the infected host. We now report that a number of gram-negative bacteria have receptors for TNFα. Using ^{125}I-labeled TNFα, we show that *S. flexneri* has 276 receptors for TNFα with a Kd of 2.5 nM. The binding of labeled TNFα to these bacterial receptors can be inhibited by cold TNFα, but not with cold TNFβ. Binding of ^{125}I-TNFα to *S. flexneri* was inhibited by trypsin treatment of bacterial cells or incubation at 52°C for 3 min., indicating a protein component for the receptor. A number of gram-negative bacteria were shown to be capable of binding ^{125}I-TNFα. In contrast, gram-positive bacteria bound significantly less ^{125}I-TNFα compared to gram-negative bacteria. Pretreatment of *S. flexneri* with TNFα resulted in enhanced bacterial invasion of HeLa cells and enhanced uptake by human and murine macrophages. This report shows for the first time that bacteria have receptors for TNFα and that a virulence property of a bacterium is altered as a consequence of cytokine binding.

E 224 STROMAL CELLS CONFER STABLE ALTERATIONS IN GROWTH FACTOR RESPONSE TO HEMATOPOIETIC CELLS,Karl Klingler[1], Martina Bögel,Christine Laker, Ursula Just, Jutta Friel, Klaus Mannweiler and Wolfram Ostertag, Heinrich-Pette-Institute, Hamburg, Germany, [1]present adress: Department of Clinical Chemistry, Medical School, University of Hamburg.

The proerythroblast cell line TF-1 has been isolated from a human erythroleukemia and grows permanently in the presence of GM-CSF or IL-3 and dies within a few days on withdrawal of the factor. In the presence of stem cell factor (SCF) TF-1 cells can only grow for a few weeks. Whereas TF-1 cells, if placed on murine stroma cells, display excellent permanent growth and an at least 10fold increased cloning efficiency, as compared to growth in GM-CSF.
TF-1 cells cocultered with stromal cells are stably altered with respect to growth in GM-CSF: Response to GM-CSF is now reduced to a transient stimulation and cells need periodic priming on stroma cells to prevent cell death which happens if these cell are kept permanently in the presence of GM-CSF. Similar results have been obtained with a murine precursor cell line (ELM-D).
Alterations in the expression pattern of several growth factor receptors will be displayed and discussed in the context of a general model of stroma cell interaction with hematopoietic cells.

E 225 INTERACTION OF G-CSF WITH ITS RECEPTOR: DISSOCIATION OF BIOLOGICAL ACTIVITY AND RECEPTOR BINDING, Judith E. Layton, Rosemary Pavlovic, Tim Osslund , Grant Shimamoto and Tom Boone , Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Parkville, 3050, Australia and Amgen, Thousand Oaks, CA.
Granulocyte colony-stimulating factor (G-CSF) is one of a family of cytokines required for hemopoiesis. Its activity is restricted essentially to the neutrophil cell lineage. The human protein has a high α-helical content with the α-helices arranged in a bundle, resembling the structures described for growth hormone, IL-4 and GM-CSF. Previously, we have mapped the binding site of neutralising anti-G-CSF antibodies to a region around the first disulphide bond (residues 20-58). We have now made alanine substitution mutations in nine surface residues in this region and tested them for receptor binding and biological activity. Three of the mutants had less than 10% of the activity of native G-CSF in the NFS-60 cell proliferation assay, confirming that the region identified by neutralising antibodies is important for biological activity. The binding affinity of the mutants was determined in competition assays using human neutrophils and equilibrium binding conditions at 4°C. The three mutants with reduced activity had only 2-4 fold reduced binding affinity. On the other hand, a mutant with 48% of native G-CSF activity had a 3-fold increase in binding affinity. Thus the binding affinity did not correlate well with biological activity, suggesting that the altered residues may be involved in aspects of signal transduction other than the initial receptor binding interaction. It will be important to test the rate of G-CSF degradation and receptor recycling over the time course of the NFS-60 proliferation assay to understand these data.

E 226 PURIFICATION AND CHARACTERIZATION OF RECOMBINANT SOLUBLE HUMAN INTERLEUKIN 4 RECEPTOR, Alan D. Levine, Linda R. Neidhart, Jean P. Favara, Department of Immunology, Monsanto Company, St. Louis, MO 63198
The Interleukin-4 receptor (IL-4R) is a Type 1 membrane-associated glycoprotein, expressed by a wide variety of hematopoietic cells which respond to IL-4 *in vitro*. cDNAs coding for both the human and mouse IL-4R have been isolated and the predicted amino acid sequence for both proteins reveals that they are members of the hemato-poietic receptor gene family, with 4 conserved cysteines in the extracellular domain and a Tryp-Ser-X-Tryp-Ser pentameric sequence proximal to the transmembrane alpha helix. To investigate the structural and binding properties of the extracellular domain of the human IL-4R, a recombinant protein, synthesized by a mammalian cell, coding for this domain has been purified. rshIL-4R is a 47 kDa glycoprotein, which binds IL-4 and neutralizes its biological activity *in vitro*. The 47 kDa polypeptide is heavily N-linked glycosylated, as is its native membrane associated form. Removal of the N-linked carbohydrates causes a molecular weight shift to 27 kDa, equal to the size of the polypeptide predicted by the modifications made in the expressed cDNA. Stoichiometric studies have shown that the monomeric ligand, IL-4, binds to a homodimeric form of the soluble receptor.

E 227 INTERACTIONS BETWEEN ONCOSTATIN M AND THE IL-6 SIGNAL TRANSDUCER, GP130
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Gp130, the signal transducer for interleukin 6 (IL-6), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), was recently identified by us and others to be the low affinity receptor for Oncostatin M. However, it is not yet clear if OM binding to gp130 requires accessory factor(s) and if gp130 alone can mediate OM signal. Here we report that: (a) expressing murine gp130 in BAF-B03 cells (BAF-m130) resulted in the appearance of single class of low affinity OM binding sites, (b) chemical crosslinking studies with ^{125}I-OM identified a 180 kDa labeled complex on BAF-m130 cells, and an additional 280 kDa species on H2981 cells, which express both high affinity and low affinity OM receptor, (c) ^{125}I-OM was specifically crosslinked to soluble recombinant gp130 (sgp130-Rg) in solution. (d) the cellular proliferation of BAF-m130 was not affected by OM treatment, and finally (e) LIF was able to compete with OM binding and crosslinking to BAF-m130 cells, but not to soluble gp130 in solution.These data suggest that gp130 can act as low affinity receptor for OM, however, gp130-OM interactions alone are unable to elicite cellular proliferation, which suggest that additional factor(s) is required to interact with OM/gp130 complex to form the high affinity and functional receptor. We propose that the 280 kDa species detected on H2981 cells is likely to be a complex of OM, gp130, and the putative β chain of OM high affinity receptor. The observation of LIF competing with OM binding to BAF-m130 cells, and not to soluble gp130 suggested that the cytoplasmic domain of gp130 may regulate LIF binding affinity. Recently, OM has been shown to be the major growth factor for Kaposi's sarcoma derived cells,Since sgp130-Rg can inhibite OM binding to its receptor on cell surface, it may be a useful inhibitor of the growth of Kaposi's sarcoma cells in vitro and in vivo.

E 228 **IDENTIFICATION OF RESIDUES IN IL-3 AND GM-CSF INTERACTING WITH THE α AND β CHAINS OF THEIR RECEPTORS**, A.F Lopez, M.F.Shannon, G. Goodall, S. Barry, T. Hercus, J. Phillips, C. Bagley, M. Dottore, B. Cambareri and M.A. Vadas, The Division of Human Immunology, The Institute of Medical & Veterinary Science, Frome Road, Adelaide, South Australia 5000.

The human hemopoietic growth factors (HGF) GM-CSF and IL-3 are pleiotropic cytokines that stimulate multiple functions in several cell lineages. GM-CSF and IL-3 appear related not only functionally but also structurally, both HGF containing four alpha helices and similar helix packing. GM-CSF and IL-3 have been shown to stimulate cellular functions following binding to cell surface receptors. The receptors for GM-CSF and IL-3 have recently been characterised and shown to each comprise two polypeptide chains, one which recognises the cognate ligand specifically and with low affinity (α chain), and a second one which is common to both receptors (β chain) and which allows high affinity binding when complexed with the α chain.

In order to understand the molecular and functional basis of GM-CSF and IL-3 actions we have performed site-directed mutagenesis and analysed the resultant mutants in multiple receptor binding and functional assays. We found that in the helix A of these HGF the Glu at position 21 in GM-CSF and at position 22 in IL-3 were required for high affinity binding and for optimal stimulation of neutrophil,eosinophil and monocyte functions. In contrast binding to the α chains was unaltered. Substitution mutagenesis in helix D of IL-3 led to the analog IL-3 Ala^{101} Val^{116} which exhibited increased biological activity. Binding experiments on monocytes indicated that this mutant exhibited enhanced binding activity to low affinity as well as to high affinity receptors.

These experiments provide data supporting a model where helix D of these HGF recognises the receptor α chain and the conserved Glu in helix A recognises the receptor β chain. In addition, the functional data suggest that (i) whilst ligand binding to the receptor β chain may be required for optimal signal transduction, signalling can occur in the absence of high affinity binding, and that (ii) both chains participate in signalling.

E 229 POXVIRUSES ENCODE SECRETED PROTEINS WHICH BIND AND INHIBIT GAMMA-INTERFERON, Grant McFadden, Karen Mossman and Chris Upton, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

Many important poxvirus-encoded virulence factors have been identified as proteins that are secreted from infected cells. We have examined proteins secreted from cells infected with myxoma virus (a poxvirus and the agent of rabbit myxomatosis) and show that the major secreted protein, an exceptionally abundant 37 kDa species, is encoded by the M-T7 open reading frame (ORF). This ORF was cloned, sequenced and shown to have homologues in Shope fibroma virus (SFV) and vaccinia virus. Furthermore, these three poxvirus ORFs were observed to share significant amino acid sequence homology to the ligand binding domain of the human and mouse receptor for gamma-interferon (γ-IFN). In cross-linking experiments it was demonstrated that the myxoma M-T7 protein specifically binds radio-labeled rabbit γ-IFN and similar activities were detected among the proteins secreted from cells infected with vaccinia virus or malignant rabbit fibroma virus (a recombinant between SFV and myxoma virus which contains only the SFV counterpart of this gene). The M-T7 protein had the greatest affinity for rabbit γ-IFN, and less for mouse and human γ-IFN. Using a bioassay of the ability of γ-IFN to induce the antiviral state in RK-13 cells, it was shown that M-T7 can quantitatively sequester and inhibit the activity of rabbit γ-IFN. Thus it appears that *in vivo* this secreted viral protein is targeted to bind and inactivate extracellular γ-IFN, one of the key regulatory cytokines in the host immune response against viral infections, and that the species specificity of binding reflects the host animal in which the virus has evolved.

E 230 MURINE T_H2 LYMPHOCYTES EXPRESS TWO IL-1 RECEPTOR ISOFORMS, D. J. McKean, R. Podzorski, M. Bell, A. Nilson, C. Huntoon, J. Slack, S. Dower and J. Sims, Dept. of Immunology, Mayo Clinic, Rochester, MN 55905 and Immunex Corporation, Seattle, WA 98101

The role of IL-1 in augmenting the antigen receptor-initiated activation program was evaluated in IL-4 producing (T_H2) $CD4^+$ murine T lymphocytes. Northern blot and ^{125}I labeled IL-1α crosslinking analyses demonstrated that T_H2 lymphocytes express both type I and type II IL-1 receptors. Previous studies have demonstrated that both IL-1 receptor isoforms bind IL-1α and IL-1β with similar affinities. The expression of both of these IL-1 receptor isoforms on the surface of the T_H2 cells is coordinately upregulated in response to anti-CD3 crosslinking in the absence of detectable accessory cells. Analyses of the kinetics of IL-1 receptor acquisition demonstrated that the peak level of type I and type II IL-1 receptor mRNA expression occurs after the peak expression of mRNA encoding IL-2 receptor α and IL4; two IL-1 responsive events in the T_H2 activation program. Type I IL-1 receptor ligand binding antagonists, IL-1 receptor antagonist (IL-1ra) and anti-type I monoclonal antibody (M15), were used to evaluate the functional significance of T_H2 cell expression of two IL-1 receptor isoforms. The addition of either IL-1ra or M15 completely inhibited the IL-1α augmented component of the proliferative response stimulated by anti-CD3 plus exogenous IL-1α. Together these studies indicate that, although T_H2 clones express inducible levels of both type I and type II IL-1 receptor isoforms, the IL-1 induced intracellular signals involved in augmenting an anti-CD3 stimulated proliferative response are mediated solely through the type I IL-1 receptor.

E 231 **THE SOLUBLE INTERLEUKIN-6 RECEPTOR IS GENERATED BY SHEDDING** JÜRGEN MÜLLBERG, HEIDI SCHOOLTINK, TANJA STOYAN, MONIKA GÜNTHER, LUTZ GRAEVE, , PETER C. HEINRICH AND STEFAN ROSE-JOHN Department of Biochemistry, RWTH Aachen, Pauwelsstraße 30, 5100 Aachen, Germany

The ligand binding subunit (gp80) of the human interleukin-6 receptor (IL-6R) was transiently expressed in COS-7 cells. The metabolically labelled protein was shown to be quantitatively released from the membrane within 20 h. Shedding of the gp80 protein was strongly induced by 4β-phorbol-12-myristate-13-acetate (PMA), indicating that the process was regulated by protein kinase C (PKC). This was further corroborated by the finding that cotransfection of a PKC expression plasmid led to enhanced shedding of the gp80 protein. Since shedding of gp80 could not be prevented by treatment of the cells with inhibitors of all known classes of proteases, a novel protease seems to be involved. As a control, an unrelated membrane protein (vesicular stomatitis virus glycoprotein) was transfected into COS-7 cells and analyzed for shedding. Since the turnover of this protein was not mediated by shedding we conclude that the release of gp80 from COS-7 cells is a specific process. The shed gp80 protein specifically binds interleukin-6 and this complex shows biological activity on human hepatoma cells. Human peripheral blood monocytes released a soluble form of the gp80 protein into the culture medium upon PMA treatment indicating that PKC-regulated shedding is the physiological mechanism of generation of the soluble IL-6R.

E 232 A TRUNCATED ERYTHROPOIETIN RECEPTOR THAT FAILS TO PREVENT PROGRAMMED CELL DEATH OF ERYTHROID CELLS, Hiromitsu Nakauchi, Norio Komatsu, and Yukio Nakamura, Laboratory of Cell Growth and Differentiation, The Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki, 305 JAPAN.

A novel form of human erythropoietin receptor (EPOR-T), whose cytoplasmic region is largely truncated by alternative splicing, has been identified. EPOR-T is the dominant form of EPOR in early-stage erythroid progenitor cells, while previously-identified EPOR (EPOR-F) becomes dominant in late-stage progenitors. Studies using transfectants showed that EPOR-T and EPOR-F were expressed on the cell surface equally well, and their EPO binding affinity were identical. In addition, EPOR-T can transduce mitotic signal in response to EPO. However, EPOR-T transfectants are far more prone to programmed cell death than those expressing EPOR-F. Our data strongly suggests that alternative splicing of the EPOR gene has an important role in erythropoiesis, and that EPOR-F can transduce a signal to prevent programmed cell death independent of mitotic signal.

E 233 EXPRESSION OF FUNCTIONAL INTERLEUKIN-4 RECEPTORS (IL-4R) ON HUMAN RENAL CELL CARCINOMA (RCC) TUMOR CELLS. Nicholas I. Obiri, *Gilda G. Hillman, *Gabriel P. Haas and Raj K. Puri. Center for Biologics Evaluation and Research, FDA, Bethesda, MD and *Wayne State University School of Medicine, Detroit, MI.

IL-4 is a glycoprotein lymphokine primarily produced by activated T helper (Th) cells and has been shown to exert a variety of biological effects on different cell types. These include in vitro and in vivo antitumor effects that are thought to involve IL-4R binding. In previous studies we described the expression of IL-4R on murine sarcoma and adenocarcinoma tumor cells and their internalization after binding to the ligand. In the present study, we have examined the expression and function of IL-4R on human tumor cells. Primary cultures of human RCC cells were established from tumor specimens obtained following nephrectomy. The cells were examined for IL-4R expression by utilizing iodinated IL-4 (^{125}I-IL-4) in a receptor binding assay. From Scatchard analysis of the binding data, we observed that RCC tumor cells from four specimens express a single class of high affinity IL-4R ranging from 1425±207 to 3831±299 (mean±SEM) IL-4 binding sites/cell with a dissociation constant (Kd) ranging from 112±11 to 283±71 pM. Northern blot analysis for IL-4R gene expression revealed that RCC cells express a single species of 4 kb IL-4R mRNA. Furthermore, we observed that IL-4 downregulated surface expression of its own receptors on RCC cells. IL-4 inhibited the growth of these cells, as measured by [^{3}H]-thymidine uptake studies, by up to 68% in a dose dependent fashion. The growth inhibitory effect was neutralized by an anti-IL-4 antibody. Although IL-4R expression was also observed on normal human fibroblasts and endothelial cells, the growth inhibiting effect of IL-4 was not observed in these cells. Instead, IL-4 caused a modest growth stimulation in these cells. These findings indicate that IL-4R on RCC cells are functional and may serve as a target for IL-4 itself, for IL-4-toxin therapy or alternatively, for gene therapy.

E 234 CHARACTERISATION OF A NOVEL HIGH AFFINITY IL-7 RECEPTOR: EXPRESSION ON T CELLS AND ROLE IN IL-7 DRIVEN PROLIFERATION. Theresa H.Page, David Taylor-Fishwick, Joanie L.Willcocks and Brian M.J.Foxwell. *The Kennedy Institute of Rheumatology, Charing Cross Sunley Research Centre, 1 Lurgan Avenue, Hammersmith, London W6 8LW, UK.*

Whilst both unstimulated and activated human T cells express receptors for IL-7, only activated T cells proliferate in response to this cytokine. These divergent responses may be explained by our observation that unstimulated T cells express IL-7 receptors of 90KDa MWt, while activated cells express an additional putative 76KDa IL-7 binding molecule. In this study the functions of these two molecules, and their relationship to the cloned IL-7R have been investigated. Scatchard analysis showed that cells expressing either receptor in isolation can bind IL-7 with both high (K_d=40pM) and low (K_d=700pM) affinity. Nevertheless, analysis of receptor expression and proliferative capability of mitogen stimulated human PBMC showed that only expression of the 76KDa receptor correlated with the ability to proliferate to IL-7. PCR analysis demonstrated that mRNAs encoding the three alternatively spliced forms of the cloned IL-7R are consistently expressed in both resting and activated PBMC, suggesting that only the 90KDa species corresponds to the cloned IL-7R. In addition, MAbs specific for the cloned IL-7R stained unstimulated T cells but not IL-7 responsive T cell clones which express exclusively the 76KDa IL-7R. These antibodies also immunoprecipitated the cloned receptor as a 90KDa species from both ^{125}-I-surface labelled resting and activated T cells, whilst being unable to precipitate a 76KDa species, further indicating that the 76KDa receptor does not represent the truncated form of the cloned IL-7R. Taken together these data suggest that the 76KDa receptor binds IL-7 with high affinity, is immunologically distinct from the cloned 90KDa receptor, and may be necessary for IL-7 driven proliferation in human T cells.

E 235 MOLECULAR IDENTIFICATION OF TWO TYPES OF IL-1 RECEPTORS IN THE MURINE BRAIN AND PITUITARY GLAND.P. Parnet, D. L. Brunke*, K. W. Kelley*, R. Dantzer.INRA-INSERM, U.176. Rue Camille St Saëns, 33077 Bordeaux Cedex, France. * Laboratory of Immunophysiology. University of Illinois, Urbana, Illinois 61801., USA

IL-1 is now known to induce a variety of biological activities including fever, anorexia, activation of the neuroendocrine system and sickness behavior. A specific 80 kDa receptors for IL-1 was originally identified on T lymphocytes and fibroblasts. Recently, a 60 kDa form of this receptor was cloned from B lymphocytes and was subsequently found to be expressed on a variety of cells, including T lymphocytes. We have used a variety of approaches to show that the pituitary, the brain and different neural cells lines express transcripts for one or the other form of the IL-1 receptor. Quantitative receptor autoradiography was used to confirm the existence of binding sites for IL-1α in the murine adenohypophysis, in the dentate gyrus of the hippocampus and in the choroïd plexus. Specific binding of IL-1α to isolated pituitary membranes and hippocampus membranes revealed a Kd of about 0.9 nM. To examine the type of receptors present in these tissues, immunocytochemistry experiments with specific monoclonal antibodies against type I and type II IL-1 receptors were performed as well as IL-1cross-linking affinity experiments. Identity of the type I IL-1 receptor was also analysed by amplification of the predicted 619 bp fragment spanning the cytoplasmic, transmembrane and extracellular domains from RNA of pituitary, clonal AtT20 pituitary cells, neurones, astrocytes and LBRM 33. Restriction fragment analysis and sequencing of the amplified cDNAs were performed. The pituitary gland, AtT20 cells, the neural cell lines C1300 and N115E also expressed transcripts for the newly identified type II IL-1 receptor as assessed by amplification of a specific 325 bp fragment, restriction fragment analysis and DNA sequencing, these transcripts were identical to those found on B lymphocytes. These results emphasize the need for identification of both types of IL-1 receptors in investigations aimed at understanding the regulation of IL-1 binding in the hypophysis and in the brain.

E 236 SOLUBLE TUMOR NECROSIS FACTOR RECEPTORS OF TWO TYPES ARE ENCODED BY COWPOX VIRUS

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Cowpox virus inhibits host inflammatory responses to viral infection. One of the viral genes necessary for this inhibition, the *crmA* gene, encodes a cytokine response modifier that can inhibit the Interleukin-1β converting enzyme, which is necessary for the activation of Interleukin-1β. Inhibition of this single cytokine, however, is insufficient to prevent inflammatory responses, suggesting that cowpox virus encodes additional cytokine response modifiers.

The identification of a soluble TNF receptor encoded by the T2 orfs of Shope Fibroma virus and Myxoma virus (Smith *et al.*, 1990, 1991; Upton *et al.*, 1991) suggested that cowpox virus might also encode modifiers of TNF. Nucleotide sequence analysis, bioassays, ligand-binding assays, *in vitro* protein synthesis, and site-directed mutagenesis procedures have been used to identify and characterize two cowpox virus genes that encode soluble forms of the TNF receptors. These genes each encode proteins that are structurally and functionally homologous to the ligand-binding domains of TNF receptors of types I and II. One is an early product similar to the products of the T2 orfs. As with cellular TNF receptors, it specifically binds both murine TNFα and TNFβ, and these ligands cross-compete. The second protein is a late product. The structure and TNF-binding properties of each of these viral proteins suggest that these receptors inhibit TNF-mediated responses to cowpox virus infection.

E 237 IL-3 RECEPTOR α SUBUNIT cDNAs AMPLIFIED FROM KG-1 LEUKEMIC CELLS: SEQUENCE ANALYSIS AND PRELIMINARY STUDIES OF FUNCTION.

A.P. Rapoport, J.F. DiPersio. University of Rochester Medical Center, Rochester, NY.

cDNAs for the α subunit of the IL-3 receptor were amplified from KG-1 leukemic cells using PCR. Oligonucleotide primers were synthesized based on the published sequence for an IL-3Rα cDNA cloned from TF-1 erythroleukemia cells (Cell 66:1165-1174). COS cells transfected with the KG-1 IL-3Rα cDNA under control of the SV40 late promoter showed no binding to ^{125}I-IL-3 up to a concentration of 1-2nM. When COS cells were co-transfected with KG-1 IL-3Rα cDNA and a cDNA for the "β" subunit from KG-1 cells, a single class of "high-affinity" IL-3 binding sites was demonstrated (Kd=520pM) by equilibrium binding and Scatchard analysis. As shown in Table 1, sequence comparisons between the KG-1 and TF-1/L-3Rα cDNAs disclosed 3 differences of the amino acid level. These changes were confirmed using another set of independently amplified KG-1 L-3Rα cDNAs. cDNAs for IL-3Rα were also amplified from normal human CD34+ marrow cells, and sequence analysis revealed the same changes noted for KG-1 IL-3Rα.

AA#(TF1 Seq.)	TF1	KG1	Normal CD34+
*126-130	val-gly-pro-gly-ala	val-gly-gly-ala	val-gly-gly-ala
*144-145	asn-arg	lys	lys
**329	leu	arg	arg

*extracellular domain **intracellular domain

While the biological consequences of these amino acid polymorphisms is unknown, they do not seem to significantly alter binding affinity since the Kd for ^{125}I-IL-3 binding to native TF1 cells was about 330pM. To begin to map functional domains of IL-3Rα, a cDNA encoding a mutant IL-3Rα receptor lacking the extracellular hematopoietic receptor consensus sequence "LSXWS" was co-transfected with the β subunit cDNA into COS cells. No IL-3 binding could be detected up to a conc. of 2nM ^{125}I-IL-3, suggesting that this sequence is a critical structural element. Further studies of this and other mutants are currently underway.

E 238 EXPRESSION AND BIOLOGICAL ACTIVITY OF IL1-RECEPTORS ON MURINE γ/δ THYMOCYTES DURING ONTOGENY,

Dominique RUEFF-JUY, Isabelle LIBERMAN, Pierre SARTHOU and Pierre-André CAZENAVE, Département d' Immunologie, Institut Pasteur, 75724 Paris. France.

We have recently shown that the response of mouse thymocytes to IL1+IL2 was maximal at birth. At this ontogenic stage, the responding cells were found to display a CD4$^-$CD8$^-$TcRγ/δ$^+$ phenotype. Unexpectedly, despite their high proportion of γ/δ$^+$ cells, fetal thymocyte populations of any embryonic stage responded only weakly to IL1+IL2. Here, we show that IL2-responsiveness of these cells could be achieved upon addition of a suitable co-stimulator signal other than IL1. Furthermore, we demonstrate that the discrepancy between the day-17.5 fetal and newborn sensitivities to the combined action of IL1 and IL2 is a consequence of the different patterns of high-affinity IL1-receptor expression displayed by these two cell subsets. Actually, high-affinity IL1-receptors are found on the TcR γ/δ$^+$ newborn cells and, by contrast, they are not detectable on the day-17.5 fetal ones. Another type of IL1-binding site, of a roughly hundred fold less affinity, has been described on CD4$^-$CD8$^-$ adult thymocytes. Our functional studies strongly support the hypothesis that high-affinity IL1-receptors on the one hand, and low-affinity ones on the other hand, are involved in the response to IL1+IL2 of the newborn and day-17.5 fetal thymocytes, respectively. In addition, the high-affinity IL1-receptors would be far more efficient than the low-affinity ones in promoting IL2-responsiveness of thymocytes.

E 239 EXTRACELLULAR DOMAIN OF TRANSFORMING GROWTH FACTOR-ß (TGF-ß) RECEPTOR TYPE II ENCODES A SOLUBLE TGF-ß BINDING PROTEIN WHEN EXPRESSED IN A BACTERIAL SYSTEM.

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There are three predominant cell surface proteins that bind TGF-ß, known as type I, type II, and type III receptors. The type II receptor has been cloned and codes for a protein of 563 amino acids [Lin et al.(1992) Cell, 68: 775 - 785]. This protein contains three discrete putative domains, a 136 amino acid extracellular domain, a 30 amino acid hydrophobic transmembrane domain and a 376 amino acid intracellular domain. The 23 amino acids at the amino terminal end of the protein are presumably cleaved off as a signal peptide after transfer through the Golgi complex. The intracellular domain bears strong homology to ser/thr kinases and has been shown to catalyze phosphorylation of ser/thr residues *in vitro* (Lin et al., ibid.). We have expressed the extracellular domain in *E.coli* and this truncated receptor (sßRII) is a soluble protein. Several assays have been developed to identify the soluble receptor. In a solution binding assay, sßRII is bound and crosslinked with ^{125}I-TGF-ß1. We find that sßRII binds TGF-ß1 specifically and with high affinity. Two antisera prepared against peptides of the carboxy-terminal region of sßRII have been characterized and identify the protein in *E. coli* extracts. A ligand blotting assay that utilizes biotinylated TGF-ß1 or TGF-ß2 has also been developed for the detection of sßRII. Recently, the soluble forms of several cytokine receptors have been applied in models of disease to reduce the level of circulating cytokine. The sßRII may be an effective therapeutic for disorders that are characterized by high levels of TGF-ß.

E 240 HIGH AFFINITY LIGAND BINDING IS NOT ESSENTIAL FOR GM-CSF RECEPTOR ACTIVATION, Armen B. Shanafelt and Robert A. Kastelein, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104, USA

The high affinity receptor of the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) is a heterodimer composed of two members of the cytokine receptor superfamily. GM-CSF binds to the α–subunit (GM-Rα) with low affinity, and to the receptor αβ complex (GM-Rαβ) with high affinity. The GM-CSF/GM-Rαβ complex is responsible for biological activity. Interactions of the N-terminal helix of mouse GM-CSF with mGM-Rαβ were examined by introducing single alanine substitutions of hydrophilic residues in this region of mGM-CSF. The consequences of these substitutions were evaluated by receptor binding and biological assays. Although all mutant proteins exhibited near wild-type biological activity, most were defective in high affinity receptor binding. In particular, substitution of Glu-21 with alanine abrogated high affinity binding leaving low affinity binding unaffected. Despite near wild-type biological activity, no detectable binding interaction of this mutant with mGM-Rβ in the context of mGM-Rαβ was observed. Cross-linking studies showed an apparent interaction of this mutant protein with mGM-Rαβ. The deficient receptor binding characteristics and near wild-type biological activity of this mutant protein demonstrate that mGM-CSF receptor activation can occur independently of high affinity binding, suggesting that conformational changes in the receptor induced by mGM-CSF binding generate an active ligand/receptor complex.

E 241 CLONING MOUSE c-MPL, A FUNCTIONAL MEMBER OF THE CYTOKINE RECEPTOR SUPERFAMILY, Radek C. Skoda, David C. Seldin, Ming-Ko Chiang, and Philip Leder, Department of Genetics, Harvard Medical School, and Howard Hughes Medical Institute, Boston MA 02115.

A mutant Friend virus called myeloproliferative leukemia virus (MPLV) was shown to contain sequences homologous to members of the cytokine receptor superfamily. We have cloned a full length spleen cDNA as well as a genomic clone for the murine c-mpl. The murine c-mpl protein has two extracellular domains, each containing the four conserved cysteines and a WGxWS or WSxWS sequence, which define this receptor superfamily. We also isolated a clone coding for a potentially secreted form of c-mpl. By ribonuclease protection assay this transcript accounts for approximately 30% of spleen c-mpl mRNA. Comparison with genomic mpl sequence shows that this form is generated by differential splicing. Anti-mpl antibodies precipitate proteins of 72 kd and 55 kd from transfected COS cells. To test if c-mpl is competent for signal transduction, we generated a chimeric receptor consisting of the extracellular domain of the human interleukin 4 receptor fused to the cytoplasmic domain of murine c-mpl. This construct was transfected into mouse interleukin 3 dependent BaF3 cells and stable clones were selected. When treated with human interleukin 4, clones expressing the chimeric receptor were able to grow in the absence of interleukin 3, indicating that the cytoplasmic domain of c-mpl can provide a proliferative signal.

E 242 THE CLONING AND EXPRESSION OF STK-1: A HUMAN RECEPTOR TYROSINE KINASE EXPRESSED IN A STEM CELL ENRICHED FRACTION, D. Small,* M. Levenstein,* E. Kim,* C. Burrow,* S. Amin,* and C. Civin. Johns Hopkins University School of Medicine, Oncology Center and Division of Nephrology, Baltimore, MD., 21287

We have cloned the cDNA for STK-1 (Stem cell Tyrosine Kinase 1), the human homologue of murine FLK-2 (Matthews et al, Cell 65, 1143-1152), and investigated its expression in subsets of normal human bone marrow. The cDNA codes for a protein of 993 a.a. with a 543 a.a. extracellular domain that includes 22 cysteine residues and 11 potential asparagine-linked glycosylation sites, a 20 a.a. transmembrane domain, and a 430 a.a. cytoplasmic domain that includes the conserved kinase domains interrupted by a 75 a.a. kinase insert. It has 85% identity with and 92% similarity to FLK-2 and is closely related to c-kit, c-fms, and PDGFr, all members of the subtype III receptor tyrosine kinase family. STK-1 is expressed on CD34+ cells, a population greatly enriched for stem and progenitor cell activity, and is not seen in a population depleted of CD34+ cells. We have also examined the expression of STK-1 in lymphohematopoietic cell lines derived from leukemias and normal cells and compared its expression to that of c-kit. STK-1 was expressed in several myeloid leukemia cell lines, including ML-1, KG1a, KG1, and HL60 cells; KMT2 cells, a line established from human umbilical cord blood; and Nalm 16 and REH cells, established from B lineage lymphoid leukemias. When compared to c-kit, STK-1 had a more restricted pattern of expression in these cell lines. It was not expressed in a number of nonhematopoietic malignancies, again in marked contrast to c-kit. Because its expression in normal hematopoietic cells is limited to the stem cell enriched population, it may be a receptor for a novel stem cell growth factor involved in regulating proliferation and/or differentiation of lymphohematopoietic stem cells. Expression of STK-1 is being evaluated in further subfractions of bone marrow cells, in leukemia patient samples, and in nonhematopoietic human tissues.

E 243 CYTOKINE RECEPTOR GENE EXPRESSION IN LYMPHOCYTIC CHORIOMENINGITIS (LCM), Anna Stalder and Iain L. Campbell, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Intracerebral inoculation of immunocompetent adult mice with the lymphocytic choriomeningitis virus (LCMV) results in a vigorous immune response in the brain culminating in death of the host around day 6 - LCM represents a prototypic model for viral meningitis. Development and progression of the disease is primarily due to the host anti-viral cytotoxic T-lymphocyte response occuring mainly in the choroid plexus and the meninges. In accordance with this, intracerebral infection by LCMV in immunodeprived, nude mice does not induce LCM and leads to a persistent infection. In recent studies we have shown that LCM is accompanied by the coordinate expression of multiple cytokine genes suggesting a likely key role for these mediators in the pathogenesis of LCM. In order to further define the role of cytokines in the molecular pathology of LCM, in the present study we analyzed cytokine receptor gene expression in mice following intracerebral infection with LCMV. PolyA$^+$RNA was isolated from brain and kidney at day 3 and day 6 post infection, and analyzed by northern blot hybridization using ^{32}P-labeled cDNA probes specific for the IL-1 type1 and type2, TNFp60 and p80, IL-6 and IFN-γ receptor gene products. In non infected mice levels of TNFp60, IL-6, and IFN-γ receptor mRNA were higher in kidney than in brain. IL-1 type1, type2 and TNFp80 transcripts were not detectable. Following infection with LCMV mRNA for IL-1 type1, TNFp60 and TNFp80, IL-6 and IFN-γ receptor were upregulated both in kidney and brain by day 3 and stayed elevated at day 6. IL-1 type2 receptor mRNA was detectable only at day 6 of infection in the brain. Parallel studies in nude mice revealed little or no change in the expression of these cytokine receptor RNA transcripts except that for IFN-γ which was downregulated in kidney and brain at day 6. In nude mice the IL-1 type2 receptor was not detectable at any time pre or post infection.

These findings indicate significant upregulation in the expression of multiple cytokine receptor genes occurs in the development of LCM. The absence of such a response in nude mice suggest the upregulation seen in LCM is intimately linked to events associated with development of the immuno-inflammatory lesion, such as mononuclear cell infiltration or production of regulatory cytokines or a combination of these.

E 244 SOLUBLE HUMAN INTERLEUKIN-6-RECEPTOR: EXPRESSION IN ESCHERICHIA COLI, PURIFICATION AND REFOLDING, Tanja Stoyan, Heidi Schooltink, Marc van Dam, Peter C. Heinrich and Stefan Rose-John, Institut für Biochemie der RWTH-Aachen, Pauwelsstr. 30, 5100 Aachen, Germany

The interleukin-6-receptor (IL-6R) consists of two polypeptide chains, an α-chain (gp 80) which binds the ligand and a β-chain (gp 130) that transduces the signal. Both molecules belong to the family of hemopoietic-growth-factor-receptors. The soluble gp 80 (sIL-6R) was recently shown to act as an agonist, i.e. it can generate a signal on cells expressing gp 130 when incubated together with its ligand. Its presence in the blood and urine of healthy patients indicates that it also plays a biological role.

A part of the extracellular domain (amino acids 145-345) of the human IL-6R was expressed in Escherichia coli and the purified protein was used to raise antibodies in rabbits. Characterization of the antiserum indicated that a single epitope of 13 amino acids close to the transmembrane region was recognized. To obtain large quantities of a soluble form of the IL-6R, its entire extracellular domain was expressed in Escherichia coli and refolded in the presence of 2 M urea, 0.2 M arginine and a glutathione redox system. The protein was purified to homogeneity using an IL-6 affinity column. Since the antiserum obtained did not interfere with IL-6 binding, it could be used to establish a cell free IL-6 binding assay. In this assay the soluble recombinant human IL-6R (srhIL-6R) bound IL-6 with an affinity of K_d = 1.5 nM as measured by Scatchard plot analysis. When ^{125}I-IL-6 was chemically crosslinked to the purified srhIL-6R and analyzed by SDS-PAGE, several ^{125}I-IL-6 containing bands were detected, indicating the possible existence of a multimeric structure of the natural IL-6/IL-6R complex. The srhIL-6R was shown to exhibit biological activity, i.e. it stimulated acute phase protein synthesis in the recently established human hepatoma cell line HepG2-IL-6 which does not express the IL-6 binding subunit of the IL-6R complex on the cell surface.

E 245 RECONSTITUTION OF THE FUNCTIONAL RECEPTORS FOR MURINE AND HUMAN IL-5, Satoshi Takaki, Yoshiyuki Murata*, Akira Tominaga*, Toshio Kitamura**, Atsushi Miyajima** and Kiyoshi Takatsu, Institute of Medical Science, University of Tokyo, Tokyo 108, Japan, *Institute of Molecular Embryology and Genetics, Kumamoto University Medical School, Kumamoto 860, Japan, **DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94303

Murine IL-5 receptor (mIL-5R) consists of two distinct subunits, α and β. The α subunit (mIL-5Rα) specifically binds mIL-5 with low-affinity. The β subunit (AIC2B) does not bind mIL-5 by itself, but forms the high-affinity receptor with mIL-5Rα. AIC2B is revealed to be the common β subunits (mβc) among the receptors for mIL-5, mGM-CSF and mIL-3. To investigate the functions of mIL-5Rα and mβc in IL-5 signal transduction, we established stable transfectants of murine IL-2-dependent CTLL-2 cells. CTLL-2 transfectant coexpressing mIL-5Rα and mβc became responsive to mIL-5 in proliferation, whereas transfectants expressing either mIL-5Rα or mβc did not. In contrast to mouse, fibroblastic cells transfected with the human IL-5Rα (hIL-5Rα) cDNA binds hIL-5 with almost same affinity compared to human eosinophils. It remains an unsolved issue how the human βc (hβc, KH97; the human homologue of AIC2B) functions in binding of hIL-5 and in transmitting IL-5 signal. IL-5 binding affinity of CTLL-2 transfectants expressing both hIL-5Rα and hβc did not differ much from that of transfectant expressing hIL-5Rα alone. Only transfectant coexpressing hIL-5Rα and hβc proliferated in response to hIL-5. Both α and β subunits are indispensable to reconstitute the functional receptors for murine and human IL-5, however, human β subunit does not contribute much in binding to hIL-5.

We next examine whether the cytoplasmic domain of the mIL-5Rα, specific subunit for mIL-5R, is necessary for IL-5 signal transduction. The mutant mIL-5Rα cDNA which lacks the whole cytoplasmic domain was constructed and was transfected into mIL-3-dependent FDC-P1 cells. The resulting transfectant did not proliferate in response to mIL-5 though it expressed the high-affinity mIL-5R. This indicates that the cytoplasmic domain of mIL-5Rα is involved in IL-5 signal transduction.

E 246 PROMISCUITY OF RANTES RECEPTORS ON HUMAN MONOCYTIC CELLS: COMPETITION FOR BINDING AND DESENSITIZATION OF RANTES RECEPTORS BY RELATED CHEMOTACTIC CYTOKINES J.M. Wang, D.W.McVicar*, J.J. Oppenheim, and D.J. Kelvin, Laboratories of Molecular Immunoregulation and * Experimental Immunology, BRMP, NCI-FCRDC, Frederick, MD 21702

RANTES is a member of the chemotactic cytokine (chemokine) ß subfamily. High affinity receptors for RANTES have been identified on a human monocytic leukemia cell line THP-1, which responded to RANTES in chemotaxis and calcium mobilization assays. Steady-state binding data analyses revealed approximately 700 binding sites/cell on THP-1 cells with a kD value of 400 pM, comparable to that expressed on human peripheral blood monocytes. The RANTES binding to monocytic cells was competed for by monocyte chemotactic and activating factor (MCAF) and macrophage inflammatory protein 1 (MIP-1) α, two other chemokine ß cytokines. Although MCAF and MIP-1α competed for RANTES binding to THP-1 cells with apparent lower affinity (with estimated Kd of 6 and 1.6 nM respectively) both of these cytokines effectively desensitised the calcium mobilization induced by RANTES. The chemotactic response of THP-1 cells to RANTES was also markedly inhibited by preincubation with MCAF or MIP-1α. In contrast, RANTES did not desensitize the THP-1 calcium mobilization and chemotaxis in response to MCAF or MIP-1α. These results, together with our previous observations on MIP-1α/ß and MCAF binding (Wang et al. J. Immunol.in press) indicate the expression of at least three distinct receptor subtypes on monocytic cells for chemokine ß subfamily members.

E 247 HUMAN INTERLEUKIN-8 RECEPTOR SUBTYPE LIGAND BINDING SPECIFICITY IS DETERMINED BY THE AMINO TERMINAL DOMAIN, M.K. White[1], K.M. Thomas[2], M., Kaufmann[1], R. Mark[1], L. Taylor[2], G. Gray[1], D. Witt[1] and J. Navarro[1], G.J. LaRosa[1],[1]Repligen Corporation, Cambridge, MA 02139; [2]Departments of Physiology and Biochemistry, Boston University Medical School, Boston, MA 02118.

Interleukin-8 (IL-8), a potent neutrophil attractant and activator secreted by many cell types, has been implicated as one of the major mediators of inflammation. In this work we report the molecular characterization of a human cDNA clone, 4Ab.il8r, encoding a receptor structurally related to the IL-8 receptor subtype encoded by the rabbit F3R cDNA (Thomas, K. M., Taylor, L. and Navarro, J. (1991) J. Biol. Chem. 266, 14839-14839). These receptors are 73% identical in amino acid sequence and exhibit seven putative transmembrane domains. Analysis of the tissue distribution of expression indicates that the message is preferentially expressed in neutrophils. CHO cells or COS-7 cells transfected with 4Ab.il8r exhibit high affinity binding to radioiodinated IL-8. The calculated apparent Ki for binding of [^{125}I] IL-8 to 4AB.il8r transfected COS-7 cells is 3.8 nM. These transfected cells also bind melanoma growth stimulatory activity protein (MGSA/GRO) with high affinity, and with lesser affinity to neutrophil activating peptide 2 (NAP-2). This novel ligand binding profile is in contrast to the receptor encoded by the F3R cDNA that exhibits binding solely to IL-8 with an apparent Ki of 3.5 nM. Chimeric receptors were constructed from the two receptor cDNAs to begin to map the molecular determinants conferring ligand specificity. Expression of the chimeras in COS-7 cells reveals that the chimeric receptors bind IL-8 with similar affinity as the parent IL-8 receptor subtypes, however, the ligand binding profile maps to the amino terminal extracellular domain; the 4Ab.il8r amino terminus confers promiscuous binding to the F3R receptor while the F3R amino terminus confers specific binding to the 4Ab.il8r.

E 248 AN ABNORMAL ERYTHROPOIETIN RECEPTOR GENE TRANSCRIPT IS EXPRESSED IN TF-1 CELLS, A HUMAN ERYTHROLEUKEMIA CELL LINE, John C. Winkelmann and Jeffery C. Ward, Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455

The erythropoietin receptor is a member of the hemopoietin/cytokine *receptor family. Receptors in* this family have been implicated in neoplastic transformation. For example, the murine erythropoietin receptor (EpoR) is involved in Friend virus induced erythroleukemia and can be activated by *in vitro* mutation into a tumorigenic form. Therefore, we are interested in the possible involvement of the EpoR in human leukemogenesis. TF-1 is a factor-dependent cell line derived from a naturally occurring human erythroleukemia. TF-1 cells over-express the EpoR and proliferate in response to Epo. Cytogenetic analysis shows that these cells have four chromosomes 19, two of which are structurally abnormal in the short arm. Because the EpoR gene maps to 19p (*Blood* 76: 24, 1990), we investigated the possibility that a structural abnormality of the EpoR gene exists in TF-1 cells. We reported previously that one of the EpoR genes is abnormal, with an apparent translocation breakpoint within exon 8, deleting the extreme 3' end of the gene (*Exp Hematol* 20:371, 1992). We used S1 nuclease analysis to determine whether the abnormal gene is expressed and, if so, to precisely map the breakpoint. A PCR-labeled probe was synthesized from EpoR cDNA from within exon 7 to the 3' end of the gene, spanning the breakpoint. The probe incorporated a small segment of plasmid to facilitate the distinction between reannealed probe and hybridization to normal EpoR mRNA. S1 nuclease analysis was performed with TF-1 cell RNA, control (+) RNA from HEL cells, and control (-) RNA (yeast tRNA). TF-1 and HEL cells expressed the normal EpoR (protected probe fragment of 909 nt). In TF-1, there was an additional 470 nt protected fragment not seen with HEL RNA. We hypothesize that this represents a transcript of the abnormal EpoR gene. Using the size of the unique protected fragment to localize the breakpoint, it maps 210 nt 5' to the translation termination codon in exon 8. This is in close agreement with the breakpoint predicted by Southern analysis. We conclude that TF-1 cells have an abnormal EpoR gene that is transcribed and that, if translated, produces a protein of altered structure. We speculate that this structurally abnormal EpoR played a role in the pathogenesis of the erythroleukemia from which TF-1 cells were derived. These results may have implications for other members of the receptor family.

E 249 STRUCTURAL ASPECTS OF IL-1 BINDING TO THE TYPE I HUMAN IL-1 RECEPTOR. Peter R. Young and Richard Einstein, Department of Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, Pa. 19406-0939.

While there have been many studies detailing the regions of IL-1 required for receptor binding, there has been little characterization of what is required in the receptor and how it responds to the binding of ligand. To examine these questions, we have cloned and expressed the extracellular domain of the human type I receptor in *Drosophila Schneider S2 cells using a copper inducible* metallothionein promoter. The secreted, partially purified receptor was found to bind IL-1β with a K_D of 1.25nM, close to the value observed upon expression of the full length receptor on human osteosarcoma cells. When the receptor was completely deglycosylated with N-glycanase, the K_D increased to 0.84μM, indicating that mannose is important for IL-1β binding. To assess the effect of ligand binding on receptor conformation, the receptor was partially digested with various proteases in the presence and absence of excess IL-1α, IL-1β, IL-1ra and the site specific IL-1β mutant T9G, which has wild-type affinity but 500x reduced bioactivity, and the results analyzed by Western blot with region specific receptor antisera. These data show that the receptor becomes considerably protease resistant *upon ligand* binding, suggesting protection by ligand and/or a more compact structure. The proteolytic patterns of the receptor differed with each bound ligand, suggesting ligand induced specific conformational changes. These changes may have implications in the different biological activities observed between ligands.

E 250 HUMAN CD69: A T CELL ACTIVATION MOLECULE THAT IS A MEMBER OF THE NKR-PI FAMILY, Steven F. Ziegler, Kathryn M. Hjerrild, Kathryn B. Hennen, Kenneth H. Grabstein, Richard J. Armitage, Fred Ramsdell, William C. Fanslow and Mark R. Alderson, Immunex Corporation, 51 University St., Seattle, WA 98101

The activation of resting T cells results in the expression of several cell-surface molecules, including CD69. CD69 is a cell-surface homodimer comprised of differentially glycosylated subunits. Cross-linking of CD69 in the presence of phorbol ester results in enhanced T cell proliferation, through the induction of IL-2 expression, as well as functional activation. Using an expression-cloning strategy we have cloned a cDNA encoding human CD69 from a human T cell clone activated through the CD3 complex. Stimulation of human peripheral blood T cells with a variety of stimuli induces the expression of a 1.6 Kb mRNA. Analysis of the predicted amino acid sequence of human CD69 showed that it is a member of the NKR-PI family of type II membrane glycoproteins. Members of this family also include several cell-surface molecules involved in NK cell function, as well as the low-affinity IgE receptor (CD23) and the asialoglycoprotein receptor. We are currently studying the expression and function of human CD69.

E 251 **ANALYSIS OF HUMAN ERYTHROPOIETIN RECEPTOR FUNCTION BY MUTAGENESIS AND GENE TRANSFER, Teresa A. Zimmers, W. David Hankins, Donna M. Williams, Ling-Mei Wang, Alan N. Schechter, and Jacalyn H. Pierce, LCB, NIDDK, and LCMB, NCI, NIH, Bethesda, MD 20892.**

The precise role of erythropoietin (Epo) and its receptor in the development and differentiation of erythroid cells is still unclear. To test whether Epo functions as a viability agent and/or as a differentiation agent, we cloned the human erythropoietin receptor (hEpoR) cDNA and transfected it into the IL-3 dependent murine myeloid precursor cell line, 32D. 32D-hEpoR cells exhibited dose-responsive dependence on Epo for viability and proliferation, while retaining IL-3 responsiveness. Also, a mutated (129R-->C) "constitutively" activated hEpoR cDNA rendered transfected cells growth-factor independent. Epo binding to both normal and mutant hEpoRs was demonstrated. Epo-induced phosphorylation was assessed by immuno-precipitation and Western blotting of Epo-triggered and untriggered cell lysates with a monoclonal antiphosphotyrosine antibody. Epo-dependent phosphorylation patterns distinct from those induced by IL-3 were observed in both normal and mutated EpoR transfectants. We also assessed the capacity of these cells to differentiate. When propagated in IL-3, 32D cells maintain an immature myeloid phenotype; upon stimulation with granulocyte-colony stimulating factor (G-CSF), however, the cells differentiate to mature neutrophils. 32D-hEpoR cells retained the ability to differentiate to neutrophils in the presence of G-CSF as determined by myeloperoxidase positivity and nuclear segmentation. 32D-hEpoR transfectants exposed to both Epo and G-CSF, however, did not exhibit these characteristic markers of myeloid differentiation. Neither cells expressing normal nor mutated hEpoR were shown to produce hemoglobin in the presence of Epo. These data indicate that hEpoR can functionally substitute for IL-3 receptor and support viability, proliferation and tyrosine phosphorylation in murine myeloid cells. As well, EpoR and its ligand, Epo, can prevent G-CSF induced differentiation of these cells.

In Vitro and In Vivo Biological Effects of Cytokines

E 300 DIVERSE "GROWTH FACTORS"/CYTOKINES INDUCE T CELL MIGRATION IN VITRO. David H Adams, Yoshiya Tanaka, Leslie Stiff, John Yannelli, Stephen Shaw, The Experimental Immunology Branch, NIH, Bethesda MD 20892

T cells migrate extensively during the process of immune surveillance under the influence of locally active chemotactic factors which include conventional cytokines. However the pleitropism of cytokines and growth factors makes it likely that other cytokines/growth factors also effect T cell migration. We examined the ability of the following structurally diverse growth factors to induce T cell migration in vitro using microchemotaxis chambers and highly purified populations of resting human peripheral blood T cells: 1) chemokine/intercrine members macrophage inflammatory protein-1β (MIP-1β), macrophage inflammatory protein-1α (MIP-1α), gro/MGSA 2) hepatocyte growth factor (HGF) 3) transforming growth factor α (TGFα).

All the factors induced dose dependent migration of resting human T cells at concentrations of 1-50 ng/ml although MIP-1α was a weak inducer of migration only at concentrations of 50 ng/ml. When highly purified T cell subsets were analysed several showed preferential activity for particular T cell subsets, although none of the factors showed complete subset specificity. Both gro/MGSA and HGF preferentially attracted CD4 and CD8 T cells with the CD45RO+ (memory) phenotype whereas MIP-1β showed preferential activity for CD45RO- CD8 T cells and TGFα attracted predominently CD45RO- CD4 T cells. All the above factors were also chemotactic for activated, cultured tumour infiltrating lymphocytes.

These results suggest that: 1) A wide range of growth factors/cytokines might be involved in the regulation of T cell migration 2) Such factors display preferential activity for T cell subsets and could contribute to the differential homing of T cell subsets 3) HGF and TGFα act on a broader range of cell types than previously thought suggesting that other factors conventionally classified as "growth factors" might also act as "cytokines".

E 301 RESPONSIVNESS OF ENDOTHELIAL CELLS TO IL-1 - CELL CYCLE ANALYSIS OF ICAM-1 EXPRESSION, Ann L. Akeson, Kendra K. Schroeder, Connie W. Woods and Terry L. Bowlin, Department of Immunology, Marion Merrell Dow Research Institute, Cincinnati, OH,45215

Vascular endothelial cells (EC) are active participants in immune and inflammatory responses. Their participation is mediated in part by IL-1. We have previously shown that unstimulated human EC derived both from aorta and umbilical vein express only the type I receptor for IL-1 (J. Cellular Physiol., In Press) with a Kd of 3 x10 $^{-10}$M and 1,000 to 3,000 receptors per cell. Yet EC respond to as little as 10pg/ml (6x10^{-16}M) IL-1β. For instance, analysis by flow cytometry shows that after 18 hr with 10pg/ml IL-1β, ICAM-1 expression by EC increases 4 to 10-fold, with 5-10% of control cells ICAM-1 positive and 43-65% of treated cells ICAM-1 positive. Analysis of mRNA levels shows that IL-1 induces a rapid increase in ICAM-1 mRNA with maximum levels 2-3 hr after stimulation. Cell cycle analysis by flow cytometry shows that a short pulse, 60 min, of IL-1β does not influence the number of cells leaving G_1 or transversing the cycle when measured 5 hr later. At this time 60-70% of both untreated control cells and IL-1-treated cells are in G1. Yet, 1 hr exposure to IL-1β can dramatically increase the number of cells expressing ICAM-1 from 10-30% of control populations to 85-95% of the treated population. Exposure of the cells to IL-1β (250 pg/ml) as briefly as 15 min significantly increases the number of EC expressing ICAM-1 with 26% of cells ICAM-1 positive 5 hr later. Cell cycle analysis of the ICAM-1 positive control EC shows that there are fewer cells in G1, 40-50%, and increased cells in S, G2, M, indicating that it is the cells transversing the cell cycle which express ICAM-1. In the IL-1 treated population there is a slight increase in the number of cells in G1 to 54-60%. The cell cycle analysis of both untreated and IL-1-treated ICAM-1 negative populations shows the majority of cells, 78-90% are in G1. These studies indicate that the majority of EC progressing through the cell cycle express ICAM-1. Treatment with a pulse of IL-1 dramatically increases the number of cells expressing ICAM-1 but does not influence the number of cells entering the cell cycle.

E 302 MODULATION OF HEPATIC DRUG METABOLISM BY CYTOKINE ADMINISTRATION IN MICE. Sherry S. Ansher*, Walter C. Thompson*, and Raj K. Puri**, Division of Bacterial Products* and Division of Cytokine Biology**, CBER, FDA, Bethesda, MD. 20892.

We have previously demonstrated that administration of vaccines and the cytokines interleukin-2 (IL-2) and interferon-α, alone and in combination, cause alterations of hepatic drug metabolism (Ansher *et al.*, Vaccine 9: 277-283, 1991; Ansher *et al.*, Cancer Res. 52: 262-266, 1992). Increased levels of serum interferon-γ (IFN- γ), tumor necrosis factor (TNF) and interleukin-6 (IL-6) were observed in mice that had received DTP vaccine (Ansher et al., Infect. Immun. 60: 3790-3798, 1992). Furthermore, we have found an induction of IL-1 and IL-6 mRNA levels in the livers of DTP-vaccine-treated mice. In order to evaluate whether these increased levels of cytokines are related to the alterations of hepatic drug metabolism, we have administered TNF, IL-1 and IFN-γ to mice. Administration of TNF or IL-1 caused dose dependent increases in hexobarbital-induced sleep times of 1.5 to 2-fold above controls, concomitant with 40-50% decreases of spectral cytochrome P-450 levels. Similar effects were observed with DTP vaccine. These changes were accompanied by specific alterations in microsomal enzyme activities responsible for the metabolism of narcotics and other drugs. Ethylmorphine demethylase activity was decreased by 20-30% after administration of IFN-γ , but not IL-1 or TNF, and cocaine demethylase activity was also decreased more by IFN-g than IL-1 or TNF. These results are consistent with a role for cytokines in the alterations observed following vaccine administration.

E 303 DIFFERENTIAL REGULATION OF NK CELL IFNγ SYNTHESIS BY IL-10 AND IL-2. GJ Bancroft, JP Kelly & S Rajagopalan. London School of Hygiene & Tropical Medicine, London WC1E, UK.

We have utilized scid mice to examine the regulation of macrophage functions by cytokines derived from natural killer (NK) cells. In the absence of T cells, NK cells were found to provide an initial defence against infection of scid mice with the bacterium Listeria monocytogenes, via secretion of the macrophage activating cytokine IFNγ. We now demonstrate that T cell derived cytokines are potent modulators of NK cell IFNγ synthesis in vitro. IL-10, a product of Th2 cells abolished IFNγ secretion by scid spleen cells triggered with heat killed Listeria, whereas the Th1 cell product IL-2 dramatically upregulated this response. The actions of IL-10 were macrophage dependent and mediated at least in part by suppression of the production of TNF. In contrast, direct activation of NK cells by recombinant TNFα plus IL-2 induced IFNγ secretion which was not susceptible to inhibition by IL-10. Thus, as in the T cell mediated IFNγ response, IL-10 inhibits IFNγ secretion from NK cells by impairing an essential macrophage accessory function in vitro. Finally, administration of IL-10 during infection of scid mice with live Listeria increased bacterial replication by 80-200 fold and resulted in overwhelming sepsis. In conclusion, IL-10 is a potent inhibitor of bacteria induced TNF and IFNγ secretion, two cytokines which are essential for resistance to infection in vivo. The contrasting effects of IL-2 and IL-10 suggest that expansion of Th1 versus Th2 T cell subsets may differentially regulate NK cell activity during infection of the immunocompetent host.

E 304 EXPRESSION OF CYTOKINE GENES DURING FETAL AND POSTNATAL HUMAN T-CELL DEVELOPMENT, Alicia Bárcena, Sheela Mohan-Peterson, Sunita Verma, Bart Vandekerckhove, Maria-Grazia Roncarolo and Hergen Spits, DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304.

Triple negative (TN) thymocytes ($CD34^+2^+7^+3^-4^-8^-$) is the most immature subset present in both fetal and postnatal thymus and undergo a poorly defined proliferation, differentiation and selection processes to give rise a mature and functional peripheral T-cell repertoire. By phenotypic analysis we identified three subsets of $CD3^-$thymocytes ($CD4^-8^-$, $CD4^+8^-$ and $CD4^+8^+$) and four subpopulations of $CD3^+$thymocytes: $CD4^-8^-$, $CD4^+8^+$, $CD4^+8^-$ and $CD4^-8^+$. To better define the role of several cytokines on T-cell development, we performed PCR analysis on fetal thymuses from 14 up to 21 weeks of gestational age and on postnatal thymus showing that IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10 and IFN-γ are constitutively expressed on unseparated thymocytes. By semiquantitative PCR method and by *in situ* hybridization we demonstrated that IL-2 and IL-4 genes are expressed very early in thymic T-cell development and these cytokines are not expressed in the $CD34^+$ $lineage^-$ population sorted from the fetal liver, which contains T-cell precursors as we have shown by intrathymic injection in an *in vitro* fetal thymic organ culture (FTOC). The highest level of expression of IL-4 is detected on $CD3^-4^+8^-$ subset and on $CD3^+4^+8^+$ immature thymocytes. In contrast, the highest level of IL-2 expression is found in mature $CD3^+$ thymocytes. IFN-γ expression is detected in TN, in $CD3^+4^-8^-$ and mature $CD8^+$ thymocytes. Very low expression of IL-10 gene is observed in immature thymocytes. These results suggest that cytokine genes are developmentally regulated during intrathymic T-cell development.

E 305 **C10, A MEMBER OF THE SIG CYTOKINE FAMILY LOCATED ON MOUSE CHROMOSOME 11, CONTAINS A NOVEL SECOND EXON NOT FOUND IN OTHER FAMILY MEMBERS**, Berger M, Kozak C, Prystowsky M, Departments of Medicine and Pathology, University of Pennsylvania School of Medicine, Phila., PA and Viral Biology Section, NIH, Bethesda, MD.

C10 is a member of the MIP-1α subfamily of the SIG (small, inducible gene) superfamily of cytokine proteins which was originally isolated from a cDNA library made from GM-CSF stimulated murine bone marrow cells. Genomic clones encoding the murine C10 gene were isolated and sequenced. The four cysteines that define the SIG superfamily occur in a spatially conserved fashion in exons two and three in other family members; in C10 these cysteines are found in exons three and four and a novel second exon of 48 nucleotides is inserted before them. The novel exon contains a high percentage of charged amino acids, and does not have homology to any known sequence on searching of computer databases. PCR analysis of C10 mRNA in several cell lines suggests that the second exon is not a splice variant. The genetic locus for C10 is closely linked to the *Sigje* locus on mouse chromosome 11. C10 contains a novel exon not found in any other members of the SIG superfamily and may have evolved from other MIP-1α family members located nearby on mouse chromosome 11.

E 306 **IDENTIFICATION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) AS AN LPS-INDUCED PITUITARY PROTEIN *IN VITRO* AND *IN VIVO***, Jürgen Bernhagen, Robert A. Mitchell, Kevin J. Tracey, Stanley B. Martin, Kirk R. Manogue, Wolfgang Voelter*, Anthony Cerami, and Richard Bucala, The Picower Institute for Medical Research, Manhasset, NY 11030, and *Physiologisch-chemisches Institut, University of Tübingen, 7400 Tübingen, Germany.

Soluble cytokines play a critical role in the systemic host response to inflammation. To investigate the role of the neuroendocrine axis in the inflammatory cytokine network, we studied the secretory response of murine anterior pituitary cells (AtT-20) to bacterial lipopolysaccharide (LPS). Pituitary cell-conditioned media was analyzed by gel electrophoresis and silver staining. A 12 kD protein, induced specifically by LPS in a time- and concentration-dependent manner was isolated and subjected to N-terminal micro-sequencing analysis. This protein was identified as the murine homolog of human macrophage migration inhibitory factor (MIF) (96% identity over 27 amino acids). To assess the role of pituitary-derived MIF *in vivo*, BALB/c mice were injected with LPS (2-5 mg/kg, ip), the pituitaries were removed at intervals, mRNA was extracted and analyzed by reverse transcription and DNA amplification. Messenger RNA levels for MIF increased with time and reached a plateau at 16-24 hr. Induction kinetics varied with the dose of LPS injected. Messenger RNA analysis of isolated pituitaries for lymphocytic markers were uniformly negative, supporting the conclusion that MIF was induced in pituitary cells rather than contaminating lymphoid cells. These studies indicate that anterior pituitary cells produce MIF *in vivo* and that MIF is likely to play a central role in the systemic response to inflammatory stimuli.

E 307 SERUM INTERLEUKIN-2 RECEPTOR CONCENTRATIONS, PHYSICAL ACTIVITY, AND MICRONUTRIENT NUTRITION IN OLDER PEOPLE. John D. Bogden, Francis W. Kemp, Benjamin L. Liberatore, Suzanne Katz, Zhengang Yang, and Adrianne Bendich, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2714

There is evidence that both dietary factors and physical activity may influence immune functions. Previous studies demonstrate that IL-2 production is decreased, but serum IL-2 receptor levels are increased, in older people. This may be a factor in the decline of cellular immunity with age, since high serum concentrations of soluble IL-2 receptors may decrease IL-2 binding to T cell receptors. We studied the influence of physical activity and supplementation with an over-the-counter formulation containing 22 essential vitamins and trace elements on serum IL-2 receptor concentrations. The formulation was given to 25 subjects aged 60-85 for one year; 23 age matched control subjects were given a placebo. Participants were asked 22 questions about daily activities that provide a quantitative assessment of physical activity. Data were evaluated by analysis of variance. Serum IL-2 receptor levels at the start of the study and after 6 months of supplementation were not significantly different for the vitamin and placebo groups. After 12 months, there was a statistically significant effect of physical activity ($p=.02$) on serum IL-2 receptor levels and a significant physical activity-vitamin supplementation interaction ($p=0.03$). Mean IL-2 receptor levels were significantly higher in the less physically active subjects (676 + 193 U/ml) than in the more active subjects (450 + 26 U/ml). In addition, there was a statistically significant correlation ($r=0.505$, $p < 0.02$) between physical activity and serum IL-2 receptor levels in the vitamin but not the placebo group. The data suggest that nutritional factors and physical activity may interact to influence serum IL-2 receptor levels. The increase in serum IL-2 receptor levels found in older people may be due in part to decreased physical activity, increased micronutrient requirements, and/or changes in nutritional status that occur with aging. (Supported in part by grants from Hoffmann La-Roche, Inc.)

E 308 T CELL PROLIFERATION WHICH CANNOT BE INHIBITED BY GLUCOCORTICOIDS IS APPARENTLY NOT DRIVEN BY IL-2, Ben Boldt, Loren Heun[1], Tom Vidmar[2], and Ann Berger. Cell Biology, [1]Laboratory Automation and [2]Research Support Biostatistics, The Upjohn Company, Kalamazoo, MI, 49001.

Glucocorticoids (GCs) inhibit human peripheral blood mononuclear cell (PBMC) proliferation induced by a variety of stimulants. The stimulant dose influences the level of inhibition seen, i.e., in the presence of an optimum GC concentration, the lower the stimulant dose, the higher the observed inhibition. Since IL-2 is required for the proliferation of activated T cells, we examined whether IL-2 levels correlated with the apparent stimulus-dose dependent inhibition of proliferation. In cultures of human PBMC stimulated with increasing concentrations of superantigen, mitogen or antibody to the T cell receptor, we identified the GC concentration required for 50% of the maximum achievable inhibition ($10^{-8.5}$M dexamethasone.) This GC concentration was the same as the K_d of the GC receptor for the GC. This GC concentration was the same for PBMC proliferation and IL-2 production and was not dependent on stimulus dose or type. This correlation suggested that GC-mediated inhibition of IL-2 production contributed to the inhibition of PBMC proliferation.
The amount of proliferation and IL-2 which remained in the presence of optimum concentrations of GC was significant (45-85% proliferation and 20-40% IL-2 production), especially at high stimulus concentrations and late time points. This proliferation and accompanying IL-2 production was, in contrast, dependent on stimulus dose and type. Further, since anti-IL-2 antibodies failed to inhibit this residual proliferation, it appeared to be IL-2 independent. The role of other cytokines in this proliferation is under investigation.

E 309 A TRANSGENIC MOUSE MODEL FOR STUDYING IL-2 EXPRESSION IN SINGLE LIVING CELLS IN VIVO, Brombacher Frank, Schaefer Thomas, Greiner Brigitte, Weissenstein Ulrike, Tschopp Claude, Mueller Kurt, Buerki Kurt and Baumann Goetz, Preclinical Research, Building 386/356, Sandoz Pharma Ltd, CH-4002-Basel, Switzerland

We developed a transgenic mouse model based on a human IL-2 promotor-driven reporter gene (*lacZ* from *E. coli*), which allows us to monitor IL-2 expression in living mouse cells directly at a single cell level by FACS-analysis. We could show, that transgene expression is faithfully regulated as compared to the endogenous IL-2 gene. Mitogen-induced expression is restricted to a subpopulation of mature T-cells, including helper ($CD4^+$) and cytotoxic T-cells ($CD8^+$), and is inhibited as the endogenous IL-2 gene by immunosuppressive agents, like cyclosporin A (Sandimmun). Further on, we were able to visualize the transitional stages during the autocrine T-cell activation process with respect to IL-2 and IL2 receptor surface expression. Mitogen-induced transgenic T-cells ($thy1.2^+$) from lymphnodes, stained for β-galactosidase expression appeared prior to the IL2R positive T-cells.
Employing this transgenic mouse model, the temporal and tissue-specific pattern of IL-2 expression is being characterized. In this context it will provide additional information on the appearance of TH1 cells and will help to study established mouse disease models (i.e. MAIDS). Additionally, we will use these mice as a pharmacological model for the in vivo characterization of immunosuppressive compounds and their side effects.

E 310 LYMPHOTOXIN-ß: A NEW MEMBER OF THE TNF FAMILY THAT FORMS A HETEROMERIC COMPLEX WITH LYMPHOTOXIN ON THE CELL SURFACE

Jeffrey L. Browning*, Richard Tizard*, Apinya Ngam-ek*, Pornsri Lawton*, Janice DeMarinis*, E. Pingchang Chow*, Catherine Hession*, Betsy Greco*, Susan Foley* and Carl F. Ware# Biogen* Cambridge Ma. and University of California, Riverside Ca.#

The lymphokine TNF has a well-defined role as an inducer of inflammatory responses; however, the function of the structurally related molecule, lymphotoxin (LT-α), is essentially unknown. Previously, we had noted that LT-α is present on the surface of activated T-, B- and LAK cells as a complex with a 33 kDa glycosylated integral membrane protein. Cloning of the cDNA encoding the associated protein, now called lymphotoxin-ß (LT-ß), revealled it to be a type II membrane protein with significant homology to TNF, LT-α and the ligand for the CD-40 receptor. The nature of the homology regions suggests that the LT-α/ß complex also retains a TNF-like trimeric structure. In transient expression experiments, LT-α is normally secreted, but when co-transfected with LT-ß, surface LT-α forms were detected. The addition of LT-ß to the family of TNF-related ligands indicates that the TNF family is more extensive than previously realized and raises the possibility that the surface LT-α/ß complex may have a specific role in immune regulation distinct from the functions ascribed to TNF.

E 311 INTERLEUKIN-4 DEFICIENT MICE AND ALLERGIC AIRWAY INFLAMMATION, Guy G. Brusselle, Claude A. Cuvelier, José Van Der Heyden, Jan H. Tavernier, Horst Blüthmann, Johan C. Kips and Romain A. Pauwels, Department of Respiratory Diseases, University Hospital Ghent and Roche Research Laboratories, Ghent B-9000, Belgium.
Elevated serum IgE levels and bronchial eosinophilia are both hallmarks of allergic asthma. To investigate the in vivo role of Interleukin 4 (IL-4) in both phenomena ,we developed a murine model of allergen-induced IgE-mediated eosinophilic airway inflammation. When C57Bl/6 mice were actively immunized to ovalbumine (OVA) and exposed repeatedly to aerosolized OVA for 7 days, differentiation of BAL cells revealed a vast increase of eosinophils ($p<0.001$) and a significant increase of lymphocytes ($p<0.01$) compared with saline (SAL)-exposed unimmunized animals.Histologic analysis of lungs of OVA-immunized and -exposed animals showed peribronchiolar inflammatory infiltrates, mainly composed of lymphocytes, plasma cells and eosinophils.
When IL-4 deficient (IL-4 o/o) mice were actively immunized and repeatedly exposed to aerosolized Ag, differential white blood cell counts of BAL cells revealed a selective and striking reduction in the number of eosinophils, compared with Ag-immunized and -exposed IL-4 wild type (IL-4 +/+) mice ($p<0.001$). Moreover, histologic examination of lungs of Ag-treated IL-4 o/o mice demonstrated clearly less peribronchiolar inflammation.
In conclusion, these results indicate that IL-4 is not only essential for IgE synthesis in vivo, but also a central mediator of allergic eosinophilic airway inflammation.

E 312 INDUCTION OF NEUROLOGIC DISEASE IN TRANSGENIC MICE AS A CONSEQUENCE OF THE ASTROCYTE-SPECIFIC EXPRESSION OF INTERLEUKIN-6, Iain L. Campbell, Michael B.A. Oldstone, Carmela R. Abraham* and Lennart Mucke, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037 and *Boston University Medical Center, Boston University, Boston, MA 02118-2394.
Altered expression of cytokines, including interleukin-6 (IL-6) has been found in a variety of CNS disorders such as multiple sclerosis, AIDS dementia complex (ADC) and Alzheimer's disease. In spite of their known pleiotropic and potent cellular actions, evidence that cytokines play a direct role in causing neuropathology is lacking. We therefore have investigated this problem by way of a transgenic approach in which the inappropriate expression of IL-6 was targeted to astrocytes in the CNS. A cDNA spanning the entire coding region for murine IL-6 was inserted in the first exon of the astrocyte specific murine glial fibrillary acid protein (GFAP) genomic DNA. Following microinjection of the chimeric DNA, a number of transgenic offspring were found to develop a phenotype characterized by growth retardation, piloerection, tremor, ataxia, hind-limb weakness and seizures. In all such mice high levels of IL-6 mRNA were expressed in the brain (northern blot analysis) particularly in the cerebellum and thalamic regions (in situ hybridization). In one case examined so far, production of IL-6 bioactive protein was demonstrated from astrocyte cultures derived from a transgenic mouse. Parallel cultures derived from control mice did not produce detectable IL-6 bioactivity. No disease phenotype was seen in non-transgenic littermates. Histopathologic examination of the brain from IL-6 expressing mice revealed occasional scattered perivasculitis and significant neovascularization; neither dysmyelination nor neuronal loss were evident. As determined by immunocytochemical staining and northern blot analysis, significant increases in the expression of GFAP and von Willebrand factor gene products revealed pronounced astrogliosis and angiogenesis respectively. These findings indicate that inappropriate expression of IL-6 in the CNS induces a number of molecular and pathologic changes in association with the development of clinical neurologic disease. This model, which recapitulates many of the features seen in some human CNS diseases e.g. ADC, should prove extremely valuable for elucidating the role of cytokines in neuropathogenesis.

E 313 VITAMIN A DEFICIENCY AND HELPER T CELL SUBSET REGULATION IN T. SPIRALIS INFECTION, Margherita T. Cantorna, Faye E. Nashold, and Colleen E. Hayes, Department of Biochemistry, University of Wisconsin-Madison.
We previously showed that lymphocytes from immune, vitamin A-deficient (A-) mice secreted (3-fold) more IFNγ than vitamin A-sufficient (A+) mice, and retinoid addition in vitro decreased IFNγ production. What cells are the target(s) of retinoid-mediated IFNγ down regulation? Vitamin A could act directly on the IFNγ secretor (TH1). Or it could control antigen-presenting cell (APC), or TH2 cell function, which then could regulate the IFNγ secretor. To investigate these possibilities, we analyzed IL-5, IL-10, and IFNγ secretion, to trace TH1 and TH2 subset development in A- and A+ Trichinela spiralis-infected mice. IL-5 and IFNγ but not IL-10 secreting cells developed differently in T. spiralis-infected mice. IFNγ ELISAs and filterimmunoplaque assays were used to show that A- MLNC cultures had higher IFNγ secretion rates then A+ MLNC cultures, although the secreting cell frequencies were the same. Furthermore, addition of retinoic acid (RA) altered IL-5 and IFNγ but not IL-10 secretion rates. RA-mediated changes in IL-5 secretion were not due to changes in IFNγ levels. Finally, the functions of A+ and A- APC and A+ and A- T cells were tested, and we determined that vitamin A deficiency increased the ability of the APC to stimulate TH1 cell IFNγ production, and T cell IFNγ secretion. Our experiments support a mechanism whereby vitamin A acts directly on IFNγ-producing T cells and on APC to decrease IFNγ secretion rates. Furthermore we present evidence that vitamin A may preferentially activate and/or expand TH2-type cells. Together our work suggests that vitamin A is a potent regulator of the immune response.

E 314 ENDOGENOUS CYTOKINE PRODUCTION IN RESPONSE TO SEPSIS IN NEUTROPENIC AND NON-NEUTROPENIC PATIENTS
Jonathan Cebon[1], Judith E. Layton[1], Darryl Maher[1] and George Morstyn[2], [1]Ludwig Institute for Cancer Research, PO Royal Melbourne Hospital, Victoria, 3050, Australia, [2]AMGEN, Thousand Oaks, California, 91320-1789, USA
A prospective survey of patients with neutropenic fever (46 episodes), bacteremia without neutropenia (20 episodes), afebrile neutropenic controls (14) and normal controls (20) was performed to define the endogenous serum colony stimulating factor (CSF) response to sepsis and neutropenia. Specific assays were used to study granulocyte (G-)CSF, granulocyte-macrophage (GM-) CSF, macrophage (M-) CSF and interleukin (IL-)6 levels in the blood.

Cytokine (ng/ml)	Febrile median, range	Non-febrile median, range
G-CSF	0.46,<0.10-142,	<0.10, <0.10-1.62,
IL-6	0.054, 0.005-24.3,	0.008, 0.002-0.024
M-CSF	18.5, 9.9-79.1,	6.45, <5.0-31.3
GM-CSF	<0.02, <0.02-8.0	0.021, <0.02-0.20

Multiple regression analyses identified the variables which were significantly associated ($P < 0.05$) with elevated cytokine levels. For G-CSF these were fever, neutropenia, pathogen type and raised bilirubin and creatinine. Although also associated with fever, Gram negative, fungal infections, raised creatinine and raised bilirubin, there was no association between IL-6 elevation and neutropenia. M-CSF elevation was associated with fever, renal failure and known pathogen. Elevated G-CSF and IL-6 levels normalized rapidly as infection resolved. In contrast, M-CSF concentrations remained elevated for up to 10 days. Cytokine levels did not normalize in neutropenic patients with uncontrolled sepsis. Endogenous cytokine levels are now being studied in febrile neutropenic patients receiving standard antibiotics and randomised to receive either G-CSF or placebo. This should help to define the value of cytokine quantitation in the treatment of sepsis.

E 315 TH1 AND TH2 LIKE CYTOKINE PRODUCTION IN ASYMPTOMATIC, HIV-SEROPOSITIVE INDIVIDUALS
Mario Clerici and Gene M. Shearer. Experimental Immunology Branch N.C.I, N.I.H, Bethesda, MD 20892
We measured the production of Interleukin-2 (IL-2)(a TH1-like cytokine) and Interleukin-4 (IL-4)(a TH2-like cytokine) in peripheral blood leukocytes (PBL) of asymptomatic Human Immunodeficiency Virus (HIV) seropositive individuals, to determine whether different profiles of dysfunction could be detected in such patients. Antigen or mitogen stimulated culture supernatants were tested in bioassay using either the CTLL IL-2 dependent or the CT.h4S (kind gift of Dr. W.E.Paul and Ms. C.Kinzer) IL-4 dependent cell lines. Three different functional patterns, independent of the number of $CD4^+$ lymphocytes, were recognized in HIV seropositive individuals: (1) high IL-2 and low IL-4 (n=16); (2) low IL-2 and high IL-4 (n=19) and (3) low IL-2 and low IL-4 (n=10). A high IL-2/low IL-4 pattern was characteristic of HIV seronegative healthy controls. The reciprocal patterns of lymphokine production could be shown as IL-2: IL-4 ratios, with the highest ratio in group (1) and progressively decreasing ratios for groups (2) and (3). IL-2 and IL-4 production from longitudinally cryopreserved specimens from a donor followed for more than three years demonstrated that the three profiles are sequential such that (1) -->(2) -->(3). To investigate a possible cross-regulation of TH2 like cytokines on production of TH1 like cytokines, PBL from patients with a low IL-2/high IL-4 profile were stimulated in vitro in the presence of an anti-IL-4 antibody. Restoration of antigen-stimulated proliferation was observed in several of such individuals. Based on these data, we hypothesize a progressive change in T helper function after HIV infection, such that a first stage in which IL-2>IL-4, is followed by a second stage in which IL-4>IL-2 and, finally by a reduction in production of both IL-2 and IL-4. These results suggest that cytokine-cross regulation is involved in the early loss of T helper function in HIV-infected individuals, and raise the possibility of cytokine-based therapy in such individuals.

E 316 PROLACTIN-INDUCED PROLIFERATION OF MURINE PRO-B LYMPHOID CELLS,
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Prolactin (PRL) was originally identified as the main hormone controlling lactation. However, recent studies suggest an immunomodulatory role for prolactin in mammals. In addition, PRL receptors were shown to be members of the recently recognized cytokine receptor superfamily. To further investigate PRL actions on hematopoietic cells, the rabbit mammary PRL receptor cDNA (PRL-R) was introduced in the BaF-3 mouse IL3-dependent pro-B lymphoid cell line by cotransfection with neomycin resistance gene. Stable transfectants were selected for G418 resistance and studied for PRL responsiveness. In contrast to parental cells, PRL-R transfectants were shown to proliferate in presence of PRL in a concentration-dependent manner in the absence of IL3. Maximal proliferation was observed at 10 ng/ml ovine PRL. In addition, PRL-R transfectants were maintained for up to 6 months in the presence of PRL instead of IL3 without any loss of proliferative responsiveness. These transfectants expressed around 5,000 PRL-R per cell and displayed only low affinity forms of PRL-R (Kd= 2 10^{-9}M). PRL-R were characterized by cross-linking studies and a PRL-R binding subunit of Mr 100,000 was identified. Depending on the cross-linking agent used, a second associated PRL receptor subunit of Mr 125,000 was also identified. Studies on the mechanism of PRL action in these cells indicated that PRL induced specific tyrosine phosphorylation of proteins associated with the PRL receptor within a few minutes at 37°C. These results indicate that the cloned cDNA coding for the long type PRL receptor chain can be functional and deliver a mitogenic signal when placed in an hematopoietic cellular context.

E 317 CYTOKINE GENE EXPRESSION IN VIVO: THE DISTINCT CONTRIBUTION OF CD4+ AND CD8+T-CELLS IN THE MEMORY RESPONSE TO *Listeria monocytogenes*.
Stefan Ehlers, Martin E.A. Mielke, Helmut Hahn. Inst. f. Med. Mikrobiologie und Infektionsimmunologie, FU Berlin, 1000 Berlin 45, Germany.

In order to elucidate the mechanisms operative during the decisive early phase of infection with L. monocytogenes, we conducted a comprehensive kinetic and quantitative analysis of cytokine gene expression in the livers of naive and immune mice using PCR-assisted mRNA amplification. The cytokine pattern characteristic of secondarily infected animals differed qualitatively by the expression of mRNAs for IL-2, IL-2Rp55, IL-3 and IL-4, demonstrating the accumulation and activation of specific T-cells in the livers as early as 1 - 2 hours after reinfection. Combined in vivo depletion of both CD4+ and CD8+ T-cells prior to reinfection almost completely abrogated the differentiated cytokine profile typical of the anamnestic response. Individual depletion of CD4+ or CD8+ T-cells revealed that the CD4+ subset alone is responsible for expression of these lymphokines. Using competitive PCR for semiquantitative determination of mRNA levels, the amount of IL-1β and IL-6 mRNAs was found to be very similar during primary and secondary infection, while TNFα mRNA was found to be increased by approx. 10-fold 2 hours and IFNγ mRNA by approx. 50-100-fold 6 hours after reinfection when compared to a primary challenge. Combined in vivo depletion of both CD4+ and CD8+ T-cells before reinfection resulted in a substantial (approx. 10-fold) decrease in IFNγ mRNA expression. Again, depletion of CD8+ cells alone had no effect on the amount of IFNγ mRNA expressed. Therefore, the expression of IL-2, IL-3, IL-4, increased amounts of IFNγ and TNFα best correlates with the presence of CD4+ cells which are known to be indispensable for granuloma formation. In addition, ex vivo PCR shows that the mechanisms by which CD8+ cells mediate their functions as the principal mediators of bacterial clearance during reinfection still remain unsolved.

E 318 EFFECT OF DISULFIDE BONDS ON THE STRUCTURE AND ACTIVITY OF ERYTHROPOIETIN,
Steve G. Elliott, David Chang, Evelyne Delorme and Tony Lorenzini, Amgen, Amgen Center, Thousand Oaks, CA. 91320

In vitro mutagenesis studies indicate that both of the disulfide bonds in human EPO, Cys^{7}-Cys^{161} and Cys^{29}-Cys^{33}, are required for proper folding and *in vitro* biological activity. The latter disulfide bond results in a 3 amino acid loop. Each of the 3 amino acids can be mutated without loss of *in vitro* biological activity which suggest that these amino acids are not part of the active site. Mouse EPO has both Cys^{33} to Pro and Arg^{139} to Cys changes. Human EPO with these changes has *in vitro* biological activity while the Cys^{33} to Pro by itself is reduced. These results suggest that a disulfide bond can form between Cys^{29} and Cys^{139} and that it can substitute for a Cys^{29}-Cys^{33} disulfide bond. We have found that mouse EPO has lower *in vitro* biological activity on human cells than on murine cells. This appears to be due to an approximately 4 to5-fold reduction in binding activity of mouse EPO to the human EPO receptor. Human EPO with both Cys^{33} to Pro and Arg^{139} to Cys changes appears to have the same binding activity to human and murine EPO receptors. This suggests that the difference in receptor binding between human and mouse EPOs is not due to the difference in disulfide bonds.

E 319 HEAT STABLE ALVEOLAR MACROPHAGE PRODUCTS STIMULATE EOSINOPHILS AND EARLY PROGENITORS IN RODENTS AND HUMANS.
***M.I. Elsas, *L.A.Silva, R.J.Lima, **D.Joseph, **N. Havet, **B.Saliou, **B.B.Vargaftig, and P.Xavier Elsas, Hospital Evandro Chagas and IFF, FIOCRUZ, and UFRJ, Rio de Janeiro, Brazil, and **Institut Pasteur, Paris, France.**

To define whether products from alveolar macrophages (AM) add to the eosinophilia of asthmatic lung, liquid and semi solid cultures of guinea pig bone marrow (BM) were seeded in the presence of LPS-stimulated culture supernatants (SN) of 95% pure AM (1% or less T cells). The SN increase eosinophil (EOS) production in liquid culture and support formation of myeloid colonies containing EOS, but not of pure EOS colonies, by acting on purified progenitors devoid of mature EOS. This effect is not duplicated by natural or recombinant sources of GM-CSF which were shown to stimulate guinea pig GM colony formation, and could not be attributed to residual LPS, even though LPS is required for induction of the activity (detectable at 30 minutes of culture, plateau levels reached at 6 h). The activity is heat resistant (30% residual activity after 30 min. heating at 100ºC detected in colony formation assays), with an apparent m.w. of 43 kDa by FPLC on a Superose 8 column, one peak being active in both liquid and semisolid culture. Trypsin digestion (1 mg/ml, 15 min., 37ºC) of serum-free, active SN did not destroy the activity and may even enhance its effect on colony formation. As the heat stable monokine, Eosinophil Cytotoxicity Enhancing Factor (ECEF) modulates EOS function (arachidonate metabolism, secretion and killing of schistosomula), its relatioship to AM heat stable products was investigated. Guinea pig AM culture SN, which react with anti-human ECEF mAbs, and mouse AM culture SN, which promote EOS production in mouse BM liquid culture, increase killing of schistosomula by human EOS as well as human BM cell proliferation. Human AM culture SN increase EOS production in human BM liquid culture, as well as human EOS antibody-dependent killing of schistosomula. These findigs suggest the presence, among AM products of rodents and humans, of heat stable cytokines able to modulate EOS function across species barriers.

SUPPORTED BY WHO (TDR), RHAE, FAPERJ and INSERM/I.PASTEUR

E 320 CD8+ RESTING T CELLS CAN BE DRIVEN TO A NON -CYTOTOXIC CD8- CD4- PHENOTYPE PRODUCING TH2 LYMPHOKINES, François Erard, Marie-Thérèse Wild, and Graham Le Gros, Department of Allergy /Immunology, Ciba Ltd, CH-4002 Basel, Switzerland.

IL4 has been recently demonstrated to play an important role in regulating lymphokine production by CD4+ TH cells. It was of interest to study the effects of IL4 on CD8 cell activation and differentiation. Microcultures of 500 FACS-sorted CD8+ lymph node cells were stimulated for 6 days, in the presence of various accessory signals. We have discovered that a combination of IL4 and calcium ionophore (Ionomycin) inhibited INFγ production and simultaneously induced production of high amounts of IL4, IL5 and IL10 in mitogen activated resting CD8+ T cells. Furthermore such IL4 + ionomycin treated CD8 cells did not exhibit any cytolytic activity and stopped expressing the α and β chains of CD8. CD3 was found to be slightly downregulated in these conditions, but not Thy1, CD44, or J11d . In similar culture conditions CD4+ T cells did not lose CD4 expression. The *in vivo* disease situations where such non cytotoxic IL4/IL5/IL10 producing CD8 cells can be identified and their potential role in the regulation of immune responses will be discussed.

E 321 IN VITRO CORRELATES OF MRL/lpr MEDIATED GRAFT-VS-HOST DISEASE: FUNCTIONAL ABNORMALITIES IN MRL/lpr, BUT NOT MRL/gld T CELL SUBSETS, Rachel Ettinger,* Julia Wang,* Kirk Papas,* Charles Sidman,# Abul Abbas,^ and Ann Marshak-Rothstein.* *Department of Microbiology, Boston University School of Medicine, Boston, MA 02118; #University of Cincinnati School of Medicine, Cincinnati, OH 45267; ^Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

Mice inheriting either the lpr or gld recessive mutations develop systemic automimmune syndromes associated with a comparable array of B and T cell immunoregulatory defects. Despite the clinical similarites in disease progression, functional distinctions in these genetic defects become apparent in adoptive transfer experiments; injection of lpr stem cells into a non-lpr histocompatible irradiated recipient results in a GVH-like disease while injection of gld cells results in lympho-proliferative disease. To elucidate the physiological mechanisms responsible for these events, we have examined the interactions between normal and autoimmune T cells in the in vivo radiation chimera model; whereas co-injection of lpr and normal stem cells into an irradiated normal host has again been shown to result in a GVH-like disease, co-injection of gld and normal stem cells led to the development of a normal, as opposed to hyperplastic, immune system. Moreover, co-culture experiments involving an allogeneic stimulator population demonstrated that lpr, but not gld, T cells invariably overgrew their normal counterparts. The TH1-mediated cytokine abnormality responsible for this growth advantage will be discussed.

E 322 A THYMIC STROMAL CELL DERIVED GROWTH FACTOR HAS CO-MITOGENIC ACTIVITY WITH ANTI-CD3 ON THYMOCYTES.

Sherree Lee Friend‡, Suzanne Hosier§, Andrew Nelson§, Diane Foxworthe‖, Douglas E. Williams‖, and Andrew Farr‡§. Departments of Immunology‡ and Biological Structure§, University of Washington, Seattle, WA 98195 and Department of Experimental Hematology, Immunex Corporation‖, 51 University Street, Seattle, WA 98101

We have isolated thymic stromal cell lines from mice in order to characterize their role in thymocyte maturation. We have previously described a murine thymic stromal cell line, Z210R.1, which supports the development of sIgM+ B cells from fetal liver (Friend, S. L. and A. Farr, 1991, J. Cell. Biochem. Supplement 15F, Abstract P-109). Conditioned medium from this cell line maintains the growth of a B cell line (NAC8/7) derived from fetal liver in the absence of stromal cells. While we have found IL-7 in the CM, it is unlikely that the biological activity detected in the Z210R.1 CM is due to this cytokine since the growth of NAC8/7 cells cannot be maintained with recombinant IL-7 and anti-IL-7 mAbs have no effect on the NAC8/7 response to CM. We have also found that Z210R.1 CM, while not mitogenic for thymocytes, does stimulate thymocyte proliferation in the presence of non-mitogenic concentrations of anti-CD3 mAbs, even in the presence of anti-IL-7 mAbs. Based on the reactivity with the 10.1.1 mAb, which detects a novel cytokine responsive 95 kDa cell surface protein and labels a minor subpopulation of medullary epithelial cells in situ, it is likely that the Z210R.1 cells represent a subpopulation of medullary epithelial cells. The interplay of cytokine effects in the thymic environment is complex and likely includes effects on thymic stromal cells as well as thymocytes. (This material is based upon work supported under a National Science Foundation Graduate Fellowship.)

E 323 TRANSIENT EXPRESSION OF A 32 kD LIGAND FOR THE CD40 ANTIGEN ON ACTIVATED HUMAN T LYMPHOCYTES, Jean Pierre Galizzi, Patrice Hermann, Blandine de Saint-Vis, François Fossiez, Béatrice Vanbervliet, Dominique Blanchard, Francine Brière and Jacques Banchereau, Schering-Plough, Laboratory for Immunological Research, Dardilly, France

In order to identify the natural ligand(s) of the human CD40 antigen, a cDNA encoding the extracellular domain of the CD40 antigen was fused to a cDNA encoding the constant region (Fc) of human IgG1. The resulting protein (CD40-Fc) was secreted by COS7 cells and purified by affinity chromatography as a homodimer of 90 kD. The CD40-Fc fusion protein was able to specifically bind to immobilized anti-CD3 activated Jurkat cells but not to many other tested cell lines of different lineages. Furthermore, it bound to a large number of CD4+ T cell clones activated with either immobilized anti-CD3 or PHA. Two out of four CD8+ T cell clones also bound CD40-Fc. The CD40 binding protein was transiently expressed on anti-CD3/PHA activated T cell clones reaching a maximum at 4-5 h post activation and then decreasing to basal level after 12-24h. Also, a proportion of CD4+ but not CD8+ mononuclear cells freshly isolated from peripheral blood and activated by anti-CD3 or PHA transiently expressed the CD40 ligand. The (^{125}I)-CD40-Fc fusion protein bound anti-CD3 activated CD4+ T cell clone (MT9) with an equilibrium dissociation constant (kd) of 10-20nM. The human CD40 binding protein expressed on the cell surface of activated T lymphocytes is a monomeric protein of ≈ 32 kD. Minor components of 29 kD and 17 kD were also detected. These results demonstrate the existence of a natural human CD40 ligand transiently expressed on activated T lymphocytes.

E 324 DUAL REQUIREMENT FOR TNF-α AND IFN-γ IN CELL-MEDIATED IMMUNE CONTROL OF TOXOPLASMIC ENCEPHALITIS, Ricardo T. Gazzinelli, Isam Eltoum , Thomas A. Wynn and Alan Sher, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD, 20,892.

C57BL/6 mice infected with the ME-49 strain of *Toxoplasma gondii*, develop a progressive encephalitis culminating in 100% mortality between 12 and 15 weeks after intraperitoneal inoculation of the parasite. High levels of lymphocyte (CD4/CD8) and macrophage/microglial cell (CD18/P22) surface antigens transcript were detected in the brains of mice throughout the infection. In addition, in the period from 2 to 4 weeks we found a significant increase in the Th1 (IFN-γ and IL-2) cytokine mRNAs which were accompanied by increases in the expression of monokine (IL-1, IL-6, IL-10, GMCSF and TNF-α) as well as inducible nitric oxide synthase (iNOS) mRNAs. Interestingly, after 8 weeks of infection with *T. gondii* we observed a dramatic decrease of Th1 cytokines, several monokines (IL-1, IL-6, GMCSF and TNF-α) and iNOS but not IL-10 mRNA levels. This down-regulation was associated with enhanced brain pathology and increased expression of parasite genes expressed specifically by the tachyzoite stage of *T. gondii* (SAG-1 and SAG-2). Finally, in vivo neutralization of either IFN-γ or TNF-α , at 4 weeks of infection resulted in accelerated and enhanced pathology, associated with decreased expression of iNOS and increased expression of SAG-1 and SAG-2. Together these results suggest that reactivation of *T. gondii* may result from a downregulation of IFN-γ and TNF-α expression leading to decreased macrophage or microglial cell activation, released parasite growth and increased tissue pathology.

E 325 INTERLEUKIN 7 ENHANCES ANTIMICROBIAL ACTIVITY AGAINST LEISHMANIA MAJOR IN MURINE MACROPHAGES

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Interleukin 7 (IL-7) is a stromal-cell derived cytokine that has growth stimulating effects on pre-B-cells, thymocytes, and mature T cells. In human monocytes IL-7 is able to induce secretion of cytokines and tumoricidal activity, as shown recently by S. Ziegler et al. and M. Alderson et al. respectively. We therefore asked, if IL-7 can be used to modulate effector functions of murine macrophages with regard to the elimination of intracellular protozoan parasites. Murine peritoneal exudate or bone marrow derived macrophages were infected with Leishmania major promastigotes and treated with IL-7 alone or in combination with interferon gamma (IFNγ). IL-7 alone reduced the percentage of infected cells as well as the parasite burden per cell in a dose dependent manner but only to a limited degree (60 % infected cells compared to 80 % in untreated controls). A synergistic stimulation of the leishmanicidal effector function was observed if macrophages were treated with IFNγ and IL-7 simultaneously. The fact, that anti-TNF alpha antibodies can revert these effects suggests, that the IL-7 effects are at least in part mediated by the stimulation of TNF synthesis.

E 326 CYTOKINE MESSAGE IN FIRST TRIMESTER HUMAN CHORIONIC VILLI. Mark K. Haynes, Rocky Tuan, Laird G. Jackson, J. Bruce Smith. Departments of Medicine and Orthopaedics, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107

There is increasing evidence that trophoblast cells produce cytokines during gestation. These factors may be important in implantation, placental growth and development, and regulating villous invasion of the endometrium. We examined cytokine expression by villous trophoblast during the first trimester using in situ hybridization techniques. First trimester chorionic villi were obtained by transcervical chorionic villus sampling (CVS) at 8-10 weeks of gestation and processed for sectioning. The cytokine mRNA profile for trophoblast layers of CVS samples was IL-1+, IL-4+, CSF-1+, TNFα+, IFNγ+, CSF-2-, and TGFb1-. IL-1, CSF-1 and IL-4 mRNA were the most prominent, and were found in both cyto- and syncytiotrophoblasts. TNFα message was primarily found in the syncytiotrophoblast whereas IFNγ message was more prominent in the cytotrophoblast. IL-1, IL-4, TNTα and IFNγ were also seen in scattered cells in the mesenchyme. CVS tissue gave similar results after 2-6 days in culture with IFNγ with the exception of TNFα message which appeared to decrease. Immunohistochemical confirmation of cytokine production is being addressed using frozen sections of CVS and anti-cytokine reagents.

E 327 INTERLEUKIN 2 ANALOGS SPECIFIC FOR THE INTERMEDIATE AFFINITY IL-2 RECEPTOR GENERATE LYMPHOKINE-ACTIVATED KILLING WITH DECREASED IL-1β, TNF-α, AND IFN-γ PRODUCTION. K.M. HEATON#, G. JU+, AND E.A. GRIMM#, #THE UNIV. OF TEXAS M.D. ANDERSON CANCER CENTER, HOUSTON, TX, AND +HOFFMANN-LaROCHE INC. NUTLEY, NJ.

Interleukin 2-stimulated peripheral blood mononuclear cells (PBMC) generate lymphokine-activated killing (LAK). Using IL-2 analogs R38A and F42K which interact primarily with the β and γ subunits of the IL-2 receptor (IL-2R), we assessed the roles IL-2Rβγ and the high-affinity IL-2R complex in LAK activation and secondary cytokine production. Although the kinetics of LAK activation were identical, lytic activity was approximately 30% lower and proliferation 50% lower in those PBMC stimulated by R38A or F42K than in those exposed to wildtype IL-2 (rIL-2). In addition, supernatants from LAK cultures stimulated for up to 7 days were tested for IL-1β, TNF-α, and IFN-γ content using ELISA methodology. As shown below, the secretion of all three cytokines was reduced compared to that produced in response to rIL-2.

	maximum reduction in secretion (%)		
	IL-1β	TNF-α	IFN-γ
R38A	28	20	75*
F42K	41*	75*	92*

*($p<0.05$, 3-way ANOVA, compared to rIL-2)

These findings suggest that interaction with the intermediate affinity IL-2R alone is sufficient for proliferation and the generation of LAK, and that stimulation of human PBMC with R38A or F42K results in significant reductions in IL-1β, TNF-α, and IFN-γ secretion. Because these cytokines are believed to mediate the toxicity seen with IL-2-based immunotherapies, these IL-2 analogs may prove to be an effective, yet less toxic, means of cancer treatment.

E 328 INTERACTION OF A FIBRONECTIN FRAGMENT WITH HUMAN ARTICULAR CHONDROCYTES: MODULATION OF PROTEOGLYCAN METABOLISM.

Hickery,M.S. and Bayliss,M.T. Kennedy Institute of Rheumatology,London,U.K.

Fibronectin (Fn) is a component of normal cartilage found throughout the matrix but predominantly localised in the superficial zone. The function of Fn has centred around its role in diseased tissue where increased levels at the articular surface may act as a local factor for the induction of pannus extension and proteolysis of the extracellular matrix may lead to an accumalation of Fn fragments in the synovial fluid. Recent studies have shown that one such fragment, the gelatin binding domain, was the most potent fragment capable of inducing chondrolysis in bovine articular cartilage explants (Homandberg *et al* 1992 J.Biol.Chem. 267:-3597). IL-1α and TNFα are also well known mediators of proteoglycan breakdown in bovine cartilage, however our studies on human articular cartilage explants have shown that rhIL-1α and rhTNFα are not capable of inducing proteoglycan catabolism (Hickery *et al* 1991 J.Orth.Trans. 15:-415). Indeed, we have found no agents capable of inducing an increased catabolic rate and maintain that the changes seen in cartilage degeneration may be a result of an inability of the chondrocytes to lay down matrix due to an inhibition of one or more steps involved in proteoglycan synthesis. The aim of this study was therefore to investigate if the gelatin binding domain of Fn could modulate proteoglycan metabolism within human articular cartilage. Measurement of proteoglycan biosynthesis by the incorporation of 100μCi/ml of ^{35}S-sulphate showed that the 45kDa Fn fragment (1μM) inhibited synthesis by 60%. No changes in the structure of either the endogenous or newly synthesised monomer species were observed when electrophoresis of 4M GnHCl extracts was carried out under dissociative conditions on agarose polyacrylamide gels. Dissociative CL-2B columns showed that the Fn fragment reduced the number of monomers but had no effect on their size (Kav 0.33). Release of proteoglycans over a 3 day culture period in the presence of the Fn fragment (1μM) showed no significant difference to control values. This data supports the hypothesis that biosynthesis of proteoglycans is more susceptible to regulation than degradation and underlines increasing evidence that suggests a number of molecules e.g thrombospondin, tenascin, laminin, produce fragments capable of influencing cellular function within the matrix (Engel 1989 FEBS Lett 251:-1-7). Extracellular matrix metabolism may therefore not only be controlled by the action of cytokines and growth factors but intrinsically by products derived from proteolysis of matrix components .

E 329 **ANALYSIS OF TUMOUR NECROSIS FACTOR BIOLOGY IN TRANSGENIC MICE,** George Kollias, Eleni Douni, Spiros Georgopoulos, Manolis Pasparakis and Lesley Probert. Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Athens 115 21, Greece.

We have established several transgenic mouse lines expressing wild type and modified tumour necrosis factor transgenes and have been able to demonstrate the following:

1. We have shown that endotoxin-responsive expression of human TNF transgenes can be established in transgenic mice and that the necessary cis-acting DNA information for this, is contained within a 3.6 Kb DNA fragment.
2. Irrespective of the gene construct used for the generation of transgenic animals, several TNF transgenic lines are found to develop defective hair-growth.
3. Previously, we presented direct *in vivo* evidence for a role for TNF in the pathogenesis of arthritis (Keffer et al. 1991, EMBO J. 10, 4025).
4. Moreover we can show that T cell-targeted production of human TNF in transgenic mice leads to local (lymphoid organ) and systemic (wasting, ischaemia) toxic effects.

TNF occupies a dominant role in the pathophysiology of a seemingly diverse range of diseases. The understanding of its pleiotropic actions necessitates the analysis of the mechanisms regulating TNF and TNF receptor production and functional potency. The use of transgenic mice to pursue this aim will provide useful information about how this complex processes are regulated and will also provide excellent models of human diseases and clinical disorders for testing new therapies and pharmacological approaches to their treatment. Our recent experiments with transgenic mice engineered to express mouse or human TNF and TNF receptor transgenes will be presented.

E 330 **INTERLEUKIN-4 IS A NEGATIVE REGULATOR OF COLORECTAL CARCINOMA GROWTH.** Harald Lahm, Zita Borbenyi, Aysim Yilmaz, Jürgen R. Fischer[1], Jean-Claude Givel[2] and Nicolas Odartchenko. Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, [1]Thoraxklinik, D-W-6900 Heidelberg and [2]CHUV, CH-1011 Lausanne.

The secretion of interleukin-4 (IL-4) is limited to rather few cell types (T cells and mast cells). In contrast, the target cells affected by IL-4 are numerous and not restricted to the immune system. Considering the pleiotropism of IL-4 activity we have investigated the potential of this cytokine to impair growth of human colorectal carcinoma cells. We have used the following cell lines, all derived from primary tumors: Co-115, HT29, WiDr, LS411N, LS513 and LS1034 and SW1116. Cells were cultured in medium with 1% FCS in the presence of serial dilutions of IL-4. Proliferation was determined every second day over a 6-day period by measuring MTT-conversion. IL-4 (100 U/ml) inhibited proliferation of HT29, WiDr, LS513 and LS1034 cells (18 to 50%). The effect of IL-4 was dose-dependent and significant inhibition was seen even with 10 U/ml of IL-4. In WiDr cells, inhibition was already evident after 4 days, while IL-6 (100 U/ml) did not influence growth at any time point. Furthermore, IL-4 was a potent inhibitor of anchorage-independent colony formation in methylcellulose. IL-4 decreased colony numbers of WiDr and LS513 by approximately 60 and 75%, respectively, while no effect was seen with HT29 and LS1034 cells. Anti-IL-4 antibodies completely neutralized the IL-4-induced reduction of LS513 colony numbers, but did not affect the decrease mediated by TGFβ$_1$. A subline of WiDr has been established through expansion of a single colony which developed in methylcellulose in the presence of IL-4. In this subline, IL-4 did not inhibit short-term proliferation and did not reduce the number of colonies in methylcellulose. Taken together, our results suggest that IL-4 may indeed be an important inhibitor of colorectal carcinoma cell growth. However, within a line, cells may respond heterogenously and the antiproliferative effect of IL-4 may vary growth conditions.

Supported by grants from the Swiss National Foundation for Scientific Research and the Ligue Suisse contre le Cancer.

E 331 REGULATION OF T CELL SUBSET ADHESION BY CHEMOKINE FAMILY MEMBERS, Andrew Lloyd, Dennis Taub, Kevin Conlon, Kouji Matsushima, Joost Oppenheim, and David Kelvin, Laboratory of Molecular Immunoregulation, BRMP, National Cancer Institute, Frederick, MD, 21702.

We have determined that several of the chemotactic cytokines (chemokines) induce the adhesion of human T lymphocytes to umbilical vein endothelial cells (HUVEC) *in vitro*. Recombinant macrophage inflammatory protein-1α (MIP-1α), MIP-1β, RANTES and interferon-inducible protein-10 (IP-10) markedly increased the adhesion of anti-CD3 activated peripheral blood T cells to IL-1 pretreated HUVEC monolayers. Phenotypic analysis revealed that MIP-1α preferentially augmented CD8$^+$ T cell adhesion whereas MIP-1β and RANTES induced a predominantly CD4$^+$ T cell adhesion. The chemokine-induced adhesion was not evident at 1-4 hours and was maximal after 6-8 hours of chemokine treatment. FACS analysis did not demonstrate alterations in the surface expression of β1 or β2 integrins, suggesting that the chemokines may alter the affinity state of one or more adhesion ligands. We are currently undertaking blocking antibody studies to further characterise the T cell-HUVEC interaction. Our results indicate that these chemokines possess the capacity to directly regulate the ability of T cells to adhere to vascular endothelium as an important initial step in the migration towards sites of antigenic challenge.

E 332 THE MULTISTEP NATURE OF LEUKEMIA: A MOUSE LEUKEMIA MODEL INVOLVING AN ONCOGENIC ERYTHROPOIETIN RECEPTOR.
Gregory D. Longmore and Harvey Lodish, Washington University School of Medicine, St. Louis MO 63110 and Whitehead Institute, Cambridge, MA 02142.
Utilizing an in vitro retroviral transduction system, similar to that utilized by retroviruses in vivo to mutate cellular oncogenes, we isolated a constitutively activating point mutation in the murine erythropoietin receptor (EpoR(R129C)). A recombinant SFFV in which the leukemogenic env gene, gp55, is replaced with EpoR(R129C) is able to induce erythroleukemia in mice. This demonstrated that mutations in members of the cytokine receptor superfamily, EpoR(R129C), can function as oncogenes in vivo. However, the biology of leukemia evolution in mice infected with SFFVcEpoR distinctly differs from mice infected with wild type SFFV, and mice reconstituted with v-fms (a tyrosine kinase containing cytokine receptor oncogene) infected bone marrow cells. The mean time to the development of erythrocytosis is 5 weeks vs 1-2 weeks. SFFVcEpoR induces massive megakaryocytosis and transient thrombocytosis, suggesting that the proposed relationship between erythropoiesis and megakaryocytopoiesis occur at the level of Epo-EpoR signal transduction. In vitro SFFVcEpoR is capable of expressing its genes in myeloid, erythroid and megakaryocytic progenitors. In the absence of any added growth factor, other than stem cell factor, EpoR(R129C) expression in BFU-E and GM progenitors can enhance their proliferation, yet like SFFV the predominant effect of SFFVcEpoR is upon CFU-E proliferation and differentiation in a growth factor-independent manner. Thus studies with an activating mutation in the EpoR have demonstrated the capacity for early trilineage manifestation of disease, with predominant erythrocytosis, a situation reminiscent of the human myeloproliferative disorder polycythemia vera. Furthermore, the Fv-2 locus in mouse can restrict the development of Friend disease. Induction of anemia in a Fv-2rr strain of mice (C57BL6), prior to infection, can overcome this genetic restriction to disease with SFFVcEpoR but not SFFV.

On the basis of cell surface phenotype and gene expression analysis we have now isolated 5 different types of transformed cells from the spleens of mice infected with SFFVcEpoR. All cell lines contain some type of mutations in p53, however, none have rearranged and activated expression of spi-1. This demonstrates that p53 mutations are essential for leukemic evolution in both SFFV and SFFVcEpoR models of leukemia, however, in stark contrast to SFFV, spi-1 is not essential for leukemic transformation in SFFVcEpoR-infected mice.

E 333 TRANSFORMED B CELL LINES EXPRESS CELL-ASSOCIATED TUMOR NECROSIS FACTOR AND MEDIATE NATURAL CYTOTOXIC ACTIVITY, Neil M. Matsui and Paul Q. Patek, Department of Microbiology, University of Hawaii at Manoa, Honolulu, HI 96822.
Natural cytotoxic [NC] activity is mediated by a cell associated form of tumor necrosis factor-alpha [TNF]. This cytolytic activity has properties which suggest a role in the immunosurveillance of tumors. NC activity has been difficult to characterize due to the heterogeneous expression of NC activity in various monocytic and lymphocytic cell populations. Here, we have characterized the NC-like properties of cloned murine transformed B cell lines. Several of these lines have the ability to cause the lysis of NC/TNF-susceptible target cells, but not NC/TNF-resistant target cells. Furthermore, lysis of targets by these B cell lines involves a non-soluble (i.e., cell-associated) mechanism which is blocked by anti-TNF antibody. Target cells selected for resistance to L10A2.J, a B lymphoma cell line expressing NC-like activity, are resistant to splenic NC-mediated lysis, resistant to TNF-mediated lysis, and have increased tumorigenicity in mice. Thus, these cells may serve as cloned effectors which lyse cells using a cell-associated TNF-dependent mechanism, analogous to NC lysis.

E 334 IDENTIFICATION OF A NOVEL HUMAN CYTOKINE INVOLVED IN MONOCYTE AND B CELL DIFFERENTIATION, Andrew N.J. McKenzie, Janice A. Culpepper, Gregorio Aversa, Rene de Waal Malefyt, Amy Sato, Atsushi Kaneda, Xu Li*, Ute Franke*, Gerard Zurawski. DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304. * Beckman Center for Molecular and Genetic Medicine, Stanford University Medical Center, Stanford, CA 94305.
A human cDNA was isolated from an activated human T cell cDNA library. The human cDNA contained a single open reading frame of 132 amino acid residues. COS-7 cells transfected with this cDNA specifically secreted a mainly unglycosylated protein with a relative molecular mass of ~10,000. Purified recombinant protein stimulated proliferation of a human pre-myeloid cell line. It also caused extensive morphological changes to, and upregulated MHC class II and FcεRII on, human monocytes. Furthermore, this protein also participated in the regulation of immunoglobulin secretion by B cells. The structure and chromosomal localization of the gene encoding this protein, and its mouse homologue, will be presented and discussed in relation to other lymphokine genes.

E 335 EFFECTS OF IL-12 ON THE GENERATION OF CYTOLYTIC ACTIVITY IN HIGHLY PURIFIED CD8$^+$ HUMAN T-LYMPHOCYTES. Priti Mehrotra, Dona Wu, James A. Crim, and Jay P. Siegel. Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892.
IL-12, formerly known as natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor, has multiple effects on both NK and T cells. IL-12 has been shown both to support the proliferation of, and augment IFN-γ production by, PHA-activated T cells. Additionally, IL-12 has recently been reported to augment the generation of cytotoxic T lymphocyte (CTL) activity in alloantigen-stimulated PBL through an IL-2 dependent mechanism. In order to delineate further the regulation of CTL generation by IL-12, we have investigated its effects on highly purified human CD8$^+$ T cells. Anti-CD3 mAb, immobilized to plastic, was used as a stimulus and hybridoma cells bearing anti-CD3 mAb were used as targets to measure CTL activity. IL-12 by itself did not activate resting CD8$^+$ T cells. However, after overnight activation of CD8$^+$ T cells with anti-CD3, further incubation with IL-12 resulted in a dose-dependent, marked increase in CTL activity per cell and a slight increase in cell number. Anti-Tac mAb to the IL-2R did not block the IL-12 effect while it did block the effects of exogenous IL-2. Thus, in contrast to the reported IL-2 dependent effects of IL-12 on PBL stimulated by alloantigen, the effect of IL-12 on purified CD8$^+$ T cells stimulated by anti-CD3 was IL-2-independent. IL-12 synergized with suboptimal concentrations of IL-2 in augmenting both proliferation and cytolytic activity. The effect of IL-12 on CTL activity of CD8$^+$ cells was first observed 2 days after IL-12 addition and peaked after 4 days. Delay of addition of IL-12 until 2 days after activation in a 5 day culture still resulted in an increase of cytotoxicity but was not associated with a change in number of CD8$^+$ lymphocytes. Taken together, these findings indicate that IL-12 can act directly on activated CD8$^+$ lymphocytes as an IL-2-independent CTL differentiation factor.

E 336 MOLECULAR CLONING AND ACTIVITIES OF A NOVEL HUMAN LYMPHOKINE, NC 30 . A. Minty [1], R. Berthier [2], P. Carayon [3], T. Defrance [4], D. Fradelizi [5], D. Caput [1] and P. Ferrara [1]. [1] Sanofi-Elf Biorecherches, 31676 Labège, France ; [2] Hemeris, 38320 Eybens, France ; [3] Sanofi Recherche, 34184 Montpellier, France ; [4]Institut Pasteur, 69365 Lyon, France ; [5] Hopital Cochin, 75674 Paris, France .

Using differential cDNA screening of an organised cDNA library , we have isolated a cDNA (NC30) corresponding to a 1.3 kb mRNA expressed in T lymphocytes activated by PMA and anti-CD28. This cDNA encodes a secreted protein of molecular mass 12.5 kDa which has 59% identity with a protein encoded by a mouse cDNA P600 (Brown et al ,1989, J. Immunol. 142, 679) that is expressed in Th2 lymphocytes. The NC30 protein shows limited sequence similarity with other Th2 lymphokines. Recombinant NC30 protein has numerous effects on human peripheral blood monocytes. In particular, it inhibits cytokine secretion and cytokine mRNA accumulation induced by LPS . On tonsil-derived B lymphocytes it modulates levels of the CD23 (FcεRII) antigen, and it stimulates the proliferation of these cells in co-stimulation with anti-Ig or anti-CD40. In common with other Th2 lymphokines such as IL4 and IL10 , it may thus serve to temper the inflammatory response while stimulating a humoral immune response. In addition NC30 inhibits the formation of granulocyte-monocyte colonies from CD34+ bone marrow cells in the presence of GMCSF, GCSF or IL3, and it increases γ-interferon synthesis from NK cells. Experiments examining a potential role for this novel interleukin in tumour immunotherapy will be discussed.

E 337 NEW HUMAN CYTOKINE, NC-30, INHIBITS HIV-1 REPLICATION IN TISSUE CULTURE DIFFERENTIATED HUMAN MACROPHAGES, Montaner L.J., Doyle A.G., Herbein G., Collin M. and Gordon S.; Sir William Dunn School of Pathology, University of Oxford, England OX1 3RE; Minty A.J., Caput D. and Ferrara P.; Sanofi-Elf Biorecherches, 31676 Labège, France

Investigation of HIV inhibitory action of cytokines has been restricted mainly to IFN-Gamma, and these results are still controversial. We present data that confirm an anti-HIV-1 action of a newly identified T-cell product, NC-30 (Minty *et al.*, this meeting), in Tissue Culture Differentiated Macrophages (TCDM).

Primary monocytes were purified from blood and cultivated *in vitro*. TCDM were then treated with NC-30 at different concentrations before HIV-1 ADA infection (m.o.i. 0.12), or 18 hours post infection. TCDM cultures were maintained for 11 days. Peripheral blood lymphocytes (PBL) were stimulated with PHA and maintained in the presence of IL-2. PBLs were treated with NC-30 as for TCDM and infected with HIV-1 ADA (m.o.i. 0.12) or HIV-1 IIIB (m.o.i. 0.03), and maintained for 7 days.

Results showed marked inhibition by 1-10 ng/ml NC-30, in a dose-dependent manner of syncytia formation, intracellular p24 (30 fold decrease) and extracellular p-24 (30 fold decrease) in HIV-1 ADA infected TCDM. NC-30 had no effect on PBLs infected by HIV-1 ADA or HIV-1 IIIB.

The inhibition of HIV in TCDM suggests NC-30 may be therapeutically beneficial by selectively inhibiting HIV replication in macrophages.

E 338 OVERCOMING RESISTANCE OF HUMAN TUMOR CELL LINES BY COMBINATION ANTI-FAS AND DRUGS/TOXINS, Hideki Morimoto, Shin Yonehara and Benjamin Bonavida, Department of Immunology and Microbiology, UCLA School of Medicine, Los Angeles, CA, 90024

Monoclonal mouse anti-Fas antibody is directed against Fas antigen, a 36 Kd encoded poiypeptide that belongs to the family of cell surface proteins which includes nerve growth factor receptor, TNF receptors, B cell antigen CD40, and T cell antigens OX40. Anti-Fas antibody mimics TNF-α in its cytolytic activity but not in other TNF-α mediated activities. We have recently shown that TNF-α synergizes in cytotoxicity with bacterial toxins (DTX and PEA), and cytotoxic drugs (CDDP and ADM) and combination treatment can overcome tumor cell resistance to TNF, toxins and/or drugs. Thus, we examined if anti-Fas antibody synergizes with toxins and drugs. The present studies demonstrate that anti-Fas antibody in combination with DTX, ADM or CDDP results in enhanced cytotoxicity and synergy and also overrides resistance to TNF, drugs or toxins when tested against a battery of human tumor cell lines. Synergy with anti-Fas and DTX requires that DTX is enzymatically active since inhibitors of DTX-mediated protein synthesis inhibition resulted in loss of synergy. When the plant toxin ricin was used, there was no synergy with anti-Fas antibody but rather additive effects. The synergy was not obtained in a TNF-receptor negative line but was achieved with other anti-Fas resistant lines. Anti-Fas antibody and ADM or CDDP resulted in enhanced cytotoxicity and synergy. Cell lines resistant either to ADM or CDDP were rendered sensitive by combination of drug and anti-Fas antibody. Further, combination treatment of anti-Fas and ADM overcame resistance of an MDR expressing ovarian line. In all cases, cytotoxicity was augmented by pre treatment of target cells with IFN-γ which upregulates Fas antigen expression.

These results show that anti-Fas antibody can synergize in cytotoxicity with toxins and chemotherapeutic drugs, and combination treatment can reverse resistance to TNF, toxins, and drugs. These findings suggest the possible application of combination therapy with anti-Fas-antibody in tumors refractory to drugs in the absence of TNF-α–mediated side-effects.

E 339 MEASUREMENT OF CYTOKINE PRODUCTION BY T CELLS IN RESPONSE TO ISLET ANTIGENS ASSOCIATED WITH INSULIN-DEPENDENT DIABETES, Roxanne Y. Morse, Margo C. Honeyman and Leonard C. Harrison, Burnet Clinical Research Unit, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

We have demonstrated proliferative T cell responses to native and recombinant pancreatic islet antigens in insulin-dependent diabetes (IDD). To further characterize T cell responses to islet antigens, we measured the levels of three cytokines produced by activated T cells: IFN-gamma, secreted by Th1 cells involved in delayed-type hypersensitivity, IL-4, secreted by Th2 cells involved in humoral immunity, and GM-CSF, secreted by all T cells. Cytokines released by peripheral blood T cells stimulated with antigen 48-72 hr were assayed by ELISA. IFN-gamma, IL-4 and GM-CSF production correlated with antigen-specific proliferation, but there was a high level of spontaneous GM-CSF production in all cultures.

We have developed a simple method (cytokine immunocapture assay, CICA) for measuring antigen-specific activation of T cells in diluted whole blood by the in situ capture and detection of cytokines produced by activated cells. Compared to the standard method which measures proliferation of T cells purified from blood and incubated with antigen for 5-7 days, this method uses small volumes of blood, is quick to set up, uses non-radioactive reagents, has a very low background, and gives a rapid read-out that may better reflect the direct activation of antigen-specific T cells. It is ideally suited to analysis of T cell reactivity to clinically relevant antigens.

E 340 EFFECT OF ISOLATED IRON DEFICIENCY ON MONOCYTE-DERIVED CYTOKINE PRODUCTION IN INFANTS. Carlos Muñoz, Manuel Olivares, Marcelo López, Valeska Simon, Angélica Letelier, Marianela Arévalo and Liana Schlesinger. INTA, Universidad de Chile, Casilla 138, Santiago 11, CHILE.

To determine whether cytokines (CK) play a role in the impaired immunity of nutritional iron deficiency, we evaluated the ability of monocytes to produce Interleukin-1 beta (IL-1ß) and Tumor Necrosis Factor-alpha (TNF-α) in 13 infants with iron deficiency (ID) who had normal weight and were free of acute infections; four of 13 presented a mild anemia (Hb <11 mg/dl). Iron deficiency was defined by the presence of at least two abnormal values in the indicators of iron status (Hb, transferrin saturation, free erythrocyte protoporphirin and serum ferritin). Nine age-matched healthy infants served as controls. Isolated blood mononuclear cells (MNC) were cultured in the presence or absence of stimulant lipopolysaccharide (LPS) for 24 hr. and levels of release/cell-associated CK were measured by using specific ELISA. No spontaneous release was detected. The amount of immunoreactive CK released by LPS-stimulated MNC is shown in the table.

Groups	IL-1ß (ng/ml)	TNF-α (ng/ml)
Iron-deficient	6.1 ± 1.7	4.4 ± 3.1
Control	6.2 ± 1.7	3.1 ± 2.0

The four mild anemic infants had values in the highest quartile of the distribution for both cytokines. These findings indicate that monocytes from iron-deficient infants possess normal capacity to produce IL-1ß and TNF-α. Further studies are needed to evaluate the effect of more severe anemia on monocyte-derived cytokine production.

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E 341 LEUKEMIA INHIBITORY FACTOR (LIF) INDUCES IL8 EXPRESSION IN HUMAN MONOCYTES, T. Musso[1], G. L. Gusella[1], M. C. Bosco[2], D. Longo[2], L. Varesio[2], [1]BCDP, PRI/DynCorp., NCI-FCRDC, Frederick, MD 21702-1201, USA; [2]BRMP, NCI-FCRDC, Frederick, MD 21702-1201, USA.

Leukemia Inhibitory Factor (LIF) is a cytokine that has diverse effects on a multitude of cell types. We studied the effect of LIF on human monocytes. We analyzed the expression of IL-8 during the stimulation of monocytes with LIF. Expression of IL-8 was determined in elutriated human monocytes activated with LIF 50 ng/ml for 1, 3 and 6 hours. IL-8 mRNA was constitutively expressed in untreated monocytes. Upon stimulation with LIF, IL-8 mRNA increased within 1 hour and became maximal between 3 and 6 hours. Dose-response experiments showed that induction of IL-8 mRNA was detectable in monocytes treated with 1 ng/ml of LIF and became maximal at LIF concentrations between 10 and 50 ng/ml. The increase in IL-8 mRNA was correlated with an increase in IL-8 secretion, as showed by ELISA assay of IL-8 secreted in the supernatants of monocytes cultured for 18 hours in the presence of LIF. These results suggest that LIF, by inducing IL-8, could play a role in the regulation of the inflammatory response.

E 342 IL-4 INDUCES THE PRODUCTION OF C10, A MOUSE β INTERCRINE, IN MACROPHAGES, Amos Orlofsky and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

The α and β intercrines comprise two related families of leukocyte chemoattractant cytokines that possess a variety of proinflammatory and hemopoietic activities. A recently identified β intercrine, arbitrarily named C10, is as yet functionally uncharacterized but shows a unique pattern of expression suggestive of a distinct set of functions. Two regulatory features, in particular, distinguish this cytokine: (1) whereas the other β intercrines, MIP-1α, MIP-1β, JE/MCP, RANTES, and TCA3, are all inducible by LPS treatment of macrophages and/or activation of lymphocytes, C10 is completely unaffected by these stimuli; (2) C10, but not the other β intercrines, can be induced in bone marrow-derived macrophages by IL-4. Induction of C10 protein parallels that of mRNA. C10 induction is blocked by cycloheximide, indicating a requirement for synthesis of an intermediate signal. The induction by IL-4 suggests a possible specific involvement of C10 in Th-2-related responses. Complicating this idea is the finding that GM-CSF, which is produced by T cells of both Th-1 and Th-2 type, also induces C10 protein production in macrophages. The complication may be resolved by preliminary results that indicate that the stimulation of C10 by GM-CSF is blocked by the Th-1-specific cytokine, IFN-γ.

E 343 **FATAL OUTCOME OF SEPTIC DISEASE IS ASSOCIATED WITH LACK OF TNF-ALPHA EXPRESSION IN PBMC**

C. Platzer, W.-D. Döcke, H.-D. Volk; Department of Clinical Immunology, Humboldt University Charite, Berlin, FRG

In order to understand the role of TNF and other immune mediators in the pathophysiological process of septic disease the functional and phenotypical state of septic monocytic cells were analyzed. A fatal outcome of sepsis was associated with a reduced HLA-DR expression on monocytes (<30%, so called "immunoparalysis") and accompanied by functional defects. Monocytes showed a diminished antigen presentation and formation of reactive oxygen species. Freshly isolated PBMC of 57 septic patients were analyzed by semiquantitative mRNA-PCR for cytokine expression. No TNF-mRNA could be detected in 16/18 cases with "immunoparalysis" (HLA-DR <30%), in 4/9 patients in the "borderline" group (HLA-DR 31-45%), but only in 4/30 patients with a nearly normal value of HLA-DR expression (>45%). According to these results monocytes from "immunoparalytic" patients showed a low spontaneous and LPS-induced TNF release in short-term culture. In contrast no significant difference in the serum TNF levels could be found between the groups of septic patients. The deactivation of the monocytic cells in some cases may be a result of high TNF serum levels as suggested by short-term treatment of mice with TNF. The mechanism leading to "immunoparalysis" is currently investigated and should contribute to a better understanding of the contradictory role of TNF in the defense against infection and as a "harmful" cytokine.

E 344 A MOLECULAR ANALYSIS OF THE TNFα ALLELE AND ITS ROLE FOR RESISTANCE TO UVB-INDUCED EFFECTS ON CUTANEOUS IMMUNITY, Jeffrey C. Richardson, Vladimir Vincek, Jan Paul Medema, and J. Wayne Streilein, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33136

In LPS sensitive inbred strains of mice, the ability of acute, low dose UVB radiation to impair the induction of contact hypersensitivity (CH) to dinitrofluorobenzene is genetically determined by polymorphic alleles at the TNFα locus. For example, certain strains fail to generate CH responses following UVB exposure (i.e. UVB-sensitive), while other strains generate CH responses comparable to un-irradiated controls (i.e. UVB-resistant). Experimental results indicate that TNFα is the major mediator of UVB effects on cutaneous immunity. This is evidenced by the ability of intradermally-injected TNFα to mimic the effects of UVB radiation and the ability of neutralizing anti-TNFα antibodies to abolish the deleterious effects of UVB on CH induction. We have analyzed the sequence and restriction fragment length polymorphism of the TNFα alleles of numerous inbred strains expressing UVB-susceptible (UVB-S) and UVB-resistant (UVB-R) phenotypes. The TNFα alleles of all UVB-R, but not UVB-S, strains contain a *BamHI* site in the first intron. Moreover, the 5' regulatory region of the TNFα allele of UVB-R mice contain a predictable $(CA)_{14}$ minirepeat that is located immediately 5' of the cytokine response element nearest the TATA box. In contrast, the TNFα alleles of UVB-S mice exhibit an unpredictable array of (CA) repeats. Data will be presented examining the role of the CA repeat with respect to the transcriptional efficiency of the TNFα gene in both UVB-R and UVB-S mice.

E 345 GM-CSF MEDIATES THE SHORT AND LONG TERM CELL SURVIVAL SUPPORTED BY IL-1 AND TNFα

J. Carlos Rodriguez and T. Hoang, Laboratory of Hemopoiesis and Leukemia, Clinical Research Institute, Montreal

The erythroleukemia cell line TF-1 undergoes programmed cell death or apoptosis when deprived of the exogenous source of granulocyte-macrophage-colony-stimulating factor (GM-CSF). The addition of GM-CSF protects cells from apoptosis and induces an immediate proliferative response, as detected by H3-Thymidine incorporation. In the absence of exogenous GM-CSF, interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) also support cell survival of TF-1 cells. IL-1 suppresses apoptosis and maintains the cell population during the first 36 h in culture. In contrast, TNF, in addition to suppress apoptosis, also promotes sustained cell growth (three-fold increase after 3 days in culture, as measured by the MTT assay). Cell proliferation in the presence of TNF was confirmed by a continued H3-Thimidine incorporation during the 3 days of follow-up. To investigate the mechanisms involved in cell proliferation and cell survival, we studied GM-CSF expression after IL-1 or TNF stimulation. In the absence of exogenous growth factor, no GM-CSF mRNA or protein (ELISA) was detected. TNFα induced a rapid and strong expression of GM-CSF mRNA, which was detectable 90 minutes after TNF addition and reached peak levels at 3h. While decreased, GM-CSF mRNA was still detectable after 3 days. In contrast, IL-1 induced GM-CSF expression was transient, with a peak at 3 h, and was undetectable after 9 h. Both cytokines stimulated the secretion of similar amounts of GM-CSF protein (50-100 pg/ml) which followed the same pattern of mRNA expression. To further study the role of endogenous GM-CSF production, neutralizing antiGM-CSF antibodies were added to the cultures. Anti-GM-CSF completely abrogated cell survival and cell proliferation induced by IL-1 and TNFα without affecting IL-3 supported growth. These results indicate an active role of GM-CSF secretion in mediating the suppression of apoptosis and cell proliferation by IL-1 and TNFα.

E 346 **CONSTRUCTION AND STABLE TRANSFECTION OF INTERLEUKIN-6 SPECIES CARRYING AN *ENDOPLASMIC RETENTION SIGNAL*** STEFAN ROSE-JOHN, LUTZ GRAEVE, HEIDI SCHOOLTINK, HILDEGARD SCHMITZ-VAN DE LEUR AND PETER C. HEINRICH Department of Biochemistry, RWTH Aachen, Pauwelsstrasse 30, 5100 Aachen, Germany

Three species of interleukin-6 (IL-6) have been constructed and stably transfected into human hepatoma cells (HepG2). An IL-6 with signal peptide was rapidly secreted as a biologically active protein. IL-6 lacking the signal peptide accumalted within the cytoplasma of transfected cells. IL-6 carrying a C-terminal extension of the amino acids Lys-Asp-Glu-Leu (KDEL) was partly retained in the endoplasmic reticulum (ER). Complete retention in the ER was achieved when the 14 C-terminal amino acids of protein disulphide isomerase (PDI) were added to the C-terminus of IL-6. This finding clearly indicates that the protein sorting signal KDEL is not sufficient for full retention of IL-6 in the ER.

All IL-6 proteins could be extracted from cell lysates in an biologically active form. IL-6 which accumulated in the cytoplasm and IL-6 retained in the ER failed to induce liver specific acute phase protein synthesis in the host cells, indicating that there is no intracellular role for IL-6 in signal transduction. Retention of IL-6 in the ER led to the retention of the IL-6 receptor protein gp80 With the consequence that HepG2-IL-6-PDI cells could not be stimulated by exogenous IL-6. The view that HepG2-IL-6-PDI cells do not express gp80 on the cell surface is further supported by the fact that these cells could be stimulated by the complex of the soluble IL-6R/IL-6.

The phenomenon that retention of IL-6 in the ER results in cells which do not express the IL-6R on the cell surface can be exploited in the future to generate transgenic animals which will become completely IL-6-unresponsive in the tissues in which they express an ER retained cytokine.

E 347 1,25-DIHYDROXYVITAMIN D_3 AND MACROPHAGE COLONY-STIMULATING FACTOR-1 SYNERGISTICALLY PHOSPHORYLATE TALIN, F. Patrick Ross, T. Meenakshi, John Martin, and Steven L. Teitelbaum, Department of Pathology and Laboratory Medicine, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110

Macrophage colony stimulating factor (CSF-1) and 1,25-dihydroxyvitamin D_3 are potent inducers of macrophage differentiation. Both appear to modulate protein phosphorylation through protein kinase C (PKC) raising the question as to whether they concurrently impact on macrophage precursors. We addressed this issue utilizing the CSF-1 dependent murine macrophage-like line BAC 1.25F5. CSF-1 treatment of these cells for 30 minutes leads to particular phosphorylation of a 165 kDa protein, the putative CSF-1 receptor and a 210 kDa moiety. $1,25(OH)_2D_3$ exposure for 25 hours prior to addition of CSF-1 enhances phosphorylation of the 165 kDA species and especially, the 210 kDa protein which, in this circumstance, becomes dominant. Phosphorylation of the latter protein is $1,25(OH)_2D_3$ dose- and time-dependent and the molecule is specifically immunoprecipitated with a rabbit polyclonal anti-talin antibody. Experiments with okadaic acid show that the enhanced phosphorylation of talin does not result from phosphatase inhibition. CSF-1 and $1,25(OH)_2D_3$, alone or in combination, do not increase talin protein expression. The tyrosine kinase inhibitor, genestein, blocks $1,25(OH)_2D_3$/CSF-1 induced phosphorylation of the putative CSF-1 receptor but has no effect on talin phosphorylation which occurs exclusively on serine. In contrast to genestein, staurosporin, an inhibitor of PKC, inhibits phosphorylation of talin. Moreover, exposure of $1,25(OH)_2D_3$ pretreated cells to phorbol 12-myristate 13-acetate (PMA) in place of CSF-1 also prompts talin phosphorylation. Finally, $1,25(OH)_2D_3$ enhances ^{3}[H]PDBu binding indicating that the steroid increases PMA receptor capacity. Thus, CSF-1 and $1,25(OH)_2D_3$ act synergistically via PKC to phosphorylate talin, a cytoskeletal-associated protein.

E 348 NON-HELICAL CONFORMATION FOR THE CARBOXY-TERMINUS OF HUMAN INTERLEUKIN 6 PROPOSED ON THE BASIS OF STRUCTURE PREDICTION AND MUTAGENESIS, Rocco Savino, Armin Laham, Marco Giorgio, Andrea Cabibbo, Anna Tramontano and Gennaro Ciliberto, Istituto di Ricerche di Biologia Molecolare (IRBM) P. Angeletti, Via Pontina Km 30.600, 00040 Pomezia, Rome, Italy

Interleukin 6 (IL-6) is a 184 amino acids polypeptide which has been previously postulated to belong to the class of helical cytokines. We constructed a three-dimensional model of human IL-6, based on homologies with other helical cytokines and on the x-ray structure of GH, IL-4 and GM-CSF. Interestingly, our model predicts that the carboxy-terminal nine amino acids might be either folded as an α-helix or adopt a non-helical conformation, interacting with residues in the A-B loop; in this region, the importance of Arg^{182} and of Met^{184} for biological activity has been already demonstrated by others. In order to distinguish between the two alternative conformations, 45 single amino acid variants were generated in positions 176-181. For each position, five to eight different substitutions were introduced. Analysis of biological activity in two systems and of receptor binding properties indicates that the entire region is involved in the composition of the receptor binding surface, but also allows to conclude that these residues are not likely to be in an α-helical conformation, as it is for GM-CSF. Interestingly, our mutagenesis gives rise to some IL-6 receptor superbinders with an affinity for the 80 kDa receptor subunit 3 to 5 fold higher than wt IL-6.

E 349 LYMPHOKINE PATTERNS OF *IN VIVO* ACTIVATED HUMAN $CD4^+$ AND $CD8^+$ T CELLS IN FETAL AND SCID-HU THYMUSES, Dominique Schols, Bart Vandekerckhove, Alicia Barcena, Hergen Spits and Maria-Grazia Roncarolo, DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304

It has been demonstrated that the peripheral T cell repertoire is a result of negative and positive selection occurring in the thymus, but it remains to be defined whether these processes involve activation and acquisition of specific lymphokine secretion patterns by thymic T cells. We investigated 1) the activation state of $CD3^+$ $CD4^+$ or $CD8^+$ single positive (SP) human fetal thymocytes, by using 4 color FACS analysis 2) the cytokines produced constitutively *in vivo* by total fetal thymic cells and by SP thymocyte subsets, by using quantitative PCR. Results were compared with those obtained in human thymuses of SCID-hu mice. Membrane markers for activation such as CD2R, CD27, CD44 and CD69 were expressed on all CD3high SP thymocytes whereas IL2Rα, which is a late activation marker, was expressed on only a fraction of the $CD3^{high}$ thymocytes. mTNFα, CD71, CD39 and CD54 were not expressed on the $CD3^{high}$ SP thymocytes. In contrast, VLA-4 and CD45RO were already expressed on the $CD3^{low}$ thymocytes and disappeared from the membrane on the $CD3^{high}$ thymocytes. Significant levels of constitutive mRNA for IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10 and IFN-γ were detected in human fetal and SCID-hu thymuses. Interestingly, the cytokine patterns in the $CD3^{low}CD4^+$ $CD8^+$, $CD3^{high}CD4^+$ $CD8^+$, and $CD4^+$ and $CD8^+$ SP populations of fetal and SCID-hu thymuses were different with regard to IL-2, IL-4 and IFN-γ. These results are compatible with the concept that positive selection is an activation process which already results in a functional differentiation between helper and cytotoxic T cells.

E 350 **CILIARY NEUROTROPHIC FACTOR INDUCES ACUTE-PHASE RESPONSE IN HEPATOCYTES** HEIDI SCHOOLTINK,TANJA STOYAN, ELKE ROEB PETER C HEINRICH AND STEFAN ROSE-JOHN Institut fur Biochemie, RWTH Aachen, Klinikum, Pauwelsstrasse 30, D-5100 Aachen, Germany

During inflammatory states hepatocytes are induced to synthesize and secrete a group of proteins called acute-phase proteins It has recently been shown that besides interleukin-6 related cytokines such as leukemia inhibitory factor, oncostatin M and interleukin-11 are also mediators of the hepatic acute-phase response. All these mediators belong to the hematopoietic family of α-helical cytokines. Here we show that an additional member of this cytokine family, ciliary neurotrophic factor, induces the hepatic acute-phase protein genes haptoglobin, α_1-antichymotrypsin α_2-macroglobulin and β-fibrinogen in the human hepatoma cell line HepG2 and in primary rat hepatocytes with a time course and dose response comparable with that of interleukin-6 Our next aim was to define the receptor components used by ciliary neurotrophic factor on hepatic cells. Using a cell-free binding assay we exclude that ciliary neurotrophic factor binds to the 80kDa Interleukin-6 receptor, a protein with significant homology to the ciliary neurotrophic factor receptor which has recently been cloned from neuroblastoma cells. When RNA from primary rat hepatocytes was analyzed on Northern blots low levels of ciliary neurotrophic factor receptor mRNA were detectable In the human hepatoma cell line Hep3B lacking the leukemia inhibitory factor receptor, ciliary neurotrophic factor was not able to induce acute-phase protein synthesis, indicating that this receptor protein may be part of the functional ciliary neurotrophic factor receptor on hepatic cells.

E 351 EXPRESSION AND STEROID-MODULATION OF I-309 AND OTHER CHEMOKINES IN HUMAN MAST CELL LEUKEMIA LINE (HMC-1), Rathinam S. Selvan, Joe H. Butterfield and Michael S. Krangel, Department of Immunology, Duke University Medical Center, Durham, NC 27710; Allergic Diseases and Internal Medicine, Mayo Clinic, Rochester, Minnesota 55905.

I-309, a member of the chemokine family, is a monocyte chemoattractant. Our initial studies have shown that I-309 gene is not transcribed during primary activation of resting T-cells but is abundantly expressed by activated T-cells only upon secondary stimulation. We have now identified human mast cell leukemia line (HMC-1) as a source for I-309 and other chemokines. Our analysis shows that I-309 and the closely related chemokines, MCP-1, RANTES and IL-8 are constitutively expressed in HMC-1. Further, the stimulation of HMC-1 by PMA greatly upregulates the expression of I-309, MCP-1, pLD78 and G-26, but not RANTES or IL-8.

We further examined the role of anti-inflammatory steroids on the expression of these cytokine genes. Treatment of HMC-1 with methylprednisolone (10^{-4} or 10^{-3} M) increases I-309 message, consistent with the presence of a glucocorticoid response element in the 5'upstream regulatory sequences of the I-309 gene. Hydrocortisone upregulates I-309 message to a lesser extent, but dexamethasone and progesterone are ineffective. MCP-1, on the other hand, is not upregulated by methylprednisolone or hydrocortisone. Dexamethasone, moreover, downregulates MCP-1 expression, consistent with reports by others. In activated T-cells, methylprednisolone downregulates the expression of I-309 induced by αCD3 antibody and PMA. Taken together, these observations suggest a complex role of corticosteroids in the regulation of expression of chemokines in HMC-1 and activated T-cells.

E 352 IN VIVO CYTOKINE EXPRESSION DURING PROGRESSION OF BCL_1 TUMOR BURDEN ANALYZED BY IN SITU HYBRIDIZATION. Nancy E. Street and J. David Farrar, Cancer Immunobiology Center, University Of Texas Southwestern Medical Center, Dallas, TX 75235-8576

The B cell leukemia/lymphoma (BCL_1) is a murine tumor which closely resembles human prolympocytic leukemia. A dormant tumor model has been developed in which the BCL_1 tumor regresses spontaneously after injection into BALB/c animals that have been pre-immunized with the tumor idiotype. >70% of these animals remain disease free for more than 100 days post-tumor inoculation, despite the fact that 2×10^6 dormant BCL_1 tumor cells are harbored in their spleens. During maintainance of dormancy, data derived from bulk splenocyte cultures revealed that the predominant cytokine secreted following Con A stimulation is IL2. However, IL10 is the dominant cytokine secreted in these cultures when dormancy is breached. Experiments to corroborate these data as well as to determine the temporal and regional expression of a variety of cytokines in vivo with respect to tumor load and the maintainance of the dormant state were undertaken. cDNA fragments corresponding to IL2, IL3, IL4, IL5, IL6, IL10, IFNγ, GM-CSF, TNFα, TGFβ, CD4 and CD8 were synthesized from endogenous sources by PCR and subcloned into pBluescript. The specificity of these inserts was determined by sequencing and Northern blot analysis. Radiolabeled cRNA was synthesized from each of these templates and used to probe splenic sections of mice during different stages of tumor growth or dormancy. Preliminary data indicate that low levels of mRNA encoding IL2 and IL4 are expressed in T-cell sheaths and absent in B-cell lymphoid follicles in both normal and non-dormant tumor bearing spleens. IL10 was expressed, albeit in low levels, in T-cell sheaths of non-dormant spleens only; additionally, high levels of IL6 were expressed in the vasculature of non-dormant tumor-bearing spleens. A model defining the role of cytokines in B cell tumor dormancy will be presented.

E 353 INTERLEUKIN-12 MODULATES THE COURSE OF DISEASE IN A MURINE MODEL OF PROGRESSIVE LEISHMANIASIS, Joseph P. Sypek, Charles Chung, Samuel Goldman, Derek Sieburth and Stanley E. Wolf, Departments of Preclinical Biology and Cellular Immunology, Genetics Institute, Cambridge, MA 02140

Resistance to Leishmania major in mice is associated with the generation of distinct $CD4^+$ Th subsets, termed TH1 and TH2. Notably, T cells from lymph nodes draining cutaneous lesions of resistant mice are primarily TH1 cells that produce gamma interferon (IFNγ), whereas T cells from susceptible mice are principally TH2 cells that generate interleukin-4 (IL-4). Although existing evidence is supportive of a role for IFNγ in the generation of TH1 cells additional factors maybe required for this to occur and for a protective response to be maintained. A potential candidate is natural killer cell stimulating factor (NKSF) or IL-12, a heterodimeric cytokine produced by monocytes and B cells that has multiple effects on T and NK cell function, including inducing IFNγ production. Employing this experimental leishmanial model we have observed that daily ip administration of 0.33 ug IL-12 per mouse (16.5 ug/kg, 5 consecutive da/wk) caused a marked decrease in footpad swelling, the site of infection, in highly susceptible Balb/c mice. Concommitant with this pronounced decrease in lesion size was an increase in the expression of IFNγ at the RNA level (10x untreated controls) in draining popliteal lymph nodes, and in its production as measured by ELISA of supernatants generated from popliteal lymph node lymphocytes and splenocytes stimulated with leishmanial antigen in vitro. These findings suggest that IL-12 may have a role in the generation of a protective TH1 response.

E 354 ANTIGEN-SPECIFIC CD4+ve HELPER T CELL LINES FROM RHESUS MONKEY VENOUS BLOOD, Bert A. 't Hart, Elisabeth Kraakman, Nicolaas P.M. Bakker, Ronald E. Bontrop and Joost J. Haaijman, Dept. of Autoimmune Diseases, ITRI-TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands.

Nonhuman primates are not commonly used in biomedical research. Molecular analysis has pointed out that some gene products from the polymorphic histocompatibility complex (MHC) are conserved across the species barriers human and nonhuman primates. Functional studies proved that transspecies presentation of certain antigens between primate APC and human T cell clones (vice versa). *Based on these data we postulate that autoimmune disease models in non-human primates are valuable for understanding the mechanism MHC-dependent genetic predisposition for autoimmune diseases.* At the ITRI-TNO a fully MHC-typed colony of 1000 rhesus monkeys is available for the study of autoimmune diseases, e.g. autoimmune-arthritis and -encephalomyelitis) and for the evaluation of therapies. Recently we have started to characterize functional helper T cell subpopulations in the rhesus monkey. We now present data on the establishment of stable CD4+ve helper T cell lines specific for the bacterial antigen streptolysin O (SLO). Based on phenotyping and IL4-dependence the lines have tentatively been characterized as Th2-like.

E 355 IL-10 INHIBIT T CELL ACTIVATION INDUCED BY ALLOANTIGENS OF MONOCYTES AND B CELLS.THE INHIBITION IS ATTRIBUTABLE TO THE SUPPRESSION OF A SYNERGY BETWEEN IL-2 AND TNFα. **WALEED TADMORI,MENG ZHANG ,LEE SULLIVAN, AND SATWANT NARULA. SCHERING PLOUGH RESEARCH INSTITUTE, BIOTECHNOLOGY/CELL BIOLOGY,BLOOMFIELD, N.J.07003**

The effect of IL-10 on T cell activation by alloantigens in primary Mixed Lymphocyte Reaction(MLR)was examined.IL-10 suppression of MLR was observed when allogeneic normal PBMC, purified monocytes or B cells was used as stimulator (APC).Monocytes and B cells from MLR containing IL-10 expressed markedly reduced levels of HLA-DR molecules .To determine if a specific cytokine synthesis inhibition caused the suppression of MLR by IL-10,the effect of exogenous cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IFNγ, or TNFα) on this suppression was examined . IL-2 and TNFα when added alone at saturating concentration were able to only partially overcome suppression by IL-10.However, When exogenous IL-2 and TNFα at subsaturating doses were added together to IL-10 suppressed MLR, complete restoration of the proliferative response was obtained.These data indicate that IL-10 is a potent inhibitor of alloantigen-induced T cells activation presented on both monocytes and B cells and that this inhibition may be attributed primerly to the ability of IL-10 ,via inhibiting the production of TNFα and IL-2,to suppress a synergy between these two cytokines.

E 356 EXPRESSION OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 IN A RAT MODEL OF BLEOMYCIN-INDUCED LUNG INJURY, Motohiro Takeya, Yuji Sakanashi, Shigeo Yamashiro, Hideo Takeshima, Teizo Yoshimura, and Kiyoshi Takahashi, Second Department of Pathology, Kumamoto University School of Medicine, Kumamoto 860, Japan, and Immunopathology Section, Laboratory of Immunobiology, NCI-FCRDC, Frederick, MD 21702, USA.

Accumulation and elicitation of monocytes/macrophages are one of the characteristic features of a number of chronic inflammatory reactions. Recent evidence suggests that the accumulation of monocytes depends in part on a family of chemotactic cytokines. Monocyte chemoattractant protein-1 (MCP-1) is a recently characterized chemotactic factor that attracts monocyte but not neutrophils. MCP-1 can be produced *in vitro* by various cells including blood mononuclear leukocytes, endothelial cells , smooth muscle cells, fibroblasts, and tumor cells. However, the *in vivo* role of MCP-1 in chronic inflammation is not fully understood. In a rat model of pulmonary injury induced by the intratracheal inhalation of bleomycin, a progressive increase in the number of alveolar and interstitial macrophages was observed during the first three days after inhalation. Thereafter the number of macrophages gradually decreased and the interstitial fibrosis began around the seventh day. Expression of MCP-1 mRNA by alveolar macrophages was examined at 1, 3, 7, and 14 days after inhalation using northern blotting. The maximum expression of MCP-1 was observed at a day after inhalation and continued to the seventh day. The expression of MCP-1 seemed to precede the increment of lung macrophages. These findings indicate that MCP-1 may play an important role in the early recruitment of monocytes/macrophages in lung tissue injured by bleomycin.

E 357 CHEMOATTRACTION OF T CELL SUBSETS BY CHEMOKINE FAMILY MEMBERS, Dennis Taub, Andrew Lloyd, Kevin Conlon, Kouji Matsushima, Joost Oppenheim, and David Kelvin, Laboratory of Molecular Immunoregulation, BRMP, National Cancer Institute, Frederick, MD, 21702.

We have determined that several of the chemotactic cytokines (chemokines) induce human T lymphocyte migration *in vitro*. Recombinant macrophage inflammatory protein-1α (MIP-1α), MIP-1β, RANTES and interferon-inducible protein-10 (IP-10) were potent chemoattractants of purified peripheral blood T cells when tested in a modified Boyden microchemotaxis assay. Migration of the T cells required prior activation with anti-CD3. Only RANTES induced significant chemotaxis of unstimulated T cells. Phenotypic analysis revealed that MIP-1α preferentially attracted $CD8^+$ T cells whereas MIP-1β and RANTES attracted predominantly $CD4^+$ T cells. In addition, anti-CD3 activated T cells of both the naive ($CD45RA^+$) and memory ($CD29^+$) phenotypes migrated in response to all of these chemokines. Our results suggest that these chemokines possess the capacity to regulate the recruitment of T cells to sites of antigenic challenge.

E 358 RAPAMYCIN HAS DIFFERENTIAL EFFECTS ON IL-4 FUNCTION: EVIDENCE FOR MULTIPLE IL-4 SIGNALLING PATHWAYS, David A. Taylor-Fishwick, Melvyn Kahan, Peter Hiestand°, Mary Ritter and Brian M.J. Foxwell.

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The immunosuppressive drug rapamycin, which inhibits the response of T cells to growth promoting lymphokines, has only been shown to act as an inhibitor of cytokine action. Our investigations into the effect of rapamycin on human IL-4, a cytokine controlling B and T cell function, show this not to be the case. Unexpectedly, rapamycin showed synergy with IL-4 in both the upregulation of CD23 expression and the downregulation of the type II(p75) TNF receptor(R) on the B cell line, Jijoye. Moreover, in the same B cell line, rapamycin simultaneously inhibited the IL-4 dependant production of TNF α and β. Thus both inhibitory and enhancing effects on IL-4 function can be demonstrated by treating the same cell line with rapamycin. These results raise the possibility that multiple IL-4 signal transduction pathways may be responsible for the pleiotropic effects of IL-4, and have important implications for both the experimental and possible clinical *in vivo* use of rapamycin as a selective immunosuppressant. The site of action of rapamycin with regard to its differential effects on IL-4 function is currently under investigation. The results of these experiments will be discussed.

E 359 MODULATION OF HUMAN ALVEOLAR MACROPHAGE INFLAMMATORY CYTOKINE GENE EXPRESSION AND SECRETION BY SYNTHETIC SURFACTANT (EXOSURF), Mary Jane Thomassen, Joyce M. Antal, Mary J. Connors, David P. Meeker and Herbert P. Wiedemann, Department of Pulmonary and Critical Care Medicine, Cleveland Clinic Foundation, Cleveland, OH 44195

Inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1-beta, and interleukin-6 have been implicated in the pathophysiology of sepsis-related adult respiratory distress syndrome (ARDS). We previously demonstrated that the synthetic surfactant Exosurf (Burroughs Wellcome Co.) inhibits endotoxin-stimulated cytokine secretion from alveolar macrophages (Am J Respir Cell Mol Biol 7:257, 1992). Endogenous cytokine secretion is not inhibited by Exosurf. The present study was undertaken to characterize the suppressive effects of Exosurf. Alveolar macrophages were obtained from normal volunteers and adhered for 1 h. After washing, the macrophages were incubated in lipopolysaccharide (LPS, 0.5 ug/ml) with/without Exosurf (500 ug/ml) for 3 or 24 h. Total cellular RNA was isolated from the macrophages and analyzed by slot blots hybridized with ^{32}P-labelled probes. At 3 h no suppression was apparent in gene expression, however, at 24 h gene expression for all three cytokines was less in Exosurf treated cells. Pretreatment of cells with Exosurf for 1-8 h before LPS stimulation had no effect on cytokine secretion. In contrast, if cells were stimulated with LPS for 1 h then washed and treated with Exosurf for 24 h, cytokine secretion was suppressed (43 ± 6, mean % inhibition $\pm$ SEM, $n = 3$). These experiments indicate that Exosurf suppresses cytokines from stimulated cells and is effective post-stimulation. Although a specific mechanism for the suppressive activity of Exosurf has not been fully elucidated, our results would suggest that Exosurf's action may be at the post transcriptional level. (Supported by Burroughs Wellcome Co. and by Grant NIH-CA 54248).

E 360 *Abstract Withdrawn*

E 361 **FLOW CYTOMETRIC ANALYSIS OF APOPTOSIS IN TNF-α DEPENDENT MONOCYTE MEDIATED LEUKEMIC CELL DEATH,** A.A. van de Loosdrecht, G.J. Ossenkoppele, A.M. Dräger, R.H.J. Beelen[1], M.G. Broekhoven, M.M.A.C Langenhuijsen. Departments of Hematology and Cell Biology[1], Free University Hospital, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands.
Little is known about the precise mechanisms of monocyte mediated cytotoxicity (MMC). Using highly purified and interferon-γ activated human monocytes (10^2 U/mL, 24 hours) we have studied cell and cytokine mediated cytotoxicity against U937 cells, a human histiocytic lymphoma cell line. At an effector to target cell ratio of 10. monocyte mediated cytolysis of 82% ± 5 after 24 hours incubation could be observed which could be partially blocked by neutralizing anti-bodies to TNF (MTT-assay). Light and electron microscopic examination showed the characteristic features of apoptosis of U937 cells after incubation with either monocytes or TNF-α. No signs of necrosis could be observed. The TNF-α induced apoptosis (10^4 U/mL) as measured by flow cytometry (propidium iodide) paralleled the functional decrease in cellular viability (MTT-assay) of 20% ± 3 after 24 hours up to a maximum of 50% ± 4 after 48 hours. Actinomycine-D (0.025-2.5 nM) and Cycloheximide (0.2-20 nM) showed a dose dependent synergistic actvity with TNF-α induced apoptosis. Quantification of apoptosis by flow cytometry of MMC was lower as compared to the percentage of MMC as measured functionally in the MTT-assay at short incubation times. Morphologically monocytes showed a highly phagocytosing activity with apoptotic bodies in phagolysosomes which may underestimate flow cytometric quantification of apoptosis. After prolonged incubation times leukemic cell death could be quantified as apoptosis by flow cytometry whereas no decrease in net cellular viability of tumor cells relative to the initial cell number could be observed. In conclusion, these data provide evidence that apoptosis is the major mode of TNF-α dependent MMC. Furthermore, we adapted a method to quantify cytokine and cellular induced apoptosis of leukemic cells by flowcytometry.

E 362 IL-10: A POTENT INHIBITOR OF THE OSTEOGENIC ACTIVITY OF MURINE BONE MARROW STROMA. Peter Van Vlasselaer, Brigitte Borremans, Rosette van den Heuvel and Rene de Waal-Malefyt*. Department of Biology, V.I.T.O., Geel, Belgium; *Department of Human Immunology, DNAX, Palo Alto, CA 94304, USA.
Cultures of 5-Fluorouracil (5-FU) treated murine bone marrow cells express in the presence of β-glycerophosphate and vitamin c, alkaline phosphatase (ALP), collagen Type I and osteocalcin in a temporal manner while they form mineralized bone-like nodules. IL-10 inhibits the expression of these bone related markers and mineralization in a concentration dependent fashion. In contrast, IL-10 had no effect on the CFU-F frequency, suggesting it interferes with the differentiation of stroma into the bone lineage. Although the mechanism by which this occurs is not clear, IL-10 induced strong morphological differences in cultured mouse stroma. Whereas control cultures contained mainly polygonal, fibroblastic cells, large numbers of rounded adherent and non-adherent cells covering underlaying stroma were found in the presence of IL-10. The latter cells are of macrophage origin as illustrated by their Mac 2^+ and Mac 3^+ phenotype. This macrophage growth and differentiation inducing activity of IL-10 correlated with its stimulatory effect on the number and growth of CFU-GM colonies. Staining for tartrate resistant acid phosphatase (TRAP), showed a 128 fold increase of large, multinucleated $TRAP^+$ cells in IL-10 containing cultures compared to controls. Although this suggests that IL-10 acts on the osteogenic lineage via the induction of osteoclasts, this is not its only way of action. Indeed, the fact that in vitro osteogenesis is suppressed on condition IL-10 is added within the first 15 days of the culture, implies its effect on early bone commitment before matrix maturation started.

E 363 MACROPHAGE DERIVED IL-1, TNF AND IL-10 ARE INVOLVED IN REGULATION OF INTERFERON-γ PRODUCTION BY MURINE NATURAL KILLER CELLS.
Kari Varkila* and Robert L. Coffman#. *Department of Bacteriology and Immunology, University of Helsinki, 00290 Helsinki, Finland and #DNAX Research Institute, Palo Alto, CA 94304.
Natural killer (NK) cells have been shown to be potent producers of interferon-γ (IFN-γ) in intracellular infections and *in vitro*. We have studied the regulation of IFN-γ production by NK cells *in vitro*. Stimulation of splenocytes from C57BL/6 or C3H/HeN or C.B-17/SCID mice with lipopolysaccharide (LPS) or *Leishmania major* amastigotes *in vitro* resulted in IFN-γ production. In this system IFN-γ is produced by nonadherent $ASGM-1^+$ cells, however, adherent cells are needed, since nonadherent cells do not respond to LPS or *L. major*. Removal of $CD4^+$ or $CD8^+$ T cells did not alter IFN-γ production in the assay. When IL-10 was neutralized from the beginning of the stimulation IFN-γ production increased by several fold. A soluble signal from LPS stimulated adherent cells is sufficient to induce IFN-γ production by NK cells, however, cell-cell contact may play an important role since coculture with adherent cells always led to higher IFN-γ production by NK cells than soluble signal alone. Further studies have revealed that neutralization of IL-1 with antibody prevents IFN-γ production and neutralization of TNF decreases the production significantly, however, incubation of purified NK cells with recombinant IL-1α or rIL-1β or rTNFα alone or in combination with the other(s) did not induce IFN-γ production. Addition of IL-10 or TGF-β in the beginning of the stimulation prevented IFN-γ production, while addition of IL-2 strongly increased production. The inhibitory effect of IL-10 could be overcome by adding both rIL-1α and rTNFα to the culture. In order to identify the soluble factor which stimulates NK cells to produce IFN-γ in this system we have tested several cytokines in addition to those mentioned above including IL-3,4,5,6,7, GM-CSF, P600 and IFN-α, however, none of them was effective. IL-2 in high concentrations induced IFN-γ production in NK cells, but IL-2 production was not critical in this system.

E 364 EOSINOPHIL PRIMING BY CYTOKINES IN VITRO AND IN VIVO. Tjomme vd Bruggen, Ruud A.J. Warringa, Rene C. Schweizer, Jan A.M. Raaijmakers, Jan-Willem J. Lammers, and Leo Koenderman. Dept. Pulmonary Diseases, University Hospital Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands.

Eosinophilic granulocytes play an important role in the pathogenesis of allergic asthma. This celltype is very sensitive for priming by cytokines in vitro. After interaction of pure eosinophil preparations from normal donors with the cytokines IL-3, IL-5 and GM-CSF, the modulation or priming of cellular responses is characterized by: (i) enhanced responsiveness towards PAF (Kd 10 nM --> 10 pM), (ii) platelet-factor 4 and well-established neutrophil chemoattractants FMLP and IL-8 become chemoattractants for eosinophils, (iii) down-regulation of GM-CSF-induced chemotaxis, (iv) enhancement of opsonized particle-induced responses (activation of the respiratory burst, release of bioactive lipids (PAF and LTC_4), and degranulation). Even when whole blood of normal subjects was incubated in vitro with GM-CSF (10 pM) or IL-5 (100 pM) prior to the isolation of eosinophils, both respiratory burst and chemotaxis were primed 8 hours after contact with the cytokines, indicating that under these conditions priming is irreversible.

When eosinophils were isolated from the blood of allergic asthmatic individuals chemotaxis of these cells was very sensitive for PAF (Kd 10 pM). Moreover, FMLP and IL-8 were chemoattractants for these cells. On the other hand, GM-CSF was a poor chemoattractant for eosinophils from allergic asthmatics. With respect to chemotaxis, eosinophils from allergic asthmatics exhibit a phenotype that resembles the phenotype of eosinophils from normal donors primed with cytokines in vitro.

E 365 SPONTANEOUS EMERGENCE OF AUTOREACTIVE T CELLS FROM LYMPH NODE CELLS OF MRL/lpr AND MRL/gld MICE, Julia Wang,* Rachel Ettinger'* Charles Sidman,# Abul Abbas^, and Ann Marshak-Rothstein.* *Department of Microbiology, Boston University School of Medicine, Boston, MA 02118; #University of Cincinnati School of Medicine, Cincinnati, OH 45267; ^Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

Autoreactive T cells (ART) have been implicated in the pathogenesis of many organ-specific autoimmune diseases. However, in systemic diseases such as SLE, the role of ART remains ill-defined. Work from several labs has shown that class II specific ART can readily be derived from lymph node cell cultures of the genetically autoimmune mouse strain, MRL/lpr. Mice exhibiting the lpr defect inherit a recessive mutation tied to defective fas antigen expression, a cell surface molecule known to mediate apoptosis. We have now found that ART can also be derived from MRL/gld mice, a strain that exhibits a clinically similar but genetically distinct autoimmune syndrome. In the current study, we have demonstrated that the outgrowth of ART from these two strains can be attributed to distinct immunoregulatory lesions. The emergence of ART in these systems is not simply due to either preactivation of ART in vivo by self antigens, to excess production of T cell growth factors, or to lack of appropriate tolerance induction to autologous class II antigens. A model involving cytokine dysregulation as the basis for this phenomenon will be discussed.

E 366 THE MURINE T CELL LINE CT6 PROVIDES A NOVEL BIOASSAY FOR INTERLEUKIN-7

Joanie L. Willcocks, Anne Hales, Theresa H. Page, and Brian M.J. Foxwell, The Kennedy Institute of Rheumatology, Charing Cross Sunley Research Centre, 1 Lurgan Avenue, Hammersmith, London, W6 8LW, England.

In common with interleukin (IL)-2 and IL-4, IL-7 has been shown to act as a growth factor for activated T lymphocytes. Upon screening a panel of growth factor-dependent T cell lines, we found that only the IL-2-dependent cell line CT6 responded to IL-7, indeed as vigorously as to IL-2. Obviously, these findings challenge previous results on IL-2 production obtained using the CT6 cell line in the absence of the appropriate blocking antibodies. However, they also demonstrate a novel and sensitive system for the bioassay of IL-7. The ability of the surveyed T cell lines to proliferate to IL-7 corresponded with the expression of IL-7 receptors (IL-7R) on the cell surface. The murine IL-7R on CT6 was shown to bind IL-7 with dual affinity and was visualized as an affinity cross-linked complex of 93 kDa. This IL-7R appears structurally similar to that seen on murine splenocytes and on 70Z/3, the pre-B cell line from which the murine IL-7R was cloned. We are currently using the CT6 cell line as a novel system to compare/contrast the signal transduction pathways of IL-2 versus IL-7 driven T cell growth.

E 367 THE RELATIONSHIP BETWEEN INTERLEUKIN-1 RECEPTOR EXPRESSION AND VASCULAR SMOOTH MUSCLE CELL PROLIFERATION, Lynn Wilson, Tim Perera, Samantha Griffith, Martin Carrier, Yamanouchi Research Institute, Littlemore Hospital, Oxford OX4 4XN.

Vascular smooth muscle cell (VSMC) proliferation is a significant component of a number of cardiovascular disease states, including atherosclerosis. A number of cytokines and growth factors have been shown to influence the growth of VSMC, including Interleukin-1 (IL-1) and PDGF. IL-1 appears to act by inducing the expression of PDGF, it has been postulated that synergism between IL-1 and PDGF expression may be required for sustained VSMC proliferation.

We have addressed the question of the role of IL-1 and IL-1 receptor in modulating VSMC (isolated from human umbilical vein) proliferation. Characterisation of the IL-1 receptor in VSMC by receptor cross-linking, Scatchard binding analysis and PCR has shown that type 1 receptor is the predominant isoform. We have carried out a number of studies measuring receptor number and mRNA in VSMC after stimulation with serum, PDGF and IL-1. This has then been correlated to proliferation using both cell counts and [^{3}H]thymidine incorporation, as well as immediate early gene expression. Similar experiments were also carried out on human dermal fibroblasts for comparative purposes. Normally growing VSMC have very few receptors (approximately 200-500), this is significantly reduced when cells enter quiescence, but is markedly up-regulated by PDGF (3 fold) and IL-1 treatment. mRNA levels are also increased as shown by PCR. Cells under these conditions are also induced to proliferate.

These data demonstrate a clear correlation between IL-1 receptor up-regulation and proliferation, and provide a possible novel insight into the role of IL-1 and IL-1 receptor in the pathobiological modulation of VSMC.

E 368 cDNA CLONING OF GUINEA PIG MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) AND THE EFFECT OF INTRADERMAL INJECTION OF THE RECOMBINANT PROTEIN, Teizo Yoshimura, Immunopathology Section, Laboratory of Immunobiology, NCI-FCRDC, Frederick, MD 21702

MCP-1 is a cytokine that belongs to a family of proteins characterized by their small molecular weights and location of four half-cystines. Although MCP-1 is a potent chemoattractant for monocytes in vitro, the in vivo role of MCP-1 has not been well established. To investigate the role of MCP-1 in vivo, animal models are necessary. In the present study MCP-1 cDNA was cloned from guinea pig spleen cells stimulated with Con A. The cDNA comprised 647 base pairs with an open reading frame that encoded for a 120 amino acid protein. The mature protein appeared to be 97 amino acids long, and the amino acid sequence similarity to human MCP-1 is about 56% (55 of the first 99 amino acids). Recombinant guinea pig MCP-1 was expressed in COS-7 cells, then purified by a three step procedure with orange A-agarose, CM-HPLC, and RP-HPLC. The purified protein was found around 25 kD as a broad band on a polyacrylamide gel under reducing conditions. Guinea pig MCP-1 attracted about 34% of input human monocytes at 5×10^{-9} M. The efficacy and potency were comparable to that of human MCP-1. Guinea pig peritoneal exudate macrophages migrated toward guinea pig MCP-1 dose-dependently, but only 1% of input cells responded to guinea pig MCP-1 at its optimal concentration of 5×10^{-9} M. Human MCP-1 attracted 1% of input guinea pig cells at its highest concentration of 2.5×10^{-8} M. Neither human nor guinea pig MCP-1 attracted guinea pig peritoneal resident macrophages. These results suggest that monocytes lose responsiveness to MCP-1 after differentiating to macrophages. Finally, the in vivo effect of MCP-1 was investigated. Intradermal injection of the recombinant protein into guinea pigs caused marked macrophage infiltration. Cloned and expressed guinea pig MCP-1 will help in studying the role of MCP-1 in vivo.

Antagonists of Cytokine Function

E 400 INHIBITION OF INTERLEUKIN 1-MEDIATED DNA SYNTHESIS OF ADULT HUMAN ASTROCYTES IN VITRO BY SOLUBLE TYPE I AND II INTERLEUKIN 1 RECEPTORS. Barbara P. Barna, Barbara S. Jacobs, and Melinda L. Estes, Departments of Clinical Pathology, and Anatomic Pathology, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Interleukin 1 (IL-1) has been implicated as a mediator of reactive astrocytosis in both acute and chronic central nervous system (CNS) injury. In experimental animals, IL-1 has been shown to stimulate astroglial proliferation in vitro and in vivo. Clinically, IL-1 becomes detectable in the CNS under diverse pathological conditions, including bacterial meningitis, Alzheimer's disease, and multiple sclerosis. The purpose of this study was to determine whether soluble Interleukin 1 receptors (gifts from Dr. S. Gillis, Immunex, Seattle, WA) would interfere with IL-1-mediated stimulation of non-neoplastic human astroglia in vitro. The astrocyte-rich culture W3N previously derived from adult human brain tissue resected for intractable epilepsy (Estes et al, J. Neurosci. Res. 27:697, 1990) was exposed to 0.05-0.5 U/ml recombinant IL-1. Cells in culture W3N were >90% positive for the astrocyte marker, glial fibrillary acidic protein. IL-1 significantly ($p < .05$) augmented DNA synthesis in a dose-dependent manner. Addition of soluble IL-1 receptors type I and type II (1-100 ng/ml) each resulted in dose-dependent partial blocking of IL-1-elicited DNA synthesis with a maximum inhibition observed of 55% ($p<.01$) at 100 ng/ml of Type II receptor. Type II soluble receptors were 10-fold more effective than type I. Soluble receptors were specific for IL-1 effects and did not interfere with DNA synthesis enhanced by Tumor Necrosis Factor (10 ng/ml). These data suggest that soluble IL-1 receptors may reduce the characteristic astroglial proliferative response elicited by IL-1. (Supported by a grant from the Epilepsy Foundation of America).

E 401 TRANSFORMING GROWTH FACTOR-β1 POTENTIATES THE STIMULATORY EFFECT OF INTERLEUKIN-4 ON THE PRODUCTION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST BY DECIDUAL CELLS, Kristina Bry and Mikko Hallman, Department of Pediatrics, University of California, Irvine, CA 92717

Interleukin-4 (IL-4) and transforming growth factor-β1 (TGF-β1) upregulate the synthesis of interleukin-1ra (IL-1ra) by peripheral blood monocytes. Interleukin-1 (IL-1) has been proposed as a signal of preterm labor in the setting of infection. In animals, preterm labor can be induced by IL-1and the cytokine-induced premature delivery can be prevented by TGF-β and IL-1ra. IL-1ra is present in high concentrations in amniotic fluid (Bry K et al., unpublished data). We studied the production of IL-1ra by amnion and decidual cells in culture. Human amnion and decidual cells from elective term cesarean sections were cultured in monolayer culture. We measured the concentration of IL-1ra in the media of cells treated for 40 h with IL-4 (100 ng/ml), TGF-β1 (10 ng/ml), the combination of these, or vehicle. The following table shows the IL-1ra production by decidual cells (% of control, n = 4):

Treatment	Without TGF-β1	With TGF-β1
Control	100	120.0 ± 10.1
IL-4	183.6 ± 19.0*	252.9 ± 27.2**

* p = 0.02 relative to control

** p = 0.01 relative to control and p = 0.04 relative to IL-4

Untreated decidual cells produced 83.0 ± 37.7 pg IL-1ra/μg protein (n = 4). Amnion cells released 25.5 ± 6.0 pg IL-1ra/μg protein (n = 5). The IL-1ra production by amnion cells was not significantly affected by treatment with IL-4 or TGF-β1. We conclude that 1) amnion and decidual cells produce IL-1ra *in vitro* and 2) IL-4 stimulates the production of IL-1ra by decidual cells; TGF-β1 potentiates this effect. The production of IL-1ra by fetal membrane cells may serve to maintain pregnancy in the face of labor-promoting cytokines.

E 402 STRUCTURAL ORGANIZATION OF THE MURINE GENE FOR IL-1β CONVERTING ENZYME (ICE). F. J. Casano, A. M. Rolando, J. Mudgett, M. J. Tocci, and S. M Molineaux. Merck Research Laboratories, Rahway, New Jersey 07065.

In monocytes and macrophages, the IL-1β precursor is processed by ICE (IL-1β converting enzyme) to mature, biologically active IL-1β. ICE is synthesized as a p45 proenzyme consisting of a prodomain and the p20 and p10 subunits that comprise the active enzyme. Murine and human ICE are closely related at the structural and functional level, suggesting that the mouse is a useful model for studying this enzyme. To further understand the role of ICE in the regulation of IL-1β mediated biological functions, we have cloned the murine gene. Five cosmid clones were isolated, each of which contained the entire coding region of ICE on two contiguous HindIII fragments 6 kb and 12 kb in size. The analysis of these clones, along with genomic Southern blot data, confirmed that ICE is a single copy gene. The gene contains at least 8 exons whose genomic organization parallels the functional organization of the ICE proenzyme. ICE mRNA is constituitively expressed in a subset of murine and human monocyte and macrophage cell lines, but appears to be induced by activation of the U937 human promonocytic cell line, suggesting that the gene is transcriptionally regulated in some cells.

E 403 MECHANISM OF DIFFERENTIAL INHIBITION OF FACTOR DEPENDENT CELL PROLIFERATION BY TRANSFORMING GROWTH FACTOR-β_1: SELECTIVE UNCOUPLING OF FMS FROM MYC, Allen R. Chen and Larry R. Rohrschneider, Divisions of Pediatric Oncology and Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Transforming growth factor-β_1 (TGF-β_1) selectively modulates hematopoietic cell proliferation. The proliferation of FDC-P1 clone MAC-11, a factor-dependent murine myeloid progenitor cell line, was inhibited differentially by TGF-β_1: strongly in M-CSF, mildly in IL-3, and not at all in GM-CSF. Flow cytometry and Western blots revealed an unexpected increase in expression of FMS, the receptor for M-CSF, in response to TGF-β_1. Metabolic labelling with ^{35}S-methionine showed that synthesis of FMS protein accelerated in response to TGF-β_1, whereas its degradation was unaffected. Northern analyses revealed a rapid increase in *c-fms* RNA after addition of TGF-β_1. TGF-β_1 did not affect kinase activity, cellular phosphotyrosine response, or internalization of FMS. However, TGF-β_1 inhibited induction by M-CSF of *c-myc* RNA, analyzed on Northern blots, and protein, detected by radioimmunoprecipitation. TGF-β_1 did not affect induction of *c-myc* expression by GM-CSF or induction of *c-fos* or *c-jun* by M-CSF. Therefore, the signal transduction pathway to *c-myc* from FMS is distinguished by TGF-β_1 from that of the GM-CSF receptor, and constitutes a mechanism of selective growth inhibition by TGF-β_1.

E 404 A PRELIMINARY STUDY INDICATES THAT CO-ADMINISTRATION OF AN INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RA) PROTECTS AGAINST CYCLOPHOSPHAMIDE (CTX)-INDUCED MYELOSUPRESSION IN A MURINE MODEL. UH Chudgar MD, CH Rundus MT, VM Peterson MD, Departments of Pediatrics and Surgery, School of Medicine, University of Colorado, Denver CO.
CTX is a radiomimetic agent which, like irradiation, causes the release of IL-1. IL-1 not only promotes movement of hematopoietic stem cells into S-phase of the cell cycle, but also augments colony-stimulating factors (CSFs) production, making these stem cells highly susceptible to the cytotoxic effects of CTX. We hypothesized that delivery of IL-1ra during CTX administration should prevent IL-1-induced initiation of cell cycling and counteract the myelotoxic effects of CTX. CF-1 female mice received a single dose of CTX, 200 mg/kg, and were then treated with twice daily i.p. injections of IL-1ra, 20 mg/kg (Synergen, Inc), or an identical volume of 0.05% human serum albumin (HSA) for 24 hours, begining immediately after CTX administration. Peripheral blood counts, bone marrow cellularity and plasma CSF activity from each mouse were assayed seperately at days 2, 7 and 10. Stem cell numbers were also quantitated in soft agar culture. IL-ra-treated mice exhibited

Rx	CSF activity.			CFU GM/Tibia.		
	D2	D7	D10	D2	D7	D10
HSA (n=9)	335 ±91	673 ±133	136 ±47	1000 ±329	1674 ±431	1455 ±291
IL-1ra (n=3)	269 ±3	240* ±112	49* ±9	2741* ±383	3832* ±352	1843 ±1106

* p<0.05 compared to same day HSA control.

lower plasma CSF levels, greater bone marrow cellularity (data not shown), and higher stem cell numbers at days 2, 7 and 10. These data indicate that IL-1ra, when administered concomitantly with CTX, appears to protect myeloid stem cells from CTX-induced cytotoxicity. This myeloprotective effect of IL-1ra could be important adjunctive therapy in cancer patients undergoing chemotherapy.

E 405 INHIBITION OF HIV ACTIVATION USING SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR, Kathleen A. Clouse[1], O.M. Zack Howard[2], Karis A. Weih[1], Craig Smith[3], R.G. Goodwin[3], and William L. Farrar4, [1]CBER, FDA, Bethesda, MD 20895, [2]PRI/Dyncorp, BCDP, NCI-FCRDC, Frederick, MD 21702, [3]Immunex Corp., Seattle, WA 98101, [4]BRMP, NCI-FCRDC, Frederick, MD 21702

The inflammatory cytokine, tumor necrosis factor-α (TNF-α), has been shown to stimulate HIV-1 replication in both chronically and acutely infected T lymphocytes and monocytes. Transcriptional activation of the HIV-Long Terminal Repeat (HIV-LTR) and subsequent increase in virus production is linked to TNF activation of the cellular transcription factor, NF-kB. In this study, we tested the ability of two forms of soluble recombinant type 1 (p80) TNF receptor to inhibit TNF-induced HIV activation in vitro. One receptor is a monomer containing the entire 236 residues of the extracellular (ligand-binding) portion of p80. The second form is a chimeric homodimer containing these latter residues fused to a truncated human IgG_1 immunoglobulin heavy chain, and thus resembles a bivalent antibody without light chains. These recombinant receptor proteins were tested for their ability to inhibit TNF-α-induced expression of HIV-1 in chronically infected human cell lines. We also examined the ability of soluble receptors to limit the activation of HIV-LTR transcription. The dimeric form of soluble TNF receptor proved more effective at blocking TNF-α-induced HIV-1 expression in both monocytic and lymphoid cells. The ratio of TNF receptor dimer to TNF-α was critical, with the most effective being at least 10 to 1. We conclude that at specific TNF to soluble TNF receptor dimer ratios, TNF-α-induced HIV-1 transcription and expression can be limited in vitro.

E 406 MOLECULAR MECHANISMS OF TNF RECEPTOR SHEDDING. Paul D. Crowe, Todd L. VanArsdale, Sammee L. Jones, Kimberly M. Dahms, Ray G. Goodwin*, and Carl F. Ware. Division of Biomedical Sciences, University of California Riverside, CA 92521 and *Immunex Corporation, 51 University Street, Seattle, WA 98101. As a model system to study molecular mechanisms regulating TNF receptor expression and signal transduction, we have constructed deletion mutants of the human 80 kDa TNF receptor ($TNFR_{80}$) that were expressed in the murine L929 cell by cotransfection with a G418 resistance plasmid. Anti-human $TNFR_{80}$ MAb (M1) was used to identify huTNFR$_{80}$ or a truncated form of $TNFR_{80}$ lacking the entire cytoplasmic region ($TNFR_{80}\Delta$cyt). Surface expression was in the range of 10^4 sites per cell. L929 transfectants expressing full length human $TNFR_{80}$ stimulated for 30 min with PMA (100 ng/ml) rapidly lost surface expression. However, expression of $TNFR_{80}\Delta$cyt was not decreased under these conditions indicating that the cytoplasmic domain may be involved in the regulation of $TNFR_{80}$ surface expression. This is in contrast to findings with the 55 kDa TNFR. To examine whether $TNFR_{80}$ is phosphorylated, a human T cell hybridoma (II-23.D7) or a promyelocytic line (HL-60) were labelled with $^{32}PO_4$. A specific 80 kDa band was immunoprecipitated in both these cell lines indicating that $TNFR_{80}$ is constitutively phosphorylated. The PKC inhibitor (chelerythrine) and the PKA inhibitor (HA1004) failed to block $TNFR_{80}$ receptor shedding indicating that phosphorylation by these kinases is not necessary for shedding. Only the immunosuppressant cyclosporine A (CsA) inhibited $TNFR_{80}$ shedding by activated T cells suggesting that gene transcription may be important in the regulation of TNFR expression by proteolytic processing. Supported by TRDRP 2RT0261 and ACS grant IM68167 (CFW).

E 407 SELECTIVE INHIBITION OF HUMAN MONOCYTE MACROPHAGE TNF-α PRODUCTION BUT NOT IL-1β, BY THE CARBOCYCLIC NUCLEOSIDE MDL201,112. Carl K. Edwards, III[1], Brenda J. Hoeper[1], Kendra K. Schroeder[1], Terry L. Bowlin[1], Michael J. Parmely[3] and David R. Borcherding[2], Departments of Immunology[1] and Discovery Chemistry[2], Marion Merrell Dow Res. Inst. Cincinnati, OH 45215-6300 and Univ. of Kansas Med. Ctr.[3], Kansas City, KS 66160-7420.

The enhanced synthesis of proinflammatory cytokines released from macrophages (Mφ) have been shown to be important in acute and chronic disorders. The overproduction TNF-α is particularly important in the development of septic shock and Rheumatoid Arthritis. We recently demonstrated that the carbocyclic nucleoside MDL201,112 (9-[(1S,3R)-cis-Cyclopentan-3-ol]adenine) was effective in inhibiting TNF-α production by primary mouse Mφ and Mφ cell lines *in vitro* and protecting D-galactosamine sensitized, LPS-challenged mice *in vivo* (Eur. Cytokine Network 3:249;1992). We have now extended these data to show that MDL201,112 is effective in inhibiting the production of human monocyte/Mφ-derived TNF-α, but not IL-1β, *in vitro*. Highly purified human adherent monocyte/Mφ, isolated from peripheral blood using LeucoPrep ficoil-hypaque, were characterized by FACs and shown to be positive for the following monocyte-Mφ markers: >80% anti-Leu-M1, anti-Leu-M3, anti-Leu-M5, 70% anti-HLA-DR/Mo2 and <2% anti-IgM/T3. Human Mφ (10^6) stimulated with *E coli*- derived LPS (1 μG/ml, 18h) produced high levels of immunoreactive TNF-α (measured by ELISA) in comparison to control Mφ (6.2+/-0.14 nG/mL vs. 1.5 +/-0.1 nG/mL, resp.). Mφ TNF-α levels were significantly reduced, in a dose-related fashion (1.0 - 100μM), by MDL201,112 (3.9+/-0.3 nG/mL; 42% inhibition at 25μM) or the methyl xanthine pentoxifylline (2.7+/-0.1 nG/mL; 55% inhibition at 25μM). LPS-stimulated Mφ IL-1β levels were not affected by MDL201,112 at similar drug concentrations (2.9+/-0.04 nG/mL vs. 3.7+/-0.1 nG/mL, resp.) but were significantly ($p<0.05$) reduced by pentoxifylline (2.6+/-0.03nG/mL; 15% inhib.). These data suggest that human Mφ-derived TNF-α and IL-1β are regulated by different molecular mechanisms. The pharmacological effects of these agents maybe important in inflammatory diseases where local and systemic modulation of cytokines is essential.

E 408 MODULATION OF ANTI-CD40-ACTIVATED B CELL RESPONSE BY PROSTAGLANDIN E2. Pierre Garrone, Laurent Galibert, Francoise Rousset and Jacques Banchereau, Schering-Plough, Laboratory for Immunological Research, Dardilly, France

Human B lymphocytes are induced to proliferate when cultured with anti-CD40 mAb89 presented by CDw32/FcgRII transfected mouse L cells. In this culture system, called CD40 system, IL-4 and IL-10 promote B cell DNA synthesis, but only IL-10 induces secretion of large amounts of IgM, IgG and IgA, whereas IL-4 is the sole cytokine inducing IgE production. Here we show that addition of PGE2 (10^{-10} M to 10^{-6} M) to tonsil B cells activated through their CD40 molecule resulted in a stimulation of their proliferation but did not promote their Ig production. In the presence of cytokine, PGE2 potentiated both IL-4- and IL-10-induced B cell DNA synthesis, but PGE2 only potentiated IL-10-dependent differentiation, thus resulting in a two-fourfold enhancement of IL-10-induced IgM, IgG and IgA production. In contrast PGE2 strongly inhibited IL-4-induced IgE secretion of anti-CD40-activated B cells. A similar blockade by PGE2 of IgE secretion was observed when B cells were cultured with a combination of IL-4 and IL-10, while the other isotypes were not affected. Furthermore PGE2 inhibited IL-4-induced IgE production of purified sIgD$^+$ (naive) B cells activated with anti-CD40, thus suggesting that PGE2 may interact with mechanisms involved in IgE switching and/or in IgE synthesis. Finally the cAMP agonist, cholera toxin, and the cAMP analogue, dibutyryl-cAMP, stimulated IL-4-induced proliferation but inhibited IgE secretion of anti-CD40-activated B cells, thus indicating that the cAMP pathway may be involved in PGE2 activities.

E 409 EFFECT OF SOLUBLE MoIFNγ-R TREATMENT ON THE DEVELOPMENT OF SPONTANEOUS AUTOIMMUNE DISEASE IN NZB/W F1 MICE. Garotta, G., Ozmen, L., Ryffel, B., Fountoulakis, M., Gentz, R. and Schmid, G., Pharmaceutical Research New Technologies F. Hoffmann-La Roche Ltd. 4002 Basel. Switzerland.

The NZB/W F1 females that develop spontaneously a SLE-like autoimmune disease were treated for 4 months with MoIFNγ ($5x10^4$ U 3x/week), mAb anti-IFNγ (100 μg 1x/week), MoIFNγ-R (100 μg 3x/week) or placebo (0.2 ml NaCl 3x/week). Up to now, the analysis of the mortality rate shows that 57% (4/7) of mice died in the placebo treated group, 87% (7/8) died in the MoIFNγ treated group whereas no death occurred neither in the mAb anti-IFNγ nor in the MoIFNγ-R treated groups. The highest score of proteinuria was reached 11, 17 and 21 weeks after the beginning of the treatment with MoIFNγ, placebo and mAb anti-IFNγ or MoIFNγ-R, respectively. These results were further confirmed by the immunohistochemistry analysis of the kidneys that showed a lower incidence of glomerulonephritis in mice treated with MoIFNγ-R or mAb anti-IFNγ. The treatments with MoIFNγ-R or mAb anti-IFNγ significantly delayed the emergence of anti-dsDNA autoantibodies. Most of the mice treated with MoIFNγ died without having developped high titers of anti-dsDNA autoantibodies. These results confirm the accelerating role of IFNγ in this pathology and show the capacity of the MoIFNγ-R in inhibiting the disease.

E 410 SOLUBLE IL-1R IN SEPSIS. J. G. Giri, M. Alderson, *C. E. McCall, W. Fanslow, J. Jackson, J. Slack, T. Bird, S. K. Dower and J. E. Sims. Immunex Research and Development Corp., 51 University St., Seattle WA 98101 and *Section of Infectious Diseases, Wake Forest University Medical Center, Winston-Salem, NC 27157.

Two types of IL-1 receptors have been identified and cloned from both human and murine sources. The most significant difference between the two receptors is the much shorter (29 aa vs. 213 aa) cytoplasmic domain of the type 2. Most available data suggests that the type 2 IL-1R (IL-1R-t 2) cytoplasmic domain may not be competent for signal transduction and there is no evidence for affinity modulation or heterocomplex formation between the two IL-1R proteins (J. Slack et al. 1992, JBC, in press), bringing into question whether IL-1R-t2 receptors have any role in modulating responses to IL-1. Many cells express type 2 receptors, and some cells such as neutrophils preferably express IL-1R-t 2 on their surface, suggesting a physiological role for this receptor. We find that the IL-1R-t2 is shed from cell lines as well as cells transfected with cDNA for type 2R. The shed receptor is similar in size to the extracellular domain of rIL-1R-t2 expressed in COS cells, as judged by immunoprecipitation and SDS-PAGE. Like some other receptors, shedding is greatly enhanced by treatment with phorbol esters. Shedding of type 2 IL-1R from peripheral blood neutrophils is induced not only by phorbol ester, but more interestingly, by the agonists, TNFα and endotoxin. Based on the observation that cell surface expression of IL-1R is upregulated on neutrophils of septic patients, correlating with disease state (J. Clin. Invest. 88: 1452), we analyzed serum from septic and non-septic patients for the presence of soluble IL-1 receptors. We find from 2.3- to 38.5-fold increase in IL-1R-t 2 protein levels in sera of patients with sepsis compared to non-septic controls, using specific antibodies for the two receptor types. Presence of elevated levels of soluble type 2 receptor is confirmed by binding of radiolabeled IL-1β. Soluble type 1IL-1R protein is also detected by ELISA, but levels do not correlate with sepsis. These results imply that together with the IL-1 receptor antagonist, soluble type 2 receptors have a role in regulating IL-1 activity *in vivo*.

E 411 IL-10 INHIBITS CYTOKINE ASSISTED EXPANSION OF IgE (+) B-CELLS IN LONG TERM CULTURES. Michael J. Grace, Loretta A. Bober, Catherine Pugliese-Sivo, Tracey A. Waters, Lee M. Sullivan and Satwant K. Narula. Schering-Plough Research Institute, Bloomfield, NJ 07003.

IL-10 is a potent inhibitor of antigen-induced T-cell clone and lectin-induced T-cell proliferation in the presence of monocytes. We have established a panel of patients with a wide range of allergen sensitivities and demonstrated that IL-10 is a potent inhibitor of allergen induced T-cell proliferation (with 50% inhibitor activity as low as 2 pg/ml). Inhibition by IL-10 was reversed with the addition of a low level of exogenous IL-2 (20 U/ml). However, the addition of IFN-gamma was ineffective in reversing the effect of IL-10 on proliferation, eventhough monocyte HLA-DR was completely repleted. Early cognate interaction between the T-cell and monocyte is exquisite as T-cell proliferation can neither be blocked by the delayed addition of IL-10 to intact cultures nor by the seperate treatment of T-cells and monocytes followed by back-addition. Long term cultures have been established that can be induced by IL-4 to increase the number of IgE+ B-cells. Incubation of these IL-4 stimulated cultures with IL-10 resulted in a substantial decrease in the number of detectable IgE+ B-cells.

E 412 **EFFECTS OF THE SERINE PROTEASE INHIBITOR, TAME, ON INTERLEUKIN-1ß IN LIPOPOLYSACCHARIDE (LPS)-STIMULATED HUMAN MONOCYTES; THE RELATIONSHIP BETWEEN SYNTHESIS AND RELEASE OF A 33 KDA PRECURSOR AND THE 17 KDA BIOLOGICALLY ACTIVE SPECIES.** **John J. Jessop, Sylvia Henry and Thomas Hoffman, FDA, CBER, Division of Hematology, Laboratory of Cell Biology, NIH, Bldg. 29, Rm. 223, 8800 Rockville Pike, Bethesda, MD., 20892.**

LPS stimulation of human monocytes induced release of the 17 kDa mature IL-1ß (mIL-1ß) but did not result in release of precursor IL-1ß (pIL-1ß). In contrast, the presence of a serine protease inhibitor, Nα-(p-toluene sulfonyl)-L-arginine methyl ester (TAME) at a concentration above 1 mM was associated with the release of the 33 kDa precursor IL-1ß as well. This effect was observed at 6 and 18 hours. At 18 hours a decrease in protein synthesis was observed in the presence of TAME, which included a decrease in total IL-1ß synthesis, most prominently reflected in diminution of the 31 kDa precursor IL-1ß. These effects were reflected in 18 hour studies measuring IL-1ß with an ELISA recognizing both the mature and precursor IL-1ß species, in that TAME inhibited total IL-1ß synthesis yet increased the percentage of total IL-ß that was actually released from lipopolysaccharide (LPS)-stimulated human monocytes. Examination of lactate dehydrogenase (LDH) release revealed that precursor IL-1ß release was unrelated to cell lysis. TNFα production was also inhibited in a dose-dependent fashion in the presence of TAME. These results show that TAME inhibitable serine proteases are probably involved in the production and proteolysis of the 33 kDa pIL-1ß in situ but that such serine proteases are probably not mechanistically related to either maturation of the IL-1ß molecule or signalling of IL-1ß release. Serine proteolysis may be a degradative pathway for excess precursor.

E 413 PRECLINICAL EFFICACY OF SOLUBLE rhuIL-1 AND TNF RECEPTORS (R) ON HEMODYNAMIC, METABOLIC, AND CIRCULATING LEVELS OF INFLAMMATORY CYTOKINES IN A NONHUMAN PRIMATE MODEL OF ENDOTOXIN SHOCK. Thomas J. MacVittie, Cornel L. Kittell, Kenneth F. Kirschner, Ann M. Farese, Jan Agosti, Douglas E. Williams, Michael Widmer. Armed Forces Radiobiology Research Institute, Bethesda, MD 20889 and Immunex Corp. Seattle, WA 98101.

Recent evidence in rodent and primate models of endotoxin and septic shock suggest a potential therapeutic utility for factors antagonistic to the inflammatory cytokines TNF and IL-1. We investigated the effect of soluble TNFR:Fc and IL-1R administration on the physiologic responses of primates to a nonlethal injection of LPS. Rhesus monkeys received rhu IL-1R, TNFR:Fc, (doses, 1 mg, 200 μg, or 40 μg/kg bw) or huIgG, (1 mg/kg bw) by subcutaneous injection 2 hr prior to injection of LPS (*E. coli* 0111:B4, 6 mg/kg bw, 10 min infusion) and then by continuous i.v. administration 5 min prior to and for 4 hr following LPS infusion. Animals were monitored at various times prior to and post LPS infusion over 72 hr for changes in mean arterial blood pressure (MAP), systemic vascular resistance (SVR), cardiac output, temperature, blood gases, serum glucose (GLUC), triglycerides, circulating neutrophil (PMN) levels and IL-1, TNF, IL-6, IL-8, TNFR (p55) and IL-1 receptor antagonist (ra) in serum with a enzyme-linked immunoabsorbent assay (ELISA). Control animals experienced significant hypotension (MAP 50%, SVR 30% decreased) hypoglycemia (GLUC 40% decreased), increased blood lactate and transient neutropenia. Administration of TNFR:Fc or IL-1R at 1 mg/kg significantly lessened the decrease in MAP and SVR, as well as blunted the LPS-induced hypoglycemia and triglyceridemia but had no significant effect on acute changes in blood gases, HCO_3^- and pH. Administration of IL-1R or TNFR:Fc did not alter the serum profile of TNF or IL-1β respectively, nor did they change the profiles of IL-8 and IL-6. Administration of TNFR:Fc did not affect the appearance of IL-1ra while IL-1R significantly delayed the appearance of IL-1ra. Soluble TNFR:Fc and IL1R, administered in a combined prophylactic and therapeutic regimen were each effective at decreasing the severity of symptoms consequent to LPS administration with maximum benefit occurring at the 1 mg/kg dose. Combined protocols using 200 μg/kg of each IL-1R and TNFR:Fc were no better than the 200 μg/kd dose of either R alone. The rhu TNFR:Fc used in these studies was developed, in part, in cooperation with Behringwerke, AG.

E 414 HUMAN BIOLOGICAL FLUIDS CONTAIN FACTORS WHICH COMPLEX WITH TNF AND INHIBIT IMMUNOREACTIVITY, P. Jeremy McLaughlin, Helen M. Davies and Stephen J. Hoiland, Department of Immunology, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

Using size fractionation HPLC the molecular size of immunoreactive TNF was evaluated in biological fluids using a monoclonal antibody based ELISA developed by us (Immunol. Cell. Biol. 68 51). Native TNF-containing biological fluids were evaluated and changes in molecular size were also monitored on incubation of recombinant TNF with human biological fluids. Recombinant TNF had a molecular size of about 50kDa, corresponding to a trimolecular form. In contrast, plasma and urine from renal transplant patients contained immunoreactive TNF of a molecular size between 100-200kDa, suggesting that naturally released TNF exists in these patients in a complexed form. Plasma and bronchial lavage fluid from patients with adult respiratory distress syndrome did not contain detectable immunoreactive TNF. Incubation of these samples with recombinant TNF led, in most cases, to a significant loss of immunoreactivity of TNF (32-75%), and to an increase in molecular size of the remaining immunoreactive TNF corresponding to complexing of TNF to a factor(s) of molecular size 30-80kDa. Incubation of recombinant TNF with plasma from healthy individuals or renal transplant patients did not result in a significant loss of immunoreactivity although molecular size of the TNF complex increased from 50kDa to 80-120kDa.

TNF complexing factors may have an influence on immunoassay of TNF and may also affect the activity, distribution and behaviour of this cytokine in vivo.

E 415 EFFECT OF AGE ON IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-8, IFN-γ, IFN-γR, TNF-α, AND TNF-β mRNA EXPRESSION BY CON A AND PHA ACTIVATED HUMAN IMMUNOCYTES, James E. Nagel, Michele M. Schoonmaker, F. Joseph Chrest and William H. Adler, Clinical Immunology Section, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

Altered cytokine production (i. e. quantity and kinetics) has been hypothesized to be responsible for age-related defects in T-cell proliferation. Reverse transcription (RT) and the polymerase chain reaction (PCR) were used to amplify specific IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-8, IFN-γ, IFN-γR, TNF-α, and TNF-β mRNAs produced by 1.5 x 10^5 unstimulated, PHA or Con A activated human mononuclear cells that were collected at 14 time points during the 24 hrs following mitogen addition. Before culture in serum-free media, the cell populations were characterized by MoAb and two-color flow cytometry to determine representations of CD4+ and CD8+ T cells, B cells and monocytes. Following RT, the cDNA from each time point was co-amplified with multiple fluorescent dye-labeled primers (multiplexing). Gene Scanner™ analysis allowed the simultaneous detection of up to eight transcripts for each PCR. Data from 12 young (mean age 26.3 ± 2.1 yrs) and 6 elderly (mean age 80.2 ± 2.1 yrs) demonstrated that unstimulated cells displayed no age differences in specific mRNA expression at any of the time points or for any of the cytokines studied. The only age-related changes detected occurred at T_0 to T_9 hrs when there was a reduced expression of IL-5 and IFN-γR mRNA by cells from the elderly. However, there were no differences noted in these or any other cytokine mRNA during the T_{10} to T_{24} period. The greatest differences in cytokine expression were between unstimulated and mitogen-stimulated cells irrespective of age. While unstimulated cells had little or no detectable mRNA for IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IFN-γ, TNF-α and TNF-β, message production increased in activated cells, particularly those treated with ConA

E 416 MECHANISMS INVOLVED IN SKEWING TOWARDS T_H1 AND T_H2 RESPONSES
Lex Nagelkerken[1,2], Kenneth J. Gollob[1] and Robert L. Coffman[1].
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[2]TNO Health Organization, Leiden, The Netherlands

Several diseases are caused by the preferential activation and expansion of T_H1 or T_H2 cells, secreting an undesired spectrum of lymphokines. The mechanism by which this skewing of CD4+ T cell responses occurs is largely unknown, although an involvement of IL-4 and IFN-γ is well-established. Interestingly, skewing can be achieved *in vitro* either by stimulating CD4+ T cells with Staphylococcus Enterotoxin B (SEB) in the presence of antigen-presenting cells (APC) and exogenous IL-2 resulting in T_H1-like responses, or with plate-bound anti-CD3 (+ IL-2) resulting in T_H2-like responses. In the present study we demonstrated that a preferential activation of T_H1 cells (defined on the basis of IFN-γ production) by SEB is prevented by the addition of IL-4, whereas inhibition of IL-4 by the antibody 11B11 has the opposite effect on anti-CD3 induced T_H2-like responses (defined by the production of IL-4, IL-5 and IL-10). Addition of SEB + APC to the anti-CD3 system stimulated rather than inhibited the T_H2-like response. It is likely that SEB + APC is incapable of inducing IL-4 production, thus generating a T_H1-like response. The skewing toward a T_H1 response was not reversed by the addition of anti-CD28 to the cultures. In the presence of exogenous IL-2, the generation of T_H2-like effector cells was more sensitive to TGF-ß or to PGE2 than the generation of the T_H1-like response. The possible role of these substances in the skewing of CD4+ T cell responses to SEB will be discussed.

E 417 FAILURE TO INDUCE FAS/APO-1 MEDIATED APOPTOSIS IN FAS-POSITIVE HUMAN OSTEOSARCOMA CELLS TRANSFECTED WITH WILD-TYPE p53, Laurie Owen-Schaub, Robert Radinsky[1], and Shin Yonehara[2], Departments of Immunology and Cell Biology[1], The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030 and The Department of Cell Biology[2], The Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo 113 Japan.

Fas/APO-1 is a cell surface protein known to trigger apoptosis in a variety of cell types upon specific antibody engagement. Previous studies using hematopoietic cells have demonstrated the dissociation of Fas/APO-1 expression and biologic function, leading to the speculation that accessory gene products may be involved in anti-Fas/APO-1 mediated cell-killing. Because wild-type p53 is known to regulate apoptosis in several tumor cell lines, we sought to determine the effects of wild-type p53 on Fas expression/function in the human osteosarcoma line SAOS-LM2. The parental SAOS-LM2 cells lacked endogenous p53, expressed low, barely detectable, cell-surface levels of Fas, and were refractory to anti-Fas mediated killing. SAOS-LM2 stably transfected with wild-type p53, however, markedly upregulated cell-surface Fas. While Fas was not increased in cells transfected with vector alone, SAOS-LM2 transfected with mutant p53 (codon 143, alanine for valine substitution) demonstrated a small, but reproducible, increase in Fas expression. Despite increased Fas expression, p53 transfectants remained refractory to the cell-killing effects of anti-Fas. These studies document that wild-type 53 expression is insufficient to render resistant, Fas-positive cells sensitive to apoptosis triggered by anti-Fas.

E 418 TAXOL INHIBITS TNF-α MEDIATED CYTOTOXICITY.
Darren J. Park and Paul Q. Patek, Department of Microbiology, University of Hawaii, Honolulu, HI 96822
Taxol was developed by the NCI as a anti-neoplastic agent. The recent discovery that taxol induces TNF-α release from macrophages may provide additional information on the antitumor activity of this cytokine. We have investigated the cytolytic and cytostatic properties of TNF-α in association with taxol. Here, evidence is presented showing that certain TNF sensitive mouse fibroblast cell lines become quite resistant to TNF lytic activity in the presence of taxol. The increased resistance to TNF lysis does not involve the cell cycle or an increased expression of a previously characterized protein synthesis dependent resistance mechanism. Rather, it appears that TNF lytic signal transduction is blocked early and permanently upon exposure to taxol. These observations show that there is no synergism between TNF-α and taxol to kill transformed cells. Nevertheless, taxol may prove to be a very powerful tool to study the elusive TNF lytic pathway.

E 419 PROSTAGLANDINS OF THE E SERIES INHIBIT GM-CSF EXPRESSION IN HUMAN FIBROBLASTS.
Ravindra R. Patil and Richard F. Borch, Department of Pharmacology, University of Rochester, Rochester NY 14642.

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) expression by fibroblasts is primarily controlled by cytokines (IL-1 and TNF-α) secreted by macrophages. Prostaglandins of the E series (PGEs), which are also produced by activated macrophages, have been shown to induce GM-CSF in murine T-cell clones and suppress it in murine bone marrow stromal cell clones with macrophage like phenotype. We found that PGEs strongly suppressed IL-1 and TNF-α induced GM-CSF mRNA and protein expression in human fibroblasts. GM-CSF protein secreted in 24 hours by maximally stimulated WI38 human embryonic fibroblasts was reduced 6 fold by 10μM PGE_1 and PGE_2. Concentration response was observed in the range 0.1μM - 10μM and 50% of suppression was observed at 0.3μM. Normal human bone marrow derived fibroblasts secreted GM-CSF at a 6-8 fold lower level than WI38 fibroblasts. Exposure to 10μM PGE_1 almost completely blocked the GM-CSF production by these cells. This suppression was specific for GM-CSF, since the simultaneous expression of G-CSF and IL-6 was not affected. The GM-CSF mRNA levels assessed after six hours exposure to IL-1β were reduced 10 fold when 10μM PGE_1 was present in the medium. PGEs have been positively linked with cyclic AMP (cAMP) production. Exposure of WI38 fibroblasts to 1μM PGE_1 resulted in a 100 fold increase in the intracellular cAMP levels. The suppression of GM-CSF mRNA and protein levels could also be mimicked by stable cAMP analogs as well as cAMP elevating agents such as cholera toxin, forskolin and IBMX. The effect was not shared by $PGF_{2\alpha}$ which does not elevate cAMP levels in these cells. Surprisingly, though PGEs caused production of large amounts of cAMP in human umbilical vein endothelial cells, they failed to suppress IL-1 induced GM-CSF expression by these cells. Our results suggest that GM-CSF induction and suppression is regulated by different cell type specific events in fibroblasts and endothelial cells.

E 420 PREEMPTIVE IL-1ra THERAPY SUSTAINS LATE POST-BURN NONSPECIFIC CELLULAR AND HUMORAL IMMUNE RESPONSES AND ATTENUATES T CELL-MEDIATED IMMUNODEPRESSION. VM Peterson, CH Rundus, M Emmett, UH Chudgar, LL Ketch, Depts of Surgery and Pediatrics, School of Medicine, and Dept of Biology (Denver), Univ. of Colorado, Denver CO.

Postburn inflammation is partly mediated by IL-1 and is followed by profound immunodepression. We hypothesized that preemptive anti-inflammatory therapy with IL-1 receptor antagonist (IL-ra) might avoid postburn immunodepression. After 20% burn injury, CF-1 female mice received IL-1ra (RA, Synergen, Boulder, CO)), 20 mg/kg b.i.d., or 0.05% human serum albumin (HSA) for 72 h. At postburn days (PBD) 1, 3, and 7, plasma colony-stimulating factor (CSF) activity and acute phase protein levels were individually measured in groups of 4 mice. Also, phagocytic stem cell numbers (CFU-GM) were assayed in soft agar cultures of bone marrow and spleen (SPL). Plasma levels of haptoglobin (HAPT), and five other hepatic acute phase proteins, and CSF bioactivity were comparable in

PBD	Rx	HAPT, %NL	CSF, U/mL	CFU-GM/SPL
1	HSA	188 + 30	520 + 40	3727 + 540
	RA	153 + 11	640 + 240	1288 + 291*
3	HSA	215 + 39	347 + 40	1408 + 539
	RA	213 + 38	400 + 27	740 + 376
7	HSA	116 + 15	360 + 93	1132 + 440
	RA	256 + 37*	627 + 67*	2189 + 494**

* $p < 0.05$, and** $p < 0.08$, versus same day control

the RA and HSA-treated mice at PBD 1 and 3, but PBD 7 levels in RA-treated mice rose above those seen with HSA treatment. Marrow CFU-GM were unaffected by RA, but splenic CFU-GM were decreased during RA treatment before rebounding above HSA controls at PBD 7. At PBD 14, cutaneous reactivity to 2,4-dinitrofluorobenzene was significantly ($p < 0.05$) restored by RA treatment (data not shown). These data suggest that a preemptive course of IL-1ra therapy sustains nonspecific cellular and humoral immunity and attenuates T cell-mediated immunodepression following a major thermal injury.

E 421 PROTECTION AGAINST ENDOTOXIC SHOCK IN MICE BY A NEW CLASS OF SYNTHETIC PHOSPHOLIPID SECOND MESSENGER INHIBITORS, Glenn Rice, Stuart Bursten, Paul Brown, William Tino, Nancy Jenkins, James Bianco, and Jack Singer. Cell Therapeutics, Inc., 201 Elliott Ave. West, Seattle, WA 98119

Septic shock is thought to be initiated by endotoxin and maintained by a cytokine cascade involving IL-1α and β, TNF-α, IL-6 and IL-8. Treatment of a variety of cell lines with either IL-1 (via the Type I receptor), TNF (probably via the Type I 55kD receptor) or LPS rapidly induces synthesis of a highly unsaturated phosphatidic acid (PA) sub-species through activation of the enzyme lyso-PA acyl CoA:acyl transferase (LPAAT). LPAAT is competitively inhibited (IC_{50}<0.5μM) by a class of newly developed synthetic heterocyclic compounds. Two of these compounds, including a reversible inhibitor (CT-1501R) and an irreversible inhibitor (CT-1541) were compared in *in vitro* and *in vivo* inflammatory and endotoxin-induced sepsis model systems. The compounds were nontoxic to normal cells *in vitro* even at high concentrations (>0.25mM) for extended periods of time (>7 days). CT-1501R and CT-1541 were well tolerated in mice and CT-1501R in dogs at high doses for prolonged intervals. The compounds blocked numerous *in vitro* LPS, TNF or IL-1-mediated proinflammatory activities including adhesion of U937 and PMN to LPS or TNF-activated endothelium and increased transcription and/or secretion of several cytokines following LPS treatment. These compounds were also examined in the Balb/C mouse model system of endotoxic shock. Mice were injected i.v. with a dose of endotoxin sufficient to produce 100% lethality within 24 hrs. The compounds were injected i.p. three times per day at 50-100mg/kg depending on the compound. When the compounds were given simultaneously with, or up to 2 hours following the endotoxin challenge, survival was 100% (measured up to 80 hrs). Significant protection was also obtained when the compounds were administered 4 hours (60% survival) and even 6 hours (30%) after the endotoxin challenge. Serum TNF-α levels in the endotoxin treated mice peaked at 1 hr (1800pg/ml) and by 6 hrs had fallen to 70 pg/ml. When a single dose of CT-1501R was administered simultaneously with endotoxin, peak serum levels were decreased to 420 pg/ml. Suppresion of TNF-α serum levels therefore did not appear to be the principal mechanism of action since CT-1501R and CT-1541 protected animals when given even 5 hrs after peak TNF-α levels. These data suggest that the LPAAT pathway may be an important proximal signaling pathway in the genesis of endotoxic shock and that small molecule inhibitors of this pathway may have significant clinical potential in the treatment of septic shock and other inflammatory diseases.

E 422 REGULATION OF IL-1 AND TNF INDUCED GM-CSF GENE EXPRESSION: POTENTIAL INVOLVEMENT OF ARACHIDONIC ACID METABOLISM. Maria Teresa Rizzo and H. Scott Boswell, Department of Medicine and Walther Cancer Institute. Indiana University School of Medicine, Indianapolis, IN 46202

IL-1 and TNF induce GM-CSF gene expression in mesenchymal cells. However the underlying mechanisms are not yet fully defined. We explored the possibility that arachidonic acid cascade may be involved in regulating GM-CSF transcription. IL-1 (500 U/ml) and TNF (500 U/ml) stimulation of the stromal cell line, +/+ LDA11 induce c-jun and GM-CSF mRNA accumulation. This was accompanied by IL-1 and TNF induce ^{3}H-arachidonate release. Pretreatment of cells with the phospholipase A2 inhibitor quinacrine not only partially blocked ^{3}H-arachidonic release but also inhibited c-jun and GM-CSF gene expression. The effect of quinacrine was specific since it did not inhibit TPA (500 nM) induced c-jun expression. Furthermore exogenous arachidonic acid (10 μM) induced expression of both c-jun and GM-CSF in a time and dose dependent manner. No effect on c-jun and GM-CSF gene expression was found when oleic or palmitic acid were used. To investigate the role of arachidonic acid on GM-CSF transcription we used a reporter vector consisting of the murine GM-CSF promoter linked to luciferase. Transfection efficiency was assayed with RSVβ galactosidase. Arachidonic acid (10 μM) induced 2.5 fold increase of GM-CSF transcription compared to control. In addition treatment with quinacrine (2 μM) prior stimulation with IL-1 and TNF partially blocked GM-CSF transcription. These results are consistent with the hypothesis that arachidonate metabolites are involved in the signalling pathway that lead to IL-1 and TNF induced GM-CSF gene expression. If arachidonic acid exerts its effect through protein kinase C dependent transcriptional factors such as c-jun/AP1 or NF-KB will need further investigation.

E 423 EFFECTS OF CYCLOSPORINE ON THE PATHWAYS OF IL-2 GENERATION AND CELL MEDIATED LYMPHOLYSIS IN A MURINE CARDIAC ALLOGRAFT MODEL

Richard D. Schulick, Matthew W. Miller and Gene M. Shearer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20892.

Cyclosporine prevents rejection by inhibiting the production of IL-2 from T helper cells. IL-2 production is considered to be important in allograft rejection by providing help to cytotoxic T lymphocytes (CTL) and is generated by three pathways. Two of these pathways are dependent on allo or stimulator antigen presenting cells (APC) and one is dependent on self or responder APC. We have used a murine heterotopic fetal cardiac tissue tranplant model to study the effects of cyclosporine on the pathways of IL-2 generation and cell mediated lysis. Hearts from newborn C57BL/6 mice were transplanted into subcutaneous pockets formed in the ears of adult male BALB/c mice treated with oil, 15, 30 or 60 mg/kg daily of cyclosporine. In oil treated recipients, the grafts started to beat within one week, but stopped and underwent rejection within two weeks of transplantation. In recipients treated with 15 mg/kg daily of cyclosporine, the grafts rejected within two to five weeks. In recipients treated with 30 or 60 mg/kg daily of cyclosporine, the grafts beat for the duration of the study (>10 weeks). In this study, we also demonstrate that splenocytes from BALB/c mice treated with 15 mg/kg daily of cylcosporine generate active cytotoxic T lymphocytes against C57BL/6 splenocytes depleted of APC. However, BALB/c mice treated with 30 or 60 mg/kg daily of cylcosporine did not generate active cytotoxic T lymphocytes against C57BL/6 splenocytes depleted of APC. When IL-2 was added during the generation of CTL, BALB/c mice treated with 30 or 60 mg/kg daily of cyclosporine regained the ability to generate active cytotoxic lymphocytes against C57BL/6 splenocytes depleted of APC. Cultures from all BALB/c mice generated cytotoxic lymphocytes against undepleted C57BL/6 splenocytes without exogenous IL-2. Thus, the ability of cyclosporine treated BALB/c mice to accept C57BL/6 cardiac tissue correlates with the absence of generation of CTL against stimulator cells depleted of APC. Our results are consistent with the rejection of cardiac allografts by a mechanism that involves self-restricted T cells that produce IL-2 and provide help for CTL effectors. We are further defining the pathways of IL-2 generation in this model to determine those that are are functional in mice given different doses of cyclosporine.

E 424 HUMAN IL-1 RA: GENE MAPPING, ISOLATION OF YAC CLONES AND CHARACTERIZATION OF THE LIVER TRANSCRIPT, Alexander Steinkasserer, [a]Nigel K. Spurr, [b]Andrew Lennard, Robert B. Sim, MRC Immunochem. Unit, Dept. of Biochemistry, South Parks Road, Oxford OX1 3QU, U.K.; [a]ICRF, Herts EN6 3LD, U.K. and [b]Yamanouchi Research Inst. (UK) Oxford

The IL-1 receptor antagonist (IL-1RA) is a protein which binds to IL-1 receptors and inhibits the binding of IL-1α and IL-1β. As a consequence, the biological activity of these two cytokines is neutralized in physiological and pathophysiological immune and inflammatory responses. Previously we described a triallelic polymorphism within the second intron of the IL-1RA gene. This length variation polymorphism was monitored in a panel of 5 CEPH families together with an IL-1α polymorphism and *subsequent linkage* analysis permitted the localization of the IL-1RA gene to chromosome 2 band q14-21, in the region of the IL-1α and IL-1β loci. This finding supports the view of an early gene duplication event resulting in the creation of an IL-1 gene family.
From the ICRF-YAC library we isolated three clones(300-620 kb) containing the IL-1RA gene. None of these clones contains the IL-1α or IL-1β genes indicating that the member of the gene family have become moderately dispersed. These YAC clones contain the additional exon for the intracellular IL-1RA and will now be used to characterize further this chromosome two region.
IL-1RA is mainly expressed in monocytes, macrophages, neutrophils and keratinocytes. In this study we report the expression of IL-1RA molecule in liver and in the hepatoma cell line HepG2. Sequence analysis of IL-1RA specific clones, isolated from a liver cDNA library, revealed that the sequence is identical to the secreted form of IL-1RA from monocytes. Finally, IL-RA was expressed in E.coli as a functionally active recombinant protein and was used to investigate if the four cysteines present are disulphide-linked. Radioalkylation and analysis of V8-derived peptides indicate that no disulphide bridges are formed.

E 425 HUMAN IL-10 DIRECTLY INHIBITS T CELL GROWTH AND CYTOKINE PRODUCTION, Kazuyuki Taga and Giovanna Tosato, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892.

Human IL-10 inhibits T cell proliferation and cytokine production in the presence of monocytes. We have investigated whether IL-10 can directly inhibit T cells. Highly purified peripheral blood T cells containing < 0.1% CD14 positive cells and unresponsive to PHA, were growth inhibited by IL-10 when stimulated with immobilized OKT3 mAb (55.4% inhibition). This effect was neutralized by the murine mAb 19F1 directed against human IL-10. IL-10 inhibited by 52.5% the proliferation of a human tetanus toxoid-specific T cell clone (TM11) induced by immobilized OKT3 mAb in the absence of antigen presenting function. T cell growth inhibition by IL-10 did not reflect a cytokine-induced change in the kinetics of T cell response to immobilized OKT3 mAb, and was observed over a wide range of cell and OKT3 mAb concentrations. Addition of monocytes to T cells resulted in the emergence of proliferation to PHA and to soluble OKT3 mAb, but did not significantly affect levels of growth inhibition by IL-10 in presence of immobilized OKT3 mAb. Similarly, addition of monocytes to the TM11 T cell clone resulted in the emergence of proliferation in response to tetanus toxoid but did not significantly influence growth inhibition by IL-10 in the presence of immobilized OKT3 mAb. When stimulated with immobilized OKT3 mAb in the absence of accessory cells, T cells secreted IFN-γ and IL-2. Secretion of IL-2 under these conditions was inhibited by IL-10 by a mean of 51.5% while secretion of IFN-γ was inhibited by a mean of 34.3%. Thus, IL-10 can directly inhibit growth and cytokine production in T cells triggered by immobilized OKT3 mAb in the absence of monocytes.

E 426 A HUMAN IL-4 TOXIN (DAB_{389}IL-4) WHICH IDENTIFIES AND SELECTIVELY ELIMINATES INTERLEUKIN 4 RECEPTOR-BEARING CELLS OF BOTH HEMATOPOIETIC AND NON-HEMATOPOIETIC ORIGIN,

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Cell surface interleukin 4 (IL-4) binding proteins have been reported on a diversity of carcinomas of both hematopoietic and non-hematopoietic origin, but few studies clearly attribute functional receptor activity to these molecules. In order to determine whether the presence of these binding proteins would render tumors susceptible to receptor-specific cytotoxic agents we utilized a genetically-engineered fusion toxin, DAB_{389}IL-4, as a probe for internalization-competent receptors which can initiate appropriate pathways of ligand processing. DAB_{389}IL-4 is a diphtheria toxin-related IL-4 fusion protein constructed by replacing sequences encoding the receptor binding domain of native diphtheria toxin with human recombinant IL-4 sequences. Receptor binding studies using neoplastic human T cell lines which constitutively express the IL-4 receptor (IL-4R) indicated that the fusion toxin was able to competitively displace (^{125}I)IL-4 binding at an effective K_I of 7.9×10^{-9} M. By contrast, IL-4 bound to the same receptor at a K_I of 7.1×10^{-12} M. Despite the alteration in binding constant, the fusion protein half-maximally inhibited protein synthesis in HUT 102/6TG T cells at an IC_{50} of 2.9×10^{-11} M. A 100-fold molar excess of human IL-4 completely blocked the interaction, indicating that DAB_{389}IL-4-mediated inhibition of protein synthesis in these cells was IL-4R-specific. Nearly half (6/14) of the B cell, a quarter (1/4) of the myelomonocytic and over half (7/12) ot the T cell continuous lines were sensitive to the IL-4 fusion toxin as defined by IC_{50} in the subnanomolar to nanomolar range. Of the 71 non-hematopoietic human tumor cell lines evaluated, 14 (20%) were sensitive to DAB_{389}IL-4. Tumors displaying the highest frequency and sensitivity to the fusion toxin included hepatocellular carcinomas, renal cell carcinomas, gastric carcinomas and gliomas. Further evaluation of the hepatoma group indicated that the intoxication process was IL-4R-specific and, as with the other non-hematopoietic cell lines studied, mediated by a high affinity receptor with a K_I similar to that found on human hematopoietic cell lines.

Cytokine Signalling/Processing

E 500 INTERLEUKIN-4 AND PROLACTIN ACTIVATE DIFFERENT TRANSCRIPTION FACTORS WHICH BIND TO THE SAME HORMONE-RESPONSIVE DNA ELEMENT,

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Prolactin is required for the induction of milk protein gene transcription in the terminal differentiation of mammary epithelial cells. A mouse mammary gland prolactin receptor isoform was cloned and belongs to the superfamily of cytokine receptors. Amongst their ligands, IL-4 was able to substitute for prolactin and induce both transcription of transfected beta-casein promoter constructs and expression of the endogenous beta-casein protein. By promoter analysis, both hormones were shown to use the same DNA element which conferred IL-4 responsiveness upon a heterologous promoter. IL-4 induced the binding of a nuclear factor (NF-IL4) to this element and a distinct, mammary gland specific factor (MGF) was induced by prolactin. Activation of the IL-4 receptor led to the modification of pre-formed NF-IL4 which was rapidly induced within 5 minutes in the absence of protein synthesis. IL-4 may act via a tyrosine kinase since NF-IL4 is phosphorylated on tyrosine and phosphotyrosine phosphatase inhibitors enhanced the induction of NF-IL4. NF-IL4 was also induced in T lymphocytes by IL-4. NF-IL4 also binds to a similar DNA element in the IL-4 responsive region of the promoter of the germ line transcript of the IgE constant region and thus could mediate IL-4 dependent immunoglobulin class switching to IgE in allergy. The study of the regulation NF-IL4 may help understand how the autocrine production of IL-4 by tumour cells which were transfected with an IL-4 expression vector, leads to the generation of a potent anti-tumour immune response.

E 501 PUTATIVE NOVEL RECEPTOR TYROSINE KINASE (EmRK0) EXPRESSION IN HEMATOPOIETIC LINEAGES

Kyunghee Choi, Marion Kennedy and Gordon Keller, National Jewish Center, 1400 Jackson Street, Denver CO. USA.

Receptor tyrosine kinases (RTKs) are believed to play an important role in the differentiation and proliferation of various cell populations, including those of the hematopoietic system. For example, c-*fms* encodes a macrophage growth factor receptor and c-*kit* encodes a receptor that is involved in the development of multiple hematopoietic lineages including mast and erythroid lineages.

To determine if novel RTKs are involved in the early development of the hematopoietic system, we have designed degenerate oligonucleotides that correspond to the conserved regions of the class III RTKs; PDGFR, CSF-1R, and the c-*kit* encoded receptors. We have carried out PCR with these oligonucleotides on both undifferentiated ES cells and on the progeny of ES cells that have been differentiated for 6 days in methyl cellulose cultures.

ES cells were chosen since the undifferentiated ES cells can differentiate efficiently into hematopoietic cells under well defined conditions in methyl cellulose cultures. In addition, precursors of the various myeloid hematopoietic lineages develop within these cultures in a defined kinetic pattern, enabling us to study the sequence of molecular events involved in the establishment of the hematopoietic system. The day 6 differentiated ES cells are at the stage where we can detect extensive hematopoiesis within the differentiated progeny. We have identified four novel transcripts in the differentiated populations that show homology to the above RTKs, and therefore likely encode RTKs.

The gene (EmRK0) corresponding to one of these receptors has been cloned and its pattern of expression has been analyzed extensively both in myeloid and lymphoid hematopoietic lineages. In the myeloid lineages, EmRK0 is expressed predominantly in erythroid cells, including both the primitive and definitive populations. Within the B lymphoid lineage, EmRK0 is expressed only in $CD5^+$ ($Ly\text{-}1^+$) population. In addition to $CD5^+$ B cells, it is also expressed in $\gamma\delta$ and $\alpha\beta$ T cells. However, within the ab T cell population, its expression appears to be restricted to immature cells of the thymus. More detailed expression pattern and the speculation of the possible role of the EmRK0 expression in the $CD5^+$ B cell population and in the T cell populations will be presented.

E 502 CONSTRUCTION OF A RIBOZYME CLEAVING HUMAN IL-6 mRNA, J. Content and M. Mahieu *(Department of Virology, Institut Pasteur du Brabant, B-1180 Brussels, Belgium).*

Interleukin-6 (IL-6) is a pleiotropic cytokine mainly involved in the regulation of inflammatory and immunological processes. Amongst its major roles, it triggers the production of acute phase proteins by hepatocytes, activates proliferation and cytotoxic differentiation of T cells, stimulates terminal differentiation of B lymphocytes, and is a growth factor for murine hybridoma and plasmacytoma cell lines. In addition it has been implicated in several pathologies as a potent growth factor for example in multiple myeloma and in Kaposi sarcoma, perhaps with other factors. In order to study the role of IL-6 production in various biological situations we have developped a ribozyme which is capable of specifically digesting IL-6 mRNA. This has been studied *in vitro*, in order to define the specificity, the kinetics and the optimal ratio between IL-6 mRNA and the ribozyme. Stable transfectants expressing this ribozyme under the control of a strong CMV promoter have been studied by PCR, Southern blot and Northern blot hybridization. Their capacity to produce IL-6 has been studied after TNF stimulation. *In vitro* the anti IL-6 ribozyme cleaved IL-6 mRNA efficiently yielding the expected size fragments even at a ribozyme/mRNA ratio of 1:4. *In vivo* suboptimal TNF-induction of IL-6 was totally abrogated in cells transfected with the ribozyme gene. This approach could be useful for studying the biological role of various cytokines and also their mechanisms of action.

E 503 **GROWTH FACTOR-STIMULATED TYROSINE PHOSPHORYLATION OF p95^{vav} IN HUMAN HEMOPOIETIC CELLS.** Robert L. Cutler, Xose R. Bustelo*, Mariano Barbacid* and Gerald Krystal. Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, Canada and *Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey.

The *vav* proto-oncogene encodes a protein, p95^{vav}, which is rendered oncogenic by the loss of a short N-terminal domain. Northern analysis of cultured human and murine cells has revealed that its expression is restricted to cells of hemopoietic origin, regardless of lineage or state of differentiation. While p95^{vav} contains an eclectic combination of functional domains including both SH2 and SH3 domains, a diacylglycerol binding site and a region of homology to the GDP-GTP exchange domains of the rhoGAP proteins *dbl*, bcr and CDC24 its function, as yet, remains obscure.

We previously demonstrated that steel factor (SF) stimulates the tyrosine phosphorylation of p95^{vav} in the human cell lines MO7e and TF-1 (Alai *et al*, J. Biol.Chem., in press, 1992) but not in the acute myeloblastic leukemic cell line, AML193 which does not express SF receptors. We have now extended these observations to include the effects of human IL-3 and GM-CSF on these cells. Phosphoamino acid analysis and Western blotting show that p95^{vav} is constitutively tyrosine ptphosphorylated in all three cell lines and that all three growth factors stimulate only tyrosine phosphorylation of p95^{vav}, with no detectable increase in the level of serine or threonine phosphorylation. Comparison of phosphorylation at 4°C and 37°C shows that tyrosine phosphorylation occurs only at 37°C suggesting that the p95^{vav} phosphorylation is a downstream event which follows the tyrosine phosphorylation of several previously documented intracellular proteins. We are currently generating phosphopeptide maps to examine whether IL-3, GM-CSF and SF stimulate tyrosine phosphorylation of p95^{vav} on the same or different tyrosine residues.

E 504 IDENTIFICATION OF NOVEL TYROSINE (Y) AND SERINE/THREONINE (S/T) KINASES IN RESTING AND ACTIVATED HUMAN STEM CELLS. J. DiPersio*, S. Luhowskyj* and J. Casnellie*. University of Rochester Medical Center, Rochester, NY.

Highly degenerate oligonucleotide primers have been designed to subdomains VI (sense) and IX (antisense) of the human Y kinase and to subdomains I (sense) and VI (antisense) of the human S/T kinase families. These degenerate primers were used to PCR cDNA obtained from both resting and cytokine activated human stem cells (CD34+, CD10-, CD38-). These cells represent only 0.005% - .01% of the starting marrow sample. These partial cDNAs were subcloned into M13 and over 300 white plaques containing kinase inserts were analyzed first using single base sequencing and subsequently by full 4 base sequencing reactions. The most frequently obtained known sequences were those of c-fms > IGF-1R > JTK14 ("tie") > csk > fes/fps > fer. Novel Y kinase partial cDNA clones were extended with a second PCR reaction using a sequence-specific 3' antisense primer and a 5' sense degenerate primer from subdomain I of the Y kinase catalytic domain. These extended clones were sequenced and used as probes to screen a normal human bone marrow λgt11 cDNA library. At least 6 novel human Y kinase partial cDNAs were identified and characterized. One was recently shown to be similar to the Y and S/T bifunctional kinase, JAK 1. Another GN22 exhibits sequence homology with TRK, JAK1, and TYRO12. GN70 was found to be homologous to the "TEC" kinase. A full-length clone has been obtained for the most frequently identified novel Y kinase, SL3. This 3.0 kb SL3 cDNA encodes a putative 894 amino acid receptor-type Y kinase with unique fibronectin and immunoglobulin type domains in the extracellular region. This kinase was found to be identical to the receptor tyrosine kinase "AXL" or "UFO" which was recently cloned from patients with CML and myeloproliferative syndromes. The expression and significance of these kinases and the technology to identify other kinases will be discussed.

E 505 SIGNAL TRANSDUCTION IN LEUKEMIC CELL DIFFERENTIATION, Angelika M.Dräger, Klaas G.v.d. Hem, Peter C. Huijgens, Gert J.Ossenkoppele, Caroline Tol and Mart M.C.Langenhuijsen, Dept.of Hematology, Free University Hospital, 1081 HV Amsterdam, The Netherlands

To clarify the role of PKA and PKC in 1,25$(OH)_2$-D3 (VD3)induced differentiation we examined a panel of differentiation markers after stimulating HL60 cells and freshly isolated leukemic blasts of 11 consecutive patients with VD3 in combination with signal transducing modulators. The parameters studied were morphology, NBT reduction, quantitative acetate-and butyrate-esterase levels, and the expression of CD14 and CD34.

The PKA agonist cAMP-Sp and the PKA antagonist cAMP-Rp had no measurable effect on VD3 induced HL60 differentiation parameters. The PKC agonist bryostatin 5 induced macrophage-like morphology with strong adherent characteristics even in the absence of VD3. In contrast the NBT reducing ability was totally depressed. Both the differentiation marker CD14 and CD34 were increased in the presence of bryostatin 5. Inhibition of PKC by staurosporin resulted in a stimulation of NBT reducing ability in HL60 cells even in the absence of VD3. Nonspecific esterase levels were decreased in the presence of staurosporin and VD3.

In patient samples varies responses were seen. In the presence of bryostatin the cells from most of the patients showed macrophage-like morphology and were strongly adherent. NBT reducing ability was strikingly reduced. The nonspecific esterase activity was increased strongly in 50% of the patients and the CD14 expression was upregulated. In the presence of staurosporin and VD3 NBT formation was slightly increased in less differentiated leukemias but depressed in AML blasts of the monocytic lineage.

In conclusion the spectrum of responses seen in patient samples suggest an heterogenous response to VD3 of signal transducing pathways in varying leukemic lineages.

E 506 DISTURBANCES IN CELLULAR DIFFERENTIATION FOLLOWING MODULATION OF *hck* TYROSINE KINASE ACTIVITY BY GENE TARGETING IN EMBRYONIC STEM CELLS.

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Mammalian *hck*, a member of the *src*-family of protein tyrosine kinases, is expressed predominantly in cells of the myeloid and B-lymphoid lineages. Here we show that *hck* is also expressed in undifferentiated embryonic stem (ES) cells although *hck* expression is suppressed when ES cells are induced to differentiate by withdrawal of leukemia inhibitory factor (LIF), or by exposure to retinoic acid. In an attempt to explore the role of *hck* during embryonic development and in haematopoiesis, we established ES cells with elevated levels of *hck* kinase tyrosine activity using gene targeting to create an *hck* "transgene" in situ.

The activity of *src*-related tyrosine kinases is negatively regulated by phosphorylation of a conserved C-terminal tyrosine (Y) residue (Y527 in *c-src*). Substitution of this Y for phenylalanine (F) causes elevated kinase activity and generates molecules with the capacity of oncogenically transform cultured cells when expressed ektopically. In order to elevate *hck* tyrosine kinase activity without influencing the transcriptional regulation of *hck* gene expression, we created an *hck* "transgene" in situ by introducing the Y499F substitution at the C-terminal Y residue using a "Hit & Run" gene targeting strategy for homologous recombination in ES cells. A number of ES cell lines containing the Y499F substitution were isolated and the fidelity of homologous recombination was confirmed by nucleotide sequence analysis from the targeted allele. On a molar ratio, *hck* tyrosine kinase activity associated with the targeted ES cells was 8-12 fold higher than that observed in parental ES cells.

To further explore the possibility that *hck* plays a role in regulating ES cell differentiation, we investigated LIF requirement and programmed cellular differentiation of ES cells containing the *hck* Y499F mutation. Targeted ES cells required 10-20 times lower concentrations of LIF to maintain an undifferentiated phenotype in vitro. Furthermore, the proportion of cells maintaining stem cell characteristics in a semi-solid culture system favouring haemopoietic cell development was 4-8 times greater for ES cells with the Y499F mutation. These observations show that the targeted introduction of an activating Y499F mutation in one *hck* allele leads to a dominant phenotype in vitro, suggesting a role for *hck* in early murine development and during haemopoietic differentiation. Currently we are also analyzing chimeric mice generated from targeted *hck* Y499F ES cells.

E 507 CHARACTERIZATION OF A 97 kDa TYROSINE KINASE ASSOCIATED WITH THE IL-2 RECEPTOR COMPLEX: LACK OF A REQUIREMENT FOR SRC-RELATED ENZYMES, [1]William L. Farrar, [2]Gonzalo G. Garcia, [2]Gerald Evans, [1]Robert Kirken and [2]Zack Howard, [1]Cytokine Mechanisms Section, Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA. [2]PRI/DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201.

IL-2 induces tyrosine protein phosphorylation in cell lines devoid of p56LCK. We have compared the tyrosine phosphorylation patterns of human YT cells and the murine CTLL-2 cell lines that do not express p56LCK against parental cells that express p56LCK. No significant differences were seen in IL-2 stimulated proliferation or in tyrosine protein phosphorylation. We next examined the IL-2 receptor associated tyrosine kinase activity and found consistently a 97 kDa phosphotyrosyl protein associated with the p75 β chain in p56LCK$^-$ cells as well as p56LCK$^+$ cell lines. The 97 kDa phosphotyrosyl protein was found to have catalytic protein kinase activity. Truncations in the cytoplasmic domain of the recombinant p75 β chain deleted regions in which src-related enzymes have been shown to interact. Nevertheless, the mutant p75 β chains still were biologically operational and associated with the 97kDa protein tyrosine kinase. These data suggest that the src-related protein tyrosine kinases are probably not involved in IL-2R signalling for proliferation and that the newly discovered 97 kDa enzyme may be a principal tyrosine protein kinase associated with regions of the p75 β chain required for IL-2 directed biological responses.

E 508 EARLY EVENT OF IL-6 SIGNALING IN HEPATOCYTES

Gerald M. Fuller, James E. Nesbitt, Yang Wang, and Nelson L Fuentes Dept. of Cell Biology, The University of Alabama at Birmingham, Birmingham AL.

Interleukin-6 is a major regulator of many plasma protein genes synthesized by hepatocytes. Molecular details of IL-6 signal generation are not currently known. Initiation of the signal begins with binding of the ligand to its cognate receptor followed by "activation" of a signal transducing protein gp130. To better understand the molecular details of IL-6 regulation of liver genes we examined some of the events which regulate the IL-6 receptor and gp130 in primary hepatocytes. Our findings reveal that IL-6R mRNA is up-regulated 2.5 fold within the first 3h of an acute inflammatory reaction, whereas the mRNA of hepatic gp130 is refractory to inflammatory mediators. The number of IL-6 receptors expressed increases nearly 10 fold but they are down modulated by either IL-6 or IL-1. Furthermore the mRNA half-life for the IL-6R and gp130 also showed significant differences indicating that the turnover of the IL-6 receptors are more rapid that that of gp130 Additionally we have cloned and sequenced the hepatocyte gp130 molecule. Nucleotide and amino acid sequence comparisons of rat hepatic gp130 with the human B-cell derived gp130 showed remarkable homology throughout the entire molecule. Examination of gp130 mRNA revealed two species, a prominent 7.5kb species, and a lesser one at 9.0 kb. These findings suggest that if different IL-6 target cells have unique responses to IL-6 they will be mediated by proteins distal to the activated gp130 molecule.

E 509 HUMAN INTERLEUKIN 1-ß mRNA TRANSCRIPTION IS ENHANCED BY THE ADDITION OF dbcAMP, Paul J. Furdon, J. David Taylor, Stephen A. Haneline, Sandra Stinnett, Gyan Chandra, G. Bruce Wisely, Indravadan R. Patel, William C. Clay, Thomas A. Kost, John G. Gray, Department of Molecular Biology, Glaxo Inc. Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709

In the human monocytoid cell lines, THP-1 and U937, transcription of interleukin 1-ß (IL1-ß) is induced by the addition of lipopolysaccharide (LPS) or LPS and phorbol 12-myristate 13-acetate (PMA), respectively. The addition of dbcAMP to LPS-treated THP-1 cells or LPS and PMA-treated U937 cells results in a synergistic increase in the rate of IL1-ß mRNA transcription measured by nuclear run-on analysis as well as an increase in the level of IL1-ß mRNA as shown by Northern blot analysis. In order to identify the *cis*-acting elements involved in this induction, we have cloned the chloramphenicol acetyl transferase (CAT) gene under the control of 4 kb of DNA sequence found upstream of the IL1-ß gene transcription start site. In transient transfection assays, this IL1-ß promoter-CAT construct responds to the inducers in a manner similar to the endogenous gene. Deletion analysis of the promoter has shown that the elements responsible for a large portion of this induction are located greater than 2.5 kb 5' of the RNA transcription start site.

E 510 STUDIES ON THE EXPRESSION OF INTERLEUKIN-12 SUBUNIT mRNAs IN MICE

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IL-12 is a heterodimeric cytokine that can act as a growth factor for activated T- and NK-cells, enhance the lytic activity of NK/LAK cells and stimulate the production of IFN-gamma by resting PBMC. The two subunits (p35 and p40) represent unrelated gene products. When coexpressed, they are assembled into the heterodimer by disulfide bonding and are then secreted as bioactive IL-12. Human and murine IL-12 subunits have been cloned and shown to be 60% (p35) and 70% (p40) identical. Human IL-12 does not act on murine cells, but murine IL-12 acts on human cells. This partial species specificity is due to sequences within the human p35 subunit.-
In the present study, semiquantitative PCR was used to monitor the changes in steady state levels of murine p35 and p40 mRNAs. The objectives of these studies were to determine i) in what cell type(s) IL-12 subunit mRNAs are expressed and ii) if changes in IL-12 gene expression can be detected during lymphocyte activation in vitro. IL-12 gene expression by both unfractionated splenocytes and isolated splenic lymphocyte subsets was examined following i) culture in the absence of an activating stimulus, ii) activation by mitogens or iii) culture of mixed leukocytes. The results generally demonstrated uncoordinated changes in p35 and p40 mRNA levels, indicating potentially complex transcriptional regulatory mechanisms for the two mRNAs.

E 511 p50 AND p65 SUBUNITS OF THE NFκB COMPLEX, BUT NOT C-REL, ARE INVOLVED IN IL-6 INDUCTION BY IL-2 IN HUMAN MONOCYTES, G. L. Gusella[1], T. Musso[1], M. C. Bosco[2], Nancy R. Rice[3], D. Longo[2], L. Varesio[2], [1]BCDP, PRI/DynCorp., NCI-FCRDC, Frederick, MD 21702-1201, USA; [2]BRMP and [3] Laboratory of Molecular Virology and Carcinogenesis, NCI-FCRDC, Frederick, MD 21702-1201, USA

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 have previously reported that IL-2 induces IL-6 mRNA expression and IL-6 bioactivity, in a dose-dependent manner. We have also shown that IL-2 can induce IL-6 directly and not through IL-1β, since antiIL-1β antibodies are not able to block IL-6 mRNA induced by IL-2. We have analyzed the level at which IL-2 induction of IL-6 occurs and the regulatory regions of the IL-6 gene involved in IL-6 induction by IL-2. Nuclear run off experiments showed that IL-6 is transcriptionally upregulated upon IL-2 treatment. Several potential transcriptional control elements, such as GRE, AP-1 binding site, MRE, NF-IL6 and NFκB binding sites were identified in the IL-6 promoter. We investigated the response of monocytes to IL-2 at the nuclear level by measuring the changes in IL-6 promoter DNA-binding proteins by electrophoretic mobility shift assays. Nuclear extracts from monocytes showed a constitutive binding to the fragment of the IL-6 promoter containing the MRE/NF-IL6 binding site. We did not detect any change in the binding to this fragment after treatment with IL-2 in comparison with medium alone. On the contrary, when we used the fragment of the IL-6 promoter containing the NFκB binding site, an increase in the binding activity was observed in nuclear extracts from monocytes treated with IL-2 in comparison to medium alone. Additional studies with antibodies prepared against the p50 and p65 subunits of the NFκB complex and against c-Rel showed that p50 and p65, but not c-Rel are involved in the binding upregulated by IL-2 treatment.

E 512 THE CRITICAL REGION FOR GROWTH SIGNAL TRANSDUCTION OF IL-4 IN THE CYTOPLASMIC DOMAIN OF HUMAN IL-4 RECEPTOR IN PRO-B CELL LINE IS ALSO ESSENTIAL FOR T CELL GROWTH AND IgE PRODUCTION, Nobuyuki Harada, Cynthia Schultz, Gloria Yang, Robert Coffman and Kenji Izuhara, Department of Immunology, DNAX research Institute, Palo Alto, CA. 94304-1104

IL-4 is preiotropic lymphokine. It acts on B cells, T cells, mast cells and several other hematopoietic cells. IL-4 manifests its biological effects through IL-4 specific, high affinity receptor on the cell surface. Previously we have identified the critical region for growth signal transduction of IL-4 in the cytoplasmic domain of human IL-4 receptor (IL-4R) in mouse pro-B cell line. In the present study, we examined whether the cricital region of human IL-4R in pro-B cells is also critical in other IL-4 dependent events, e.g. growth of T cells and IgE class switching in B cell. To address these questions, we transfected several mutated hIL-4R cDNAs into CTLL cells and Abelson murine leukemia virus transformed B cell line, A20 cells. Both transfected CTLL cells and A20 cells which express mutated IL-4R exhibited high affinity binding sites for human IL-4 on the cell surface. Among these transfectants, transfectants expressing hIL-4R lacking the critical region for growth signal transduction identified in pro-B cells were not able to respond to human IL-4. These results suggest that both the growth signal pathway of IL-4 in pro-B cells and T cell, and the differentiation signal pathway of IL-4 for class switching to IgE, are transduced by same molecule(s) which may associate with IL-4R.

E 513 DIFFERENTIATION INDUCTION BY LEUKEMIA INHIBITORY FACTOR (LIF) CORRELATES WITH THE OCCURENCE OF A SPECIFIC LIF-RESPONSE FACTOR THE LIF-RF, Gertrud M. Hocke, Roland Piekorz, Georg Fey, Lehrstuhl für Genetik, University Erlangen, Staudtstr. 5, Germany

Leukemia Inhibitory Factor (LIF) is a growth factor for ES1 murine embryonal stem cells and a differentiation promoting factor for other cell types. In hepatocytes it induces a similar set of acute phase genes as interleukin 6 (IL6). A cis-acting LIF-response element (LIF-RE) of the acute phase rat α_2-macroglobulin (α_2M) gene was previously mapped and shown to coincide with its IL6-response element (IL6-RE). The specific DNA-binding capacity of a nuclear LIF-Response Factor (LIF-RF) was induced in HepG2 human hepatoma cells by treatment with LIF. This protein was similar or identical with the IL6-Response Factor (IL6-RF) and formed a characteristic protein-DNA complex (complex II) with the LIF-RE. A corresponding complex II was also formed between the LIF-RE and nuclear extracts from LIF-treated proliferating ES1 cells. Withdrawal of LIF caused the differentiation of ES1 cells and the loss of their ability to form complex II as well as a progressive approximately 10-fold loss of LIF receptors. Differentiating cells of the myeloblastic murine cell line M1 showed a protein DNA complex which migrated with the same mobility as complex II of ES1 cells. Thus, a strong correlation between the induction or inhibition of differentiation by LIF and the specific DNA-binding ability of the LIF-RF was observed in different cell lines.

E 514 HUMAN INTERLEUKIN-1β CONVERTING ENZYME: A MUTATIONAL ANALYSIS OF PROENZYME ACTIVATION.

A. D. Howard, G. J-F. Ding, A. M. Rolando, O. C. Palyha, E. P. Peterson, F. J. Casano, E. K. Bayne, S. Donatelli, J. M. Ayala, L. A. Egger, D. K. Miller, S. M. Raju, T. T. Yamin, J. Jackson, K. T. Chapman, N. A. Thornberry, J. A. Schmidt, M. J. Tocci, S. M. Molineaux.
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Interleukin-1β is a potent inflammatory cytokine released from activated monocytes and macrophages. In human blood, IL-1β production has been shown to be dependent on the action of a novel, highly specific and selective protease, termed interleukin-1β converting enzyme (ICE). Currently, there is strong interest in elucidating the maturation pathway and cell biology of ICE since it may be important in the production of active IL-1β. Active ICE is a heterodimeric, cysteinyl protease with subunit masses of 20 (p20) and 10 (p10) kDa derived proteolytically from a common precursor of 45 kDa (p45) which also contains an N-terminal prodomain of 119 residues. Since ICE-like cleavage sites (Asp-X, where X is Ser, Asn, etc.) flank the mature subunits, and p45 is a substrate for the p20:p10 ICE complex, it has been suggested that ICE maturation proceeds by an autocatalytic mechanism. In the present study, we sought to (1) identify the human ICE cDNA sequence(s) responsible for generating active ICE in transfected COS-7 cells, (2) determine the catalytically-active ICE forms produced, and (3) assess the functional role of the ICE-like cleavage sites. Our results indicate that maximal levels of active ICE are generated using expression constructs containing p45. The catalytically-active form was found to be exclusively the p20:p10 complex through inefficient conversion from the p45 proenzyme. No other active forms were detected, including p45, which was the predominant form detected by immunoblotting. Mutation of the catalytically-essential thiol (^{285}Cys) abolished ICE activity and prevented the generation of the p20 subunit. Deletion of the N-terminal prodomain preceding the p20 and p10 subunits gave a recombinant protein with greatly decreased activity while the individual subunits expressed singly or in combination did not reconstitute enzymatic activity. A series of mutant p45 constructs containing single Asp to Ala substitutions in p45 ICE-like cleavage sites were assessed for their ability to generate active enzyme. Surprisingly, single mutations at the Asp-X bonds defining the amino or carboxy terminus of the p20 protein did not affect ICE activity, whereas mutation of the Asp-X bond at the amino terminus of p10 markedly decreased the generation of active ICE. In addition, a mutation of an Asp residue in the prodomain profoundly decreased ICE activity. We are currently generating p45 constructs with multiply mutagenized Asp-X bonds to further elucidate the processing pathway of ICE. Finally, indirect immunofluoresence studies colocalize recombinant ICE and its natural substrate IL-1β to the cytoplasm of transfected cells, as previously described for IL-1β in human monocytes. The above findings strongly suggest that active ICE is generated by proteolysis of an inactive precursor (p45) and argue that the N-terminal prodomain and certain ICE-like cleavage sites serve a functional role in proenzyme activation.

E 515 NUCLEAR REGULATORY CASCADE INDUCED BY INTEERLEUKIN-6, Wei Hsu and Selina Chen-Kiang, Molecular Biology Center, Mount Sinai School of Medicine, New York, NY 10029

Interleukin-6 (IL-6) is a pleiotropic cytokine, which is involved in cell growth and differentiation depending on the nature of the target cells. We present evidence here that the *oct-1* gene, which is ubiquitously expressed and has been shown to be regulated post-translationally, can also be regulated at the level of mRNA and protein synthesis by IL-6 in human embryonal carcinoma and T cells. NF-IL6, a human transcription factor of C/EBP family and an intermediate in the IL-6 signal transduction pathway, can confer this regulation. The abundance and the molar ratio of the three forms of NF-IL6 proteins initiated in frame from different AUG's appear to be regulated by IL-6. Furthermore, the regulation of NF-IL6 and Oct-1 synthesis are also induced by another signal, retinoic acid (RA). These results suggest that the overlapping regulation of NF-IL6 and Oct-1 in two signal transduction pathways and that Oct-1 may be downstream of NF-IL6 in the shared regulatory cascade.

We have previously shown that NF-IL6 can substitute for the adenovirus E1A proteins in transactivating E1A-responsive promoters in transfection and in complementing an E1A-deletion mutnat virus in viral infection. The enhanced Oct-1 synthesis correlates with one of its functions: stimulation of adenovirus DNA replication. It provides an example of functional consequence of IL-6 and RA signalling that is mediated by NF-IL6 and Oct-1 regulation.

E 516 OKADAIC ACID, A PHOSPHATASE INHIBITOR, REGULATES THE EXPRESSION OF THE IL-1ß GENE BY INCREASING THE AP-1 ENHANCER ACTIVITY IN MYELOID LEUKEMIA CELLS. M. Hurme and S. Matikainen, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland.

Okadaic acid (OA) is a specific inhibitor of serine-threonine phosphatases 1 and 2A. In myeloid cells it has similar effects as the protein kinase C (PKC) activating phorbol esters, like induction of the *c-fos* and *c-jun* genes, downregulation of the *c-myc* expression and cessation of cell proliferation. Now we have examined the capability of OA to induce IL-1ß gene expression in THP-1 myeloid leukemia cells. OA alone was a weaker inducer of IL-1ß mRNA than the phorbol ester PMA. OA also had a weaker effect on the induction of the AP-1 enhancer activity (as detected by transfecting the cells with 5x AP-1 binding site CAT-reporter plasmid). The same was true also in cells transfected with a construct containing the AP-1 element of the IL-1ß gene. Thus these data demonstrate that the effect of OA on IL-1ß expression is mediated directly via the AP-1 element of the gene. The effect of PKC inhibitors was, however, clearly different on OA and PMA induced responses. PKC inhibitor H7 downregulated, as expected, the PMA induced IL-1ß mRNA expression but the OA induction was greatly potentiated implying that the endogenous PKC activity has a suppressive effect on the OA induced AP-1 enhancer activity. Analysis of the different components of the AP-1 indicated that OA induced the *c-fos* expression as strongly as PMA but with slower kinetics. In contrast to this, *c-jun* expression could be detected earlier after OA stimulation. Induction of *junB* expression resembled that of *c-fos*. It remains to be established which one of the AP-1 components is negatively controlled by the endogenous PKC activity.

E 517 IL-4 INDUCES THE PROTEIN TYROSINE PHOSPHORYLATION IN FIVE PROTEINS INCLUDING IL-4 RECEPTOR IN MOUSE T CELL LINES, Kenji Izuhara, Nobuyuki Harada, Department of Immunology, DNAX Research Institute, Palo Alto, CA. 94304-1104

IL-4 is known to be a pleiotropic factor which causes proliferation and differentiation on B cell, T cell and other hematopoietic cells. Previously we and others have cloned human and mouse IL-4 receptor (IL-4R) cDNAs, and we have further identified the critical region for growth signal transduction in the cytoplasmic domain of human IL-4R. However, despite these structural studies, the biochemical mechanism of the signal transduction of IL-4 is still unclear. In this study, we analized the tyrosine phosphorylated proteins upon the stimulation of IL-4 using mouse T cell lines, CTLL and HT-2. By western blotting using anti-phosphotyrosine antibody, IL-4 appeared to induce protein tyrosine phosphorylation in five different proteins which molecular sizes are 140KDa, 110KDa, 96KDa, 92KDa and 85KDa. By immunoprecipitation with anti-mouse IL-4R antibody, we identified the 140 KDa protein to be mouse IL-4R. In other growth factor receptor systems, it is well known that tyrosine phosphorylation induces the association of PI3 kinase and receptor. Also the molecular wight of pp85 is same as one of subunit of PI3-kinase. For these reasons, we are now analizing PI3-Kinase activity in immunoprecipitates to know whether PI3-Kinase physically associates with IL-4R.

E 518 UNSPLICED TNF-ß mRNA PRODUCTION IN THE NORMAL AND CHEMICALLY CAUTERIZED MURINE CORNEA. Bradford E. James, Howard W. H. Lee, and Gordon K. Klintworth, Departments of Pathology and Ophthalmology, Duke University Medical Center, Durham, NC 27710.

In studies evaluating the role of various cytokines in the pathogenesis of corneal angiogenesis we have examined cytokine mRNA production in corneal tissue of CD-1 mice by PCR amplification. RNA was prepared from normal corneal tissue as well as from corneas which had been exposed to silver/potassium nitrate cautery (0-96 hours post cauterization). This RNA was reverse transcribed to cDNA and amplified by PCR using primers specific for the 5' region of the TNF-ß mRNA, which spans two introns. The amplified PCR product from this region corresponded in length to that of the unspliced genomic sequence, rather than the spliced mRNA. The unspliced product was present in all uninjured corneas and at all post-cautery time points tested. The size of the TNF-ß amplified product was not affected by DNase treatment of the RNA preparations and other inflammatory response cytokine and control mRNAs produced PCR products which corresponded to the size expected for their normal mRNAs, indicating that DNA contamination did not account for the genomic size of the TNF-ß amplified product. Therefore it seems that the mRNA for TNF-ß is produced in an unspliced form in the normal and uninjured murine cornea. To the best of our knowledge an unspliced form of TNF-ß mRNA has not been observed in any other tissue.

E 519 INTERLEUKIN-1 INDUCIBLE EXPRESSION OF *gro*-ß VIA NF-kB ACTIVATION IS DEPENDENT UPON TYROSINE KINASE SIGNALING, Swati S. Joshi-Barve[1], Vidya V. Rangnekar[2], Stephen Sells[1], and Vivek M. Rangnekar[1,2]*,Department of Surgery, Division of Urology[1], and Department of Microbiology and Immunology[2], University of Kentucky, Lexington, KY 40536.

To elucidate the early intracellular signals associated with IL-1 action, we are studying the second messenger signals and transcription factors required for induction of *gro*-genes. Here, we present evidence that in human melanoma cells, A375-C6, IL-1 inducible *gro*-gene expression is dependent on tyrosine kinase signaling. Using gel retardation and transient expression assays, we show that IL-1 causes protein tyrosine phosphorylation-dependent activation of an NF-kB-like enhancer binding protein, which then induces transcription of the *gro*-genes via an NF-kB site located 76 bp upstream from the cap site. IL-1 activated protein tyrosine phosphorylation is also required for *gro*-gene induction in human cervical carcinoma cells, HeLa; human fibroblast cells, WI-38; and mouse fibroblast cells, L929. Thus, in diverse cell types IL-1 induces *gro*-genes via tyrosine kinase-dependent signals.

E 520 CHARACTERIZATION OF *C-MET* AND RELATED mRNAs IN NORMAL BONE MARROW CELLS AND FACTOR-DEPENDENT PROGENITOR CELL LINES. T. Kmiecik[1,2], G. Vande Woude[2], F. Ruscetti[3], and J. Keller[3]. [1]Biological Response Modifiers Program, Laboratory of Molecular Immunoregulation, [2]ABL-Basic Research Program, and [3]PRI/DynCorp, Inc., Biological Carcinogenesis and Development Program, NCI-FCRDC, Frederick, MD 21702.

The protooncogene is a member of the tyrosine kinase receptor family. A 7 kb *met* mRNA is detected in epithelial cells. The *met* protein is synthesized as a 165 kd precursor which is proteolytically cleaved to give disulphide-linked 135 kd and 40 kd chains. Hepatocyte Growth Factor (HGF), which has homology to plasminogen, has been shown to function as a ligand for *met*. We have recently shown that HGF can act as a synergistic factor for bone marrow colony formation*. HGF increased colony formation from lin- bone marrow progenitors up to 100% when added with limiting amounts of IL-3 and GM-CSF. Further characterization of the expression of *met* in murine myeloid progenitor lines was performed. In addition to a predominantly expressed 7 kb *met* mRNA in murine myeloid progenitor lines and in lin- and unfractionated murine bone marrow, we have detected *met*-hybridizing mRNA species of 9 kb and 3 kb in the NFS-58, NFS-61 and DA-3 myeloid progenitor lines. In order to characterize the novel *met*-related messages in murine bone marrow, we have identified a 1.8 kb cDNA from a murine bone marrow library using a low-stringency screen with a *met* probe. This cDNA has a stretch of sequence which is identical to a portion of the *met* extracellular domain, but also contains sequences which are not homologous to *met* or any other sequence in Genbank. This novel message may represent an alternately spliced form of *met*.

*T. Kmiecik, J. Keller, and G. Vande Woude, Blood, in press.

E 521 HUMAN 55kDa RECEPTOR FOR TUMOR NECROSIS FACTOR COUPLED TO SIGNAL TRANSDUCTION CASCADES, Martin Krönke[1], Katja Wiegmann[1], Stefan Schütze[1], Eva Kampen[1], Adolf Himmler[2], and Thomas Machleidt[1], [1]Institut für Medizinische Mikrobiologie und Hygiene, Technische Universität München, Trogerstrasse 4a, 8000 München 80, [2]Ernst Boehringer Institut, Bender&Co GesmbH, 1121 Vienna.

The numerous biological activities of tumor necrosis factor (TNF) appear mediated by two types of receptors of 55kD (TR55) and 75kD (TR75) molecular weight. To test TR55 for its individual role in signaling across the membrane, a cDNA coding for the human TR55 was stably expressed in murine 70Z/3 pre-B cells, which lack binding sites for, and proved non-responsive to human TNF. The transfected TR55 showed high-affinity ligand binding and active internalization. Further, expression of recombinant p55 TNF receptors can be downmodulated by PKC activators. Thus, the transfected TR55 appears indistinguishable from its wild-type counterpart. It is demonstrated that the TNF signaling cascade, i.e. stimulation of protein kinase C, sphingomyelinase and phospholipase A_2, production of the second messengers diacylglycerol and ceramide, can occur completely through exclusive binding of TNF to TR55. When compared to TNF, lymphotoxin (LT) induced NF-*k*B at similar concentrations, indicating that TR55 transduces signals from both ligands. The p55 TNF binding site thus functions as an autonomous TNF receptor that mediates activation of most of the signalling pathways so far ascribed to TNF action.

E 522 THE MEMBRANE PROXIMAL SERINE/ACIDIC-RICH REGION OF THE HUMAN β COMMON CHAIN IS ESSENTIAL FOR BOTH IL-3 AND GM-CSF INDUCED CELL PROLIFERATION. A. Laurie, M. Weiss, D.G. Nathan, and C.A. Sieff. Dana-Farber Cancer Institute and Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115.
Interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulate hematopoietic cells by binding with high affinity to a receptor (R) complex comprised of either a ligand-specific IL-3Rα chain or GM-CSFRα chain, and a common β (βc) chain. To define the minimum requirements for the reconstitution of functional human IL-3R and GM-CSFR heterodimers, we used PCR to construct a series of GM-CSFRα and IL-3Rα cytoplasmic truncation mutants and βc cytoplasmic truncation mutants with transmembrane (tm), serine-rich (s), and serine/acidic-rich (sa) domains, while a fourth encoded approximately half of the cytoplasmic (c/2) domain. Stable co-transfections into the murine factor dependent cell line, Ba/F3, were used to determine the effects of these mutations on ligand binding and human IL-3 and GM-CSF dependent growth. Co-transfection of the wild-type IL-3Rα or GM-CSFRα and βc subunits into Ba/F3 cells followed by selection in hIL-3 or hGM-CSF respectively, led to expression of functional heterodimers that conferred high affinity ligand binding and dose-dependent proliferative responses to hIL-3 or hGM-CSF in the high-affinity (picomolar) range. Expression of the α chain truncation mutants with βc led to high-affinity binding but no proliferation. Substitution of the wild type βc by either βc(c/2) or βc(sa) in similar cotransfections also led to hIL-3 and hGM-CSF dependent cell lines; in contrast, hIL-3 and hGM-CSF dependent lines could not be derived from IL-3Rα + βc(tm) or βc(s) transfectants or GM-CSFRα + βc(tm) or βc(s) transfectants even though they bound ligand with high affinity. In conclusion, these results show first, that only the extracellular and transmembrane regions of the α chains are sufficient, with βc, for high-affinity ligand binding; none of the cytoplasmic domain is necessary. Second, the membrane proximal serine/acidic-rich region of the βc subunit is essential for signal transduction through both the human IL-3 and GM-CSF receptor heterodimers.

E 523 REGULATION OF GENE EXPRESSION OF CYTOKINE-INDUCIBLE RNA TRANSCRIPTS FROM A MOUSE MONOCYTE MACROPHAGE CELL-LINE, RAW 264.7 Caroline G.L. Lee and William E. O'Brien Department of Biochemistry and Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.

Macrophages can be activated *in vitro* by bacterial lipopolysaccharide (LPS) or cytokines such as γ-interferon. Three genes (IRG1, IRG2 and IRG3) from a LPS-activated library were selected for analysis. All three genes were induced more than 20 fold and maximal induction occurred between 3 and 3.5 h following LPS (50ng/ml) and 2 h following γ-IFN (10U/ml) treatment. LPS induction for all three genes was cycloheximide sensitive. IRG2 encoded a 1.8 kb transcript homologous to human interferon-induced 54 KD (94%) and 56 KD (81%) protein. IRG1 and IRG3 encoded transcripts of 4 and 4.6 kb respectively. A genebank search revealed no significant homology to any known proteins.

Agents that perturb various second messengers signal transduction pathways were administered to activated RAW264.7 cells to evaluate their involvement in the regulation of LPS induced mRNA expression of all three clones. Increasing cytosolic cAMP with forskolin caused inhibition of all three genes by two to three fold. However, preliminary studies using other cAMP modifiers (dibutryl cAMP; HA1004) revealed no significant change in expression. Activation of PKC with phorbol esters (PMA), on the other hand, stimulated the expression of the three genes. Trifluoperazine dichloride (TFP), an inhibitor of calmodulin (CaM)-dependent enzymes, inhibited the expression of IRG1 but not IRG2 or IRG3. Removal of extracellular calcium with EGTA inhibited the expression of all the genes while increasing intracellular calcium with calcium ionophore A23187 stimulated their expression. Activation of ADP-ribosylation of G-proteins with cholera toxin were without effect.

E 524 EXON JUNCTION PRIMER IN A rTth POLYMERASE BASED RT-PCR TO DETECT mRNA OF CYTOKINES, Wai-Choi Leung, W. Frank Lawrence, Jing-Zhou Hou and Maria F.K.L. Leung, Division of Molecular Pathology, Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA 70112

The molecular action of a cytokine can be measured by the expression of a target gene sequence into mRNA in a recipient cell. Coupled reverse transcription and polymerase chain reaction (RT-PCR) is a sensitive and convenient method to detect the presence of mRNA. We have developed an improved method for RT-PCR for cytokine mRNA. The salient features are: (1) Using one single thermostable enzyme, i.e. rTth polymerase, for both RT and PCR reactions, instead of the commonly used two enzyme method, (2) The melting temperature of primers were set at 75°C to avoid nonspecific reannealing at low temperature, (3) The reaction temperature of RT-PCR never fell below 75°C to avoid folding of RNA template. This RT-PCR method yielded a single band of predicted size for a number of human and rodent cytokines, (i.e. IL-6, TNF, etc.) as well as cellular and viral mRNA. Nonspecific PCR products were not observed in conditions known for their production, i.e. high concentrations of template, primers, etc.

We aim to adopt this protocol for *in situ* RT-PCR. As a first step, we increased the specificity of RT-PCR to detect only the mRNA, but not the corresponding gene sequence in genomic DNA or mRNA. Our rationale is that primers with sequence corresponding to an exon junction will fit the above criteria. Human IL-6 mRNA contains 4 exon junctions. We then evaluated all of the six possible combinations of primer pairs in our RT-PCR. Models 2 and 3 exhibited a single DNA band. None of these models were able to detect genomic DNA or other contaminating sequences. In template titration experiments, model 2 demonstrated to be a more efficient primer pair than model 3. It can be explained by the presence with a region of extensive secondary structure in model 3, but not in model 2. More interesting, RT-PCR using a combined primer pairs from model 1 and 6 resulted in the rescue of the predicted DNA products of model 1 and model 6, while model 1 and model 6 alone yield negative RT-PCR. It suggested an intricate interplay of molecules during the reverse transcription and/or amplification process.

E 525 COUPLING OF THE HUMAN GM-CSF RECEPTOR TO BIOLOGICAL RESPONSES BY THE PROTO-ONCOGENE HCK, Diana Linnekin, O. M. Zack Howard*, Linda Park#, William Farrar, Douglas Ferris*, Dan L. Longo, Biological Response Modifiers Program, National Cancer Institute, *Biological Carcinogenisis and Development Program, Program Resourses, Inc./Dyn Corp, Frederick MD, and #Immunex Research and Development Corp., Seattle, WA.

The human myeloid cell line HL-60 expresses approximately 300 high affinity GM-CSF receptors. Interestingly, GM-CSF stimulation of these cells does not result in enhanced cellular proliferation or increases in protein tyrosine phosphorylation. In contrast, GM-CSF induces rapid increases in protein tyrosine phosphorylation and proliferative responses in HL-60 cells pretreated for 3-5 days in DMSO. One event associated with DMSO treatment of HL-60 cells is an increase in expression of the proto-oncogene HCK. To determine if HCK has a role in the coupling the GM-CSF receptor to proliferative responses, we have overexpressed HCK in HL-60 cells. The resulting cell line, HL-60/HCK, expresses HCK mRNA and protein at levels comparable to HL-60 cells cultured 3-5 days in DMSO. Further, the HL-60/HCK cell line has similar numbers of GM-CSF receptors as the untransfected HL-60 cells. Treatment of the HL-60/HCK cell line with GM-CSF results in increases in proliferative response as well as stimulation of protein tyrosine phosphorylation. In addition, treatment of HL-60/HCK cells with GM-CSF rapidly induces autophosphorylation of HCK. Thus, GM-CSF activates the src-like tyrosine kinase HCK, and activation of HCK correlates with the capacity for GM-CSF to elicit a biological response. These observations demonstrate that expression of GM-CSF receptors can be dissociated from expression of signal transduction components necessary for coupling to biological responses. Further, these data suggest that cytokine receptors can exist in an "uncoupled" form and that regulation of signal transducer expression is one mechanism mediating cellular response to cytokines.

E 526 STEEL FACTOR STIMULATES THE SERINE/ THREONINE PHOSPHORYLATION OF THE INTERLEUKIN-3 RECEPTOR.

Ling Liu, Alice L.F. Mui, Brent Kennedy & Gerald Krystal, The Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., V5Z 1L3.

Steel factor (SF) (also called stem cell factor, mast cell growth factor, or c-kit ligand) is a recently cloned hemopoietic growth factor that is produced by bone marrow stromal cells, fibroblasts and hepatocytes. In both mouse and man it acts synergistically with several colony stimulating factors, including interleukin-3(IL-3) and granulocyte macrophage-colony stimulating factor (GM-CSF) to induce the proliferation and differentiation of primitive hemopoietic progenitor cells. In order to explore the molecular basis for this synergistic activity we examined the proteins that become phosphorylated in response to SF and IL-3 in the mouse myeloid cell line, B6SUtA$_1$. In terms of tyrosine phosphorylations, the two growth factors stimulate, in addition to their own cell surface receptors, an overlapping set of proteins, with the notable exception that only IL-3 induces the appearance of a major tyrosine phosphorylated protein with a molecular weight of 95 kd. Interestingly, SF and the phorbol ester, TPA, both increase the apparent molecular weight of the IL-3 receptor β chain, but not its tyrosine phosphorylation. Moreover, pretreatment of the cells with compound 3, a potent and specific inhibitor of protein kinase C, prevents this mobility shift, suggesting that SF and TPA stimulate the serine/threonine phosphorylation of the IL-3 receptor via protein kinase C. Pretreatment of the cells with TPA, which inhibits IL-3 induced B6SUtA$_1$ cell proliferation, or SF, which enhances IL-3 stimulated proliferation, had little or no effect on IL-3 receptor number. We are currently investigating whether this phosphorylation event affects intracellular signaling.

E 527 IDENTIFICATION, MOLECULAR CLONING AND CHARACTERIZATION OF MURINE INTERLEUKIN-1β CONVERTING ENZYME.

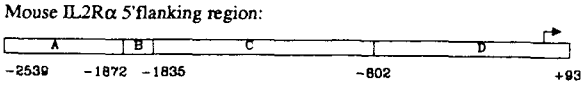
S. M. Molineaux, F. J. Casano, A. M. Rolando, E. P. Gaffney, G. Limjuco, J. Chin, P.R. Griffin, J. R. Calaycay, G. J.-F. Ding, T. T. Yamin, O. Palyha, S. Luell, D. Fletcher, D. K. Miller, A. D. Howard, N. A. Thornberry, and M. J. Kostura. Merck Research Laboratories, Rahway, New Jersey 07065.

ABSTRACT. Human IL-1β converting enzyme (ICE) is a cysteinyl protease that generates mature IL-1β in stimulated monocytes by cleavage of the precursor at the Asp^{116}-Ala^{117} bond. The active form of the enzyme is a heterodimer composed of p20:p10 subunits that are derived proteolytically from a p45 precursor. Validation of ICE as a therapeutic target requires the development of IL-1β driven animal models of inflammatory disease in which to test the efficacy of ICE inhibitors. To this end, we have identified murine IL-1β converting enzyme (ICE) in extracts of *P. acnes* elicited peritoneal exudate cells. Murine ICE, like human ICE, cleaves both the murine and human IL-1β precursors to 28 kDa and 17.5 kDa products. The peptide AcYVAD-AMC is a substrate for murine ICE and the tetrapeptide aldehyde AcYVAD-CHO inhibits murine ICE (K_i = 3 nM) with a potency similar to that previously observed for the human enzyme (K_i = 0.76 nM). A 1.4 kb full length murine ICE cDNA was cloned which encodes a 45 kDa proenzyme of 402 amino acids. At the amino acid level, the murine proenzyme is 62% identical to the human, with the p20 and p10 subunits having 60% and 81% identity, respectively. The active site Cys lies within a completely conserved stretch of 18 amino acids, but the Ser residue that aligns with the catalytic region of serine and viral cysteinyl proteases is not present in murine ICE. Four ICE-*like* cleavage sites are conserved in the proenzyme, suggesting that active murine ICE consists of p20 and p10 subunits that are derived, in part, by autocatalysis. Recombinant murine ICE, which has the expected p20:p10 subunit structure, generates mature murine IL-1β by cleavage at the Asp^{117}- Val^{118} bond. Ac-YVAD-CHO prevents the processing of IL-1β precursor in LPS-stimulated murine macrophages (IC_{50} = 20 μM), demonstrating that murine ICE is required for the generation and release of mature 17.5 kDa IL-1β.

E 528 MAPPING OF NEW CIS-ACTING RESPONSIVE ELEMENTS IN THE MOUSE IL2Rα 5'FLANKING REGION, Markus Nabholz, Peter Sperisen, San-Ming Wang, Sonia Barange, Elisabetta Soldaini, Anne Wilson[1] and H. Robson MacDonald[1], ISREC and [1]LICR, CH-1066 Epalinges, Switzerland

The published data mapping cis-acting elements controlling human IL2Rα gene transcription are difficult to reconcile with our work on the mouse gene [Plaetinck et al. (1990) J. Immunol. *145*, 3340-3347]. This incited us to start a comprehensive analysis of the regulatory elements in the mouse IL2Rα 5'flanking region in different cells, and in response to different stimuli, as well as in transgenic mice. So far we have found (1) that the -2539/+93 (*A-D*) but not the -802/+93 (*D*) segment of the mouse gene is sufficient to confer inducible or constitutive expression on two reporter genes in a variety of T cell lines which express the genomic IL2Rα gene, including antigen-dependent CD8$^+$ clones. (2) The segment between nt -1835 and -802 (*C*) is required for expression in antigen stimulated CD8+ T cells and in IL1+IL2 stimulated cells of a CD4$^-$CD8$^-$ thymic lymphoma line. (3) In this cell line the expression pattern of constructs containing segment *A-D* closely resembles that of the genomic IL2Rα gene. We have previously shown that segment *C* has cytokine inducible enhancer activity in these cells. Now we have found that the IL2 response of the *A-D* region strictly depends on a 160 bp region in segment *C*. (4) Deletions between nt -584 and -49 in segment *D*, including the nucleotides homologous to the NF-κB binding site in the human gene, affect baseline expression in CD4$^-$CD8$^-$ cells but not cytokine responsiveness. Our results show that crucial cis-acting elements in the mouse IL2Rα gene are located upstream of the previously analysed region (-471/+105) in the human gene.

Mouse IL2Rα 5'flanking region:



E 529 TRANSCRIPTIONAL REGULATION OF THE RANTES GENE, Peter J. Nelson and Alan M. Krensky, Department of Pediatrics, Stanford University, Stanford, CA 94305

RANTES is a member of a large supergene family of pro-inflammatory cytokines called beta intercrines or CC chemokines which appear to play a fundamental role in inflammatory processes. The RANTES protein causes release of histamine from basophils and is a chemotatic factor for CD45RO/CD4+ "memory"-helper T lymphocytes, monocytes and eosinophils. In order to gain a better understanding of the molecular mechansims which regulate expression of RANTES locus we have characterized the RANTES gene and determined a putative promoter region. The DNA sequences of the intron/exon boundaries are conserved relative to the other CC intercrine/chemokine family members. Approximately one kilobase of DNA from the immediate 5' upstream region of RANTES was sequenced and found to contain a large number of potential regulatory consensus elements including those for T cell/heamatopoietic specific factors, ets-1, TCF-1, myb, PU.1 and CD28 responsive element; myeloid specific factor, NF-E2; muscle specific factors, MyoD, MEF-2 and CTCT; and ubiquitously expressed factors, NFkB, Ap-1, UBP-1, NF-S, and AP-2. RANTES-promoter-luciferase reporter gene fusion experiments demonstrated high levels of luciferase-reporter activity in the "mature" T cell line, Hut 78, and the rhabdomyosarcoma cell line, RD, with little or no activity in the "early" T cell line, Jurkat, or the pre-erythroid cell line, K562. Because RANTES is expressed in diverse tissue types under different conditions, it is likely that different transcriptional mechanisms control its expression in the various tissues.

E 530 CHARACTERIZATION AND ACTIVATION OF THE MURINE INTERLEUKIN-1β (IL-1β) CONVERTING ENZYME, Michelle A. Nett-Fiordalisi, Douglas R. Berson, and David D. Chaplin, Department of Medicine and Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

IL-1β is an important regulator of immune and inflammatory responses. The cytokine is initially synthesized as an inactive 33 kD pro-molecule which in mice is cleaved between Asp-117 and Val-118 to generate active 17 kD IL-1β. This proteolytic maturation is catalyzed by the IL-1β converting enzyme (or IL-1β convertase). IL-1β lacks a signal peptide and is not released via the classical secretory pathway. Rather, release of IL-1β is induced by cell injury. When injury leads to cellular necrosis, pro-IL-1β is released without processing. In contrast, injuries leading to cellular apoptosis cause efficient release of processed mature IL-1β. In order to study how apoptosis regulates IL-1β convertase activity, we have isolated murine convertase cDNA. These cDNA predict a 402 residue protein which is highly conserved with the human convertase cDNA. The murine convertase is constitutively expressed in a wide variety of tissues, including spleen, heart, and brain, as well as in cultured mononuclear phagocytes and T lymphocytes. Preliminary data indicate that the convertase is synthesized as an inactive pro-enzyme. Convertase activity was demonstrated in phagocytic cells by incubating lysates of J774 cells with *in vitro*-translated pro-IL-1β. Lysates from unstimulated J774 cells cleaved pro-IL-1β to its 17 kD mature form. Of considerable interest, lysates from apoptotic J774 cells (treated with 5 mM ATP in the medium) showed a dramatic increase in convertase activity. These studies suggest that the IL-1β convertase is activated during apoptotis.

E 531 SIGNAL TRANSDUCTION BY THE INTERLEUKIN 2 RECEPTOR FOLLOWING EXPRESSION OF P75 AND P55 RECEPTOR CHAINS IN IL3-DEPENDENT CELLS, Gordon R. Perkins, Claudia L. Flemming, David Kabat and Mary K.L. Collins, Chester Beatty Laboratories, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, England.

Murine IL3-dependent cell lines were established which expressed the human p75 IL2-binding protein, in the absence or presence of the human p55 IL2-binding protein. Whereas p75 expression was sufficient to confer response to an intermediate (half-maximal stimulation at 50pM) concentration of IL2, additional expression of p55 increased the sensitivity of the cells to half-maximal stimulation at 3pM IL2. A mutant IL2 molecule, Lys20 IL2 which is known to be defective in p75 interaction, was unable to stimulate cells expressing only p75; p55 co-expression could restore its activity. Under conditions of low p75 expression, Lys20 IL2 could act as an antagonist of wild-type IL2 action. These data support a role for p55 in the enhancement of responsiveness to IL2. The stimulation of tyrosine and serine/threonine kinases by IL2 is currently under investigation and data will be presented comparing the rapid signals elicited by IL2 in cells expressing different receptor forms.

E 532 THE REGULATION OF THE AUTOCRINE PRODUCTION OF CYTOKINES IN HUMAN MONOCYTES, Adrian Puren, Angela Flagg and Nerina Savage, Department of Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg, South Africa, 2193

The pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF) α are produced *de novo* in response to various stimuli. These latter cytokines act in an autocrine manner to induce the further synthesis of the cytokines. Recent evidence suggests that the autocrine pathways may play a role in perpetuating the production of cytokines in response to exogenous stimuli.IL-4, a possible anti-inflammmatory cytokine, has been shown to down-regulate the production of monocyte cytokines in response to endotoxin. We wished to establish the role of IL-4 in the regulation of IL-1-induced production of monocyte cytokines *viz.*, IL-1β, IL-6 and TNFα.

Isolated human peripheral blood mononuclear cells were stimulated with IL-1α (1 ng/ml) in the presence and absence of IL-4 (1 ng/ml). Total RNA was isolated and analysed for IL-1β mRNA production after a 3 hour period of stimulation. Cell supernatants and lysates were collected separately and tested by means of RIAs for the production of IL-1β, IL-6 and TNFα.

The results show that IL-4 is able to down-regulate the production of IL-1β mRNA induced by IL-1α. In addition we show that there is the down-regulation by IL-4 of the protein products for IL-1β and IL-6 in both the cell supernatants and lysates. TNFα production was not influenced by IL-4. The effect of IL-4 on IL-1-induced production of IL-1 and IL-6 may be at a transcriptional or post-transcriptional level or both. In addition IL-4 has a differential effect in the regulation of monocyte cytokine production. IL-4 may play a role in modulating the autoregulatory production of cytokines but that this effect is complicated by the differential nature of its action.

E 533 ANALYSIS OF TNF SIGNAL TRANSDUCTION Mike Rothe and David V. Goeddel, Molecular Biology Department, Genentech, Inc., South San Francisco, CA 94080

The biological activities of tumor necrosis factor (TNF) are mediated by two trans-membrane receptors, TNF-R1 (55 kd) and TNF-R2 (75 kd). TNF-R1 initiates signals for cytotoxicity and many other TNF activities in diverse cell types. Direct signaling by TNF-R2 has so far been restricted to a small subset of TNF activities in some lymphoid cells such as the stimulation of thymocyte proliferation and proliferation of the murine cytotoxic T-cell line CT6.

In a search for potential targets of TNF-R2 signaling we have found that in CT6 cells TNF induces the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) through TNF-R2. Interestingly, GM-CSF induction in fibroblasts is initiated through TNF-R1 indicating that this TNF activity can be mediated by the individual TNF-receptors in a cell type specific manner.

E 534 RAPID LIGAND-INDUCED TYROSINE PHOSPHORYLATION OF A 120 kDa PROTEIN ASSOCIATED WITH PROLACTIN AND GROWTH HORMONE RECEPTORS. [1]Hallgeir Rui, [2]Gerald A. Evans,[3]Julie Y. Djeu,[4]Paul A. Kelly and [1]William L. Farrar, [1]Cytokine Molecular Mechanisms Section, LMI, Biological Response Modifiers Program and [2]Biological Carcinogenesis Development Program, PRI/DynCorp, National Cancer Institute, FCRDC, Frederick, Maryland 21702,[3]H. Lee Moffitt Cancer Institute and Research Center, Univ. of South Florida, Tampa, FL 33612, [4]Inserm Unité 344, Endocrinologie Moleculaire, Faculte de Medicine Necker, Enfants Malades, 156, rue de Vaugirard, 75730 Paris Cedex 15, France.

A protein of M_r 120,000 (p120) was found to undergo rapid and transient tyrosine phosphorylation in response to ligand activation of receptors for prolactin (PRL) and growth hormone (GH), as revealed by SDS-PAGE and anti-phosphotyrosine immunoblotting. The effect was observed in all target cell lines examined, including prolactin-responsive rat pre-T lymphoma cells (Nb2) and human breast carcinoma cells (T47D) and GH-responsive rat hepatocarcinoma (H4) and human B-lymphoblastoma (IM-9). Maximal stimulation occurred at hormone concentrations of 100 nM, and peak phosphorylation levels were reached within 5 minutes. Detectable tyrosine phosphorylation of p120 was also observed at physiological hormone concentrations (0.1-1 nM). A major portion of the tyrosine phosphorylated p120 co-purified with the detergent-solubilized receptors from activated cells, using either anti-receptor antibodies or anti-ligand antibodies, thus indicating a tight receptor-association of the phosphorylated p120. Furthermore, tyrosine kinase assays performed on affinity-purified receptor-complexes, demonstrated time-dependent tyrosine phosphorylation of p120. Receptor-complex tyrosine kinase assays were carried out using both immunoblotting technique as well as analysis of incorporation of [^{32}P] from γ-labeled ATP. Our observations demonstrate that p120 is a prominent phosphotyrosine substrate involved in both PRL and GH receptor signalling, and indicate that p120 may constitute an autophosphorylating tyrosine kinase shared by PRL and GH receptors.

E 535 THE INHIBITION OF TUMOR NECROSIS FACTOR RESISTANCE MECHANISM BY THE TYROSINE KINASE INHIBITOR GENISTEIN, Carl Y. Sasaki and Paul Q. Patek, Department of Microbiology, University of Hawaii at Manoa, Honolulu, HI 96822.

Previous studies have demonstrated that many cytokines activate a pathway involving protein phosphorylation. Tyrosine phosphorylation is a rare post-translational event accounting for less than 0.01% of phosphorylated amino acids. Nonetheless, phosphotyrosine kinase activity is associated with several cell surface receptors and is presumed to be involved in intracellular signaling. Based on this, we investigated the effects of genistein, a tyrosine kinase inhibitor, on tumor necrosis factor (TNF) mediated cytolysis. We show that TNF resistant cell lines become sensitive to TNF mediated cytolysis when treated with genistein. In contrast, genistein has no effect on TNF sensitive cell line. In addition, when target cells are treated with genistein before the addition of TNF, there is no increase in TNF sensitivity; however, when genistein was added after TNF treatment, the genistein-induced TNF sensitivity was detectedable. Therefore, genistein inhibits a TNF resistance mechanism that is activated after TNF binding, and it appears that the TNF-inducible resistance mechanism involves a tyrosine kinase.

E 536 REGULATION OF IN VIVO IgE RESPONSES BY IL-4 AND IFN-γ, Huub F.J. Savelkoul, René van Ommen and Robert L. Coffman*, Department of Immunology, Erasmus University, P.O. Box 1738, Rotterdam, The Netherlands and *DNAX Research Institute, Palo Alto, CA 94304

Continuous IL-4 treatment for 3 months by implanting IL-4 transfected CV-1 cells encapsulated in alginate resulted in a "transient IL-4 transgenic" mouse. These IL-4 mice displayed after ending of the treatment increased expression of CD23+ B cells, no change in MHC class II expression, unchanged production of IL-4 but decreased production of IFN-γ, increased numbers of IgG1 and IgE memory cells. In the serum, these IL-4 mice displayed a persistent but IL-4 independent IgE level that lasted up to 4 months after ending of the treatment. The increased IgE memory could be transferred to naive recipients and again proved to be IL-4 independent. Reverse transcription PCR analysis was performed to detect differences in the expression of IL-4, IL-6, IFN-γ, IL-10 cytokine mRNA. In vitro analysis of secondary B cell responses in IL-4 treated mice showed an increase in surface IgG1-IgE double positive B cells, providing circumstantial evidence for sequential switching from IgG1 to IgE via IgG1-IgE double positive B cells. IgE low responder SJL mice display an IFN-γ-mediated inhibition of antigen-specific IgE responses. The induced expression of IFN-γ mRNA is delayed by four days in comparison to the IL-4 expression. Anti-IFN-γ treatment on day 6 will therefore increase and prolong the resulting antigen-specific IgE response. The defective IgE production in SJA/9 mice could be restored by application of exogenous IL-4, despite the evidence for IL-4 production in vivo. These data show that the outcome of IgE responses in vivo are mainly directed by the relative amounts of IL-4 and IFN-γ present in these mice.

E 537 INTERLEUKIN-7 SIGNAL TRANSDUCTION MECHANISM. Philippe Seckinger and Michel Fougereau. CIML - Case 906 - 13288 Marseille Cedex 9 France

Interleukin-7 (IL7) has first been identified as a specific pre-B cell growth factor. It has now been shown to extend its biological activity on T lymphocytes, thymocytes, monocytes and NK cells. We have investigated IL7 signal transduction mechanism on the human pre-B cell line Nalm-6 and have found, by using a specific antibody to phosphotyrosine (PY) residues that IL7 stimulates tyrosine phosphorylation of various proteins termed pp27, pp43, pp46, pp54, pp64, pp78, pp90, pp105 and pp120. Neither IL1 nor IL4 were capable to induce tyrosine phosphorylation, suggesting that the phenomenon is specific of IL7. Activated tyrosine-kinases autophosphorylate in the presence of ATP and Mn^{++}. Indeed, αPY immunoprecipitates from IL7 stimulated Nalm-6, shows two major proteins with a mol. wt. of 55 and 63 kDa capable to autophosphorylate in a transient manner as soon as 1 min. following stimulation and peaking at 10 min. Kinase autophosphorylation returns to base line after 30 min of stimulation. Hence, αPY immunoprecipitates of IL7 stimulated cells also increase tyrosine phosphorylation of the exogenously added substrat histone H2B. Furthermore, by using a polyclonal αIL7R antibody in western blotting analysis we observe that αPY immunoprecipitates of digitonine solubilized protein complexes, immunoprecipitate also the IL7 receptor (IL7R) in a transient manner. These data indicate that the IL7R associates with tyrosine phosphorylated proteins since the amino acid sequence of the IL7R is devoid of a putative site of tyrosine phosphorylation. The results are confirmed as at least five ^{32}P labelled proteins (pp64, pp74, pp84, pp110 and pp120) are visualized after immunoprecipitation of the digitonine solubilized complexes by using the polyclonal αIL7R. Preliminary data indicates that the IL7R associates in a transient manner with a tyrosine-kinase. The kinetics of IL7-induced protein tyrosine phosphorylation, autophosphorylation and association with tyrosine phosphorylated proteins are all comparable, suggesting that they are linked by one or more tyrosine-kinase(s) that also associates with the IL7R and whose nature remains to be determined.

E 538 UP-REGULATION OF CYTOKINE MESSENGER RNA ASSOCIATED WITH THE ONSET OF INTESTINAL REGENERATION AFTER IRRADIATION. J.G. Sharp, J. Hester, J.A. Rogers and S.K. Saxena, Dept. of Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198-6395

Damage to the hematopoietic system by high dose cancer therapy can largely be overcome by the transplantation of stem cells from marrow or peripheral blood. Toxicity can be further ameliorated using cytokines. High dose cancer therapy also causes damage to the intestinal mucosa which has resulted in intestine and mucus membrane damage becoming a new dose limiting factor. However, whether cytokines are capable of accelerating mucosal recovery is unknown. Epidermal growth factor appears only to have a partial, possibly minimal role since its binding is more closely associated with epithelial cell migration than proliferation. Surprisingly, interleukin 3 appears to have the effect of increasing epithelial cell proliferation. In an attempt to define which other cytokines which might be involved in mucosal regeneration we have begun to examine cytokine mRNA expression in murine intestine following high doses of irradiation (13 Gy whole body or whole abdomen). Regeneration based on stathmokinetic analysis of the intestinal epithelium commences on the third day post-irradiation. Techniques were developed to separate the villus from the crypts and muscle layer so that each could be probed separately. As an example, tumor necrosis factor mRNA was upregulated in the villus in temporal correspondence to the onset of crypt epithelial cell proliferation. Candidate cytokines identified by this approach can then be tested *in vivo* for efficacy.

E 539 MOLECULAR CLONING OF RAT INTERLEUKIN - 1β - CONVERTING ENZYME: DISTRIBUTION AND REGULATION, Brenda D. Shivers, David A. Giegel* and Karen M. Keane, Departments of Neuroscience Pharmacology and Biochemistry*, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48105

Interleukin - 1β (IL - 1β) is an important mediator of inflammation. Bioactive 17.5 kD IL - 1β arises from a 31 kD precursor through cleavage by interleukin - 1β - converting enzyme (ICE). The cDNA sequence for human ICE was recently determined[1,2]. To study the role of cytokines in rat models of nervous tissue inflammation, we cloned rat ICE cDNA using primers based on the human ICE sequence. For PCR cloning, total mRNA was extracted from rat spleen obtained 5 hours after an LPS injection (0.2 mg / 1 kg, i.p.). The predicted protein sequence of rICE cDNA showed >60% identity to hICE. Northern blot analysis revealed two rICE transcripts - 2.2 and 1.45 kb, with the smaller transcript predominating. The relative tissue content of rICE mRNA was as follows: spleen>>testis, intestine > brain. rICE mRNA content was LPS-induced in spleen and brain (other tissues remain to be examined). The distribution of rICE mRNA is consistent with ICE being co-expressed in tissues producing large amounts of IL-1β. rICE may be regulated by the same stimuli that induce its substrate.

[1] Cerretti, D. P., *et al.*, Science 256:97, 1992.
[2] Thornberry, N.A., *et al.*, Nature 365:768, 1992.

E 540 TUMOR NECROSIS FACTOR ALPHA STIMULATES THE ACTIVITY OF THE HUMAN CYTOMEGALOVIRUS IMMEDIATE-EARLY-ENHANCER / PROMOTER

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Human cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in immunocompromised patients. CMV infection commonly results from reactivation of a latent infection in these patients. Peripheral blood mononuclear cells (PBMNC), especially monocytes, are believed to play a central role in the pathogenesis of CMV infection. Only very little is known about the regulation of CMV gene expression in PBMNC and the factors inducing reactivation of latent virus.

Using a set of mAb's recognizing distinct CMV antigens, we found CMV immediate early (IE)-antigen expression in PBMNC, particularly in monocytes, in 83 of 203 organ transplant recipients and 27 of 37 septic patients (> 750 samples were investigated). Positive reults could be confirmed by detection of CMV-IE-DNA using in situ hybridization or polymerase chain reaction. The incidience of CMV infection in PBMNC was clearly associated with elevated cytokine serum levels. Anti-T cell antibodies induce a systemic cytokine release, particularly following the first infusion. Sequential investigations of these patients showed that CMV infection appeared several days after cytokine release. Remarkably, anti-CD 4 mAb did not induce cytokine release as well as did not reactivate CMV in PBMNC.

The hypothesis that cytokines play a significant role in the process of reactivation of latent CMV infection was supported by our findings that tumor necrosis factor-alpha (TNF) was able to stimulate the activity of CMV-IE-enhancer/promoter region in the human monocytic cell line, HL-60. In contrast, the interleukins 1, 2, 3, 4, 6, 8, 10, TGF-ß and GM-CSF did stimulate the CMV-IE-enhancer/promoter activity. As shown in mice, TNF is also able to inhibit the cell-mediated immunity. Thus, TNF seems to play a key role in the pathogenesis of CMV infection by inducing virus replication and inhibition of anti-viral immune response resulting in CMV disease.

E 541 IL-4 ACTIVATES A DISTINCT SIGNAL TRANSDUCTION CASCADE FROM IL-3 IN FACTOR-DEPENDENT MYELOID CELLS, Ling-Mei Wang[1], Achsah D. Keegan[2], William E. Paul[2], Mohammad A. Heidaran[1], J. Silvio Gutkind[3] and Jacalyn H. Pierce[1], [1]Laboratory of Cellular and Molecular Biology, NCI, NIH, [2]Laboratory of Immunology, NAIAD, NIH and [3]Laboratory of Cellular Development and Oncology, NIDR, NIH, Bethesda, MD 20892

Interleukin-4 (IL-4) was shown to induce a potent mitogenic response in the IL-3-dependent myeloid progenitor cell line, FDCP-2. Although IL-4 could not sustain long-term growth of FDCP-2 cells, it enhanced their growth in serum-free medium containing IL-3. IL-4 triggered prominent tyrosine phosphorylation of a substrate(s) migrating at 170 kilodaltons (kD) and less striking phosphorylation of several other proteins including the IL-4 receptor. By contrast, IL-3 induced distinct tyrosine phosphorylation of proteins migrating at 145, 97, 70, 55 and 52 kD in the same cell line. IL-4 treatment of FDCP-2 cells caused the dramatic association of phosphatidylinositol-3 (PI-3) kinase with both the 170 kD tyrosine-phosphorylated substrate and with the IL-4 receptor, itself. By contrast, IL-3 triggered only weak association of PI 3-kinase activity with the 97 kD substrate. While IL-4 did not affect cellular *raf*, IL-3 stimulation did induce a shift in its mobility presumably due to serine/threonine phosphorylation. Taken together, our results indicate that IL-4 and IL-3 activate distinct phosphorylation cascades in the same cell background which may reflect a difference in the biological function of these two cytokines.

E 542 ROLE OF C-TERMINAL REGION OF MURINE INTERLEUKIN-6 IN BIOLOGICAL ACTIVITY AND STRUCTURAL CONFORMATION, Ward L.D., Hammacher A., Zhang J-G, Discolo J., Tu G-F, Moritz R.L., Morton C.J. , Norton R.S.*. and Simpson R.J., Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research/Walter and Eliza Hall Institute For Medical Research, Parkville, Vic 3050, Australia and *NMR Laboratory, Biomolecular Research Inst., Parkville, Vic,3050 , Australia.

Interleukin-6 (IL-6) is a multifunctional cytokine with both growth promoting and differentiation effects depending on the nature of the target cell. IL-6 exerts these effects by binding to a specific cell surface receptor (IL-6R) which in turn activates an associated signal transducing subunit (gp-130). IL-6 has been postulated to belong to a family of cytokines which possess a four α-helical bundle tertiary structure. To probe the proposed C-terminal α-helix for its role in maintaining the correct structural conformation and biological activities of mIL-6, we generated substitution and deletion mutants of mIL-6 using PCR technology. Native and mutated forms of mIL-6 and hIL-6 were purified as β-galactosidase fusion proteins from E.coli following our purification protocol for recombinant mIL-6[1]. To differentiate whether changes in activities of mutants were due to direct effects at the IL-6-IL-6R interface or were due to induced conformational changes, the conformation of all engineered proteins were probed by circular dichroism, NMR and the ability to bind conformational dependent monoclonal antibodies. Structural and biological characterisation of native and mutated mIL-6 will be presented.
The ability to monitor the kinetics and equilibrium binding of purified forms of cytokines and their receptors would greatly improve the understanding of the interactions responsible for high affinity binding and transmission of the biological message. A biosensor employing surface plasmon resonance detection[2] is being used to study, *in vitro*, cytokine-receptor interactions. Preliminary data will be presented.

(1) Zhang et.al.(1992) Eur.J.Biochem. 207, 903-913.
(2) Ward et.al.(1992) Biochem.Int. 26, 559-565.

E 543 ACTIVATION OF ADENYL CYCLASE BY INTERLEUKIN-1: A ROLE FOR cAMP IN INTERLEUKIN-1 SIGNAL TRANSDUCTION, M. Neale Weitzmann & Nerina Savage, Dept. of Medical Biochemistry and the Brain Function Research Unit, University of the Witwatersrand Medical School, York Rd, Parktown, 2193, Johannesburg, South Africa.

The signal transduction pathways through which IL-1 action is directed, are highly controversial.
In this study the role of intracellular cAMP and adenyl cyclase activity in IL-1α mediated cytostasis of K562 cells, was investigated.
It was found that the anti-proliferative effects of IL-1 on K562 cells could be mimicked by the cAMP modulators forskolin, and dibutyryl-cAMP, as well as the PKC modulators staurosporine and PMA.
IL-1, forskolin and staurosporine at concentrations reducing proliferation by ± 28%, were found to activate adenyl cyclase by ± 6 fold. Although the activation of adenyl cyclase by staurosporine is consistant with it's anti-proliferative effects, this constitutes a non-specific action of this inhibitor distinct from it's effects on PKC.
PMA had no effect on adenyl cyclase activity. Dibutyryl-cAMP resulted in a 7 fold increase in total cAMP at a concentration producing a 17 % reduction in proliferation.
A dose-dependent elevation of adenyl cyclase activity was elicited by IL-1 within the range 3 - 25 pM in isolated K562 membranes. Total cAMP concentrations were similarly raised in whole cells, peaking within 5 minutes after IL-1 addition.
This work demonstrates a link between IL-1 activation of adenyl cyclase and cytostasis of K562 cells.

E 544 SUBSTITUTION MUTAGENESIS OF MOUSE INTERLEUKIN-2 IDENTIFIES THREE SEPARATE SURFACES FOR SPECIFIC INTERACTION WITH EACH RECEPTOR SUBUNIT, Sandra M. Zurawski, Felix Vega Jr., Ellen L. Doyle, Bernard Huyghe, Kevin Flaherty*, David B. McKay*, Gerard Zurawski. DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304 and *Department of Cellular Biology, Stanford University Medical Center, Stanford, CA 94305.

A completed extensive substitution analysis of mouse interleukin-2 (mIL-2) is presented. Several different substitution mutant proteins at each previously unanalyzed mIL-2 residue (essentially positions 44-142) were analyzed in a two part biological assay. These data defined residue positions that were structurally or functionally unimportant (i.e. tolerated many different side chains) vs. important. A subset of the important residues tolerated only conservative hydrophobic changes. The remaining residues were shown by biological and receptor-binding analyses to be important for specific interactions with either the α, β, or γ subunits of the IL-2 receptor. mIL-2 structure was modeled on the basis of the refined human IL-2 X-ray crystallographic structure. On this model all the important hydrophobic residues were located in the protein core and the other important residues were localized to three separate and distinct surfaces involved in receptor subunit-specific interactions.

Late Abstracts

ATTENUATION OF ALLERGIC AIRWAY INFLAMMATION IN IL-4 DEFICIENT MICE

Horst Bluethmann*, Guy Brusselle¶, Manfred Kopf§, Georges Köhler§, and Jan Tavernier$, *Department of Biology PRTB, F. Hoffmann-La Roche Ltd., Basel, Switzerland, ¶Department of Respiratory Diseases, University Hospital, Gent, Belgium, §Max-Plank-Institut für Immunbiologie, Freiburg, Germany, $Roche Research Gent, F. Hoffmann-La Roche Ltd., Gent, Belgium

To investigate *in vivo* the role of the cytokine IL-4 in allergic asthma, we developed a murine model for allergen-induced airway inflammation. C57BL/6 mice were immunized with ovalbumin (OVA) and exposed daily to aerosolized OVA for 7 days. This treatment induces the production of Ag-specific IgE antibodies in the mice. In bronchoalveolar-lavage (BAL) 24 h after the last aerosol exposure, a vast increase in eosinophils was detectable compared to saline (SAL) - exposed unimmunized control animals. Histological analysis of lungs of OVA-challenged animals showed peribronchial and peribronchiolar inflammatory infiltrates, mainly composed of lymphocytes, monocytes and eosinophils.

When IL-4 deficient ($IL\text{-}4^{0/0}$) mice were treated in the same way, no IgE was detectable in the serum. In addition, differential white blood counts of BAL fluid revealed a selective and striking reduction in the percentage and the total number of eosinophils, compared to OVA-challenged IL-4 wild type ($IL\text{-}4^{+/+}$) mice. Moreover, histological examination of lungs of OVA-treated $IL\text{-}4^{0/0}$ mice demonstrated clearly less peribronchiolar inflammation. In conclusion, these results indicate that IL-4 is not only essential for IgE synthesis *in vivo*, but also a central mediator of allergic airway inflammation.

ROLE OF INTERLEUKIN-2 (IL-2) IN THE GENERATION OF A NEGATIVE REGULATORY SIGNAL. Bruce H. Devens, Satoshi Fukuse, Merideth Kelley, and David R. Webb. Institute of Immunology and Biological Sciences, Syntex Research, Palo Alto, CA. 94303.

It has been recently documented in both murine and human CD4+ lymphocytes that there exist two subsets based on cytokine production profiles, Th-1 and Th-2. At least two of these cytokines (IFN-γ, and IL-10) may reciprocally regulate the capacity of the subsets to function in a variety of immune response models. We now report that IL-2 stimulates the production of an immunosuppressive cytokine, soluble immune response suppressor (SIRS), by CD8+ cells. The generation of SIRS by IL-2 occurs in a dose dependent fashion and requires 3 or more days of culture. The SIRS producing suppressive cells generated are not acting via a cytolytic mechanism. They block *in vitro* generation of both antibody and cellular immune responses. Further, injection of the suppressive cells into mice decreases the induction of antigen specific humoral and cellular responses *in vivo*. Animals rendered non-responsive via this pathway have been shown to remain unresponsive to antigenic stimulation for up to 200 days. Induction of non-responsiveness with IL-2 can be inhibited with antibody to the 55 kd subunit of the IL-2 receptor. The anergy inducing activity is inhibited with antisera to SIRS, an 8-10 kd protein of known amino terminal sequence. Induction of anergy with IL-2 requires CD8+ cells plus adherent cells. IFNγ also activates this pathway by stimulation of SIRS production. However, exposure of splenic cells to IL-1, 3, 4, 6, 7, or 10, as well as TNFα or TGFβ fails to induce SIRS production. The pathway is also triggered by exposure of lymphocytes to alloantigen or anti-CD3 suggesting that regulatory cells arise during the course of a normal immune response. Thus in addition to IL-10 and IFN-γ, IL-2 must now be considered to function as both a growth factor and as a mediator of cellular non-responsiveness via the production of a specific inhibitor, SIRS.

IL-4 AND PKC REGULATION OF GENE (PERFORIN) EXPRESSION AND PROTEIN (BLT-ESTERASE) SYNTHESIS IN T CELL ACTIVATION, Chou-Chik Ting*, Shu-Mei Liang**, Yang-Yang Chen**, and Myrthel E. Hargrove*. National Cancer Institue* and Center for Biologics Evaluation and Research**, Bethesda, MD 20892.

Activation of T cell by αCD3 induced a cascade of events leading to T cell proliferation and generation of activated killer cells (CD3-AK). We showed that the major pathway for CD3-AK generation is protein kinase C (PKC)-dependent and is regulated by IL-2 and IL-4. There is also a minor PKC-independent pathway which is primarily regulated by IL-4. Adding a PKC antagonist staurosporine into CD3-AK cells cultured in IL-2 or depletion of PKC by prolonged treatment with PMA did not affect the proliferative response, indicating that activation of T cells by αCD3 can be induced in the absence of PKC. However, generation of killer cells was abrogated, which was associated with reduced perforin gene expression and production of BLT-esterase. Adding exogenous IL-4 at the begining of culture restored the lytic activity of CD3-AK cells, which was associated with the restoration of perforin gene expression and BLT-esterase production. Furthermore, At 10 days after initial αCD3 activation, switching the lymphokines in these cultures reversed their cytolytic activity: switching from IL-2 to IL-4 restored the cytolytic activity and switching from IL-4 to IL-2 reduced the cytolytoc activity. Adding IL-4 at this late time also restored the expression of perforin gene and BLT-esterase production. These findings indicate that, independent from PKC, cytokine (IL-4) may directly switch on and off the gene (perforin) expression and protein (BLT-esterase) synthesis in resting and in preactivated T cells, and this effect is reversible, and thus appears to be different from the cytokine (IL-4) regulation of the gene expression and immunoglobulin (IgE) production in B cells.

IP-10, A GAMMA-INTERFERON INDUCIBLE "INTERCRINE", ELICITS A POTENT THYMUS-DEPENDENT ANTITUMOR RESPONSE *IN VIVO*, Andrew D. Luster and Philip Leder, Department of Genetics, Howard Hughes Medical Institute, Harvard Medical School, Boston MA 02115

IP-10 is a member of a superfamily of PF-4 related pro-inflammatory chemotactic cytokines that have been grouped under various names including "intercrines" and "chemokines". This family includes the neutrophil and lymphocyte chemotactic and activating protein IL-8, the platelet α-granule protein PF-4, and the monocyte and eosinophil chemotactic and tumoricidal activation protein MCP-1. IP-10 was isolated as the predominant mRNA induced in IFNγ and LPS treated monocytes. To date, the biological function of IP-10 remains unknown.

We have utilized a tumor cell transplantation assay to explore the *in vivo* biological function of IP-10. Murine IP-10 was stably transfected into the J558L plasmacytoma cell line and the K485 mammary adenocarcinoma cell line under the control of both the murine Moloney leukemia virus LTR and the IgM heavy chain promotor. Stable subclones that constitutively secrete high levels of IP-10 (20-120 ng/ml per $2x10^6$ cells/48 hrs) were used to demonstrate that in an immunocompetent syngeneic host IP-10 secretion leads to inhibition of tumor growth. Histological analysis of regressing tumors revealed a host response composed of neutrophils and mononuclear cells. IP-10 was able to exert its effect in a non-cell autonomous fashion and led to killing of wild-type tumor cells if they were mixed with IP-10 expressing tumor cells. However, IP-10 had no effect on tumor growth in the congenitally athymic *nu/nu* mice, suggesting that this effect is T-cell dependent.