MOLECULAR PATHWAYS OF CYTOKINE ACTION
Organizers: Stanley Cohen and Charles H. Evans
January 27-February 3, 1990

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Molecular Pathways of Cytokine Action

Keynote Address (joint)

CD 001  THE T CELL REPertoire, Philippa Marrack and John Kappler,
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National Jewish Center, Denver, CO 80206

The T cell repertoire in any given mammal is controlled by 4 phenomena.
the germ line receptor genes available in that animal, positive
selection for self-MHC associated antigen recognition in the thymus,
tolerance induction and exposure to environmental antigens. There is
evidence in laboratory and wild mice that all of these phenomena
operate, sometimes to surprising extents. For example, in mice self
superantigens can cause the deletion of a high percentage of all T cells
leaving some laboratory or wild animals with diminished T cell
repertoires. Large deletions of this type are less striking in man but
may occur in some circumstances. There is evidence that T cell
tolerance can be acheived either by clonal deletion or by clonal anergy.
The circumstances under which these 2 mechanisms operate, and their
consequences, will be considered.

Control of DNA Synthesis and Cell Proliferation

CD 002  CYTOPLASMIC FACTORS MODULATING DNA REPLICATION,
Kerin L. Fresa, Frederick D. Coffman and Stanley Cohen,
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Philadelphia, PA 19102.

Cytoplasmic extracts prepared from proliferating normal or transformed lymphocytes
can induce DNA synthesis in isolated, quiescent nuclei, whereas extracts prepared from
resting lymphocytes fail to do so. This activity is mediated through the action of a
cytoplasmic protease (ADR). ADR activity can be induced in resting cytoplasmic extracts, in
a dose-dependent fashion, by brief incubation with a membrane-enriched fraction from
spontaneously proliferating MOLT-4 cells, suggesting that ADR is already present in the
resting cytoplasm in a inactive or precursor form. This precursor or inactive ADR is
precipitated between 30 and 50% ammonium sulfate saturation, similar to active ADR isolated
from dividing lymphocytes. Furthermore, ADR activity induced in resting cytoplasmic
extracts by MOLT-4 membranes can be inhibited by the protease inhibitor, aprotinin,
suggesting that this induced ADR has a similar mechanism of action to the factor normally
present in cytoplasmic extracts from actively dividing lymphoid cells. Thus, the induced ADR
activity appears similar to or identical with "spontaneous" ADR activity. The interaction
between MOLT-4 membranes and resting cytoplasm that generates ADR activity appears to be
dependent upon protein kinase activity, as this reaction is inhibited, in a dose-dependent
manner, by H7, which is an inhibitor of cAMP-dependent protein kinases and protein kinase
C, but not by a synthetic peptide, IP-20, which appears to be a specific inhibitor of cAMP-
dependent protein kinases. These results suggest that a membrane-associated protein kinase C
participates in the conversion of an inactive or precursor form of ADR already present in the
resting cell cytoplasm to one that is capable of inducing nuclear DNA synthesis.
Supported by NIH Grant R35-CA-39723.
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CD 003  EXPRESSION AND EXTERNALIZATION OF EGF-RECEPTOR: ACCELERATION OF PRE- AND POST-TRANSLATIONAL EVENTS BY EGF, Manjuri Das, Prabakaran Kesavan and Jayant Khire, Department of Biochemistry & Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059.

The EGF receptor belongs to a class of growth regulatory proteins in which a single membrane-spanning segment separates the extracellular (ligand-binding) domain from the intracellular (tyrosine kinase) domain. We have studied the expression and stability characteristics of two forms of receptor mRNA - a 5.6 kb form that encodes for the intact transmembrane 170 kDa receptor and a smaller 2.6 kb form that encodes for a 100 kDa secreted EGF-receptor. The latter is identical (up to nucleotide 2079) to the normal intact EGF-receptor mRNA of 5.6 kb, but it diverges downstream in the 3' region, and lacks the regions encoding for transmembrane spanning and cytoplasmic kinase domains. The studies indicate that synthesis and stability of EGF-receptor gene transcripts are stimulated by EGF and cycloheximide. The stability of the 5.6 kb transcript is enhanced by EGF and cycloheximide in a non-additive manner, suggesting certain common features in their pathways of action. The smaller 2.6 kb mRNA shows higher basal stability in comparison with the 5.6 kb entity. It is further stabilized by cycloheximide, especially in combination with EGF acting in a synergistic manner. The results imply the involvement of 3' located structural features in the control of receptor mRNA stability, and suggest the involvement of labile proteins in the regulation of EGF-receptor mRNA expression.

Next we tested whether EGF influenced receptor expression at the level of post-translational maturation and externalization. We used the 100 kDa EGF-receptor as the model. This receptor is identical to the external domain of the transmembrane EGF-receptor in its polypeptide structure, glycosylation state and conformation; but because it lacks a membrane anchor, biosynthetic transport and externalization of this soluble receptor is easier to monitor than that of the membrane-bound receptor, and is moreover uncomplicated by any possibility of internalization. The studies indicate that exocytosis of the 100 kDa protein is constitutive, but slow, and dependent upon events within endoplasmic reticulum (ER) that are rate-determining from the viewpoint of transport. One of the ER-located events - core N-linked glycosylation - although essential for transport, occurs soon after the translation of the protein. However, a subsequent slow event(s) controls its maturation, processing, exit from ER and entry into golgi. The rate determining step may be regulated by the ligand EGF, which is now found to have an accelerating effect upon receptor maturation, transport and secretion. This EGF-induced acceleration of biosynthetic transport occurs independent of ongoing translation.

CD 004  CYTOKINE ACTIVATION OF TARGET CELL PROGRAMS, Nancy H. Ruddle, Marc C. Levesque, Janet D. Geiger, Sarah Fashena, and Cheryl M. Bergman, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510

Lymphotixin (LT; TNF-β) and tumor necrosis factor (TNF-α) are functionally and genetically related molecules. Though originally described as cytoxic factors, they can affect target cells in several different ways including killing, induction of proliferation, and induction of differentiation. LT and TNF's effects are dictated in large measure by the differentiated state of the target cell itself. LT and TNF can induce release of target cell DNA. This induction of DNA fragmentation termed apoptosis or programmed cell death is characteristic of many differentiating systems including digit formation, resorption of the tadpole tail, and glucocorticoid and anti-CD3 induction of thymocyte suicide. The role and mechanism of LT and TNF in several of these systems is under investigation. It has been postulated that the laddered pattern of DNA isolated from cells undergoing apoptosis is due to fragmentation at internucleosomal intervals suggesting the possibility that histone proteins might be released in association with the DNA fragments. We have determined that the kinetics of histone release from nuclei of cells undergoing apoptosis corresponds to the kinetics of DNA release. The size of these fragments suggests that the DNA and histones are associated in the form of intact chromatin. We have determined that LT and TNF are not themselves endonucleases but must activate or release cellular endonucleases perhaps through their effects on lysosomes. The effect and mechanism of LT and TNF induction of lymphocyte differentiation is under investigation. They may act as autocrine and paracrine growth factors or as inducers of suicide. A variety of murine B cell lines representing stages of B cell differentiation from fetal liver to plasmacytomas have been studied. LT and TNF expression is associated with early B cells which expand by responding to proliferating signals or may undergo programmed cell death in the case of inappropriate gene rearrangements.
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Oncogenesis

CD 005 POSITIVE AND NEGATIVE GROWTH REGULATORY SIGNALS, Judith Campisi,
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The proliferation of higher eukaryotic cells is regulated by positive and negative signals,
provided by the cellular environment, and by the state of cellular differentiation. Positive and
negative-acting cytokines regulate proliferation ultimately by altering the expression of specific
genes. Among these are the cellular protooncogenes, which are often critical intracellular regu-
lators of the growth response. However, it is the state of differentiation that dictates whether
and how a specific gene, and subsequently the cell, responds to a particular cytokine. We de-
scribe recent data on two cell systems in which extracellular signals, protooncogene expression
and differentiation interact to regulate cell proliferation. First, we studied human fibroblasts at
early passage, when the cells proliferate well, and at late passage or senescence, when the cells
have irreversibly arrested growth. This growth arrest is thought to constitute a mechanism for
curtiling transformation, and is exceedingly resistant to reversal by growth factors, carcino-
gens, radiation and oncogenic viruses. An exception is the SV-40 T antigen. At late passage,
human fibroblasts show several qualitative and quantitative changes in gene expression which,
together, suggest that senescence is a process of terminal differentiation. The senescent cells
have functional growth factor-signal transducing systems for inducing the expression of many
genes, including the c-myc and c-ras-Ha protooncogenes. However, there is a selective, trans-
criptional block to induction of the c-fos protooncogene which cannot be overcome by growth
factors. Since c-fos expression is required for the proliferation of fibroblasts, this repression may
be critical to the irreversible growth arrest and overcome by T antigen. We also studied c-fos
expression in primary rat lung epithelial cells (alveolar type 2 cells). These highly differen-
tiated cells have a very limited division potential in vivo and in culture. Although c-myc and
c-ras-Ha are expressed by type 2 cells, c-fos is neither expressed nor inducible by growth factors.
However, immortalization by SV-40 T antigen fully restores c-fos inducibility by growth factors.
Thus, the differentiated state can cancel the action of growth factors by selectively repressing
one or more positive acting protooncogene, and some dominant-acting oncogenes may drive
proliferation by overcoming this repression.

CD 006 FUNCTIONAL ANALYSIS OF COMPLEX FORMATION BETWEEN THE RETINO-
BLASTOMA SUSCEPTIBILITY GENE PRODUCT AND SV40 LARGE T ANTIGEN, David
M. Livingston, James A. DeCaprio, Mark E. Ewen, William G. Kaelin, Jr., and John W.
Ludlow. The Dana-Farber Cancer Institute and The Harvard Medical School, Boston, MA
02115.

The product of the RB-1 locus is a 928 residue nuclear, DNA binding polypeptide
believed to exert some measure of suppression of neoplastic growth in multiple cell
types. It exists as a complex family of unphosphorylated (pRB) and differentially
phosphorylated (pRBphos) species. A series of DNA tumor viral transforming proteins,
including the adenovirus E1A products, SV40 large T antigen (T), and HPV E7, all form
stable complexes with the RB-1 product, and complex formation likely participates in
the mechanisms underlying the transforming functions of these proteins. Specifically,
the data suggest that each of these elements can modulate (?) inhibit) the growth
suppressing function of the RB-1 product. In this presentation, we will review
evidence from our laboratory pointing to a possible cell cycle regulatory role for pRB
which is conditioned by its cyclical phosphorylation and dephosphorylation. At least
part of the effect of T-pRB complex formation may be to by-pass checkpoint(s) in the
cycle at specific point(s) which depend, in some measure, upon the maintenance of pRB
cell cycle regulatory function. Moreover, evidence describing the existence of discrete
T and pRB domains which, in part, control T-pRB complex formation will be described,
along with data pointing to a role for the quarternary structure of T in this process.
By sequence comparison, GRO can be placed in a family of genes which encode secretory proteins involved in the inflammatory response. Previous studies of GRO in fibroblasts have shown that it is an early response gene. GRO mRNA is elevated by serum, shortly after \textit{fos} but before \textit{myc}, when serum-starved cells enter the cell cycle. GRO mRNA is also elevated in starved or growing cells in response to TNF or IL-1, suggesting that GRO has a role in cytokine signalling. New findings support and extend these studies.

In order to determine other functions of GRO, recombinant protein has been produced through harvest of conditioned medium from cos cells transiently transfected with a plasmid containing GRO cDNA. A purification scheme has been developed which allows for production of homogeneous protein. The recombinant protein has an apparent molecular weight of 6.5 kDa, in close agreement with the predicted molecular weight. Current studies demonstrating the biological activity of recombinant GRO protein will be presented. In addition, a polyclonal antibody has been raised against a peptide sequence of GRO and has been shown to be specific for the GRO protein. It is capable of recognizing GRO in Western analysis, immunoprecipitation, and RIA. Using these three assay systems it is possible to monitor GRO protein production as an adjunct to expression levels noted by Northern analysis.

CAT-assays and gel retardation analysis shows that the TNF and IL-1 responses are mediated by the NFkB binding site located at -65 to -75 5' to the GRO coding region. A protein which is induced by IL-1 and TNF is capable of binding to a fragment containing the NFkB site. In competition experiments this binding is eliminated by a 72 base pair SV40 DNA containing the NFkB sequence. Competition experiments with oligonucleotides containing wildtype or mutated NFkB sites are in progress.

Regulation of Immune Function

\textbf{CD 008} \textbf{LYMPHOHEMATOPOIETIC GROWTH FACTOR REGULATION OF PROTEIN KINASES AND GENE EXPRESSION}, William L. Farrar\textsuperscript{1}, Gonzalo Garcia Garcia\textsuperscript{1}, Gerald A. Evans\textsuperscript{2}, Dennis F. Michiel\textsuperscript{1} and Diana Linnekin\textsuperscript{1}, Cytokine Mechanisms Section, Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, National Cancer Institute, Frederick, Maryland 21701-1013 and \textsuperscript{2}BCDP, Program Resources, Inc., NCI-FCRF, Frederick, Maryland 21701-1013. Proliferation and differentiation of lymphohematopoiesis and the regulation of the mature immune system is under the exquisite control of a variety of polypeptic factors collectively referred to as cytokines. With the exception of CSF-1/c-fms, all the cytokine receptors cloned to date have no cytoplasmic catalytic domains, thus separating them from previously described growth factor receptors such as those found for insulin, EGF, PDGF, etc.

We have examined the protein kinase coupling mechanisms and cellular substrates for a number of lymphohematopoietic growth factors including IL 2, IL 3, and GM-CSF. Each factor possesses a unique receptor but nevertheless, trigger many similar biochemical events and program of gene expression. All the proliferative cytokines appear to regulate a similar pattern of gene expression including, c-fos, c-myc, c-myb, ODC, cyclin, IL 2R alpha, and some members of the heat shock gene family. The cytokines appear to regulate both serine and tyrosine kinase activity. One substrate at 68 kDa is rapidly phosphorylated on serine by all mitogenic cytokines tested. The p68 protein is highly conserved and appears central to the proliferative response. Human IL 3 and GM-CSF stimulate the catalytic activity and autophosphorylation of two tyrosine kinases at 160 and 92 kDa. The 92 kDa tyrosine kinase also appears to be regulated by the IL 2R beta chain suggesting a conservation between myeloid and lymphoid cell types. The data suggests that certain cytokine receptors may use generic kinases common to several receptors in mediating the signal transduction process.
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CD 009  CYTOKINE REGULATION OF CYTOTOXIC LYMPHOCYTE INDUCTION.
Elizabeth A. Grimm, Departments of Tumor Biology and General Surgery, University of Texas M.D. Anderson Cancer Center, Houston, TX.

The adaptive immune response contains two major functional classes of cytotoxic lymphocytes: (1) MHC-restricted antigen specific CTL and (2) MHC-unrestricted ones, commonly called lymphokine activated killers or "LAK." Neither function is expressed constitutively, but must be induced. Although the activation processes are poorly understood, it is known that interleukin-2 is an obligate cytokine for differentiation of both classes of cytotoxic lymphocytes. It is possible that the available level of IL-2 regulates their quantity: antigen-primed CTL precursors require 100X less IL-2 (10^{-1}M) to interact with the high affinity bimolecular IL-2 receptor complex, than is necessary for the induction of LAK via a unimolecular intermediate affinity (10^{-9}M) receptor. IL-2 alone, in serum-free chemically-defined conditions, is sufficient for LAK activation, and to date, no requirement for any other exogenous factor has been revealed.

IFN-γ does not induce LAK, however, in the presence of monocytes, its synergy with low levels of IL-2 results in optimal LAK. Two major products of IFN-γ stimulated monocytes are tumor necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1). Subsequent studies have revealed that each of these factors synergize with suboptimal IL-2 for LAK activation, probably through distinct pathways. Recent analysis of the TNF-α pathway indicates that IL-2 directly regulates induction of both the expression of TNF-α receptors and the TNF-α message in lymphocytes, thereby initiating an autocrine loop. Monocytes are not required for this level of regulation. Although TNF-α does not appear to be involved in the direct lysis of any of the tumor targets employed, its steady state mRNA expression in TIL, appears temporally related to the cytotoxic capability of these cytotoxic lymphocytes. In conclusion, our results indicate a role for autocrine and paracrine extra-IL-2 regulation of cytotoxic lymphocyte induction.

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T Cell Traffic and Endothelial Cell Interactions (joint)

CD 010  THE ADHESION OF NORMAL AND NEOPLASTIC CELLS TO ENDOTHELIUM.
Marion C. Cohen, Michael Bereta, Scott J. Antonia, and Stanley Cohen, Department of Microbiology and Immunology and Department of Pathology, Hahnemann University, Philadelphia, PA, 19102.

The adherence of inflammatory or neoplastic cells to endothelium is recognized as an event which appears to be mediated by molecules on the circulating cells which recognize specific sites on endothelial cells. The adherence of lymphocytes, macrophages, neutrophils and mast cells to endothelium has been identified as important in the generation of inflammatory reactions. The identification of the factor(s) which causes adhesion to have been approached in several ways: molecules on the surface of the circulating cell involved in the process have been identified as well as molecules on the endothelial cell surface. Some of the former have been shown to be specific to a cell type while others are shared by several types. Studies of endothelium have suggested that some of these cells express organ-specific ligands on their surface. Other studies have focused on conditions in the local microenvironment which result in increased binding. Of particular interest in this regard is the role played by cytokines in cell binding phenomena.

It has also been suggested that the first critical step in metastasis is arrest and adhesion of the circulating tumor cell in the microvasculature followed by extravasation. It appears that this process involves mechanisms similar to those operative in normal inflammatory cell-endothelial cell interactions. We have therefore studied the role of cell adhesion in tumor cell metastasis. We have found that adherence to endothelium is mediated by glycoproteins on the surface of the tumor cell and that binding is cation-dependent. To purify adhesion molecules on the tumor cell we utilized methods that had been successful in identifying adhesion molecules on neural and liver cells. In preliminary studies we have been able to achieve approximately 450-fold purification of adhesion molecules from membrane preparations of tumor cells. In other experiments we incubated endothelium with cytokines and found increased numbers of neoplastic cells bound to endothelial monolayers which had been incubated with TNF, LPS, or PMA. Unlike the situation for inflammatory cells, IL-1 and IFN-γ produced no changes in binding. In addition, exposure of tumor cells to these factors prior to incubation with endothelium had no effect. This suggests that there is an active process by which surface molecules on endothelial cells may be induced (or normal molecules increased) to mediate adherence of neoplastic cells and that some cytokines may play an important role in their expression. Whether these structures are identical to those involved in inflammatory cell adhesion remains to be determined.

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The Role of Homing Receptors in Recirculation of Activated T Cells

In Primates, W. Michael Gallatin, Patricia Hoffman, Dennis Willerford, Stephen Rosenman, Joey Meyer, and Thomas St. John, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Many cells of hemopoietic origin, particularly lymphocytes, move continuously between the various lymphoid tissues to reach specialized microenvironments presented by each organ. Transmigration across post-capillary venules in organized lymphoid organs and at sites of inflammation is a multistep process that can be divided conceptually into (1) attachment to endothelial cells (EC), (2) lateral movement on the surface of EC, (3) transmigration between EC, (4) penetration of the basement membrane, (5) release, and (6) intraorgan sorting into appropriate microenvironments (e.g. T vs. B). The initial phase of this movement, attachment, is orchestrated by organ-specific adhesion of the trafficking cells to the endothelial lining of the microvasculature at each site. Migration competence is modulated as a function of lymphocyte activation by varied expression of at least three distinct classes of lymphocyte cell adhesion molecules (CAMs): integrins (e.g. LFA-1), the CD44 glycoproteins, and selectins (e.g. Mel-14, LAM-1). For example, lack of Mel-14/LAM-1 on IL-2 dependent, CD8+ cytotoxic T cells propagated in vitro, may contribute to poor recirculation and limit therapeutic utility in vivo. We have used a primate model to analyze the structure and function of both CD44 and Mel-14 (LAM-1) type CAMs. In macaques expression of these structures by normal PBL, correlated with their ability recirculate in vivo and to adhere to lymphokine induced cultured EC in vitro. T cells could be placed in a maturational sequence of noncycling virgin, actively cycling, and resting memory cells according to expression of these receptors. These stages of activation/maturation were anatomically partitioned in vivo. High-level CD44 expression was also correlated with an increased responsiveness to mitogenic stimuli transmitted via CD3. This may involve a direct contribution of CD44 to the activation cascade. To facilitate structure/function analyses of CD44 in each of these processes and to test the relative contributions of CD44 and Mel-14/LAM-1 type CAMs to EC binding, we isolated cDNAs encoding the macaque homologues of each. In both cases cross-species comparison of primate, human, and murine genes reveals a high degree of conservation. Functional analyses of cells expressing CD44 or macaque LAM-1 (LAM-1mac) de novo following plasmid and retrovirus mediated gene transfer indicate that CD44 expression per se is insufficient to mediate lymphocyte adhesion to EC. Nonetheless, CD44 can promote cell-cell adhesion in other contexts and may serve as a facilitating CAM for lymphocyte-EC attachment. In contrast, a more direct functional correlation with LAM-1mac expression exists. Constitutive expression of the latter in IL-2 dependent T cell clones may provide a means to enhance recirculation and effector function in a clinical setting.

Organ Specificity of Metastasis is Determined by Adhesive, Invasive and Growth Properties of Unique Tumor Cells and Host Environments. Garth L Nicolson, Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The sites of distant metastases in many clinical cancers and animal tumors are nonrandom, and their distributions cannot be rationalized by anatomical or mechanical hypotheses. Using animal models for metastasis based on murine melanoma, large cell lymphoma and fibrosarcoma and rat mammary adenocarcinoma we have examined tumor cell and host organ properties, such as differential tumor cell adhesion to organ-derived microvessel endothelial cells or their subendothelial basement membrane-like matrix and organ parenchymal cells, differential tumor cell invasion of host organ tissues and extracellular matrix and their expression of degradative enzymes, and differential tumor cell responses to organ-derived growth-stimulatory and growth-inhibitory factors. These appear to be collectively important in determining the organ specificity of metastasis. In the interactions of tumor cells with microvessel endothelial cells we have identified several tumor cell and endothelial cell surface components involved in organ-preference of adhesion. These include endogenous lectins, integrin-like, calcium-dependent and other adhesion molecules. Malignant cells possess another set of cell surface adhesion components for basement membranes, including receptors for fibronectin, laminin, collagen, heparan sulfate proteoglycan, and other components. During invasion metastatic cells release and display on their cell surfaces specific degradative enzymes, such as metalloproteases, proteinases and endoglycosidases, that are used to dissolve basement membranes and other structures. Finally, we have found that highly metastatic cells differentially respond to organ-derived, secreted, paracrine growth factors which are present in dissimilar amounts in different organs. One of the most potent of these paracrine growth factors for lung-colonizing epithelial tumor cells has been purified to homogeneity and shown to be a unique Mr = 68,000 glycoprotein that has been isolated from rodent and porcine lungs and binds to a specific receptor on lung-metastasizing tumor cells. Another differentially expressed growth factor that stimulates highly metastatic tumor cells is transferrin. These and other tumor cell surface and host properties may eventually be used to predict and explain the unique metastatic distributions of certain malignancies. [Supported by NCI grant R35-CA44352].
Non MHC-Restricted Recognition and Lysis (joint)

CD 013 THE ROLE OF HLA MOLECULES IN THE SUSCEPTIBILITY OF TUMORS TO NATURAL KILLING. Jeffrey R. Dawson, Walter J. Storkus; Jeffrey Alexander, J. Alan Payne and Peter Cresswell, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710, USA. The cytotoxicity mediated by the natural killer (NK) cell is not restricted by class I major histocompatibility complex (MHC) antigens; target cells may be lysed across allogeneic and, in some instances, xenogeneic barriers. Recent reports suggest that reduced target cell class I expression may coincide with an increase in sensitivity to NK. This hypothesis was tested indirectly by examining a series of related lymphoblastoid cell lines differing in the expression of class I HLA antigens; sensitivity of these cell lines to NK was inversely proportional to the cell surface expression of class I antigens. The expression of class I HLA antigens can be reduced following infection of human lymphoblastoid cell lines with certain strains of Adenovirus; the sensitivity of Adenovirus-infected target cells to NK-mediated conjugation and cytolyis also showed a similar inverse correlation to cell-surface expression of class I HLA molecules.

The hypothesis was tested directly by transfection of NK-sensitive cell lines with class I MHC genes. The NK-sensitive, class I-deficient, human B-cell line CILR was transfected with class I, MHC genes. Expression of transfected HLA, but not H-2, class I gene products resulted in loss of susceptibility to human NK-mediated conjugation and cytolyis. Regions of the class I HLA molecule involved in providing this protective effect were mapped to the alpha-1 and alpha-2 domains by exon shuffling the HLA-B7 and H-2-D^{b} genes and examining target cell NK-susceptibility following transfection with these constructs. Further refinement of the mapping was possible through the use of class I HLA alleles that did or did not confer protection following transfection. Three residues in the first alpha helix and a single residue in the second alpha helix (of the Bjorkman et al. (1986) monoclonal antibody) were identified by comparative amino acid sequence analyses of protective (HLA-A3, Aw68, B6, B27, Bw58) versus poorly protective (HLA-A1, A2) class I alleles.

CD 015 MECHANISM OF LYSIS BY HUMAN NATURAL KILLER CELLS. John R. Ortaldo, Steve Anderson, Ko Okamura, Casuo Sugamura, Joyce Frey, Mark Smyth, and Howard A. Young, IRMP, NCI-FCRF, Frederick, MD 21701; Biotech. Research Inst., Montreal, Quebec, CAN; Univ. of Toronto, Mount Sinai Hospital Research Institute, Toronto, Ontario, CAN; Juntendo Univ., Sch. of Med., 2-1-1 Hongo, Bunkyo-ku, Tokyo, Japan 113; Fukushima Univ., Sch. of Med., Sendai, Japan. Natural killer (NK) cells and killer (K) cells mediating ADCC have been shown to be a lymphocyte subset designated large granular lymphocytes (LGLs). The mechanism of cytotoxicity by LGLs can be divided into two distinguishable stages. The first stage involves recognition and binding to target cells, while the second involves post-binding events that lead to target cell lysis. Studies in this laboratory are proceeding to define the receptors and structures involved in NK recognition. We have developed an anti-idiotypic antibody (anti-ID) against a monoclonal antibody that blocks LGL binding and LGL-mediated NK target cell lysis. This anti-ID antibody is reactive with 85-100 and 150 kD proteins and blocks LGL binding and NK target cell lysis. LGLs pretreated for 18 hr with the F(ab')2 anti-ID antibody significantly enhanced lysis of target cells and production of interferon-γ by LGL suggesting triggering through this molecule that results in intracellular signalling events. Biochemical characterization of this proposed NK receptor and its cloning are in progress. The role of LGL soluble and intracellular factors, such as pore forming protein (PFP) and natural killer cytotoxic factor (NKCF), in this cell-mediated lysis has been examined to gain an understanding of the mechanism of lysis. We have analyzed PFP mRNA expression in resting and stimulated human peripheral blood CD3+ LGL, CD3- T cells and their CD4+ or CD8+ subsets. Increases PFP mRNA in T cells occurred in 2-4 hours, with peak levels occurring after 6 hours in the absence of DNA or protein synthesis. After stimulation of CD3+ lymphocytes with IL-2, the CD8+ T cell subset was predominantly induced to express PFP mRNA. Experiments utilizing monoclonal antibodies to the p75 IL-2 receptor indicated that IL-2 signaling was via the p75 IL-2 receptor. The cytotoxic potential of peripheral blood T cells and LGL induced in response to IL-2 correlated with IL-2 induced PFP mRNA levels in these cells and was consistent with PFP being one of several important molecules involved in the effector function of cytotoxic lymphocytes. Using monoclonal antibodies we have demonstrated NKCF to be a factor responsible for LGL-mediated lysis of a variety of tumor targets. Elucidation of the site and mechanism of action of these lytic factors should expedite our understanding of NK cell-mediated killing.
Macrophage activation for tumor cell killing is a multiphasic reaction sequence in which responsive macrophages require sequential interaction with multiple stimuli for the development of full lytic capability. Interferon gamma (IFN-γ) has been recognized as the prototypic activating factor responsible for initiation of this complex pathway and, in combination with bacterial lipopolysaccharide (LPS), this molecule can stimulate the development of macrophage anti-tumor activity. The multi-step nature of this pathway suggests the induction of this functional activity requires the completion of a series of strictly regulated biochemical and molecular events. However, investigation of these mechanisms has been hindered by the lack of objective markers for identification of intermediate stages in the activation pathway. Our experimental approach to the analysis of the process of activation for tumor cell killing involves the use of murine macrophage cell lines as model systems for the study of normal macrophage function. In our recent work, we have demonstrated that, unlike heterogeneous populations of normal cells, a given macrophage cell line will express a distinct subset of functional activities in response to a defined activating signal such as IFN-γ. Thus, the RAW 264.7 cell line exhibits a strict requirement for interaction with IFN-γ and LPS for the development of tumor cytolytic activity. In contrast, the WEHI-3 cell line, although IFN-γ-responsive as assessed by the induction of MHC antigen expression, does not develop cytolytic activity. The identification of homogeneous macrophage populations capable of differential cellular responses to IFN-γ provides a novel system for analysis of the mosaic of changes associated with the activation process. As a first step toward understanding that process at the molecular level, we have produced monoclonal antibody probes which identify cell surface proteins changes occurring in macrophages during activation. We have used a variety of molecular techniques to detect and clone gene products that are uniquely expressed in cells activated by IFN-γ, in order to assess the molecular profile of macrophages activated for particular functions. Finally, our third approach to the analysis of macrophage activation has been through the identification of an alternative activation stimulus, gamma radiation, which has many effects on macrophage function similar to those initiated by IFN-γ. Our efforts in this area are centered on the identification of signal transduction mechanisms and protein or molecular genetic changes expressed in common by cells activated by these distinct stimuli. These combined approaches should allow dissection of the functional roles of genes and proteins induced in macrophages activated for tumor cell killing.
Neuroattenuated, demyelinating mutants of MHV-4 were selected for resistance to monoclonal antibodies against the major spike glycoprotein, S. These mutants and their wild type parents have been characterized by northern and western blotting and by direct RNA sequencing. Neuroattenuated mutants were shown to contain large deletions of over 450 nucleotides in the S gene sequence, and these deletions were consistently mapped to the N-terminal domain of the S polypeptide. Replication of the mutant and wild type viruses was compared in culture and in the mouse brain, and it was observed that the deletion mutants grew more efficiently in culture but not in vivo. Sequences and biological properties of S proteins of additional MHV-4 point and deletion mutants as well as other MHV strains have been compared in an effort to understand the molecular and biologic basis for the observed polymorphism in S.

Using in situ hybridization we have studied the trafficking of virus during infection and find that in contrast to wild type MHV-4 which causes massive early infection of gray matter tracts, the neuroattenuated mutants only mildly affect gray matter, but spread avidly in white matter tracts. Animals surviving chronic infection with the variant viruses show evidence of viral genome in cells in the white matter 6 weeks and longer after infection by in situ hybridization and show persistent chronic demyelination and remyelination. We are currently studying the state of the persistent viral genome as well as activation of host MHC and myelin associated genes in these persistently infected mice in an effort to understand the pathogenic mechanisms.

Neuropeptides and structural variants of neuropeptides are generated by diverse cells of the immune system, and affect proliferative and synthetic functions of T- and B-lymphocytes. Eosinophils generate substance P (SP) and VIP, identical to those in the neuroendocrine system. In contrast, other immune cells produce variants of neuropeptides, such as the VIP, which are derived from mast cells through alternative genetic mechanisms, and the VIP, which come from lymphocytes through post-translational peptidolysis. Some blood lymphocytes and cultured lines of S- and T-lymphocytes express 2 x 10^3-40 x 10^3 receptors for VIP with binding constants of 0.5-13 nM. The VIP variant binds with the same affinity as VIP, and VIP binds with lower affinity than VIP to lymphocyte, gastrointestinal cell and neural cell receptors. Both VIP and VIP increase chloride flux in monolayers of 184 human colonic carcinoma cells. In contrast, VIP, but not VIP, stimulates adenylate cyclase activity in membranes prepared from cultured lines of lymphocytes. The distinctive peptide structural requirements for VIP receptor binding and biochemical activation of cells in different systems thus determines the extent to which VIP and other neuropeptides serve as mediators in multisystem networks.
INTERACTIONS BETWEEN THE IMMUNE and CENTRAL NERVOUS SYSTEM: IMPLICATIONS FOR DISEASE, Jean E. Merrill, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024

While interactions between the central nervous system (CNS) and immune system have been documented over 100 years ago, the molecular biological, biochemical, and anatomical bases for these interactions have been elucidated only in the last decade. The function of shared receptors and their ligands in the CNS and immune systems can be examined by illustrating similarities and differences in the biological events in the two systems resulting from signal transduction or cell-cell interactions. As examples, interleukin 1 (IL1), tumor necrosis factor alpha (TNFα), prostaglandin E (PGE), and interleukin 2 (IL2) are immunomodulators which are found in normal brain and may play a role in disease states subsequent to increased production either by inflammatory cells or resident glial cells and an upregulation of their receptors. IL1, TNFα, and PGE may be critical in regulating neurophysiological and inflammatory events in the CNS of multiple sclerosis (MS) and AIDS patients. IL2 and IL2 receptors are prominent in brains of patients with a variety of neurological diseases including MS and Alzheimer's disease and have recently been detected in normal human, rat, and mouse brain white matter and grey matter. IL2 affects a variety of brain cell types, both glial and neuronal, and may have an endogenous counterpart in the CNS. The study of lymphokines, monokines, and their endogenous brain-derived analogues will elucidate their participation in the homoeostatic response of brain cells to injury and abnormalities resulting from chronic infections, tumors, and autoimmune disease of the CNS.

Biotherapeutic Targeting Strategies

BIFUNCTIONAL MONOCLONAL ANTIBODIES IN ACTIVATION OF LYMPHOCYTES FOR CYTOKINE PRODUCTION AND LYSIS OF HUMAN RENAL AND OVARIAN CANCER CELLS. Dr. R.L.H. Bolhuis, Dept. of Immunology, Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands; Dept. of Tumor Immunology, Radio-Biological Institute, TNO-Health Organization, Rijswijk, The Netherlands. Under physiological conditions T cells become activated when their T cell receptor (TCR) binds to the MHC/antigen complex on another cell resulting in multiple links between these structures and consequently in stable conjugates between the T cell and the target cell. For cytotoxic lymphocytes the result is delivery of a lethal hit to its conjugated target cell. The activation of cytotoxic lymphocytes can also be induced by monoclonal antibodies (mAbs) that recognize the CD3/TCR complex on a target cell surface structure. This can be accomplished by using bifunctional mAbs which recognize activation sites on the lymphocytes and target cell structures respectively. Therefore the use of bifunctional mAbs allow to "retarget" the specificity of CTL, thereby endowing them with a laboratory chosen immune specificity. The activation of T cells by mAbs via T cell activation sites also results in the production of cytokines. In addition to the TCR/CD3 complex activation signals can also be transduced via CD2 and CD16. Our recent data also demonstrate that the lymphocyte function associated antigen-1 (LFA-1) coactivates the TCR complex, and under physiological conditions the LFA-1/ICAM-1 interaction is a prerequisite which however can be circumvented by the use of bifunctional mAbs. Using bifunctional mAbs, which recognize TCR/CD3 and renal cell or ovarian cell carcinoma associated antigens, we have shown that this retargeting of CTL represents a powerful system for adoptive immunotherapy of these cancers. Experiments were performed to investigate the recycling capacity of bifunctional mAb retargeted (cloned) CTL. It appears that reactivation of the retargeted CTL requires the addition of "fresh" bifunctional mAbs.
Molecular Pathways of Cytokine Action

CD 019 LYMPHOKINE ENHANCED DRUG DELIVERY. Charles H. Evans, Laboratory of Biology, National Cancer Institute, Bethesda, Maryland 20892.

Cytokines have great potential for the development of new approaches to improve drug targeting and pharmacologic action. Leukoregulin is a lymphokine that selectively increases the permeability of many tumor cells. The kinetics of membrane destabilization and high degree of specificity for tumor cells suggest that leukoregulin treatment might be useful for increasing the intracellular entry of pharmacologically active molecules into leukoregulin-sensitive cells. The ability of leukoregulin to facilitate the uptake of tumor inhibitory drugs, therefore, was assessed by flow cytometry measurement of the entry of the fluorescent anthracycline doxorubicin and several other antitumor antibiotics into leukoregulin-treated human K562 erythroleukemia cells. K562 cells were exposed to 0.25-0.5 µg of the metabolic inhibitors/ml for up to 60 minutes at 37°C. Commencing within 15 minutes, leukoregulin increased the entry of doxorubicin approximately two-fold and the uptake of mitomycin, mithramycin, and propidium iodide two-fold to ten-fold. The P value for the 1.9-fold increase in uptake of doxorubicin in the presence of 1 unit of leukoregulin was <0.05 and <0.005 for the 2.3-fold increase produced by 4 units of leukoregulin. A leukoregulin concentration-dependent enhancement of doxorubicin uptake was also obtained in U937 histiocytic lymphoma and in RPMI 2650 squamous carcinoma cells. Neither interferon, tumor necrosis factor, IL-1, nor IL-2 alone or in combination influenced doxorubicin uptake. Leukoregulin enhancement of doxorubicin uptake was also specific for tumor cells in that no significant increase was observed in leukoregulin-treated, freshly isolated human mononuclear leukocytes. No significant increase in doxorubicin uptake was observed in leukoregulin-treated proliferating lymphocytes activated by 3-day exposure to mitogenic monoclonal antibody to the CD3 epitope or by culture for 7 days in the presence of interleukin-2. A synergistic decrease in proliferation was observed in K562 cells treated for 30 minutes with leukoregulin and doxorubicin in comparison to cells treated with either agent alone. This indicates that leukoregulin enhances both the cellular uptake and the tumor cell growth inhibitory action of doxorubicin. Identification of cytokines that increase the entry of pharmacologically active molecules into the desired target cells will greatly facilitate the pharmacotherapeutic goal of achieving sufficient intracellular uptake of the pharmacologically active agent to obtain the desired cellular action. The increased permeability of the plasma membrane and concurrent enhanced uptake of metabolic inhibitors into leukoregulin-treated target cells constitute a new approach in this direction.

CD 020 LEUKOREGULIN MODULATION OF TARGET CELL SENSITIVITY TO NK AND LAK CELL KILLING. Paulette M. Furbert-Harris and Charles H. Evans. Laboratory of Biology, National Cancer Institute, Bethesda, Maryland. 20892.

The ability of cytokines, e.g., interferon (IFN), interleukins (ILs) and colony stimulating growth factors (CSFs), to stimulate the proliferation and activity of leukocytes including natural killer (NK) and lymphokine activated killer (LAK) lymphocytes is well recognized. Cytokines also influence the net outcome of cytotoxic reactions by directly altering the sensitivity of target cells to the effector lymphocytes. Two cytokines possessing anti-thetical actions at the target cell level are leukoregulin and interferon. Leukoregulin, a 50 Kd cytokine up-regulates the sensitivity of tumor cells and human cervical epithelial cells - immortalized by human papilloma virus 16 DNA transfection - to NK and LAK lymphocyte cytotoxicity. Tumor cells, ranging in sensitivity from resistant to highly sensitive, become more sensitive after one hour pretreatment with 2.5 µ/ml leukoregulin in a 4hr 51Cr release assay. Moreover, leukoregulin renders the tumor cells two to six-fold more sensitive to LAK. This up-regulation does not occur when the cells are treated with other cytokines, e.g., rGM-CSF, rIL-1(88) rIFN , rTNFa or combinations of the latter two. In fact treatment with rIFN for 16-18 hr, or with GM-CSF and IL-1,6 for 1 hr makes the cells resistant to both NK and LAK lymphocyte cytotoxicity. Leukoregulin may be a significant regulatory factor in NK and LAK lymphocyte immunocytotoxicity. Its biotherapeutic role in the prevention and control of cervical dysplasia and neoplasia is being studied using the HPV16 DNA-immortalized cells developed as a model for studying the role of HPV16 in the development of cervical neoplasia and lymphokine modulation of cervical epithelial cell sensitivity to natural immunocytotoxicity.
Molecular Pathways of Cytokine Action

CD 021  NEW ADVANCES IN CANCER THERAPY WITH RADIOLABELED ANTIBODIES, David M. Goldenberg, Robert M. Sharkey, and Rosalyn D. Blumenthal. Center for Molecular Medicine and Immunology, UMDNJ, Newark, NJ 07103. Anticancer antibodies have been conjugated with gamma-emitting radionuclides for external scintigraphy (radioimmunodetection, or RAID), or with beta- or alpha-emitters, drugs, or toxins for antibody-guided therapy. A major limitation for therapy, however, is the very low uptake of antibody in tumor, usually less than 0.01% injected dose per g tumor (1). In order to compensate for the low tumor uptake of antibodies in therapy, first generation therapeutic immunoconjugates are more likely to be radioimmunoconjugates, because the energy of the isotope will kill bystander cells. However, the high blood levels of radioisotopes deliver high rad doses to the bone marrow, which is the major organ of toxicity. Nevertheless, radiosensitive tumors have responded to radioimmunotherapy (RAIT) (1,2). Another approach is to combine high-dose radioimmunoconjugates with methods to protect or rescue the marrow elements. Animal studies have shown that use of antibodies directed against the primary antibody (anti-antibodies) can reduce bone marrow toxicity (3). Also, certain cytokines, such as Interleukin-1, can prevent or reverse the leukocytotoxicity of radioimmunoconjugates, thus enabling the administration of higher body rad doses (4). (Supported in part by USPHS grant CA39841 from the NIH.)

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CD 022 MODULATION OF 5-FLUOROURACIL (5FU) EFFECTS ON TWO HUMAN COLON CANCER CELL LINES BY RECOMBINANT INTERFERONS (rIFN). Scott Wadler, Deborah Thompson, Peter H. Wiernik. Department of Oncology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY 10467. rIFN-alpha, -beta, and -gamma augment the cytotoxic effects of the fluorinated pyrimidine, 5FU, against HT-29 and SW480 cells in a dose- and schedule-dependent manner (1). Tests for drug interaction by median effects analysis demonstrated that the combination of 5FU and rIFNs exhibited synergy whether assumptions of mutual exclusivity or non-exclusivity of action were made. For rIFN-alpha and -beta, synergy was noted over nearly the entire range of drug concentrations tested; for rIFN-gamma synergy was noted only when Fa>0.8. To determine whether rIFN augments 5FU activity by effects on the 5FU target enzyme, thymidylate synthase (TS), TS levels were measured by a binding assay employing (6-3H)FdUMP in extracts from control and rIFN-treated cells. Levels of free enzyme were 19-fold higher in SW480 cell extracts than HT-29 extracts (10.5 v 0.6 fmol/mg protein). In the presence of rIFN-alpha, -beta, or -gamma, (6-3H)FdUMP binding to TS increased by 6- to 13-fold in SW480 cell extracts. There was no significant change in HT-29 cell extracts, however. To determine whether rIFN can stabilize the covalent ternary complex formed by TS, FdUMP, and N5, N10 methylene tetrahydrofolate, stability of the complex was determined by SDS-PAGE analysis of extracts from cells incubated with (3H)FdUrd chased with cold FdUrd for varying time periods. Pretreatment of SW480 cells with rIFN-alpha for 24 h did not alter the T1/2 of the complex. Conclusions: (1) rIFNs synergistically enhance the cytotoxicity of 5FU against two human colon cancer cell lines; (2) rIFNs enhance (6-3H)FdUMP binding to TS in SW480, and not HT-29, cells, but do not stabilize the complex.

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Differentiation

CD 024 CYTOKINES MEDIATE NEURAL CELL GROWTH AND DIFFERENTIATION,
Barbara Detrick, Charles H Evans, Gerald J Chader and John J Hooks, National
Eye Institute, National Cancer Institute, NIH, Bethesda, Maryland 20892.

Retinoblastoma, the most common primary intraocular tumor of childhood, is an
important model for exploring human malignancy and differentiation. Although the
histogenesis of this tumor remains controversial, current data support the concept that
retinoblastomas originate from neuroectodermal tissue of the retina and consist of
multipotent embryonic cells capable of differentiating into neuronal, glial and
epithelial-like components. Using analytical flow cytometry we have been able to
quantitate cellular proteins associated with the identification of selected cells growing
within the retinoblastoma population. The undifferentiated human retinoblastoma cell
line, Y-79, was used in this study. Cytokines, multifunctional proteins which modulate
many vital biological processes, were evaluated for their potential to alter
retinoblastoma growth and development. We found that the cytokine, Interferon
(IFN)-gamma, can selectively alter both MHC class I and II antigens as well as the
neuronal protein, S-antigen. Gene transcription techniques indicate that IFN-gamma is
acting at the level of post-transcriptional control to enhance S-antigen expression.

This system was also used to monitor and quantitate attachment cultures of
retinoblastoma cells differentiated by butyrate. Butyrate-poly-D-lysine treatment results
in enhanced expression of neuronal cellular markers while butyrate-laminin treatment
results in enhanced expression of retinal pigment epithelial cellular markers. Herein,
we describe that modifications in the developmental pathway of these cell types can be
achieved by co-joining such cytokines, as IFN-gamma, IFN-alpha, IFN-beta and
TNF-alpha, to butyrate. This study demonstrates that cytokines can influence the
expression of selected cellular proteins and it underscores the possibility that
cytokines may be potent modulating agents in cellular development.

CD 026 SIGNAL TRANSDUCTION IN MONOCYTIC DIFFERENTIATION, D. Kufe, R. Datta,
M. Mohri, T. Nakamura, M. Sherman, R. Stone, and H. Yamada,
Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Harvard
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Tumor necrosis factor (TNF) and 12-0-tetradecanoylphorbol-13-acetate (TPA)
duce monocytic differentiation of HL-60 cells and other human leukemic cell
lines. The cytoplasmic and nuclear signaling events induced by these agents
remain unclear. TPA activates protein kinase C, while TNF exposure is
associated with stimulation of phospholipase A2 and eicosanoid production.

Recent studies indicate that both TNF and TPA induce expression of the c-jun
protooncogene during monocytic differentiation of HL-60 cells. Run-on
analysis has indicated that c-jun expression is regulated in part by
transcriptional activation. The half-life of c-jun mRNA in induced HL-60
cells was approximately 30 min. Furthermore, the finding that this half-life
is significantly prolonged during exposure to cycloheximide has indicated
that c-jun expression is also regulated posttranscriptionally by a labile
protein. Previous studies have indicated that c-jun expression is regulated
by activation of protein kinase C. However, preliminary findings indicate
that the cyclooxygenase metabolite PGE2 acts as an inducer of c-jun
transcripts in HL-60 cells and that cAMP can, in part, regulate expression of
this gene. AP-1, the polypeptide product of c-jun, binds to specific DNA
sequences that stimulate transcription of genes responsive to certain growth
factors and phorbol esters. Expression of the TNF and macrophage-specific
colony stimulating factor (CSF-1, M-CSF) genes during monocytic
differentiation involves activation at the transcriptional level. These
genes have AP-1-like sites in their promoter/enhancer regions. Studies are
underway to determine the role, if any, of AP-1 in transducing nuclear
signals that regulate transcription of these genes during induction of
monocytic differentiation.
Molecular Pathways of Cytokine Action

CD 028 ACTION OF COLONY STIMULATING FACTORS ON TERMINAL DIFFERENTIATION OF MYELOMONOCYTIC CELLS, Giovanni Rovera, Brent Kreider and Neelham Shirshat, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

The events that occur after the interaction of granulocyte colony stimulating factor (G-CSF) with target cells were analyzed in the interleukin (IL)-3 dependent 32DC13(G) hemopoietic progenitor cells. These cells differentiate into granulocytes 12 days after G-CSF treatment and, at the molecular level, this phenomenon is associated with appearance of myeloperoxidase (MPO) mRNA at day 4, disappearance of cmyb mRNA at day 7, appearance of lactoferrin (LF) mRNA at day 8-10 and increases of beta actin and ras associated (rho) B mRNAs at day 7-10. G-CSF treatment also results in the activation of a number of early response genes including the rapid transient increase of both c-fos and c-jun mRNA between 30' and 1 hr of G-CSF exposure.

Following the early response gene activation and prior to the activation of late response genes the receptor for granulocyte-macrophagic (GM)-CSF becomes expressed on the surface of 32D cells. The expression of this new receptor is an irreversible phenomenon that provides the cells with an alternative option: to differentiate not only into granulocytes but also into macrophages upon addition of GM-CSF.

The orderly program of terminal differentiation in 32DC13(G) cells can be disrupted by the introduction of viral oncogenes into these cells. v-erb B and v-abl transformed 32D cells do not require growth factors for proliferation and they become unresponsive to G-CSF and unable to differentiate. In the case of v-abl transformed cells G-CSF still elicits the early response of c-fos activation, indicating that the v-abl oncogene acts downstream of the early events in interfering with the response to G-CSF. v-Ki-ras also interferes with the growth factor dependent proliferation and differentiation by interfering with the signal transduction pathway. Ki-ras 32D are blocked at the promyelocytic stage of myeloid differentiation and proliferate rather than differentiate in the presence of G-CSF or GM-CSF but atypically differentiate and stop proliferating in IL3.

Resistance to Bacterial and Parasitic Disease

CD 029 INTERFERON GAMMA-INDUCED ENHANCEMENT OF THE PHAGOCYTE RESPIRATORY BURST: DEFINING COMPONENTS OF THE PHAGOCYTE OXIDASE, Harry L. Malech, Thomas L. Leto, Karen J. Lomax, Stuart L. Abramson, and John I. Gallin, Bacterial Diseases Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Phagocytic cells contain a multicomponent, latent superoxide-generating NADPH oxidase which is assembled into an active complex at the cell membrane following cellular interaction with opsonized particles or certain soluble stimuli. Activated oxidase catalyzes synthesis of superoxide leading to generation of potent oxidants (respiratory burst). Chronic granulomatous diseases of childhood (CGD) results from defects in activation of the phagocyte respiratory burst, leading to recurrent infections and granuloma formation. Normal monocytes exhibit a physiologic loss of respiratory burst capacity in culture that can be restored by interferon-gamma (IFN-γ), suggesting that components of the oxidase system may be responsive to IFN-γ and IFN-γ might be useful in CGD. In vitro IFN-γ restored considerable oxidase activity to cultured monocytes from most autosomal recessive and some X-linked CGD patients (1). Similarly, IFN-γ administered subcutaneously enhanced the oxidase activity of neutrophils and monocytes from some CGD patients. IFN-γ associated increases in normal monocyte membrane cytochrome b558 (terminal electron donor of the NADPH oxidase complex) has been reported and such increases were also seen in neutrophils from some IFN-γ treated CGD patients. Since monocytes from CGD patients not deficient in cytochrome b558 were particularly IFN-γ responsive, this and other factors suggested that non-cytochrome components of the oxidase are also regulated by the oxidase. We have defined and cloned cDNA encoding two phagocyte oxidase factors (p47-phox and p67-phox) located in cytosol and missing from phagocytes of patients with either of two autosomal forms of CGD (2,3). The p47-phox is a basic protein of 390 amino acids containing a tandem repeat of a 51 amino acid motif in the middle of the molecule with similarity to the control region A of the src family of oncogenes. This motif is present in phospholipase C gamma, fodrin, and p21-ras GTPase activating protein. The p47-phox carboxyterminus is arginine and serine-rich, containing several potential phosphorylation sites, and phosphorylated p47-phox appears to be the 47 kD phosphoprotein associated with respiratory burst activation. The p67-phox is an acidic protein of 526 amino acids which shares with p47-phox a src control region A motif at its carboxy terminus. Using cDNA probes and recombinant protein antibodies to these oxidase components, further studies are in progress to determine the effects of IFN-γ on these cytosolic oxidase components.

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Molecular Pathways of Cytokine Action

**CD 030 THREE ANTIMICROBIAL SYSTEMS IN PHAGOCYTES: REGULATION BY CYTOKINES.**
C Nathan, D Campanelli, A Ding, M Fuortes, J Gabay, N S Kwon, S Srimal, and
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Phagocytes kill microbes either by depriving them of nutrients, such as tryptophan or iron, or by exposing them to toxic products. Three classes of phagocyte-derived molecules toxic to microbes have been identified: reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI), and antibiotic proteins (AP).

**ROI:** Capacity of macrophages to release ROI can be enhanced in vitro and in vivo, in mice and man, by IFNγ, and in vitro by TNFa, TNFβ, CSF-GM, and CSF-M. Activation of normal macrophages can be explained by a decrease in the Km for NADPH of the respiratory burst oxidase, without a change in its Vmax or content of its cytochrome b558 component (though IFNγ can increase cytochrome b558 in phagocytes with partial cytochrome b558 deficiency). ROI releasing capacity is suppressed by TGF-β1, TGF-β2, and macrophage deactivation factor (MDF). MDF increases the Km for NADPH of the respiratory burst oxidase. The mechanism of deactivation by TGF-β is unknown.

**RNI:** Capacity to release RNI is enhanced by IFNγ, LPS, IFNγ + LPS, IFNγ + TNFα/β, and IFNα/β + LPS. IFNγ activates RNI-producing capacity by inducing expression of the enzyme that oxidizes N0-L-arginine to nitric oxide (NO), and may also enhance cellular levels of tetrahydrobiopterin, a cofactor of the NO-producing enzyme. Induction of RNI release is suppressed by TGF-β’s and MDF. AP in PMN include: defensins, lysozyme, major basic protein (MBP), cathepsin G, elastase, bactericidal/permeability increasing protein (BPI), cationic antimicrobial protein of 31 kD, and two apparently novel proteins of 29 kD termed azurocidin and p29b. Mononuclear phagocytes in man make lysozyme but reportedly lack defensins, MBP, cathepsin G, PMN-type elastase, and BPI. The possible presence in human monocytes of a protein related to azurocidin, a broad spectrum antibiotic, is under study. Also under study is the impact of IFNγ therapy on levels of AP in PMN. Complex interactions among ROI, RNI and AP can be predicted but remain to be defined.

Administration of IFNγ has helped treat and/or prevent protozoan and bacterial infections in mice and man. It has not been established if enhanced production or action of ROI, RNI, or AP participate in these beneficial responses.

**CD 031 RESISTANCE TO MALARIA SPOROZOITES.**
V. Nussenzweig, Department of Pathology, New York University Medical Center, New York, NY 10016.

Malaria sporozoites are introduced into the host’s circulation by mosquito bite and within a few minutes they enter hepatocytes. There they multiply by schizogony and develop into the exoerythrocytic forms (EEFs). After a few days each EEF contains thousands of newly formed parasites (merozoites) which invade red cells. The EEF development is inhibited by γ-interferon and by tumor necrosis factor, raising the possibility that lymphokines, together with other effector mechanisms, mediate resistance to malaria in individuals living in endemic areas.

Antibodies to the repeat domain of the circumsporozoite (CS) protein inhibit entry of the parasite into liver cells. However, to achieve satisfactory protection, antibody titers must be high. We recently reported the identification of an epitope contained within amino acids 249-260 of the Plasmodium berghei CS protein, which is recognized by H-2Kd-restricted CD8+ cytotoxic cells (CTL). Passive transfer of CTL clones directed against this epitope conferred a high degree of protection against sporozoite challenge. The CTLs are most likely attacking the EEFs, either by destroying the infected hepatocytes, or by releasing inhibitory lymphokines. Either mechanism implies recognition of processed CS peptides in association with class I major histocompatibility complex molecules.

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**CD 032** THE IMPACT OF CYTOKINES ON PROTOZOAAL INFECTIONS IN VITRO AND IN VIVO, Steven G. Reed, Seattle Biomedical Research Institute, Seattle, WA 98109

Infections with intracellular protozoan parasites *Trypanosoma cruzi* and *Leishmania* are characterized by proliferation of the organisms within host macrophages. Here they are protected from the host antibody response, but are susceptible to killing by "activated" macrophages. Delivery of the appropriate activation signals to the infected host cells is an alternative approach to conventional chemotherapy which is toxic and often inefficient. A second aspect of infection with these parasites is an accompanying depression of immune responsiveness to parasite and/or non-parasite antigens. Thus the marked decrease in T cell responsiveness which occurs during infection provides further rationale for choosing an immunotherapeutic approach in these infections.

In vitro, the most effective cytokine for the activation of mouse macrophages to inhibit *T. cruzi* or *Leishmania* is IFN-γ. In vivo administration of IFN-γ was also shown to be effective in reducing parasite numbers in infected mice. In mice with visceral leishmaniasis, liposome encapsulation of the IFN-γ was necessary for effectiveness. The most dramatic effects were seen in mice infected with a uniformly fatal inoculum of 10^6 parasites. When these animals received repeated injections of IFN-γ during the course of infection, 100% of the mice survived. Furthermore, treated mice did not have depressed immune responsiveness. Several other cytokines mediated some degree of intracellular killing of these parasites, particularly in human monocytes and monocyte-derived macrophages. This included GM-CSF, CSF-1, and, to a lesser extent, IL-3. In some cases, combinations of IFN-γ and a CSF had additive effects for parasite killing. The inhibitory effect of IFN-γ was found to be partially blocked when the macrophages were pre-incubated with TGF-β. Pre-incubation of human macrophages with TGF-β also significantly reduced their respiratory burst activity. The induction of parasite killing by IFN-γ also occurred in HIV-infected macrophages.

Other studies have focused on therapy of depressed immune responses in mice with parasitic infections. In vitro, the inability of spleen cells to respond to non-parasite antigens by producing antibody is a dramatic aspect of *T. cruzi* infection which begins during the acute phase and persists through the chronic phase of infection. Although the splenic B cells are capable of antibody production, the necessary T cell signals appear to be absent or blocked. Cytokine therapy is very effective in restoring depressed antibody production in infected mice. Most effective are GM-CSF, IL-1, and IL-2, all of which function both in vitro and in vivo. Anti-IL4 antibody (1D1) was also effective in restoring normal antibody production in vitro. Mechanisms relating to cytokine action in this system of depressed immune responsiveness will be discussed.

**DNA, RNA, HIV and Other Retrovirus Expression**

**CD 033** PHYSIOLOGIC INFLUENCES OF HUMAN RETROVIRAL EXPRESSION, V. L. Perez, S. Justement*, T. S. Butera, C. June**, and T. M. Folks, Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, Georgia 30333, *Lab Immunoregulation, NIAID, NIH, Bethesda, Maryland 20892, **Naval Medical Research Institute, Bethesda, Maryland 20892.

Regulation of viral expression is a major focus of research which has currently gained great momentum. Understanding the role contributed by cellular communication factors, such as cytokines, on this regulation of viruses in general has just begun. Using a T-cell model system, we have derived a cytokine inducible HIV producing clone. Because of the nature of the clone, cytokine induction of HIV can be abrogated following cell membrane ligand cross-linking with monoclonal antibodies directed at the T-Cell receptor (CD3).

Decreases in both supernatant p24 and total cellular HIV protein by Western Blot were observed after the addition of cytokine and then followed by anti-CD3 treatment. Cytokine induction of viral mRNA remained the same in the presence or absence of the anti-CD3 treatment. IL-2 secretion was increased in both the HIV-infected clone and the uninfected parental T-cell line following stimulation by anti-CD3 antibodies while such anti-CD3 treatment failed to mobilize Ca^2+ in the infected cell as compared to the uninfected control. This cell represents a dual regulatory pathway model to study both positive and negative influences on HIV expression.
Molecular Pathways of Cytokine Action

CD 034  THE ANTI-VIRAL ACTIVITIES OF TNF: ADENOVIRUS STRIKES BACK  Linda R. Gooding and William W.S.M. Wold  Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322 and Institute for Molecular Virology, St. Louis University Medical Center, St. Louis, MO 63110. When adenovirus infects human or rodent cells in vitro, expression of products of the viral E1A region, which contains the functions required by the virus for the earliest phases of its replicative process, alters many cells in such a way that they become susceptible to the lytic effects of TNF. Adenovirus encodes at least two, and possibly three, other proteins elsewhere in its genome that function to prevent TNF-mediated destruction of virus-infected cells. The functioning of these proteins is highly dependent on the infected cell. Thus, most mouse fibroblasts become susceptible to TNF when infected with adenoviruses that delete only a single gene in the E3 region that specifies a 14.7kD protein (E3-14.7K). Infection of these cells with viruses that produce E3-14.7K prevents TNF-mediated lysis. In contrast, infection of many human fibroblasts (HEL299, for example) or cultured human tumor cells with viruses that delete only E3-14.7K does not produce the TNF-sensitive phenotype despite the fact that E1A induces TNF sensitivity in these cells. In addition, these cells are actively protected from TNF lysis when susceptibility is induced by other means, such as inhibition of protein synthesis. Using double deletion mutants we have mapped a second gene involved in prevention of TNF lysis to the E1B 21kD protein. Although E3-14.7K appears to protect the vast majority of cells from TNF, E1B-21K is only effective in a subpopulation of cells. We have recently identified but have not precisely mapped a third gene, within the E3 region, that prevents TNF lysis of yet another distinct subpopulation of cell lines. The two identified genes, E3-14.7K and E1B-21K, appear to act by completely different mechanisms in preventing TNF lysis.

CD 035  CYTOKINE REGULATION OF HIV EXPRESSION IN CHRONICALLY INFECTED CELLS. Guido Poli and Anthony S. Fauci, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. The immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), frequently establishes in CD4+ cells of the immune system a state of chronic infection which is characterized by low levels of virus expression. In order to investigate the physiologic mechanisms that convert a latent to a productive infection we have established a panel of chronically infected cell lines of both T lymphocytic and monocytic origin, reflecting the two immune cell targets of HIV infection. Using these systems, we have demonstrated that certain cytokines can upregulate the expression of HIV. In particular, we first observed that tumor necrosis factor alpha (TNF-α) was capable of inducing HIV expression in both chronically infected T lymphocytic (ACH-2) cells and promonocytic (U1) cells. In addition, colony stimulating factor such as GM-CSF selectively upregulated virus expression in U1, but not in ACH-2 cells. More recently we have identified an autocrine mechanism of HIV upregulation which is mediated by the synthesis and release of endogenous TNF-α, which contributes both to the constitutive and to the inducible expression of virus in these lines. At the molecular level, we and others have identified the cellular transcription factor NF-kB as the mediator of TNF-α induced effects on virus expression. Furthermore, we have also correlated the viral inductive activity of certain cytokines, such as TNF-α, with the activation of a protein kinase C dependent pathway of second cellular messengers. Another pleiotropic cytokine, interleukin-6 (IL-6), similar to GM-CSF, was capable of inducing HIV expression in monocytic cells, but not in the T lymphocytic line ACH-2. Of interest, in promonocytic cells, TNF-α, GM-CSF and IL-6 synergized in the induction of viral expression. The molecular basis of these synergistic effects is currently under investigation. Preliminary results suggest that different cytokines can affect different steps of viral replication or expression. In fact, in contrast with TNF-α, stimulation of infected cells with IL-6 results in a significant induction of HIV expression measurable at the protein/virion level, which is not associated with a significant transcriptional activation of HIV. In conclusion, our in vitro studies support the concept that HIV has evolved to infect and persist in the CD4+ immune cells where virus expression is modulated by the cytokine network which normally regulates the homeostasis of the immune system.
Molecular Pathways of Cytokine Action

Aging and the Immune System

CD 036 ACTIVATION DEFECTS IN T LYMPHOCYTES FROM OLD MICE, Richard A. Miller, Boston University School of Medicine, Boston, MA 02118.

We have carried out a series of studies that ask why T lymphocytes from old mice, like those from old humans, respond poorly to mitogenic lectins and to antibodies specific for components of the T cell receptor complex. Our results show:

a) that many T cells from old mice fail to produce an increase in cytoplasmic free calcium concentration after stimulation with either Concanavalin A or anti-CD3 antibody;

b) that the inability of most T cells from old donors to generate this calcium signal largely accounts for the decline, with age, in tests of helper, cytotoxic, and proliferative T cell function;

c) that the defect in calcium signal generation is not caused by defects in the production of inositol phosphate second messengers;

d) that T cells from old mice are relatively resistant to increases in [Ca^{2+}] even when these are caused by agents that bypass the signal transduction pathways (e.g. ionomycin challenge); and

e) that the defective T cells that accumulate in old mice express high levels of the Pgp-1 surface antigen, a marker for memory cells.

These findings have led to a model for senescent decline in T cell function, in which thymic involution leads to a decrease in production of virgin T cells, leading to the relative enrichment of memory T cells that resist changes in intracellular Ca^{2+} levels, possibly by a change in the plasma membrane calcium extrusion pump.

CD 037 DEFECTS IN LYMPHOKINE EXPRESSION IN AGING HUMANS, James E. Nagel, Rajesh K. Chopra and William H. Adler, Clinical Immunology Section, Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224.

The age-associated decline of the immune response is well documented, but the cellular and molecular basis of this decline is not well characterized. While animal models have suggested age-associated alterations in the synthesis of the lymphokines interleukin-2 (IL2), interleukin-3 (IL3), interleukin-4 (IL4), and interleukin-6 (IL6), data in the human is less clear. Decreased IL2 production and expression of the signal transducing high affinity interleukin-2 receptor (HA-IL2R) by mononuclear cells from aged individuals activated with cell membrane dependent mitogens such as phytohemagglutinin (PHA) or concanavalin A is decreased, as is the amount of specific IL2 and p55 IL2R mRNA. While p70 IL2R is an essential and limiting component of the HA-IL2R, its mRNA expression does not undergo a significant age-related decline. Conversely, T lymphocytes activated with agents that do not require binding to the cell membrane, such as the combination of the protein kinase C activator phorbol myristate acetate (PMA) and the calcium ionophore A23187, demonstrate no age-related differences in either IL2 or p55 IL2R specific mRNA accumulation. Yet these T cells, while producing up to 100 times more IL2 than PHA stimulated cells, proliferate less than comparably treated cells from young individuals. This decreased proliferation, as quantified by [3H]TdR incorporation into cellular DNA, appears confined to an as yet uncharacterized subset of T cells that either die during culture or do not respond to mitogen or antigen stimulation. This results in fewer IL2 synthesizing and IL2 responding T cells. While present data indicates that the specific genes for IL2 and p55 and p70 IL2R are not constitutionally defective in the elderly, there is not sufficient information to account for either the decreased proliferation observed in T cells from both aged animals or elderly humans or for the distinctive effects of different activating agents on T cell mRNA expression. Despite demonstrating important interactions with IL2 and in some cases age-related changes in animal models, the production and utilization of other important lymphokines such as IL3, IL4, and IL6 have not been systematically examined at the molecular level in humans.
EFFECT OF AGING ON LYMPHOKINES. LYMPHOKINE RECEPTORS AND OTHER EARLY ACTIVATION ANTIGENS. David N. Ernst, Monte V. Hobbs and William O. Weigle, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Splenocytes from aged mice synthesized less DNA than young counterparts following stimulation in culture with antibodies against the T cell receptor/CD3 complex. This difference was not due to gross changes in anti-CD3 dose optima, response kinetics, accessory cell function, numbers of T cells cultured, CD4\(^+\);CD8\(^+\) cell ratios or levels of CD3\(\varepsilon\) membrane molecules. Studies using two-color immunofluorescence (IF) staining and multiparameter flow cytometry (FCF) revealed that smaller proportions of both CD4\(^+\) and CD8\(^+\) splenic T cells from aged versus young mice were stimulated to enter and progress through the cell cycle. This was reflected by their lower expression of pre-S phase, early activation molecules including IL-2, membrane RL388 Ag, and receptors for IL-2 and transferrin. Importantly, cells that did respond to stimulation were found to express the full range of cell-associated, early activation characteristics regardless of donor age. Multicolor IF and FCF analyses of CD4\(^+\) cells have revealed substantial phenotypic differences between CD4\(^+\) subpopulations obtained from young or aged mice. A larger proportion of CD4\(^+\) T cells from aged donor spleen was found to express high levels of membrane Pgp-1 (CD44) molecules, but lower levels of CD45RB and 3G11 molecules when compared to young splenic CD4\(^+\) cells. The phenotypic pattern of splenic CD4\(^+\) cells from aged mice was consistent with the pattern that defines Th2 cells in young mice. Consistent with this finding, splenic CD4\(^+\) cells from aged mice were found to produce significantly greater levels of IL-4 and lower levels of IL-2 upon primary stimulation with anti-CD3 in culture than young counterparts. These latter results indicate that analysis of age-related changes in immunocompetence should take into account shifts in the proportion of phenotypically and functionally distinct subsets within the major CD4\(^+\) (and CD8\(^+\)) T cell subpopulations.
Late Addition

CD 039  RETROVIRUS MEDIATED TRANSFER OF THE IL-3 GENE INTO HUMAN HEMATOPOIETIC CELLS. A.A. Fauser, P. Laneuville, R. Tellier. Med. Klinik, Department of Hematology, Albert-Ludwigs-Universität Freiburg, FRG, and Division of Hematology, Royal Victoria Hospital, McGill University, Montreal, Canada.

The ability to transfer new genetic information into hematopoietic cells provides a new and promising approach to address questions concerning stem cell commitment and proliferation. It should be possible, for instance, to introduce developmentally regulating genes, oncogenes and genes encoding for growth factors into various hematopoietic cells, thereby modulating the system in a precise way. In addition, certain human genetic defects may be corrected by the insertion of a functional gene into primitive bone marrow cells of the hematopoietic system. Growth factors are soluble polypeptides that interact with cellular receptors controlling cell proliferation and differentiation. A number of oncogenes have been found to encode for growth factors or for the receptor of such a growth factor. Some receptors exhibit tyrosine-kinase activity, which might lead to an overstimulation of cells due to increased autophosphorylation. Unregulated growth by the autocrine production of growth factors has been proposed as a mechanism of tumorgenesis. Colony stimulating factors (CSFs) are known to be essential for the survival, proliferation and differentiation of hematopoietic cells "in vitro". The exact physiological role still remains unclear but seems, for example, to include a proliferative response to an infection challenge. Autocrine loops involving CSFs in leukemia have generated an immense interest, in particular for GM-CSF and Interleukin-3 (IL-3). Retroviral mediated gene transfer of IL-3 into normal murine cells resulted in myeloid proliferation "in vitro" under serum free conditions, and demonstrated "in vivo" an elevated white cell count with normal differentiating myeloid cells, a hypercellular bone marrow, and infiltration of the liver and the spleen. Exploiting the CSF-activity of a gibbon lymphosarcoma cell-line a gibbon-IL-3 and its human homolog could be cloned. The function of the gibbon-IL-3 is similar to that of human IL-3 with a significant homology between the two genes. Studies of the leukemogenic potential with regard to the autocrine production of human IL-3 have to be evaluated, since experiments in the murine system indicated that IL-3 alone is able to induce abnormal proliferation of mature myeloid cells, and tumorigenicty can be obtained in conjunction with other transforming events. The current working hypothesis is that in the M07E cell line, internal stimulation of receptors is qualitatively different than when stimulated on the cell surface. Suggesting that another IL-3 receptor associated component normally present at the cell surface is not operational internally. This data demonstrate that autocrine stimulation might result in autonomous growth of the human cell line M07E. The results also suggest that direct internal stimulation may also occur as has been demonstrated in the murine system.
Molecular Pathways of Cytokine Action

Cytokines in Control of DNA Synthesis, Cell Proliferation and Oncogenesis

CD 100 EFFECTS OF LIPOXYGENASE (LO), CYCLOOXYGENASE (CO) AND MIXED LO/CO INHIBITORS ON INTERLEUKIN-1 (IL-1) ACTIVITY AND BIOSYNTHESIS, Laurel M. Adams, William L. Baeder, Lisa J. Yonno, Joseph Y. Chang, Wyeth-Ayerst Research, Princeton, NJ 08543

Recent investigations have provided evidence that lipoxigenase and cyclooxygenase products may play an important role in immunoregulatory processes. In particular, leukotrienes have been shown to augment, while prostaglandins have been shown to inhibit IL-1 biosynthesis. Likewise, lipoxigenase inhibitors have been shown to inhibit and cyclooxygenase inhibitors reported to augment IL-1 biosynthesis. Further studies have suggested an obligatory role for lipoxigenase products in the functional activities of interleukin-1 or interleukin-2. Two difficulties in interpretation of these data are the use of a bioassay (known to be down-regulated by PGF2α) to measure interleukin-1 and the lack of a highly selective LO inhibitor (i.e., one having little or no CO inhibitory activity). The purpose of the present studies, therefore, was to determine whether a selective LO inhibitor would inhibit interleukin-1 biosynthesis, as measured in a receptor assay for IL-1 activity. Compounds tested included the CO inhibitor, indomethacin, the mixed LO/CO inhibitor, tenidap, and the selective 5-LO inhibitor, WY-50,295. When culture supernatants from murine peritoneal macrophages were assayed for IL-1 activity in a receptor assay on BALB/c 3T3 fibroblasts, indomethacin did not potentiate and WY-50,295 did not inhibit IL-1 biosynthesis at concentrations 10-30x greater than their CO or LO inhibitory concentrations. Tenidap had no effect at its CO inhibitory concentration but inhibited IL-1 biosynthesis by 50% at 30 μM, five times its LO inhibitory concentration. Neither WY-50,295 nor tenidap enhanced or inhibited mitogen-induced splenocyte proliferation or IL-1- or IL-2- induced thymocyte proliferation at concentrations up to 30 μM. We conclude that at the LO or CO inhibitory concentrations for these compounds there is no direct effect on either the biosynthesis or activity of IL-1.

CD 101 IL-1 AND TNF INDUCED INCREASE IN GRO MESSAGE IN HUMAN FORESKIN FIBROBLASTS IS MEDIATED BY AN NF-KB BINDING SITE IN THE GRO ENHANCER, Anthony Anisowicz, Mark Messineo, Sam Lee and Ruth Sager, Division of Cancer Genetics, Dana-Farber Cancer Institute, Boston, MA 02115

IL-1 and TNF induce a rapid and pronounced increase in the level of GRO message in human fibroblasts whether growing or serum-starved confluent cells are induced. The growth status independent inducibility of GRO by TNF and IL-1 is similar to the inducibility of IL-6 and in contrast to the growth status dependent inducibility of fos. This suggests that GRO may play a role in the inflammatory response to TNF and IL-1 as well as the growth response. To better understand the control of GRO expression by cytokines, we have isolated and sequenced a 3 kb of the GRO gene 5’ region. We found an NF-KB binding site GGGAATTTCC in the GRO enhancer at position -74 to -65. CAT constructs driven by the GRO enhancer are inducible by IL-1 and TNF. Competition studies with oligonucleotides representing wild type and mutant NF-KB sites are in progress.

CD 102 GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) AND DIMETHYL SULFOXIDE (DMSO) INDUCE EARLY CHANGES IN HL-60 CELL PHOSPHOPROTEINS WHICH PRECEDE EFFECTS ON CELL CYCLE PROGRESSION, J.K. Brennan, J. Amos, K.S. Lee, and M. Frazel, University of Rochester School of Medicine, Rochester, NY 14642.

High and low passage HL-60 populations containing at least 90% G1 cells were obtained by centrifugal elutriation, exposed to 100 u recombinant human GM-CSF and/or 0-1.25% DMSO and phosphoprotein changes quantified on autoradiograms of [32P]-orthophosphate-labeled cell proteins separated by giant 2-D gel electrophoresis. Results were correlated with intracellular pH, determined by measurement of BCECF fluorescence; [32P]-orthophosphate uptake; and cell cycle progression, determined by flow quantitation of DNA content, cell volumes, and cell concentrations. GM-CSF stimulated, and DMSO inhibited the phosphorylation of two proteins (460 kDa, p.i. 5.6; 50 kDa, p.i. 5.1) within one minute of exposure. These changes were sustained for at least four hours, were not associated with changes in intracellular pH and preceded similar antagonistic effects on phosphate uptake (15-30 min), cell volume change (18-20 hr), and cell concentration increase (28-32 hr). GM-CSF accelerated, DMSO inhibited G1 to S transit with the most marked antagonism observed in the second cycle following synchronization (28 to 40 hrs). Cell maturation (morphology, NBT reduction) was dominated by DMSO and not antagonized by GM-CSF. These results suggest that GM-CSF and DMSO exert antagonistic effects on growth through a common mitogenic pathway not activated by change in intracellular pH; that protein phosphorylation is an important early event, and that the major locus of cytokinetic effect is on G1 to S transit.
TNF competed with the binding growth inhibitory effects. The relative ability of TNF and lymphotoxin [LT] to inhibit the growth of five human tumor cell lines was examined both in the presence and absence of IFN-γ. Two adenocarcinoma lines, HT-29 and SK-CO-1, were 20- to 320-fold more sensitive to the inhibitory effects of TNF than LT in 3- to 4-day proliferation assays. In contrast, the breast carcinoma line BT-20 showed only a one- to twofold difference. The MCF-7 and ME-180 cell lines exhibited intermediate behavior. These results parallel the reported disparate potencies of TNF and LT in their effects on endothelial cells, hematopoietic development and their abilities to sustain a mixed lymphocyte response. Radioiodinated TNF binding studies showed two classes of receptors (Kd 0.04 to 0.15 nM and 0.2 to 1.0 nM) on the highly sensitive SK-CO-1 line. HT-29 cells also appeared to possess some high affinity-binding sites, whereas the BT-20 line completely lacked the high affinity form. Thus the presence of high affinity-binding sites correlated with increased sensitivity to the antiproliferative effects of TNF. Cold TNF competed with the binding of radioiodlated human TNF three- to fivefold better than LT for binding to all three lines. These relatively small differences between the TNF and LT receptor-binding characteristics are insufficient to explain the dramatic disparity in their antiproliferative properties. Thus, the receptor binding data conflict with the growth inhibitory effects.

Cold TNF or lymphotoxin (LT) in three- to fivefold better than LT for binding to all three lines. These relatively small differences between the TNF and LT receptor-binding characteristics are insufficient to explain the dramatic disparity in their antiproliferative properties. Thus, the receptor binding data conflict with the growth inhibitory effects.

**CD 104 INTERLEUKIN-6 CAN PRIME THP-1 MONOCYTIC LEUKEMIA CELLS FOR ENHANCED PRODUCTION OF TUMOR NECROSIS FACTOR α IN RESPONSE TO LPS.** F.R. Cochran and M.B. Finch-Arletta, Department of Allergy and Inflammation, Hoffmann-La Roche, Nutley, NJ 07110. Although interferon-γ has been shown to effectively prime macrophages to secrete tumor necrosis factor α (TNFα) in response to trigger stimuli such as LPS, it is reasonable to assume that other cytokines present in the extracellular environment may cooperate with bacterial products to activate cytokine production by macrophages. Interleukin 6 (IL-6), for example, has been detected in rheumatoid synovial effusions and is produced by synovial cells and tissues. Therefore, the purpose of the present study was to examine whether IL-6 may serve as a priming stimulus for macrophage activation as assessed by TNFα production. THP-1 monocytes were differentiated by treatment with phorbol ester (TPA) for 30 minutes, then “primed” for 18 hr in the presence of 0.1 - 10 ng/ml IL-6, followed by overnight incubation with 200 ng/ml Sal. minnesota LPS to “trigger” TNFα secretion. TNFα contained in cell supernatants was quantitated by specific ELISA. Although IL-6 alone was observed to be a weak stimulus for TNFα secretion, IL-6 demonstrated a marked and dose-dependent potentiation of TNFα production when added prior to LPS. The priming effect of IL-6 could be reversed by the addition of a neutralizing antibody against IL-6. Taken together, these data demonstrate that IL-6 can prime macrophages for enhanced TNFα release when exposed to a sufficient trigger signal, such as LPS. Although IL-6 exerts diverse regulatory effects within and outside of the immune system, this is the first report to describe the macrophage-activating properties of this cytokine.

**CD 105 TUMOR NECROSIS FACTOR (TNF) STIMULATES PHOSPHORYLATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) ON TYROSINE RESIDUES IN A431 CELLS.** Nicholas J. Donato, Michael G. Rosenblum, Department of Clinical Immunology, M.D. Anderson Cancer Center, Houston, TX 77030. Previous studies of TNF action on tumor cells revealed a role for tyrosine phosphorylation of EGF-R in the growth modulatory actions of this cytokine. (Donato, et al. J Biol Chem, in press). Immunoprecipitation of EGF-R from 32P-equilibrated A431 cells demonstrated that TNF treatment (10 nM) resulted in a time-dependent stimulation of EGF-R phosphorylation which was maximal (3-fold) after 10-20 min of TNF exposure. Equal concentrations of EGF resulted in a similar stimulation of EGF-R phosphorylation and phosphoamino acid analysis revealed that both factors resulted in increased phosphorylation on tyrosine residues. Tryptic phosphopeptides of EGF-R isolated from A431 cells treated with TNF, EGF or phorbol ester demonstrated that TNF induced phosphorylation of EGF-R which was quite distinct when compared to the actions of phorbol ester but was similar to the effects of EGF on receptor phosphorylation. However, TNF stimulated phosphorylation of tyrosine residues on a distinct phosphopeptide when compared to the tyrosine phosphopeptides resolved from EGF-treated cells. Unlike EGF, TNF was unable to directly stimulate EGF-R tyrosine kinase activity in membranes prepared from A431 cells. Our results suggest that TNF stimulates phosphorylation of EGF-R in a manner distinct from that of both phorbol ester and EGF. Future studies of the differential phosphorylation of EGF-R by TNF may aid in the understanding of the complex process of the molecular mode of TNF action.
Molecular Pathways of Cytokine Action

CD 106 THE EFFECTS OF BIOLGIC RESPONSE MODIFIERS ON C-SRC KINASE ACTIVITY IN HUMAN COLORECTAL CARCINOMA CELL LINES, Gary E. Gallick, R. Lotan, D. Lotan, H. Sayay, R. Garcia and C. Smith. Depts. of Tumor Biology and Neuro-oncology, M.D. Anderson Cancer Center.

Modulation of the tyrosine kinase activity of pp60c-src has been implicated in several cellular processes. In human colorectal carcinomas the kinase activity of pp60c-src is greatly elevated with respect to normal colonic epithelial cells, leading to the hypothesis that increased activity may be associated with loss of growth control. We have studied the relationship of kinase activity to growth control by analyzing the effects of several biologic response modifiers, including tumor necrosis factor alpha (TNF), retinoic acid (RA), and the antibiotic herbimycin A (herb A) on the growth and soft-agar colony formation of colon cells. In cells growth-inhibited in monolayer by TNF, c-src kinase activity was decreased by greater than 19 fold within 10min; in cells resistant to TNF, no effect on pp60c-src kinase was observed. RA exerted different growth-control effects, decreasing pp60c-src kinase activity in some cell lines in the absence of growth-inhibition in monolayer. However, in these cell lines, a decrease in soft agar colony formation was observed. While TNF and retinoic acid affect pp60c-src kinase activity indirectly, herb A specifically inhibits tyrosine kinases, and directly inhibits pp60c-src kinase in vitro. We have found that herbimycin A inhibits every colon carcinoma cell line thus far examined, and rapidly reduces c-src kinase activity, but not EGF receptor kinase activity. These studies suggest that specific modulation of the tyrosine kinase activity of pp60c-src is likely to directly affect growth properties of colon tumor cells.

CD 107 ONTOGENY OF INTERLEUKIN-1-LIKE FACTORS IN NORMAL RAT TISSUES. Tina Granholm and Olof Soder, Pediatric Endocrinology Unit, Karolinska Hospital, S-104 01 Stockholm, and Department of Pediatric Surgery, S:t Gøran's Children's Hospital, Stockholm, Sweden.

Introduction: Interleukin-1 (IL-1) was originally described as a potent inflammatory mediator secreted by activated macrophages. IL-1 has also been demonstrated in the skin, and we have recently detected IL-1-like factor(s) in the testis, esophagus, stomach and tongue of adult rats. In the present project we have studied the ontogeny of IL-1-like factors in the rat.

Methods: Tissues from Sprague-Dawley rats were collected at fetal day 18, and day 7, 17, 30 and 42 postnatally. IL-1 activity of aqueous tissue extracts was measured in a murine lymphocyte proliferation assay.

Results and conclusions: Fetal esophagus, stomach, tongue and skin were devoid of IL-1 bioactivity, whereas the liver showed high activity. 7 days after birth all studied tissues were positive. The activity increased during postnatal development, and reached plateau levels in adult animals, except in the liver, where the activity decreased. Fetal liver is active in hematopoiesis, during which IL-1 has been shown to act as a growth factor. Our findings of IL-1-like factors in other tissues is as yet unexplained, but since these tissues have a high proliferation rate it might be suggested that they function as growth factors.

CD 108 EXPRESSION OF INTERLEUKIN-6 GENE IN PRIMARY CENTRAL NERVOUS SYSTEM TUMORS AND POSSIBLE ROLE AS AN AUTOCRINE GROWTH FACTOR, William N. Harrington, Roberta L. Hayes, Section of Neuropathology, Yale University School of Medicine, New Haven, CT 06510 and Department of Neurosurgery, NYU School of Medicine, New York, NY 10016.

Expression of the IL-6 gene was studied in 12 cases of primary human CNS neoplasia by RNA dot blot hybridization. Transcription of the IL-6 gene was detected in RNA isolated from fresh tumor tissue in one case of meningioma, 1 of 2 cases of primary neuroectodermal tumor, and 2 of 4 cases of high grade glioma. In addition, one case of low grade and 2 of 4 cases of high grade glioma in tissue culture expressed IL-6. In order to establish the influence of IL-6 upon the proliferation of primary CNS neoplasms, the same five cultured tumors were challenged with increasing concentrations of recombinant human IL-6 (rIL-6) up to 100U/ml and ^3H-thymidine incorporation was measured at various intervals up to 7 days. Results indicate that only the cultured cells that did not express the IL-6 gene were stimulated by exogenous rIL-6. If the tumors transcribing the IL-6 gene were maximally stimulated by endogenous production of this cytokine, these results are consistent with IL-6 serving as an autocrine growth factor for some primary CNS neoplasms and suggests that not all gliomas are transformed by the same mechanism.
Molecular Pathways of Cytokine Action

**CD 109** INVOLVEMENT OF NO AND SUBSEQUENT IRON-NITROSYL FORMATION IN CELLULAR DYSFUNCTION INDUCED BY CYTOTOXIC ACTIVATED MACROPHAGES (CAM’S) AND BY TUMOR NECROSIS FACTOR (TNF), Jack R. Lancaster, Jr.*, Michael A. Sweetland*, Amy Clark* and J.B. Hibbs, Jr.*, * Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300, and † Division of Infectious Diseases, University of Utah Medical Center, Salt Lake City, Utah 84148. It has been shown previously that some mammalian cells, including CAM’s, produce nitric oxide (NO) from L-arginine and that N-monomethylarginine (NMMA) is an inhibitor of NO production by this pathway. Previous studies have also shown that CAM’s induce loss of intracellular iron and iron-containing enzyme function in target cells. We show here the production of a signal detected by electron paramagnetic resonance (EPR) spectroscopy in CAM’s attributable to iron-nitrosyl (Fe-NO) complex formation. The production of this signal is enhanced by L-arginine addition and prevented by NMMA. Hyperfine splitting differences are observed in the iron-nitrosyl signal when 15N is substituted for 14N in the guanidino group of L-arginine. These data show conclusively that NO arises from the guanidino group of L-arginine and that this NO forms a tight complex with intracellular iron. We also have previously shown that TNF alone causes loss of target cell mitochondrial electron transfer. EPR examination of mouse fibroblast C3HA cells reveals the presence in these cells of the iron-nitrosyl signal when they are killed by TNF plus cycloheximide. These data suggest that iron-nitrosyl complex formation via the L-arginine-dependent pathway and consequent loss of iron-containing enzyme function plays an important part in cell killing by immune effectors.

**CD 110** THE EFFECT OF GLUTATHIONE ON THE BINDING AND INTERNALIZATION OF INTERLEUKIN-4, Shu-Mei Liang, Nancy Lee, David Finbloom and Chi-Ming Liang, Division of cytokine Biology and the Division of Blood and Blood Products, Food and Drug Administration, Bethesda, MD 20892. We have shown recently that glutathione (GSH) regulates the activity of interleukin-2 (IL-2) on murine cytotoxic cells such as CT-4R (J. Biol. Chem. 264, 13519). Since CT-4R cells are also highly responsive to interleukin-4 (IL-4), we examined whether GSH regulates the cellular actions of IL-4. Thymidine incorporation assay showed that incubation of CT-4R cells with GSH (0.64-3.2 mM) for 72 h enhanced the proliferative effect of IL-4. This effect of GSH was concentration dependent and down modulated by L-buthionine-(S,R)-sulfoximine, an inhibitor of de novo GSH synthesis. Receptor binding studies showed that GSH treatment caused a 2-fold increase in the amount of radiolabelled IL-4 bound to the CT-4R cells. In addition, GSH increased the percentage of bound IL-4 that was internalized into the cells. These results suggest that GSH treatment increases the level of intracellular GSH and potentiates the binding and internalization of IL-4 which may in turn affect the growth and proliferation of the cells.

**CD 111** CYTOKINE EFFECTS IN A MACROPHAGE-MEDIATED ANTIBODY DEPENDENT ANTI-TUMOR CYTOTOXICITY SYSTEM, Jane L. Liesveld, Karen M. Frediani, Reggie E. Duerst, Jill Winslow, Camille N. Abhoud, Departments of Medicine and Pediatrics, University of Rochester Medical Center, Rochester, NY 14642. The monocyte-macrophage system is thought to contribute to host anti-tumor immunological defense. To investigate the role of cytokines on human macrophage-mediated tumor cytotoxicity in vitro, we have utilized a model system employing the human colon cancer cell line target, SW116. This cell line is recognized by an IgG2a monoclonal antibody, 17-1A (Centocor, Inc., Malvern, PA) which mediates effective antibody dependent cellular cytotoxicity (ADCC). Monocytes isolated from normal donor buffy coat preparations were kept in continuous culture with maximum ADCC capability occurring after 5-7 days of culture as measured in a H-thymidine release assay. The most effective E:T ratio was 30:1 to 50:1, and cytotoxicity was determined after 18-20 hours. Nonspecific cytotoxicity was on average less than one fifth that observed in ADCC assays. Augmentation of ADCC was not seen with interleukin-1 alpha or interleukin 6. Human recombinant macrophage CSF in doses up to 1000 u/ml did not increase ADCC above levels seen with 10% fetal calf serum alone. Recombinant human (rh)CSF-CSF, rhIL-4, and rhIL-3 were all capable of increasing ADCC in this system in a dose-dependent fashion during the 7 days of monocyte culture. These effects were seen whether or not the cytokine in question was present during the ADCC incubation. These data indicate that macrophage-mediated ADCC against a human solid tumor cell line can be augmented by several hematopoietic cytokines. Whether such augmentation will be seen in in vivo or with other tumor types remains an open area of investigation.
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**CD 112**

REGULATION OF CELL PROLIFERATION AND GROWTH ARREST BY AN AUTOCHONOUS SOLUBLE Vertebrate Lectin, Livio Mallucci and Valerie Wells, Laboratory of Cellular and Molecular Biology, Department of Microbiology, UMDS, Guy's Campus, London Bridge. SE1 9RT UK

We have cloned and expressed in recombinant form a cell growth regulatory molecule which can cause growth arrest at nanogram concentrations. The factor, which belongs to a class of proteins classified as soluble vertebrate lectins, plays a part in the autocrine system of cell growth control as a negative regulator. Both as a regulatory molecule and as a growth arrest factor it controls exit of cells from G0 and traverse from S phase through G2 with a mode of action attributable to that of a cytokine rather than that of a lectin.

**CD 113**

INDUCTION OF ENDOTHELIAL CELL (EC) MIGRATION AND PROLIFERATION BY G AND GM-CSF:
ROLE OF THE Na+/K+ EXCHANGER, Alberto Mantovani, Federico Bussolino, Istituto di Ricerche Farmacologiche "MARIO NEGRIT", Via Eritrea 62, 20157 Milan, Italy, Università di Torino, Via Santena 5, 10126 Torino, Italy.

G and GM-CSF are hematopoietic growth and differentiation factors which have been considered restricted to the hematopoietic lineage. We observed that G and GM-CSF induce migration and proliferation of EC. Endothelial cells have receptors for G and GM-CSF similar in number and affinity to those present in myelomonocytic cells. The rapid intracellular events initiated by these cytokines on binding to their receptors on HEC are not defined. Addition of G- or GM-CSF to HEC produced a rapid activation of Na+/H+ exchanger resulting in an increase in intracellular pH (pHj). Both cytokines induced an alkaline displacement in the pHj dependence of the exchanger, without affecting the affinity for external Na+ (Na,) and the rate of exchanger. Ethylisopropil-amiloride (EIPA), a specific inhibitor of Na+/H+ exchange, inhibited the intracellular alkalinization, the migration and proliferation induced by G- and GM-CSF. The data indicate that G- and GM-CSF initiate a rapid exchange of Na+ and H+ by mean of the Na+/H+ exchanger and that this EIPA-sensitive ions flux is important to the biological effects of these cytokines on HEC.

**CD 114**

EXTRACELLULAR CALCIUM AND PROTEIN KINASE C ARE NECESSARY FOR IL2 PRODUCTION BY MITOGEN-ACTIVATED HUMAN T CELLS, J. Modiano, R. Kolp, & P. Nowell, Pathology Dept., Univ. of Pennsylvania, Philadelphia, PA 19104.

Increased concentration of Ca++ and activation of protein kinase(s) C (PKC) seem critical in the triggering of human T cells. We used human peripheral blood lymphocytes (PBL) and human T cell lines H33 and J32 to study further the role of extracellular Ca++ and PKC in production of interleukin 2 (IL2), which ultimately drives proliferation of these cells. PBL stimulated by PHA as well as either cell line stimulated by PHA + PMA, produced IL2. Chelating extracellular Ca++ from 300 uM to 1 uM, to lower the out-in Ca++ gradient 100-fold, abrogated IL2 gene expression in PHA-stimulated PBL; it was fully reconstituted by restoring the gradient. MgCl2, but not choline chloride, could partially replace Ca++ to allow IL2 production by PHA-stimulated PBL. Inhibiting PKC with Staurosporine ("Ss") decreased IL2 gene expression in mitogen-stimulated cells by about 50% at a concentration of >5 nM. IL2 production by mitogen-stimulated PBL or either cell line was also inhibited by Ss with an I50 of 5-10 nM. Also, with purified PKC from H33 cells, 10 nM Ss inhibited autophosphorylation by 70% and histone phosphorylation by 50%. Thus, an out-in Ca++ gradient >10 (i.e., [Ca++]_[>>1 nM]) is required for IL2 production by mitogen-stimulated human T cells, and PKC activation also affects IL2 gene expression. Preliminary observations suggest that PKC may also influence post-transcriptional events leading to secretion of IL2.
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CD 115 MAb 425 ANTI-EGF RECEPTOR MONOCLONAL ANTIBODY INHIBITS EGF/TGF-α-INDUCED SECOND MESSENGERS AND DISRUPTS AUTOCRINE GROWTH STIMULATION OF CARCINOMA CELLS, Ulrich Rodeck, Uma Murthy, and Meenhard Herlyn, The Wistar Institute, Philadelphia, PA 19104 and Department of Cell Biology, Smith, Kline & French Laboratories, King of Prussia, PA 19406. Monoclonal antibody 425 binds to a protein epitope of the human EGF receptor and blocks EGF dependent functions such as, EGF receptor phosphorylation and mitogenesis (Murthy et al., Arch. Biochem. Biophys.252, 549, 1987). In this study, we showed that MAb 425 also blocks binding of TGF-α to the EGF receptor and inhibits EGF/TGF-α induced generation of inositol1,4,5 triphosphate in carcinoma cells. In addition, MAb 425 inhibited growth stimulation of carcinoma cells induced by exogenous EGF/TGF-α. Using a culture medium free of exogenous growth factors we found that MAb 425 inhibited growth of 5 out 7 carcinoma cell lines that express surface EGF receptors and produce TGF-α-like mitogens. This effect was independent of the level of EGF receptor expression, dose-dependent and could be overcome by exogenous EGF/TGF-α. Our results suggest that tumor-derived, secreted TGF-α is an autocrine growth factor for carcinoma cells under growth factor-deprived conditions.

CD 116 The Acidic FGF Receptor is Related to flg Tyrosine Kinase M. Ruta*, N. Neiger*, J. Epstein*, W. Burgess+, and J. Schlessinger** Laboratory of Retrovirology, FDA, +American Red Cross, and **Rorer Biotechnology Inc. We have previously isolated a novel human gene from an endothelial cDNA library encoding a putative tyrosine kinase which we designated flg (flg-like gene). In order to analyze the gene product(s) of flg we generated rabbit polyclonal antibodies directed against a synthetic peptide from flg and used it to immunoprecipitate biosynthetically labeled flg protein(s) from a variety of human cell lines. This antiserum recognizes specifically glycoprotein(s) of 100-130 kd with a protein core of 90,000 and 110,000 daltons. Acidic fibroblast growth factor stimulated tyrosine kinase activity of flg protein both in vitro and in living cells, suggesting that flg encodes the membrane receptor for acidic FGF. Further supporting evidence came from cross-linking experiments on intact cells with the covalent cross-linking agent disuccinimidyl suberate and ¹²⁵I-labeled acidic FGF as a specific probe. The cross-linked ¹²⁵I-labeled-FGF receptor complex was specifically immunoprecipitated with flg anti-peptide antibodies. It appears therefore that the receptor(s) for FGF is related to flg gene product.

CD 117 EXPRESSION AND BIOLOGICAL ACTIVITIES OF TRANSFORMING GROWTH FACTOR-α AND EPIDERMAL GROWTH FACTOR RECEPTOR IN HUMAN GLIOMAS. Peter A. Steck and W.K. Alfred Yung, Department of Neuro-oncology, University of Texas M.D.Anderson Cancer Center, Houston, TX 77030. Recent studies have implicated the overexpression of EGF-R in combination with the expression of TGF-α leads to transformation in model systems. We have examined the expression of EGF-R and TGF-α by several independent methods in primary human brain tumors. The amplification of EGF-R gene was observed in about 40% of the glioblastoma multiforme tumors examined, which is in agreement with previous reported studies. The TGF-α gene was found amplified in some anaplastic astrocytomas. TGF-α-like material was found to be present in the majority of tumors examined, albeit at different levels by competitive binding assays with iodinated EGF. Western blot analysis confirmed the presence of TGF-α, although the majority of the immunoreactive protein material appeared to be of large (> 10 Kd) molecular weight. Alternatively, the activity of EGF-R from the various tumors was examined in immunocomplex kinase autophosphorylation assays. Quantitation of the kinase activity per receptor, as determined by Western blotting, revealed three distinct patterns, 1) low basal activity with no stimulation by exogeneously added EGF; 2) increased basal level of activity with stimulation with EGF; and 3) increased basal level and no stimulation with EGF. The increased basal kinase activity was independent of the expression of EGF-R, although all samples exhibiting high levels of kinase activity were derived from glioblastoma tumors. These results suggest the TGF-α is expressed in many glial-derived brain tumors, and that the intrinsic tyrosine protein kinase activity per receptor varies dramatically among the tumors examined.
Molecular Pathways of Cytokine Action

CD 118  ERYTHROPOETIN INHIBITORY AND POTENTIATING ACTIVITIES. G. Steiner, E. Pavlicek, U. Barnas and W. Woloszczuk, 2nd Dept. of Medicine, University of Vienna, and L. Boltzmann-Institute for Clinical Endocrinology, Gamisongasse 13, A-1090 Vienna, Austria. Erythropoietin (EPO) is considered as the key regulator of red blood cell formation. Other growth factors, such as lymphokines and colony stimulating factors (CSF) may modulate the effects of EPO. IL-1 is of interest because it can induce the production of CSFs both in vitro and in vivo, but this lymphokine is also suspected to be partially responsible for anemia in some rheumatoid arthritis patients. To measure EPO-modulating activities we developed a cellular in vitro assay using EPO-dependent erythroid precursor bone marrow cells from rabbits treated with phenylhydrazine and actinomycin D. Cells were grown in serum-free medium containing between 3 and 100 mU/ml EPO. Cell proliferation as measured by ³¹H-thymidine incorporation was strictly dependent on the presence of EPO CSFs (GM-CSF, G-CSF, M-CSF, IL-3) and lymphokines (IL-1, IL-2, IL-4, IL-6, TNF-alpha, IFN-gamma) did not have stimulating activity either alone or in combination with EPO. EPO-induced growth was reduced in the presence of 100 U/ml IL-1 or IL-6 and almost completely inhibited by 50 U/ml IL-2 or 5 mU/ml succinylacetone (an inhibitor of heme biosynthesis). To measure EPO-inhibitor or -potentiating serum activities, cells were incubated with 25 mU/ml EPO and 10% to 0.1% human serum. At 10% serum concentration proliferation was completely abolished by most sera, inhibition disappeared when the serum concentration was reduced and at 1% concentration sera from healthy subjects EPO-potentiating activities (EPO-P) caused a 1.4 to 2-fold increase in proliferation. In contrast, only 50% of sera from patients with impaired kidney function exerted such activities. Sera from 10 hemolyysis patients treated with recombinant human EPO were assayed over 5 months of treatment. No change in inhibitory activities could be observed at 10% serum concentration. However, a rise in EPO-P was observed in seven cases during the initial phase of therapy which was followed by a pronounced decrease after two months treatment when hemoglobin levels were >12g/ml. These data have to be interpreted carefully, but we may draw the following conclusions: serum contains activities which inhibit or potentiate the effects of EPO on erythroid precursor cells, lymphokines IL-1, IL-2 and IL-6 inhibit EPO-induced growth in vitro; therapy of anemic patients with antibodies to EPO-P stimulates erythropoiesis and potentiates and inhibits serum activities. These serum activities may be cytokines or cytokine-like factors and could be important in regulation of erythropoiesis.

CD 119  GROWTH CONTROL BY MONOCYTES IN SERUM-FREE MEDIUM. Jürgen van der Boch, Stephan Rüller and Daniel Horn, Forschungsinstitut Borstel, 2061 Borstel, Fed.Rep.Germany.
In view of the supposed role of mononuclear phagocytes in growth control and tumor defense an important question concerns the principles which determine the quality and quantity of a cell’s response to the complex cytokine milieu created during interaction with these effector cells. In an approach to this question the population development of 8 adherent human tumor cell (TC) lines, which grow continually in a defined medium, was studied under the influence of elutriated, unstimulated human monocytes (MO) or of supernatants from MO/TC-co-cultures. The analysis, performed by electronic counting of tumor cell nuclei and flow cytometry yielded the following results: At subconfluent TC-density MO can cause growth stimulation in EGF-dependent cell lines, which have been deprived of EGF, whereas in the presence of EGF growth inhibition is the general TC-response observed to a challenge by MO. On the other hand, at confluent and higher TC-density the induction of cell death is a prominent feature of the interaction, leading to maximum cell loss at rather low effector:target-ratios (E:T=1:2), whereas high MO-numbers (E:T=10:1) cause a reversible G1-arrest of the cell cycle, and prevent the induction of cell death. EGF-withdrawal decreases the G1/S-transition rate in EGF-dependent TC populations and concomitantly renders them less susceptible to the induction of cell death. Addition of hydrocortisone reduces the rate of cell loss. The data suggest, that in MO/TC-co-cultures the decision on target cell lysis is made by the target cell and that the criterion for this decision is the target cell’s ability to respond to a MO-challenge by arresting the cell cycle in G1. This ability can be modulated by growth factors and hormones. Interactions between target cells play an important role in determining the result of this decision process.

CD 120  RESISTANCE OF U937 VARIANTS TO TNF-INDUCED DNA FRAGMENTATION AND CELL LYSIS IS NOT DUE TO INABILITY TO ACTIVATE NF-KB. Susan C. Wright, Madhu Varma, Albert W. Tan, and Poornima Kumar, Genelabs Incorporated, Redwood City, CA 94063. This study has employed TNF-resistant tumor cell variants to analyze the mechanism by which TNF lyse sensitive tumor cells. TNF resistant variants were selected by the prolonged culture of U937 tumor cells in the presence of TNF. Experiments were designed to test the hypothesis that TNF-induced activation of the DNA binding protein, NF-KB, results in DNA fragmentation and cell death. However, gel mobility shift assays demonstrated that TNF will activate NF-KB in the resistant variant as well as in the sensitive U937. Two possible models could explain these results: one is that activation of NF-KB is not involved in the TNF lytic pathway. Alternatively, if activation of NF-KB is essential, then some subsequent steps must be blocked to prevent cytolyis in the U937 variant. It is concluded the activation of NF-KB alone is not sufficient to lead to TNF-induced DNA fragmentation and target cell lysis. (Supported by grant CA 47669).
Regulation of Immune Function

CD 200 THE ROLE OF THE IMMUNE SYSTEM IN VACCINE-INDUCED INHIBITION OF HEPATIC DRUG METABOLISM, Sherry Ansher*, Raj Puri**, Walter Thompson*, and William Habig*, Division of Bacterial Products* and Division of Cytokine Biology**, CBER, FDA, Bethesda, MD. 20892

Some vaccines have been shown to alter hepatic drug metabolism. The mechanism of this inhibition is unknown. Studies suggest that the immune system may be involved in the mediation of the inhibition of drug metabolism. Increased serum levels of interferons (IFN) have been reported following administration of vaccines, and IFN inducers such as poly I:C are known to inhibit several drug metabolizing enzymes. In order to elucidate the mechanism by which vaccines alter hepatic detoxication and the role of immunomodulators, we have studied hexobarbital-induced sleep time, as well as cytosolic and microsomal enzyme activities in mice treated with DTP vaccine, poly I:C, and several cytokinas. Sleep times were significantly increased in mice which had received DTP vaccine, poly I:C, or IL-2. Treatment with IFN-α alone did not affect sleep time, but in combination with IL-2 it prolonged the sleep time over that of animals treated with IL-2 alone. Cytochrome P-450-dependent enzyme activities were significantly inhibited (up to 75%) in mice treated with DTP vaccine, poly I:C, or IL-2. IFN-α alone caused marginal (10-20%) decreases in cytochrome P-450-dependent enzyme activities. Mice pretreated with gamma-irradiation to suppress the immune system reduced the inhibition of drug metabolism following administration of cytokinas but not the inhibition following administration of DTP vaccine. These studies suggest that cytokinas may play a complex role in the inhibition of drug metabolism and further suggest that radiosensitive cells may contribute to the IL-2 induced suppression of drug metabolism.

CD 201 IL-4 INDUCED GENERATION OF LECTIN-TRIGGERED CYTOTOXICITY IN MURINE THYMOCYTES REQUIRES IL-2 AND IL-6. M. Bertagnolli, V. Pinto, S. Herrmann, Depts. of Surgery and Pathology Harvard Medical School and Dana-Farber Cancer Inst. Boston MA 02115

The generation of active cytotoxic T lymphocytes (CTL) requires both antigen and lymphokine signaling. To investigate lymphokine requirements we cultured thymocytes from C57BL/6 mice for three days in the presence of 3 μg/ml of Con A plus various lymphokines. In a typical experiment lectin mediated target cell lysis by responders cultured in the presence of IL-4 was 4±2% compared to 17±1% lysis for responders cultured in the presence of IL-2 and 31±1% lysis for responders cultured in the presence of IL-2 + IL-6. To understand the relationship between the different lymphokines we added to the thymocyte culture mAbs able to block the activity of: IL-4 (11B11); IL-2 (S4B6); or IL-6 (6B4). Anti-IL-4 mAb, able to block the response to IL-4, had no effect on the generation of CTL in the presence of IL-2 + IL-6. Thymocytes cultured in the presence of IL-2 + IL-6 gave 28±2% lysis while culture in the presence of IL-2 + IL-6 + IL-11 gave 30±1% lysis. In contrast mAb to either IL-2 or IL-6 was able to inhibit the generation of CTL in the presence of IL-4. In this assay culture with IL-4 resulted in responders eliciting 46±2% lysis which fell to 0% lysis when S4B6 was included in the thymocyte culture. In another assay, culture in IL-4 generated 76±13% lysis while culture in IL-4 + 6B4 gave 25±13% lysis. Using lymphokine-dependent cell lines we have shown that thymocytes cultured in Con A were capable of producing low levels of IL-2, IL-6, and possibly IL-4. Our data suggest that the response generated in the presence of exogenous IL-4 is dependent upon the presence of endogenous IL-2 and IL-6.

CD 202 MULTIPLE LEVELS OF MURINE GM-CSF GENE REGULATION. Matthias Bickel, Roger B. Cohen and Dov H. Pluznik, Division of Cytokine Biology, Center for Biologics, Evaluation and Research, Food and Drug Administration, Belheada, MD. 20892

Phorbol esters (TPA) are known to induce the expression of a number of cytokines in T cells by transcriptional mechanisms. We noted that TPA induced the expression of GM-CSF mRNA in EL-4 thymoma cells, a mouse T cell line >100 fold without a corresponding increase in transcription as measured in nuclear run-on assays. Furthermore, we noted that untreated EL-4 cells contain detectable amounts of GM-CSF mRNA. Actinomycin D chase experiments show that GM-CSF mRNA half-life in untreated cells is <30' and after TPA treatment it exceeds 6 hours. Changes in mRNA t1/2 appear to be the principal explanation of the large increase in GM-CSF mRNA after TPA treatment of these cells. We have begun using RNA mobility shift gel assays to characterize cytoplasmic proteins which bind to the 3'UTR of the GM-CSF message that may modulate the changes in message stability. We also have noted the presence of unspliced and partially spliced GM-CSF mRNA precursors in nuclear RNA from untreated cells. Interestingly, these GM-CSF mRNA precursors rise > 5-fold after 30 minutes of TPA treatment and then fall below their initial levels after 3 hours at which point mature GM-CSF mRNA appears in the cytoplasm. The rapid rise in GM-CSF precursor RNA is out of proportion to the increase in transcription which occurs after phorbol ester treatment. These latter data suggest that GM-CSF mRNA levels are also modulated by stabilization and processing of nuclear RNA precursors.
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**CD 203** IL-1 RESPONSIVENESS AND RECEPTOR EXPRESSION BY Th1 AND Th2 CELLS, Brian R. Champion, Nick Smithers and Roberto Solari, Departments of Molecular and Cellular Sciences, Glaxo Group Research Ltd, Greenford, Middlesex, UB6 OHE, UK.

We have been studying the IL-1 responsiveness and receptor expression by murine T cells. The Th2 cell line D10.G4.1 will proliferate in response to both IL-1α and IL-1β in the presence of suboptimal concentrations of ConA and this proliferation is dependent upon the production of IL-4. Over a period of several months of repeated stimulation with antigen/APC, the sensitivity of D10 cells to IL-1 triggered proliferation was found to increase approximately 50-fold. This increased sensitivity could be reversed by a 2d culture in the absence of any added stimuli and was lost on cryopreservation. Binding studies revealed a single class of binding site and showed that the number of these IL-1 receptors (IL-1R) was upregulated following stimulation, although the affinity remained the same. Crosslinking studies using 125I-IL-1 and the homobifunctional crosslinking agent DSS revealed 2 forms of IL-1R: an ~80kDa form similar to the well characterized IL-1R found on EL-4 cells and a ~60kDa form. Partial peptide map analysis suggested that these 2 forms represent differently processed forms of the same receptor. Early passage, less sensitive, D10 cells expressed predominantly the ~60kDa IL-1R whereas the more sensitive, continuously passed cells showed a marked increase in ~80kDa IL-1R expression. Resting of such sensitive cells led to a down-regulation of the ~80kDa IL-1R but had little effect on the ~60kDa form. In contrast, stimulation with ConA or anti-CD3 in the absence of APC led to a 10-fold increase in ~60kDa IL-1R but had no effect on expression of the ~80kDa form. The maintenance of both IL-1R forms on resting cells was blocked in the presence of anti-IL-4 antibody. A Th1 cell line, MTg12B, was also studied. This cell line failed to respond to IL-1, either alone or in the presence of ConA or anti-CD3, and did not express ~80kDa IL-1R. However, this cell line did express the ~60kDa form of the IL-1R in a stimulation dependent manner. Although expression of the ~60kDa form of the IL-1R can clearly be regulated in both Th1 and Th2 cells, whether it can transmit biological signals to these cells remains to be determined.

**CD 204** EXPRESSION OF CYTOKINES BY INFILTRATING T-CELLS
Amos Cohen, G. Mills and H. Martinez-Valdez, Division of Immunology-Rheumatology, Research Institute, Hospital for Sick Children, and Department of Immunology, University of Toronto, Toronto, Ontario, Canada, MSG 1X8.

Lymphocytes infiltrating human ovarian carcinoma were isolated from intraperitoneal ascites of patients with ovarian tumors. The presence of mRNAs of T cell-associated cytokines was determined by the polymerase chain reaction using specific primers after reverse transcription of cellular RNA. Using different numbers of amplification cycles, it was possible to quantitate the level of mRNA in the T-lymphocyte preparations. We have identified the presence of large amounts of mRNAs for IL-2, IL-3, IL-6, INF-γ and GM CSF in the tumor infiltrating T-lymphocytes. These cytokines may be involved in paracrine and/or autocrine regulation of the cellular immune response directed against the ovarian tumor.

**CD 205** CRYSTAL STRUCTURE OF RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR, William J. Cook, Steven E. Ealick, & Charles E. Bugg, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, AL 35294 and Paul Reichert, Gerald S. Hammond, Hung V. Le, Tattanahalli L. Nagabhushan, & Paul P. Trotta, Schering Corporation, Bloomfield, NJ 07003. Crystals of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) have been grown using either polyethylene glycol 8000 or solutions of sodium/potassium phosphate. The protein used in this study was derived from E. coli and is similar to naturally-occurring human GM-CSF. The crystals are orthorhombic, space group P212121; the axes are a = 45.5(1), b = 58.7(1) Å and c = 127.3(1) Å. Although the molecule exists as a monomer in solution, it crystallizes with two or three molecules in the asymmetric unit. Based on a molecular weight of 14,477 daltons as predicted from the cDNA, this corresponds to solvent volume fractions of 5% and 39% for two or three molecules, respectively.The crystals are stable to x-rays at room temperature for at least three days, and x-ray diffraction data for the native crystals have been collected to 2.8 Å resolution using an area detector. Crystallographic analysis of the structure of GM-CSF is in progress, and one potential derivative has been identified. Current structural results will be presented.
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**CD 206**  
CHARACTERIZATION OF THE TUMOR NECROSIS FACTOR-α RECEPTOR ON ACTIVATED HUMAN PERIPHERAL BLOOD LYMPHOCYTES.  
William L. Crump III, Laurie Owen-Schaub and Elizabeth Ann Grimm,  
Department of Tumor Biology, University of Texas M.D. Anderson Cancer  
Center, Houston, TX 77030.

TNF-α is postulated to play an obligatory role in the IL-2 driven  
generation of cell mediated, MHC unrestricted cytotoxicity. Furthermore, we  
have recently reported that IL-2 regulates lymphocyte production of TNF-α  
and expression of tumor necrosis factor receptors (TNF-R) on lymphocytes.  
Our present work is directed towards characterizing TNF-R on activated  
human peripheral blood lymphocytes. Using PBL stimulated for 12 days with  
500 U/ml IL-2, Scatchard analysis demonstrates a Kd of 40-60pM and  
approximately 2500 TNF binding sites per cell. 125I-TNF crosslinking  
studies with the DSS analog BS₂, demonstrate a receptor:ligand complex of  
approximately 109.5 kDa. An additional band at 46 kDa, which we postulate  
to be crosslinked trimeric TNF, is also observed. Photoaffinity  
crosslinking with 125I-ASD-TNF demonstrates a band of 95 kDa. The absence  
of the 46 kDa band in photoaffinity crosslinking studies supports our  
conclusion that this band is trimeric TNF. Lectin precipitation  
experiments demonstrate that the TNF-R is a glycoprotein.

**CD 207**  
PRODUCTION OF TNF BY STAPHYLOCOCCAL ENTEROTOXIN A (SEA) ACTIVATED T CELLS.  
Dohlsten, M., Hedlund, G., Fischer, H., Ericsson, P.E,  
Hansson, J., *Andersson, U and Sjogren, H-O.  
Dept. of Tumor Immunology, The Wallenberg Laboratory,  
University of Lund, Sweden and *Department of Immunology,  
Stockholm University.

SEA-induced T cell activation has been shown to be  
strictly accessory cell dependent and requires the  
binding of SEA to the MHC Class II molecule on the  
accessory cell and subsequent interaction with the TCR-  
CD3 complex on the responding T cells. We now demonstrate  
that SEA is a potent inducer of TNF in cultured human T  
cells and monocytes of peripheral blood. SEA at 1 pg/ml  
induced TNF activity with peak value after 48-72 hours of culture. IL-2 and IL-4 were growth promoting for human T  
cells, but only IL-2 induced TNF. TNF-alpha was produced  
already after 6 hours, whereas TNF-beta was produced  
after 48-72 hours of culture. TNF-alpha was detected in  
monocytes and T cells, whereas TNF-beta was seen  
exclusively in T cells. Production of TNF-alpha and TNF-  
beta required the presence of both monocytes and T  
cells. CD4+ and CD8+ T cells produced TNF-beta, but the  
frequency of TNF producers was higher among the CD4+  
cells. The CD4+CD8- T cell subset produced TNF and  
interferon, whereas the CD4+CD8+ cells produced only TNF.

**CD 208**  
TRANSCRIPTIONAL REGULATION OF HUMAN TUMOR NECROSIS FACTOR GENE  
EXPRESSION. J. Economou, K. Rhoades, W. McBride. Division of  
Surgical Oncology, UCLA Medical Center, Los Angeles, CA 90024.  
TNF is an  
important immunoregulatory cytokine. The 615 bp 5' flanking region was  
ligated to the luciferase (Luc) reporter gene and a series of 5' truncations (from 615 to 56) generated by exonuclease III digestion. The  
full-length construct, when transfected into U937 cells, could be induced  
with phorbol ester (1-fold), PGE₂ (22-fold), 8 br cAMP (10-fold) and  
cAMP (2-fold). The inducibility was retained in constructs retaining 95  
but not 56 bp 5' to the transcription start site. This short inducible  
region of the TNF promoter contains Sp-1, AP-1 and AP-2 consensus  
sequences which may confer phorbol ester and cyclic nucleotide inducibility.
Three different antibodies against a human TNF receptor (htr-1, htr-5, and htr-9) have been examined for their binding pattern to U937 cells and ability to mimic TNF-α activity in U937 cells, F54 fibroblasts and human endothelial cells. Flow cytometric analysis revealed that htr-5 and htr-9 bound specifically to a TNF receptor on U937 cells which could be blocked by pretreatment with rTNF-α. Pretreatment of U937 cells with rTNF-α blocked the binding of htr-9, but to a lesser extent htr-5 binding. Pretreatment with htr-5 inhibited the binding of htr-9 to U937 cells while pretreatment with htr-9 did not inhibit htr-5 binding. These results indicate that htr-5 and htr-9 recognize distinct but overlapping epitopes of a human TNF receptor on U937 cells and that htr-5 may be close to a TNF-α specific domain of the binding site. Pretreatment with htr-5 or htr-9 only minimally reduced binding of rTNF-α to U937 cells, however, these antibodies were more effective in inhibiting rTNF-α binding to HL-60 cells. Furthermore, it was found that htr-1 and htr-9, but not htr-5, had TNF-α activity on U937 cells, on F54 fibroblasts and on endothelial cells and that the TNF-α activity induced by htr-9 was completely inhibited by htr-5. However, the cytotoxic activity of TNF-α was only partially inhibited by htr-5 on U937 cells while htr-5 had no effect on TNF-α activity on F54 cells. The data suggested that a common epitope is involved in inducing TNF activity in three different cell systems.

**CD 209 CHARACTERIZATION OF BINDING AND BIOLOGICAL EFFECTS OF MONOCLONAL ANTIBODIES AGAINST A HUMAN TUMOR NECROSIS FACTOR RECEPTOR**

Terje Espevik, Manfred Brockhaus*, Hansruedi Loetscher*, Unni Nonstad and Rezaat Shalaby, Institute of Cancer Research, N-7006 Trondheim, Norway, and F. Hoffman-LaRoche, Basel, Switzerland.

Three different antibodies against a human TNF receptor (htr-1, htr-5, and htr-9) have been examined for their binding pattern to U937 cells and ability to mimic TNF-α activity in U937 cells, F54 fibroblasts and human endothelial cells. Flow cytometric analysis revealed that htr-5 and htr-9 bound specifically to a TNF receptor on U937 cells which could be blocked by pretreatment with rTNF-α. Pretreatment of U937 cells with rTNF-α blocked the binding of htr-9, but to a lesser extent htr-5 binding. Pretreatment with htr-5 inhibited the binding of htr-9 to U937 cells while pretreatment with htr-9 did not inhibit htr-5 binding. These results indicate that htr-5 and htr-9 recognize distinct but overlapping epitopes of a human TNF receptor on U937 cells and that htr-5 may be close to a TNF-α specific domain of the binding site. Pretreatment with htr-5 or htr-9 only minimally reduced binding of rTNF-α to U937 cells, however, these antibodies were more effective in inhibiting rTNF-α binding to HL-60 cells. Furthermore, it was found that htr-1 and htr-9, but not htr-5, had TNF-α activity on U937 cells, on F54 fibroblasts and on endothelial cells and that the TNF-α activity induced by htr-9 was completely inhibited by htr-5. However, the cytotoxic activity of TNF-α was only partially inhibited by htr-5 on U937 cells while htr-5 had no effect on TNF-α activity on F54 cells. The data suggested that a common epitope is involved in inducing TNF activity in three different cell systems.

**CD 210 KINETICS OF THE INDUCTION OF LEUKOREGULIN SECRETION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR LEUKOCYTES**

Esme K. Farley, Anna C. Wilson & Charles H. Evans, Laboratory of Biology, National Cancer Institute, Bethesda, MD 20892.

This investigation sought to determine whether leukoregulin secretion is up-regulated or truly inducible following mitogenic stimulation. The kinetics of leukoregulin secretion were examined to define the optimal time for collection of mRNA for cloning of the gene for leukoregulin synthesis. Human peripheral blood leukocytes were isolated on a discontinuous density gradient and cultured at a concentration of 10⁶ cells/ml at 37°C. Stimulation with 10 μg/ml PHA revealed the inducible nature of this lymphokine as no constitutive secretion was detectable. Leukoregulin secretion commences at 4-6 hours, reaches a peak about 8 hours and plateaus by 18-24 hours. Stimulation with 10 μg/ml human K562 erythroleukemia cells/ml in place of PHA, although accompanied by a continued rise in secretion for 48 hours, is at a lower level than that obtained with PHA stimulation. The lower level of secretion may reflect a lower initial degree but continuing recruitment by tumor cell stimulation. The biochemical nature of K562 cell induced leukoregulin was analyzed to determine whether it is the same molecule as that produced with PHA stimulation by studying its molecular size, isoelectric point and biological activities. An examination of the possibility of negative feedback of leukoregulin secretion was made to determine whether the kinetics of leukoregulin secretion is a function of the lymphokine itself or that of the stimulus. Culture of leukocytes in the presence of conditioned media containing leukoregulin showed no down-regulation of leukoregulin production during 18 hours culture in the presence of PHA.

**CD 211 INDUCTION AND REGULATION OF INTERLEUKIN-4 RECEPTOR EXPRESSION ON MACROPHAGES BY INTERFERON-GAMMA**

Gerald M. Feldman and David S. Finlbloom, Division of Cytokine Biology, Food & Drug Administration, Bethesda, MD 20892.

Interleukin 4 (IL-4), is a T cell-derived cytokine that regulates the induction of proliferation of resting B cells, and appears to act on various other cells involved in the immune response. The pluripotential effects of IL-4 are dependent upon the interaction of IL4 with its receptor (IL-4R). IL-4R have recently been reported to be present on the surface of a variety of inflammatory cells, both human and murine. Although the regulation and metabolism of these receptors have been examined on human and murine B and T cells, little is known about the metabolism or regulation of the IL-4R on macrophages. In studying the dynamics of IL-4R expression on the murine macrophage-like cell line J774.A1, we detected the presence of low numbers of high affinity IL-4 receptors on the surface membrane. However, upon exposure to interferon-γ (IFN-γ), a potent macrophage activating cytokine, there was a rapid induction of IL-4R on the cell surface. The increase in IL-4R density was first noted within 45 minutes after exposure of the cells to IFN-γ, reaching its peak within 4 hours. No further increase in IL-4R expression occurred over the next 48 hours. This induction of receptor required the continued presence of IFN-γ. In experiments wherein the cells were treated for short periods of time with IFN-γ, washed, and continued in culture, only those cells that were exposed to IFN-γ for at least 2 hours displayed maximum IL-4R expression. The induction of IL-4R by IFN-γ was dose-dependent: at least as little as 0.5 ng/ml of IFN-γ was capable of inducing IL-4R expression, with optimal induction at 10 ng/ml. Addition of metabolic inhibitors prior to the addition of IFN-γ indicated that both RNA transcription and protein translation are required for this induction to occur.
Molecular Pathways of Cytokine Action

CD 212 DIFFERENT 5' SEQUENCE REQUIREMENTS FOR CLASS II INDUCTION BY IFN-γ AND TNF-α IN A MURINE MACROPHAGE CELL LINE. Yvonne R. Freund, Department of Immunology & Infectious Diseases. Research Institute, Palo Alto Medical Foundation, Palo Alto, CA. 94301.

The expression of murine class II genes can be induced on the surface of macrophages by both IFN-γ and TNF-α. This induction is mediated via increases in levels of class II mRNA. We have demonstrated in WEHI-3 cells, using the bacterial chloramphenicol acetyl transferase reporter gene, that the promoter of the Aα gene is transcriptionally activated by IFN-γ and TNF-α. Using 5' deletion and nested linker-scanner mutations, we have demonstrated that 105 nucleotides 5' of the initiation site are required for induction by each cytokine. This region includes the X, Y and H boxes. In addition, a unique sequence has been found, located between -31 and -17, which appears to play a role in TNF-α-mediated, but not IFN-γ-mediated Aα promoter activation. This sequence contains an SV40 core enhancer element and is also found in the human HLA-DQα and the rat RT1.Ba genes, both Aα homologues. The core enhancer sequence is found in the Εα and the HLA-DRα genes at the same distance 3' of the Y box. The -31 to -17 sequence, which we have tentatively called the T box, also has homology to a region in the second intron of the c-myc genes of mouse, human and rat. Gel mobility shift and methylation interference assays are in progress to analyze trans-acting factors which bind to this region and to determine whether the SV40 enhancer or the entire T box region is essential for binding.

CD 213 REGULATION OF LYMOPHINE PRODUCTION BY LYT-2+ CELLS IN MURINE ACUTE GRAFT VERSUS HOST REACTIONS. James J. Gibbons, Jr., Carmen Raventos-Suarez, Judy Lucas and Richard Hernandez, Oncology and Immunology Research Section, American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River NY 10965.

Early in the course of an acute graft versus host (aGVH) reaction, generated by injection of parental spleen cells into Fl recipients, there is a marked accumulation of Lyt-2+ cells in the spleen. It has been reported that spleen cells from aGVH mice are deficient in both IL-2 production and IL-2 receptor expression. We were interested in studying whether all lymphokine production by GVH spleen cells was similarly depressed. Using the lymphokine dependent cell lines FDCPL and 32DCl5 we found that IL-2, IL-3 production is depressed in aGVH spleen cells but GMCSF and, by ELISA, gamma interferon production is enhanced. Lyt-2+ cells from aGVH mice sorted by FACS produced increased amounts of GMCSF on a per cell basis and decreased amounts of IL-3 in response to Con-A. Using allele specific anti-Lyt-2.1 or 2.2 antibodies, we found that the increase in gamma interferon production was caused by donor Lyt-2+ cells. Treatment with anti-Lyt-2.2 did not diminish GMCSF production. Cell sorting experiments showed that removal of Lyt-2+ cells from aGVH spleen resulted in increased IL-2 and IL-3 production relative to unfractonated aGVH spleen cells. The data suggest that donor Lyt-2+ cells suppressed IL-2, IL-3 and GMCSF production of Lyt-2+ cells in aGVH spleens but are themselves able to produce large amounts of gamma interferon and GMCSF.

CD 214 DEFINING THE MINIMAL REQUIREMENTS FOR ANTIBODY PRODUCTION TO PEPTIDE ANTIGENS. Gail Goodman-Snitkoff, Leslie E. Eselle, and Raphael J. Mannino. Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.

Understanding how to present specific, defined epitopes to the immune system in order to induce both humoral and cell mediated immune responses is a major challenge in the design of modern vaccines. The approach we have taken has been to attempt to determine minimal essential components required to construct a well defined immunogenic composite. Synthetic peptides, representing immunologically interesting epitopes from pathogenic microorganisms, i.e. HIV and Malaria, are provided with a hydrophobic tail through crosslinking to a phospholipid and then assembled into a peptide-phospholipid composite. We have found that an immunogenic mixture is comprised of two essential components: 1. Each composite must contain peptides representing both B-cell determinants and T-helper (Th) determinants presented either contiguously, i.e. part of the same synthetic peptide, or on individual peptides. 2. To be immunogenic these peptides must be covalently coupled to phospholipid and incorporated as a phospholipid conjugate. No other carriers or adjuvants are required to induce an immune response. Additionally, trace amounts of a detergent extract of the envelope of Sendai virus to act as a "generic" provider of Th-cell determinants can be included in the composites. Following immunization with these preparations, antibody titters increase with each subsequent inoculation. Additional parameters being studied include dose of immunogen, route of immunization, the nature of the crosslinker used to couple the peptides to phospholipid, and the phospholipid composition. The ability of peptide-phospholipid composites to induce antigen specific cytolytic T lymphocytes is also under investigation.
Molecular Pathways of Cytokine Action

CD 215  MODIFICATIONS OF A SYNTHETIC MATURE HUMAN TUMOR NECROSIS FACTOR GENE BY SITE-DIRECTED MUTAGENESIS, Pamela Huff, Mary E. Fling, Jane Bynum, and Frederick C. Kull, Jr. Divisions of Molecular Genetics and Microbiology and Cell Biology, Burroughs Wellcome Co., Research Triangle Park, NC 27709. The carboxy-terminus of tumor necrosis factor (TNF) is a highly conserved, hydrophobic region. The role of the carboxy-terminal region in the structure and function of TNF was examined by designing changes in the codon that in the natural human TNF gene codes for the carboxy-terminal residue, leucine, (Leu156). Codons for other hydrophobic residues (Val, Phe, Ala, Ile) as well as for residues with hydrophilic side chains (Asn, Thr, Lys, Glu) were introduced at this position using an in-vitro site-specific mutagenesis method. The modified genes were expressed in an E. coli T7 expression system. Mutant TNF in crude bacterial extracts was tested for cross-reactivity with polyclonal anti-TNF antiserum, competitive binding with recombinant mature human TNF (rhTNF), and cytotoxicity against murine L-M cells. Substitution of Leu156 by amino acids with short hydrophilic side chains (Thr or Asn) produced TNF that was present as a 17 Kdalton protein in Western blots; however, these mutants were not competitive with rhTNF in a radioimmunoassay or a radioreceptor assay, and had no cytotoxic activity. On the other hand, mutant TNF in which hydrophobic residues (Val) or residues with long, charged side chains (Lys) were substituted for Leu156 displayed characteristics identical or very similar to those of control TNF.

CD 216  DOWN REGULATION OF INTERLEUKIN-1 (IL-1) RECEPTOR EXPRESSION ON DIOS, A SUBCLONED MURINE T-HELPER CELL LINE, BY IL-1, C.C. Huntenburg, L. Lum, D. Walker and C.A. Dinarello. Baxter Healthcare, Round Lake, IL 60073 and Tufts University School of Medicine, Boston, MA 02111. The subclone DIOS is a stable murine helper T-cell line (LJF4+). ELA.6.1 is a cloned murine T-cell line (L3T4+). These cell lines are used for biosays and as a model for elucidating the effects of IL-1 on T-cell proliferation. DIOS and ELA.6.1 cells were incubated with IL-1 (5 to 5000 pg/ml) for 6, 24, 48 and 72 hours. IL-1 receptor expression was evaluated using a monoclonal antibody to the IL-1 80 kD receptor (provided by R. Chizzonite) and the FACStar™ Plus flow cytometry system. DIOS cells showed decreased IL-1 receptor expression at all concentrations and time points tested. At concentrations greater than 50 pg/ml, the receptor expression was reduced to less than 10% of the control population. Similar treatment of ELA.6.1 cell line had no effect on the IL-1 receptor expression. Treatment with IL-1 had no effect on the L3T4 receptor expression of the DIOS cell line. It has been reported that administration of IL-1, 24 hours prior to fatal insult, has protective effects. The results presented here may help explain one mechanism of protection through the down regulation of IL-1 receptors.

CD 217  CYTOKINE REQUIREMENTS FOR ALLOGENEIC T CELL STIMULATION BY L428 CELLS. Mark J Inglis, Judith L McKenzie and Derek NJ Hart. Haematology Department, Christchurch Hospital, Christchurch, New Zealand. The precise cellular origin of the L428 cell line derived from a patient with nodular sclerosing Hodgkins Disease is unclear, but our recent data suggests notable similarities with cells of the human dendritic cell (DC) series. The L428 cell line shares a similar cell membrane phenotype with human DC and stimulates allogeneic T cells strongly in MLRs during which L428-T cell clustering is evident. We have shown that tonsil DC stimulate allogeneic and autologous T cells in the absence of interleukin 1 α or β (IL-1α or β). Therefore the expression of cytokine mRNA in LPS stimulated and unstimulated L428 cells was investigated by Northern Blot analysis using specific probes for IL-1α and IL-1β. No IL-1β mRNA was detected in LPS stimulated or unstimulated L428 cells although small amounts of IL-1α message were detected (approx. 20% of control monocyte preps). However no IL-1 activity was identified in L428 supernatants using either biological or ELISA assays. Furthermore, the possible involvement of membrane bound IL-1α in T cell activation was excluded by the finding that L428 cells stimulated allogeneic T cells effectively in the presence of polyclonal antiserum to IL-1α and IL-1β, which were known to block functional cytokine activity. We conclude that IL-1 is not essential for primary T cell activation by either DC or the L428 cell line. Instead, it appears from experiments on isolated human cells that IL-1 may increase the antigen presenting capability of DC for autologous immune responses.
Molecular Pathways of Cytokine Action

CD 218 ACTIVATION OF MURINE HELPER CELL CLONES WITH ANTIBODY TO THE T CELL RECEPTOR DECREASES THE β/α RATIO OF IL2 RECEPTOR SUB-UNITS, Ronald M. Karpati and Richard J. Hodes, Experimental Immunology Branch, NCI, NIH Bethesda, MD 20892

Previous work from Fitch and co-workers (Nau, G.J., et. al., J. Immunol. 141:3557-3563, 1988) has shown that stimulation of murine helper cell clones through the T cell receptor (TCR) can result in inhibition of the proliferative response to exogenous IL2. The mechanism underlying this inhibition is unclear since it has been shown that inhibition is not associated with a decrease in endogenous IL2 production, release of soluble inhibitory factors, or a decrease in expression of IL2 receptor α-chain (IL2R-α, p55). We have studied cell surface expression of IL2R-α and IL2R-β (p70-75) by covalent cross-linking of radiiodinated IL2 to its receptor, followed by protein electrophoresis. We have found that the ratio of IL2R-β to IL2R-α is decreased in murine TH1 and TH2 clones stimulated in vitro with immobilized 145-2c11, an antibody to murine CD3-ε. The ratio of IL2R-β/IL2R-α expression was increased slightly (1.21 times the ratio observed in unstimulated controls) in cells stimulated with IL2 alone, and was reduced to 0.48 times control in cells stimulated with anti-CD3, and 0.56 times control in cells stimulated with a combination of anti-CD3 and IL2. Since IL2R-β is an integral component of the high affinity receptor, and may be necessary for signal transduction, we propose this finding as a possible mechanism for the inhibition of the proliferative response to exogenous IL2 seen with supra-optimal TCR mediated stimulation. In addition, because endogenous IL2 plays a role in TCR mediated stimulation, this finding may also explain the inhibition of proliferation noted in response to supra-optimal TCR stimulation in the absence of exogenous lymphokines.

CD 219 IL4 INHIBITS HIGH AFFINITY IL2 RECEPTORS EXPRESSION ON MONOCLONAL HUMAN B CELLS, Saoussen Karray, *Alice Dautry-Varsat and Pierre Galanaud, Institut National de la Santé et de la Recherche Médicale U 131 and *Unité de génétique somatique, Institut Pasteur, France.

In the presence of anti-μ antibodies (anti-μAb), monoclonal B lymphocytes from patients suffering from B type chronic lymphocytic leukemia (B-CLL) can respond to interleukin (IL) 2, B cell growth factor and interferon alpha. In contrast to the effect it exerts on normal B cells, IL4 does not promote DNA synthesis by B-CLL lymphocytes. Rather this interleukin inhibits the response to IL2 in all patients' cells which responded to this interleukin. We thus examined whether IL4 would modulate the number and/or the affinity of IL2 receptors. A 3 day activation of cells by anti-μAb induced a few hundred high affinity IL2 receptors (HA-IL2R) on B-CLL cell surface, as determined by Scatchard analysis. Treatment of cells with IL4 caused a marked decrease in the number of HA-IL2R without affecting the amount of p55 chain of IL2 receptor (determined by flow cytometry). Moreover, after HA-IL2R were induced in a 3 day culture in the absence of IL4, one hour incubation at 37°C with IL4 still causes a decrease in their number, without interfering with the binding ability of IL2 at 4°C. Thus, IL4 appears able to influence the binding function of HA-IL2R.

CD 220 REGULATION OF LYMPHOKINE PRODUCTION DURING ANTIGEN SPECIFIC INDUCTION AND EXPANSION PHASES OF THE IMMUNE RESPONSE. Paul M. Kaye, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. U.K.

Lymphokine (LK) production following exposure to foreign antigen is a central, but poorly understood determinant of the subsequent immune response. Naive splenic lymphocytes exposed to Leishmania parasites proliferate weakly during 1st (3-5 day) culture and produce IL2, barely detectable γ-IFN and no IL3 or IL4. 1st blasts proliferate in response to exogenous IL2 but weakly to IL4 or IL4+IL1. γ-IFN production is unaltered under such conditions. Cells recovered from this 1st culture mount a vigorous 2nd proliferative response when provided with fresh antigen and APC (3000R ¥ Thy1 spleen cells). This is accompanied by IL2, IL3 and γ-IFN production but still no IL4. Depletion of CD8+ cells during the 1st culture led to elevated 2nd proliferation and increased IL2, IL3 and γ-IFN production, but IL4 was still not detected in 1st or 2nd culture supernatants. Increased responsiveness to IL4 was seen in CD8+ 1st blasts, but without any synergistic effect of IL1. These results were not due to an increased proportion of CD4+ cells in the 1st cultures. Such data illustrate the restricted LK repertoire of the developing immune response and that CD8+ T cells, though downregulating expansion of the CD4+ compartment, do not appear to influence the balance of γ-IFN vs. IL4 production by them. Experiments in progress using defined APC and anti-LK MAb will determine whether this balance reflects inappropriate APC signalling or LK (e.g. γ-IFN) mediated regulation. These considerations will be of importance if restricted activation of T effector pathways is required for efficient vaccination against this and other parasites.
CHARACTERIZATION OF T CELL SIGNALS INVOLVED IN INDUCTION OF IL-1 SYNTHESIS BY HUMAN MONOCYTES, R. Clive Landis, Richard I. Fisher and Thomas M. Ellis, Section of Hematology and Oncology, Loyola University School of Medicine, 2160 S. 1st Ave., Maywood IL 60153. We have previously shown that induction of IL-1 mRNA in normal human monocytes during anti-CD3 mitogenesis occurs rapidly (1 h), requires activated T cells and is not mediated by binding of anti-CD3 mAb/T cell complexes to Fc receptors on monocytes. We have further characterized the IL-1 inductive signal and now demonstrate that cell contact between T cells and monocytes is required for optimal early (≤ 4 h) induction of IL-1 α and β mRNA, although a later phase (> 12 h) of low level IL-1 mRNA induction occurs as a result of soluble factors released during anti-CD3 mitogenesis. The late phase of IL-1 mRNA induction coincides with the appearance of detectable IL-2, IFN-γ and TNF-α in culture supernates. Class I restricted (CD8+) and class II restricted (CD4+) T cell populations induce comparable levels of IL-1 β synthesis in the anti-CD3 mitogenesis model. Furthermore, both naive (CD45RA+) and memory (CD45RO+) T cells are able to induce IL-1 β synthesis. These studies demonstrate that the T cell IL-1 inductive signal 1) involves direct cell contact between T cells and monocytes, 2) does not require obligate binding of class II MHC antigen on monocytes for signal transduction and 3) can be mediated both by naive and previously primed T cells. Studies are in progress to determine the molecular identity of the T cell IL-1 inductive signal involved in anti-CD3 mitogenesis.

A NOVEL TECHNIQUE TO STUDY MOLECULAR PATHWAYS OF CYTOKINE RELEASE AND ACTION: MESSAGE AMPLIFICATION PHENOTYPING (MAPP). JW Larrick, MJ Coloma, K Fry, N Leung, C Levrat, JG Wong-Lee, PA Nelson, CA Brenner, SC Wright. Genelabs Inc. 505 Penobscot Dr., Redwood City, CA. A highly sensitive technique [MAPPing] has been developed to simultaneously analyse the array of messenger RNAs made by small numbers of cells. MAPP utilizes a micro-procedure for isolating RNA from one to one million cells followed by two enzymatic steps: reverse transcription of total cellular RNA to produce cDNA followed by enzymatic amplification of cytokine-specific DNA fragments using specific primers and the polymerase chain reaction. Application is made to populations of lymphoid cells (T, B, NK) and macrophages. Thirty cytokines (and their receptors) and other inflammatory proteins were simultaneously MAPPed; [including: interleukins 1-8, TGFs, TNFs, interferons, various growth factors, heat shock proteins, extracellular matrix proteinases, etc.]. The presence of individual mediators was compared to the production of specific cytokines using functional bioassays and ELISAs. IL-2 and interferon gamma can be detected from single T lymphocytes. The role of various second messenger pathways in the expression of cytokines is easily studied using the technique. The patterns of cytokine mRNAs expressed by cells derived from tissue of patients affected with inflammatory diseases such as rheumatoid arthritis and ARDS will be displayed. MAPPing permits definition of complex mixtures of cell activation molecules without the need to perform individual functional assays. This will facilitate an understanding of which factors alone or in specific combinations mediate the inflammatory response.

REGULATION OF THE EXPRESSION AND TurnsRUER OF INTERLEUKIN-2 RECEPTORS BY GLUTATHIONE, Chi-Ming Liang, Jay Epstein, Nancy Lee and Shi-Mei Liang, Division of Blood and Blood Products and Division of Cytokine Biology, Food and Drug Administration, Bethesda, MD 20892. Glutathione (GSH) regulates the binding, internalization and degradation of interleukin-2 (IL-2). In this study, we examined the mechanism of GSH actions. We found that both the level of intracellular GSH and the cellular actions of IL-2 were dependent on the duration of GSH treatment. Northern blot and slot blot analysis for mRNA of IL-2Rαp55 and IL-2Rαp70, the two major components of the high affinity IL-2 receptors, showed that the IL-2Rαp55 mRNA level reached maximum 4 h after GSH treatment and then declined, whereas the IL-2Rαp70 mRNA level increased 1 h after GSH treatment and then remained relatively constant. GSH treatment shortened the half-life (from 5 h to < 3 h) and increased the turnover of surface high affinity IL-2 receptors. These results suggest that GSH may potentiate the binding, internalization and degradation of IL-2 by enhancing the expression and turnover of the IL-2 receptors.
Molecular Pathways of Cytokine Action

CD 224  **EFFECTOR INTERACTIONS OF LYMPHOKINES PRODUCED BY T₁ AND T₂ HELPER CELLS.** Michael Lohoff, Ernst Marsig, Frank Sommer and Martin Röllinghoff, Institute for clinical Microbiology, Wasserturmstr 3, 852 Erlangen, FRG.

The interactions of lymphokines produced by T₁ and T₂ cells were assayed in various test systems. In the vesicular stoflatitis Ffirus system, murine fibroblast cells are protected from viral lysis when they are preincubated with the T₁ derived lymphokine IFNγ. As we show, the protective effect was completely abrogated in the simultaneous presence of supernatant (SN) of a T₂ cell clone. Closer analysis demonstrated that the blocking effect of the T₂ cell SN was eliminated by anti-IL-4 antibodies and that r IL-4 was similarly able to block the effect of IFNγ. In addition, rIL-4, like the T₂ SN, was able to abrogate the virus-protective effects of IFN-alpha and IFN-beta in the same test system.

The interactions of lymphokines produced by the two T-cell subsets were also tested on the IL-4 and/or IL-5 dependent B-lymphoma cell line BCL-1. No proliferation inducing effect on BCL-1 cells was observed in supernatants containing IFNγ in addition to IL-5. Proliferation took place in the presence of anti-IFNγ antibodies. Thus, when BCL-1 cells are used to measure IL-5, conclusions on the IL-5 content of a test supernatant containing IFNγ can only be drawn, if the test is performed in the presence of anti-IFNγ and anti-IL-4 antibodies.

CD 225  **EFFECTS OF CYTOKINES ON T CELL GROWTH.** Londei., M, Verhoef, A, Hawrylowicz, C., Groves, J. and Feldman. M.

The Charing Cross Sunley Research Centre, Lurgan Avenue, W6 8WL, London U.K.

Interleukin 2 (IL-2) was originally thought to be the only T cell growth factor, but subsequent work has shown that IL-4 also has this property. IL-1, GM-CSF, IL-6 and TNF have also been shown to influence the proliferation of some mature T cell populations. Recently we observed that IL-3, a growth factor that acts on multilineage precursor and progenitor cells, stimulates a subpopulation of peripheral T cells with the rare phenotype of CD4⁻ CD8⁻ TCRαβ. We therefore tested whether IL-7, a newly cloned cytokine, which similarly stimulates lymphoid precursors of the B and T lineage, acts on mature human T cells. We observed that T cell clones of four different phenotypes, namely CD4⁺ TCRαβ, CD8⁺ TCRαβ, CD4⁻ CD8⁻ TCRαβ and γδ were stimulated to proliferate by recombinant IL-7, as were peripheral blood mononuclear cells (PBMC) and purified T cells, with or without costimulation with anti CD3 antibody. Our results thus show that IL-7 acts on both the mature and the immature pool of human T cells. T cell growth may be influenced by cytokines either directly, or indirectly by stimulating the production of other growth factors and their receptors. There is evidence that IL-7 may act trough an IL-2 dependent mechanism. In view of the potency of the IL-7 effect on T cell growth, we investigated whether this may be mediated indirectly by IL-2 acting on IL-2 receptor. However experiments performed in the presence of saturating concentration of anti IL-2 antibody, anti IL-2 receptor antibody and/or Cyclosporin A were unable to block the proliferative response mediated by IL-7. These data indicate that other lymphokines, besides IL-2 or IL-4, control the clonal expansion of human mature T cells.

CD 226  **INTERLEUKIN 1 INHIBITION OF COLLAGEN BIOSYNTHESIS BY FIBROBLASTS. A POSSIBLE IMPLICATION OF ADENYLATE CYCLASE.** MAUVIEL, A., REDINI, F. and PUJOL J.-P.. Laboratoire de Biochimie du Tissu Conjonctif, C.H.U. Côte de Nacre, 14033 CAEN Cédex, FRANCE.

Pharmacological agents that increase cellular contents of cyclic AMP were studied for their ability to affect collagen production by human dermal fibroblasts, either in the presence or absence of Interleukin 1, during 24 h-incubations. Forskolin and cholar toxin, two activators of adenylate cyclase, as well as 3-isobutyl-1-methylxanthine (IBMX), a cyclic nucleotide phosphodiesterase inhibitor, and Prostaglandin E2 (PGE₂), an arachidonate metabolite known to increase the levels of cyclic AMP in fibroblasts, induced a dose-dependent inhibition of collagen synthesis and had additive effects with IL-1. The inhibition of collagen synthesis exerted by Interleukin 1 was accompanied by a huge secretion of PGE₂ in the culture medium ; however, the inhibitory effect of IL-1 on collagen production persisted when PGE₂ release was blocked by indomethacin. This latter drug could not further prevent the inhibitory effect exerted by forskolin, cholera toxin or IBMX, suggesting the involvement of cyclic AMP, even when PGE₂ secretion was abolished.

Taken together, these results suggest that IL-1 inhibition of collagen synthesis may be mediated in part by PGE₂ and subsequent cAMP accumulation, but also by a direct action of IL-1 on intracellular cyclic AMP levels, through a direct activation of adenylate cyclase or through Gs-coupled proteins.
Molecular Pathways of Cytokine Action

CD 227  INDUCTION OF CYTOKINE PRODUCTION FROM HUMAN MONOCYTES STIMULATED WITH ALGINATE. Marit Otterlei*, Kjetill Østgaard, Gudmund Skjåk-Bræk, Olav Smidsrød and Terje Espevik*, *Inst. Cancer Research and Inst. Biotechnology, University of Trondheim, Norway.

Alginates are polysaccharides composed of mannuronic acid blocks (M-blocks), guluronic acid blocks (G-blocks) interspaced with regions of alternating structures (MG-blocks). Alginates can be used for immobilization of different cell types, and it is therefore important that they are not giving rise to unwanted immune reactions. In this study we have examined the ability of alginates and their components to stimulate human monocytes to produce tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6). Alginates stimulated the monocytes to produce high levels of all three cytokines. The M-blocks and the MG-blocks, but not the G-blocks, were potent stimulators of TNF-α, IL-1 and IL-6 production. The results demonstrate that the mannuronic acid residues are the active cytokine inducers in alginates.

CD 228  TNF-β UPREGULATES TNF-α GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES, Laurie B. Owen-Schaub and Elizabeth A. Grimm, Department of Tumor Biology, Box 79, Univ. TX M.D. Anderson Cancer Center, Houston, TX 77030. TNF occupies a central role as a potent modulator of NK, LAK, and cytotoxic T cell function and proliferation. Evidence suggests that constitutive TNF production occurs in several lymphoid malignancies. This aberrant cytokine production is thought to play a role in the autocrine growth of these cells (B lymphoblastoid) and contribute to the pathophysiology of disease presentation (osteolytic bone lesions in adult T cell leukemia). In the course of studying the cellular and molecular basis for TNF-α gene regulation in normal IL-2 activated lymphocytes, we observed that exogenous TNF-β strongly upregulated TNF-α gene expression and protein secretion. For these studies, total cellular RNA and culture supernatants were obtained from lymphocytes activated with hrIL-2 (ranging from 10-500 U/ml) and rhTNF-β (ranging from 0-5000 u/ml). Steady state TNF-α mRNA was measured 1-3 days after the addition of rhTNF-β using S1 nuclease analysis and protein secretion determined by TNF-α specific ELISA. The data show a superinduction of TNF-α with a 2-5 fold increase in TNF-α mRNA and a 5-20 fold increase in secreted protein. These experiments demonstrate that TNF-β can regulate TNF-α expression and suggest that cellular dysregulation involving autocrine TNF production may be exacerbated by this positive feedback pathway.

CD 229  A POSSIBLE ROLE FOR LYMPHOKINES IN THE ACTIVE SUPPRESSION MECHANISM OF NEONATALLY-INDUCED CLASS II TOLERANCE. T. J. Powell and J. W. Streilein. University of Miami School of Medicine, Miami, FL 33133.

Neonatally-induced tolerance of Class II alloantigens in mice of the A strain background is maintained primarily by active suppression, with little or no contribution from clonal deletion. While a majority of Class II-tolerant mice (A.TL to1 A.TH, A.TH to1 A.TL) retain tolerogen-reactive lymphocytes detectable in the mixed lymphocyte reaction, they fail to develop tolerogen-specific cytotoxic lymphocytes (CTL). The MLR responding cells of tolerant mice differ from those in the normal mice, in that the tolerant MLR is dominated by a high frequency of IL-4-producing cells while the normal MLR is dominated by IL-2-producing cells. Recent experiments have suggested that this shift in the functional properties of Th cells may play a role in the active suppression of specific immune effector function. In coculture MLR experiments, lymphocytes from tolerant mice suppressed two important effector functions of normal lymphocytes responding to tolerogen: the production of tumor necrosis factor (TNF) and the generation of CTL. The addition of recombinant IL-4 to normal MLR was shown to suppress the production of TNF, but not the generation of CTL (in the presence of exogenous IL-2). Therefore, it appears that suppression in neonatally-induced Class II tolerance is mediated by a unique profile of lymphokines secreted by tolerogen-specific cells. Current experiments are exploring the possibility that suppression of tolerogen-specific CTL responses is dependent on a lymphokine other than IL-4.
Molecular Pathways of Cytokine Action

CD 230 NOVEL IMMUNOMODULATORS WITH PRONOUNCED IN VIVO EFFECTS CAUSED BY STIMULATION OF CYTOKINE RELEASE, L.-T. Rasmussen and R. Seljelid, Institute of Medical Biology, University of Tromsø, 9000 Norway.

Aminated αl-3D polyglucose (AG) and αl-3D polyglucose attached to plastic microbeads (GDM) are immunomodulators with strong effects in vivo and in vitro. (Seljelid, R. Tumor regression after treatment with aminated αl-3D polyglucose is initiated by circulatory failure. Scand. J. Immunol. 29, 181-192, 1989; Rasmussen, L.-T., Seljelid, R. The modulatory effect of lipoproteins on the release of interleukin 1 by human peritoneal macrophages stimulated with αl, 3-D-polyglucose derivatives. Scand. J. Immunol. 29, 477-484, 1989.)

AG causes total regression of Meth A sarcoma in mice. This effect can be understood in terms of a concerted action of local and systemic cytokines. AG and GDM protect against otherwise lethal infections in mice. Also this can be explained partly by the effect on AG and GDM on the release of cytokines and prostaglandin E2.

CD 231 IL-1 ENHANCES HUMORAL IMMUNE RESPONSE TO HEPATITIS B SURFACE ANTIGEN. STEVEN M. ROSEN AND NEIL GOLDSTEIN, IMCLEONE SYSTEMS INCORPORATED, NEW YORK, NEW YORK 10014.

In order to evaluate the role of IL-1 as a potential adjuvant for vaccines we determined the ability of this cytokine to enhance the humoral immune response to Hepatitis B surface antigen (HBs Ag). Groups of Balb/c mice (8-10 weeks old) each were injected with one dose of Hepatitis B surface antigen (10 mcg/animal) with 2, 20 or 200 ng of IL-1. Control groups were vaccinated with S Ag emulsified in complete Freunds adjuvant (CFA) or with S antigen alone. Animals were bled at four and eight weeks and the sera were tested for anti-HBs Ag activity by ELISA. At four weeks all of the IL-1 concentrations were more effective in generating a humoral response to S antigen when compared to sera from animals immunized with S antigen alone. However, none of the IL-1 concentrations were as effective as CFA. At eight weeks, however, IL-1 at 20 ng per animal was found to be the most effective approaching that seen with CFA. The adjuvant hierarchy was 20 ng > 2 ng > 200 ng. These experiments appear to indicate that IL-1 may be useful as an adjuvant for vaccines. The lymphokine seems to have a window of effectiveness.

CD 232 INDUCTION OF TUMOR NECROSIS FACTOR-ALPHA AND INTERLEUKIN-1-BETA BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR TREATMENT IN PATIENTS WITH LUNG CANCER. Mary Jane Thomassen, Ronald Bukowski, Joyce Antal, Barbara Barna, David Meeker, Herbert Wiedemann, Vicky Gibson, Muzaffar Ahmad. Cleveland Clinic Foundation, Cleveland, OH 44195.

The effect of recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) (Hoechst-Reesel) treatment on monocyte activity was studied in five patients with lung cancer. Treatment consisted of constant intravenous infusion of 60 µg/m²/day (n = 3) or 125 µg/m²/day (n = 2) for 14 days. Because of limitations in cell yields not all studies were carried out with each patient. All patients demonstrated increased numbers of circulating monocytes with treatment. Monocytes were obtained from patients prestudy, day 4, day 11 and post treatment and evaluated for expression of mRNA for tumor necrosis factor-alpha (TNF) and interleukin-1-beta (IL-1) as well as secretion of TNF and IL-1. RNA was extracted from monocytes before culture and evaluated by Northern blot analysis. Monocytes from all patients obtained during treatment demonstrated enhanced TNF and IL-1 mRNA expression versus prestudy. TNF and IL-1 were measured by enzyme immunoassay (sensitivity = 40 pg/ml TNF and 20 pg/ml IL-1) in supernatant fluids from 24 hr cultures. All monocyte cultures demonstrated 10-15 fold enhancement of TNF secretion versus prestudy levels and IL-1 levels were enhanced 5-10 fold. These data demonstrate that GM-CSF treatment not only increases the effector cell pool but also enhances monocyte TNF and IL-1: cytokines associated with enhanced tumoricidal activity.
CD 233 HUMAN BLOOD-DERIVED DENDRITIC CELLS DO NOT PRODUCE IL-1, IL-6 OR TNF-α, Jukka M. Vakkila, Marja Sihvola and Mikko Hurme, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland. Little is known about the necessary non-antigen specific signals delivered to T cells by DC. Recent data shows that several monocyte-derived factors like IL-1-α, IL-1-β, IL-6 and TNF-α have enhancing activity in T cell proliferative responses. We studied the production of the above-mentioned cytokines and their mRNA by DC and monocytes separated from human peripheral blood. The intracellular expression of the proteins were studied at a single cell level, which allowed a precise identification of the cytokine producing cell. The supernatants and cell lysates were studied with ELISA (IL-1-α, IL-1-β and IL-6). Several approaches were taken to stimulate the production of IL-1-α, IL-1-β, IL-6, and TNF-α by DC. These included the incubation of the DC either in the presence of LPS, rlL-1 or non-antigen specific signals delivered to T cells by DC. Recent data shows that several

Neuronal Modulation of Multidrug Resistant Glycoprotein - P170 Expression

Patricia D. Baker and Charles H. Evans, Laboratory of Biology, National Cancer Institute, Bethesda, MD 20892

P-glycoprotein (P170) is a 170 kD cell membrane protein associated with multidrug resistance. The quantity of this protein in tumor cells may be important in the sensitivity of cells to drug uptake and metabolism. The doxorubicin drug sensitive human myeloma cell line, 8226/S, and its doxorubicin resistant derivative cell line, 8226/Dox<sub>40</sub>, provide an excellent model to study the relationship of P170 to chemotherapeutic drug resistance. Leukoregulin (LR), a 50 kD cytokine, increases plasma membrane permeability and uptake of doxorubicin within minutes in many tumor cells. Membrane permeability is increased within 5 minutes and doxorubicin uptake is increased within 15 minutes in LR treated K562 human erythroleukemia cells. 8226/S and 8226/Dox<sub>40</sub> cells are also sensitive to the membrane permeabilizing effect of leukoregulin. The sensitive cell line (8226/S) requires LR and less time to achieve maximum membrane permeability than the resistant cell line (8226/Dox<sub>40</sub>). P170 glycoprotein expression was studied by flow cytometry using P-glycoCHEK FITC-C21S,(Centocor, Inc., Malvern, PA), a fluorescein labeled murine monoclonal antibody which reacts with the P170 epitope expressed on the internal surface of mammalian cell membranes. K562 cells incubated with 2 units of LR for 2 hours showed a 40% decline in the expression of P-glycoprotein. The kinetics and reversibility of LR modulation of P-glycoprotein in K562 and 8226 cells and the effects of other cytokines on epitope expression were also examined.

CD 301 NEUROBLASTOMA CELLS PRODUCE IL-6, Florence M. Hofman, Neil Sidell, David R. Hinton, Jean E. Merrill, Department of Pathology, USC, Los Angeles, CA 90033 and Departments of Pathology and Neurology, UCLA, Los Angeles, CA 90024.

Human IL-6 is a 26 Kd protein originally identified as a T cell derived lymphokine inducing antibody production in B cells. Recent studies have shown that glial cells of the central nervous system can also produce IL-6. In this study we report that in the two out of three human neuroblastoma cell lines tested, IL-6 was produced constitutively, and that following differentiation induced by retinoic acid (RA), the number of cells producing this cytokine was greatly reduced. LA-N-5, IMR32, and SHF were either left unstimulated or exposed to 5X10<sup>-7</sup>M RA. LA-N-5 and IMR32 when exposed to the RA demonstrated typical neurite outgrowth, while unstimulated cultures remained in cluster formation. SHF did not exhibit neurite outgrowth in the presence of RA. IL-6 production was quantitated using immunocytochemistry with a polyclonal anti-human IL-6 reagent on cell preparations grown in culture chambers or flasks. The results show that for LA-N-5 and IMR32, there was significant staining for IL-6 (30% and 10% respectively). In the presence of RA, the number of IL-6 positive cells dropped markedly (5% and 0% respectively). No IL-6 positive cells were detected in SHF cultures in the presence or absence of RA. Cryostat sections of normal human adult brain did not exhibit IL-6 positive neuronal cells. These data indicate that primitive, poorly differentiated neuroblastoma cells produce IL-6, while the more differentiated cells show a reduced production of this cytokine. This suggests that IL-6: (1) may be a marker for the immature neuroblastoma cells; and (2) may be involved in tumor cell growth and dissemination.
CD 302  OPTIMAL CONDITION OF BISPECIFIC mAb TARGETING OF IN VITRO EXPANDED PBL FOR IMMUNOTHERAPY. C.H.J. Lamers*, C.P.M. Ronteltap, E. Braakman, R.L.H. Bolhuis., Dept. of Immunology, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands, Dept. of Tumor Immunology, Radio Biological Institute, NWO Health Organization, Rijswijk, The Netherlands. We studied the prerequisites for the in vivo therapeutic application of in vitro expanded lymphocytes, retargeted with the bispecific mAb (bsAb) against CD3 and a human ovarian carcinoma antigen MOV18. Cytotoxic T cells (CTL) were generated from PBL (starting with $10^6$ lymphocytes) derived from both healthy donors and ovarian carcinoma patients by repeated stimulation with PHA or anti-CD3 mAb (OKT3) and IL-2. Between days 12–20 (medium, day 14) yield was $5*10^9$ lymphocytes required for therapy. The triggerability of lymphocytes for cytolysis was tested using anti-3 expressing hybridoma cells and bsAb plus ovarian carcinoma cells (IGROV) or fresh ovarian carcinoma cells. Lytic activity was optimal between days 10–20 of culture. Removal of excess bsAb prior to cytolysis does not affect the level of target cell lysis. Moreover, triggerability of the lymphocytes is not inhibited when the lysis takes place in human serum or in ascitic fluid derived from ovarian carcinoma or non-ovarian carcinoma patients (upto 100% concentration).

CD 303  A DOMINANT PERMISSIVE ROLE FOR GLUCOCORTICOIDS IN IL-1 INDUCTION OF ANGIOTENSINOGEN: INTERACTION BETWEEN INDUCIBLE ENHANCERS. David Ron, Allen R. Brasier, and Joel F. Habener, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02114. We demonstrate by direct-site mutagenesis that IL-1 activates transcription of the rat angiotensinogen gene in hepatoma cells through a NFkB binding cis-active element located adjacent to the glucocorticoid response element (GRE), 545 base-pairs upstream from the transcription start site. Monocyte conditioned media induces a 50kd, NFkB-like protein in HepG2 cells. This induction occurs in the absence of new protein synthesis and is not dependent on glucocorticoid stimulation. However transcriptional activation of the endogenous angiotensinogen gene, as well as reporter constructs, containing the NFkB-binding site and the GRE, occurs only in the presence of glucocorticoids, suggesting an interaction between the IL-1 inducible, NFkB binding enhancer and the GRE.

CD 304  ALTERNATIVE PATHWAYS FOR GENERATING CYTOTOXIC LYMPHOCYTES IN VIVO USING ANTI-CD3, INTERLEUKIN-2 (IL-2) AND TUMOR NECROSIS FACTOR-a. Stephen C. Yang, Elizabeth A. Grim, and Jack A. Roth, Department of Thoracic Surgery and Tumor Biology, M. D. Anderson Cancer Center, Houston, TX 77030 The purpose of this study was to generate lymphokine-activated killer activity (LAK) via alternative pathways using combinations of biologic agents. The effect on established pulmonary metastases (PM) produced from B16, K175M2 or MCA-203 cell lines was assessed using the monoclonal antibody anti-CD3, followed by IL-2 and TNF in B6C3F1 mice. Treatment of PM began with a single 5 µg i.p. dose of anti-CD3 on day 3, followed by either IL-2 alone or 4-fold less IL-2 with TNF (25,000 U/day) given at 3 day intervals. PM were counted on day 14. A single dose of anti-CD3 followed by low dose IL-2 and TNF caused the greatest reduction of metastases compared to higher doses of IL-2 alone, or IL-2 + TNF. Reduction of metastases (median 91%) using the three agents in combination was observed in all tumor models (p < 0.006), and was equal to or exceeded that achieved by 9-fold higher concentrations of IL-2 alone. Treatment with anti-CD3/IL-2/TNF significantly prolonged survival, and resulted in 60% of mice with B16 or K175M2 PM achieving long-term survival 120 days. This was superior to single agents or other combinations. LAK and natural killer activities of splenocytes in vitro increased following anti-CD3/IL-2/TNF treatment, and were consistently greater than that generated with 4-times more IL-2 alone. The anti-CD3 activated splenocytes were a heterogeneous population of T-cells, with more Lyt2' cells than splenocytes from mice treated with doses of IL-2 alone. Analysis of tumor infiltrating lymphocytes (TIL) obtained from MCA-203 PM showed a greater proportion of Lyt2' cells in anti-CD3 treated mice compared to IL-2 alone (30.7% vs 38.1%), but a lower proportion of Lyt4' cells (19.0% vs 46.6%). These results indicate that the sequential use of anti-CD3, IL-2 and TNF for LAK induction and maintenance potentiated anti-tumor activity, and suggests novel strategies for combination immunotherapy.
Molecular Pathways of Cytokine Action

**CD 400**

**CYTOKINE EXPRESSION BY THE MURINE PLACENTA**, Mary Crainie, Larry Gullbert* and Thomas G. Wegmann, Department of Immunology, University of Alberta, Edmonton, Alberta, T6G 2H7 *Canadian Red Cross Blood Transfusion Service, Edmonton, Alberta, T6G 2K8

Immune interactions can be attributed to the release of cytokines, a group of soluble molecules which act as intercellular activation signals between cells of the immune system. It has become apparent that cytokines are neither produced nor utilised exclusively by immune cells. An excellent example is the placenta, which both produces and responds to what were formerly considered to be exclusively hematopoietic system cytokines. The human placenta is documented to produce a diverse number of immune regulatory molecules and growth factors including IL-1, IL-2, TGF-β, GM-CSF and CSF-1. The latter two are directly implicated in promoting placental function and fetal survival. In contrast to the human placenta there is little information available on cytokine production by the murine placenta. We have analysed the expression of cytokine genes in the murine placenta using a panel of probes which detect IL-1, IL-2, IL-3, IL-5, TNF, GM-CSF and CSF-1. Transcripts for IL-1 and CSF-1 were identical in size to those found in macrophage and fibroblast cell lines. On the other hand, mRNA transcripts for GM-CSF and TNF were larger. Present the significance of these high molecular weight cytokine transcripts is unclear but may reflect alternative promoter utilisation by the placenta during development.

**CD 401**

**A MONOKINE THAT REGULATES EOSINOPHIL ARACHIDONIC ACID METABOLISM IS A MYELOID CELL SURFACE MARKER ASSOCIATED WITH MONOCYTE DIFFERENTIATION** P. Xavier Eilas, M. S. Pombo de Oliveira, B. Curi, A. Bercho, D Goldberg Tabak and M. I. Gaspar Eilas, Instituto Oswaldo Cruz and Instituto Nacional de Cancer, Rio de Janeiro, Brazil

The Eosinophil Cytotoxicity Enhancing Factor (ECEF) is secreted by human monocytes and by PMA- and LPS- stimulated U937 histiocytic lymphoma cells, and regulates eicosanoid production and cytotoxic activity of eosinophils. Monoclonal antibodies to ECEF recognize a 13 kDa acidic protein in secretion products as well as an integral membrane protein of macrophages and resting U937 cells. Membrane ECEF (mECEF) expression correlates with acquisition of mature macrophage characteristics by U937 cells after PMA stimulation. Positive selection of mECEF-positive U937 cells led to the isolation of a variant cell line that responds more efficiently than unselected cells to PMA as a differentiation stimulus, as shown by a average 4-fold increase in the number of fully differentiated, adherent macrophages in cultures of PMA-stimulated, positively selected cells relative to PMA-stimulated controls. This is associated with an increased sensitivity to PMA and with a faster kinetics of response. mECEF is a surface marker strongly expressed in some myeloid leukemias, both of the monocytic and granulocytic lineages, but absent from a variety of other hematopoietic malignancies. In peripheral blood, strong mECEF expression is found in 70% of monocytes; weak expression was seen in purified, resting T lymphocytes; positivity is therefore associated with cell types known to secrete ECEF. These findings suggest that, besides regulating arachidonate metabolism and cytotoxicity in target cells, this monokine, in a surface-associated form, plays a role in myelomonocytic differentiation and behaves as an activation marker.

**CD 402**

**EXPRESSION OF IL-10 mRNA AND IL-1 RECEPTOR DURING MONOCYTIC DIFFERENTIATION INDUCED BY DIBUTYRYL CAMP OR PHORBOL MYRISTATE** Mikko Hurme, Tapani Ronni, and Elina Serkkola. Department of Bacteriology and Immunology, University of Helsinki, SF-00290, Helsinki, Finland. Both elevation of cAMP and activation of protein kinase C function as differentiation inducing signals in the promyelocytic HL 60 leukemia cells, resulting in appearance of cells with monocytic markers. We have now used these two differentiative signals to study the appearance of the interleukin-1 (IL-1) receptor as well as the IL-18 mRNA during monocyte differentiation. The data obtained demonstrated that both dibutyryl (db) CAMP, a soluble structural analog of CAMP, and protein kinase C activating phorbol myristate (PMA) were able to induce the expression of the IL-1 receptor (as detected by the binding of 125I-labeled IL-1). In contrast to this, only PMA treatment induced the expression of IL-18 mRNA. However, when the effect of the PMA plus dbCAMP combination was tested, it was evident that dbCAMP greatly enhanced the PMA-induced IL-18 mRNA expression. Taken together, these data indicate that expression of IL-1 and IL-1 receptor are differentially regulated during the differentiation of monocytes.
CD 403  CYTOKINE EFFECTS ON CD14 ANTIGEN EXPRESSION IN MONOCYTES AND MACROPHAGES, Regine Landmann, Reto Obrist, Jean-Paul Obrecht, Division of Oncology, Department of Research, University Hospital Basel, Switzerland

CD14 antigen expression increases in monocytes differentiating into macrophages, and it is reduced by rIFNγ in monocytes in vitro (Landmann, Cell Immunol 117: 45, 1988). In the present study CD14 membrane expression was investigated in cultures of human mononuclear leukocytes (PBL), of elutriated, purified monocytes and of blood monocyte derived macrophages which had been incubated with rIL1, rIL2, rIL3, rIL4, rIL6, rTNFα, rGM-CSF, rIFNα and rIFNγ. rIFNα and rIL2 reduced CD14 in PBL cultures, their effect was mediated by endogenous IFNγ, because it was abolished by simultaneous addition of an anti-IFNγ antibody. rIFNα was ineffective in purified monocytes or macrophages. rIL2 slightly enhanced CD14 expression in purified monocytes. rIFNγ and rIL4 reduced CD14 expression strongly in purified monocytes, weakly in macrophages. rIL1, rIL3, rIL5, rIL6, rTNFα and rGM-CSF did not change CD14 expression in monocytes or macrophages. In summary, CD14 antigen expression is modulated only by rIFNγ, rIL4 and rIL2, the cytokine effect is stronger in monocytes than in differentiated macrophages.

CD 404  CHARACTERIZATION OF LONG-TERM CSF-1 STIMULATED HUMAN MONOCYTES. *Steven G. Reed, *Donna M. Russo, ‡John Ho and §Kenneth H. Grabstein. *Seattle Biomedical Research Institute, Seattle, WA 98109, ‡Cornell University Medical College, New York, NY 10021 and §Immunex Corporation, Seattle, WA 98101.

We have cultured peripheral blood monocytes (PBM) from normal donors and from patients with the parasitic infection, leishmaniasis, for several months in the continued presence of CSF-1. A 100-fold increase or greater in cell numbers has been obtained with several donors. Cells have been maintained in culture for up to three months. We have measured several monocyte functions, including surface antigen expression, antigen presentation and oxidative burst activity. Progressive changes have been noted in monocyte functions with time in culture. Fresh monocytes have high respiratory burst activity upon stimulation with PMA, ionomycin and F-met Leu-Phe (FMLP). With time in culture, the ability of these cells to undergo increased oxidative metabolism in response to ionomycin and FMLP was significantly reduced although the response to PMA remained strong. Antigen presentation by long-term (3 month) cultured monocytes from a leishmaniasis patient was also observed. Cultured monocytes from this patient were effective in presenting leishmania antigen to an autologous T-cell line in a proliferation assay. The ability of these cells to present antigen did not decrease with time in culture. FACS analysis of cultured monocytes revealed that the expression of monocyte markers varied. After approximately 8 weeks in culture, the cells became UCHM1 negative, and approximately 50% of the cells were LFA-1 positive, and 60% expressed DR.

CD 405  DIFFERENTIAL REGULATION OF LEUKEMIA INHIBITORY FACTOR- AND INTERLEUKIN-6-INDUCED EXPRESSION OF SURFACE MARKERS IN DIFFERENTIATING MYELOID CELLS, Stefan Ruhl, Matthias Bickel and Dov H. Pluznik, Division of Cytokine Biology, Center for Biologics, Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

Recombinant leukemia inhibitory factor (rLIF) and recombinant interleukin-6 (rIL6) induce expression of Fc receptors (FcR) and interleukin-2 receptors (IL2R) during the process of differentiation of murine myeloid leukemia M1 cells to mature macrophages. In the present study, we show that the expression of these surface markers is regulated by distinct mechanisms involving metabolites of the arachidonic acid pathway. Cytofluorometry utilizing monoclonal antibodies specific to FcR and IL2R revealed different kinetics of expression of these surface markers. Expression of FcR increased continuously after induction with rLIF or rIL6 and plateaued at 48 hours. In contrast, expression of the IL2R reached its peak 24 hours after induction and declined thereafter almost to background levels at 48 hours. Addition of prostaglandins PGE2, PGD2, and PGF2α with these cytokines suppressed IL2R but not FcR expression at all time points. Addition of indomethacin, an inhibitor of prostaglandin synthesis, together with rLIF and rIL6 enabled continuous IL2R expression up to 48 hours, presumably by blocking prostaglandin synthesis in differentiating M1 cells. Interestingly, indomethacin had no effect on FcR expression. Adding back PGE2, PGD2, and PGF2α after inhibition of prostaglandin synthesis restored the original kinetics for IL2R expression, while FcR expression was not affected. Our data suggest that expression of IL2R in differentiating M1 cells is influenced by arachidonic acid metabolites and follows a different regulatory pathway than FcR expression.
Resistance to Bacterial and Parasitic Disease

CD 407 IFN-\(\gamma\) INDUCES IgM LYMHPHOMA CELL LINE 29M10 TO DIFFERENTIATE TO IgGl BEARING CELLS, AND TO SECRET IgGl ISOTYPE, Israel Zan-Bar, Irit Altboim and Yael Porat, Department of Human Microbiology, Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Murine B cell lymphoma 1.29 cell lines can be stimulated by LPS, IL-4, IL-5 and other reagents to secrete Ig and to differentiate from Ig\(\mu\) to IgG2a, IgG1, IgE, or IgA cells. In the present work the effect of IFN-\(\gamma\) on the Ig secretion, proliferation, and differentiation of 1.29 cell lines were examined. No direct or indirect effect of IFN-\(\gamma\) on the cell growth were detected. IFN-\(\gamma\) induces in LPS stimulated 1.29 cells isotype switch and secretion of IgGl. In addition IFN-\(\gamma\) induces augmentation in the secretion of IgGm and IgA, and has no effect on the secretion of IgG2a, IgG2b or IgG3. Simultaneous stimulation of the lymphoma cells with IL-4, IFN-\(\gamma\), and LPS reveals that IFN-\(\gamma\) antagonized the effects of IL-4 in elevation of IgGm and total Ig secretion and abrogates the secretion of IgE. IFN-\(\gamma\) synergizes IL-4 in IgG1 secretion. Maturation of the cells with IFN-\(\gamma\) for 48 hrs prepares them to secrete IgGl on subsequent stimulation of the cells with LPS. Similar observation of prerecognition of the lymphoma cell lines with IL-4 or IL-6 were obtained.

CD 406 CHARACTERIZATION OF PLACENTAL-DERIVED GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTORS. Kathleen T. Shiverick, Theresa Medrano, Susan Ovilie and James R. Zucali*, Department of Pharmacology and Therapeutics and Department of Medicine*, University of Florida College of Medicine, Gainesville, FL 32610, USA

Recent evidence suggests a role for hematopoietic colony stimulating factors (CSFs) in placentation growth and development. The present study investigated whether rat placental tissue secretes factors which stimulate granulocyte-macrophage colony formation (GM-CSF) in vitro. Gestation day 15 placentas were dissected into separate decidua basalis, basal zone, labyrinth and yolk sac tissues which were cultured in Eagle's Modified MEM for 24 hours. Placental-conditioned media was then bioassayed for CSF activity using rat bone marrow cells grown in 2% methylcellulose with 10% fetal calf serum; positive controls for CSF activity were endotoxin-stimulated rat serum. Decidua-conditioned media (DEC-CM) had the highest CSF activity, which was retained after dialysis. The morphology of DEC-CM-stimulated colonies showed 60% M, 6% G and 34% mixed GM. Explant culture of DEC in the presence of tunicamycin resulted in a concentration-dependent loss of CSF activity in DEC-CM. Ammonium sulfate fractionation of DEC-CM isolated CSF activity in the 80% precipitate and supernatant fractions. A possible paracrine role for DEC-CSFs was further examined in experiments which characterized the effects of recombinant CSFs on trophoblast cell synthesis. Explants of basal zone tissue were cultured with [\(^3\)H]methionine in the presence of 100 U/ml rec murine GM-CSF or rec human CSF-1 for 24 hours. Analysis of radiolabeled proteins in the culture medium by 2-dimensional polyacrylamide gel electrophoresis showed that mCSF-CSF markedly increased the secretion of M: 22,000 rat placental lactogen IL, while hCSF-1 increased secretion of the M: 30,000 prolactin-like protein-A. Thus, data support the hypothesis that maternal decidua tissue produces CSFs which may have regulatory effects on adjacent fetal trophoblast cells in the rat placenta.

Resistance to Bacterial and Parasitic Disease

CD 500 ROLE OF IL-1 IN HEMATOPOIETIC RECOVERY AND HOST RESISTANCE TO INFECTION FOLLOWING CYTOTOXIC THERAPY. R. Gladue, A. Cunningham, and I. Otterness. Department of Immunology and Infectious Diseases, Pfizer Central Research, Groton, CT 06340. The relationship between IL-1, peripheral blood neutrophils, and host resistance was examined. Mice were treated with 150 mg/kg of 5-fluorouracil (5-FU) and examined daily for blood neutrophil numbers and hypersusceptibility to infection with S. aureus (IP) and P. aeruginosa (aerosol). Hypersusceptibility to infection was observed through day 8, although normal levels of blood neutrophils did not return until day 11-12. Mice IL-1 \(\alpha\) (0.2 - 1.0 ug/animal) accelerated neutrophil recovery (day 6 vs day 11) and enhanced host resistance to infection. Similar results were obtained with mIL-1 \(\beta\). Administration of IL-1 \(\alpha\) for the 3 days following 5-FU gave an identical response to animals treated with 8 daily injections. Treatments initiated 4 days after 5-FU increased blood neutrophil numbers, but only after day 8; a time when placebo-treated animals were no longer hypersusceptible to infection. Administration of anti-IL-1 \(\alpha\) or anti-IL-1 \(\beta\) inhibited the effects of IL-1 on neutrophil restoration and anti-infective activity. Treatment with anti-IL-1 \(\alpha\) or treatment with anti-IL-1 \(\beta\) did not impair normal recovery following 5-FU (day 11). Titters of anti-IL-1\(\alpha\) and \(\beta\) antibody measured in treated mice were determined and found to remain high and to neutralize exogenously administered IL-1 in normal mice through the 11 day period. Therefore, although exogenously administered IL-1 enhances neutrophil recovery and increased host resistance to infection, failure of neutralizing antibody to inhibit normal recovery after 5-FU suggests that IL-1, (or at least circulating IL-1), may not play a role in the recovery of normal hematopoiesis after cytoreductive therapy.
INTERFERON-γ CONTRIBUTES TO THE LETALITY OF ENDOXEMIA, Frederick P. Heinze, Department of Medicine, UCSF Medical Center, San Francisco, CA 94143. Experimental animals injected with lipopolysaccharide (endotoxin) develop widespread and fatal tissue damage that is mediated by proinflammatory cytokines. Pretreatment of C57BL/6J mice with 20 μg of rMuIFN-γ for 2 consecutive days significantly diminished survival compared to control mice upon challenge with 1 mg of Salmonella enteriditis LPS (60% vs 0%). MuIFN-γ pretreated endotoxemic mice had significantly greater serum levels of TNF (10.4 ± 3.1 units) compared to control endotoxemic mice (1.2 ± 1.5 units). Spleen, lung and liver TNF mRNA levels were either unchanged or slightly diminished in rMuIFN-γ pretreated mice relative to endotoxemic controls, suggesting that rMuIFN-γ enhanced the translation or secretion of TNF; IL-1 βa mRNA levels were consistently decreased in these same tissues. Recombinant MuIFN-γ did not induce monokine message during endotoxemia in formerly nonexpressive tissues (brain, intestine and kidney). Because the modulatory functions of administered rMuIFN-γ may not predict the function of IFN-γ produced endogenously after endotoxia, we pretreated animals with monoclonal anti-IFN-γ. This significantly increased survival (71% vs 14%), suggesting that IFN-γ may mediate both harmful modulatory and effector functions during endotoxemic shock. Preliminary studies show that the induction of short-term endotoxin tolerance correlates with greatly decreased IFN-γ expression in vivo following endotoxin challenge, further supporting a deleterious role for this factor during sepsis.

PRODUCTION OF MONOKINES IN RESPONSE TO AN ARENAVIRUS INFECTION, T.R. Jerrells, V. Brown, and J. Aronson, Dept Path, Univ Texas Med Branch, Galveston, TX 77550 Infection of strain 13 guinea pigs with Pichinde virus results in a progressive infection characterized by cachexia and ultimately death. Studies have shown that this virus has a predilection for macrophages (Mφ). The present study was done to determine if infection of susceptible animals results in production of monokines, especially tumor necrosis factor (TNF) and interleukin-1 (IL-1), and to correlate monokine production with viral pathology. At various times after infection of strain 13 guinea pigs with virus levels of TNF and IL-1 in sera and Mφ culture supernatants were determined. It was found that progressive viral infection was associated with increasing levels of serum monokines. Further, Mφ isolated from infected animals produced demonstrable levels of TNF and IL-1 without further stimulation. Mφ isolated from noninfected guinea pigs or mice were found to support viral growth that was associated with the production of TNF. Our data support the idea that uncontrolled viral growth in permissive Mφ results in over production of TNF and IL-1. It is possible that these monokines in large amounts are at least partially responsible for the pathology associated with arenavirus infections. Supported in part by a McLaughlin Fellowship to JA.

TUMOR NECROSIS FACTOR PRODUCTION DURING EXPERIMENTAL LEISHMANIASIS. Heidrun Moll, Christian Bogdan, Kerstin Binöder and Martin Röllinghoff, Institut für Klinische Infektionshologie, Universität Erlangen-Nürnberg, Wasserturmstrasse 3, 8520 Erlangen, Federal Republic of Germany. We have assessed the role of tumour necrosis factor-α (TNF) during murine cutaneous leishmaniasis. No TNF activity could be detected in the serum of mice infected with Leishmania major but significant levels of TNF were released by spleen cells from infected mice after in vitro restimulation with L.major promastigotes. After challenge with bacterial endotoxin, TNF activity could also be demonstrated in the serum of L.major-infected mice and the titers appeared to correlate with the course of cutaneous disease in susceptible and resistant mice. The evidence for TNF production by L.major-activated spleen cells in the absence of endotoxin suggests that TNF is produced in response to infection and thus is involved in the immunoregulation during cutaneous leishmaniasis. Furthermore, our study indicated that the elicitation of L.major-induced TNF activity by macrophages is dependent on the presence of T cells. TNF did not exert a direct leishmanicidal effect in vitro. In combination with IFN-γ, TNF induced elimination of parasites in infected macrophages, whereas it promoted parasite burden in the presence of IL-4. These findings suggest that TNF acts in concert with other cytokines produced during L.major infection and that its role depends on the composition of T cell subsets and cytokines present.
CHARACTERIZATION OF GAMMA DELTA POSITIVE T-LYMPHOCYTES IN LEISHMANIASIS

Patients. *Donna M. Russo, †Richard Armitage, ‡Manoel Barral, §Kenneth H. Grabstein and
*Steven G. Reed. *Seattle Biomedical Research Institute, Seattle, WA 98109, †ImmuneX Corporation, Seattle, WA 98101, ¶Federal University of Bahia, Salvador, Brazil. Recent evidence suggests that T-lymphocyte expression of γδ T-cell receptors (Tcr) may play a role in certain chronic infectious diseases particularly those involving the skin. In addition, these cells have been shown to reside in autoimmune lesions associated with these and other diseases. In order to determine the importance of these cells in immune responses to the intracellular parasite leishmaniasis, we examined the differential expression of γδ versus αβ Tcr on the surface of T-lymphocytes from patients with active or cured cutaneous, mucosal or visceral leishmaniasis. Tcr was evaluated by FACSANALYSIS on resting PBL as well as on cells stimulated by leishmania antigens. Cells from two patients, one with active cutaneous disease and another with cured visceral leishmaniasis were analyzed in depth. Each patient had 30-12% γδ + Tcr cells in resting PBL. However antigen-specific T-cell lines generated from these individuals displayed markedly different Tcr profiles. The line generated from the cutaneous patient contained >50% γδ positive cells. Approximately half of these cells were CD8+, the remaining γδ + cells were CD4-CD8-. Proliferative responses as well as cytokine secretion profiles were analyzed in cells from the line as well as clones derived from the line. In contrast, a T-cell line generated from the visceral patient contained no γδ + cells. This line was predominately CD8+. A survey study of patient PBL demonstrated that following antigen stimulation the percentage of T-cells expressing γδ Tcr was significantly higher in cutaneous and mucosal patients than in recovered visceral patients. Further analysis of visceral patients demonstrated that the majority had higher than normal control numbers of circulating γδ + cells which did not expand with antigen stimulation. These data suggest that T-cells expressing γδ TCR may play a role in parasitic infections.

ROLE OF INTERFERON-y IN HOST RESPONSE TO INFECTION WITH PLASMODIUM CHABAUDI AS, M. M.

Stevenson, E. Ghaediri, M. Tam, M. Belosevic*, P. H. van der Mede** and J. E. Podoba, Centre for the Study of Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada, *Department of Zoology, University of Alberta, Edmonton, Alberta, Canada and **TNQ Primate Centre, Richwej, The Netherlands. Acquired immunity to blood stage infection with certain murine malaria species has been demonstrated to occur by an antibody-independent, cell-mediated mechanism which requires T cells and an intact spleen. The role of interferon-γ (IFN-γ), a pluripotent lymphokine capable of activating macrophages, in acquired immunity leading to control and elimination of the intraerythrocytic parasites was investigated during infection with P. chabaudi AS. C57BL-derived, C57B1/10ScNhsd (B10), which are resistant to infection with P. chabaudi AS, were treated with anti-IFN-γ monoclonal antibodies (mAbs). Two mAbs were used: R4-6A2, a rat anti-mouse, neutralizing IgG1, which was prepared against natural, murine IFN-γ and DB-1, a murine anti-rat IgG1 prepared against recombinant rat IFN-γ which can neutralize the murine as well as the rat molecule. B10 mice were injected intraperitoneally (IP) with 200 µg of R4-6A2 one day before infection and every 3 days through day 21. Control mice were treated with normal rat serum. In separate experiments, DB-1 (1.0 mg/week for 3 weeks) was administered IP to B10 mice beginning on the day of infection; control mice were untreated. Control and mAb-treated mice were infected IP with 106 P. chabaudi AS parasitized red blood cells (PRBC) and the course of infection determined. Control B10 mice exhibited a course of infection which was characterized by a peak parasitemia between 30-40% PRBC on day 9-10 and elimination of the parasite by 4 weeks. mAb-treated mice exhibited a significantly greater parasitemia on day 7 as well as a significantly greater peak parasitemia. However, mAbs-treated animals also completely cleared the infection by 4 weeks. Thus, these results suggest that IFN-γ plays a role in control of parasite multiplication and peak parasitemia but does not play a role in elimination of the parasite. (Supported by The Thrasher Research Fund)

ASSESSMENT OF LYMPHOKINES IN PLASMODIUM BERGHEI SPOZOITE-IMMUNE SPLENIC CULTURES. Katherine White, Dan Jarboe, and Urszula Kryzych. Dept. of Biology, The Catholic University of America, Washington, DC 20064, and Dept. Immunology and Gastroenterology, WRAIR, Washington, DC 20307.

Understanding of the cellular mechanisms responsible for protective immunity against malaria is imperative for development of an effective malarial vaccine. Previously we have demonstrated that the immune protection induced with irradiated P. berghei sporozoites and the proliferative responses of sporozoite-induced splenic lymphocytes vary not only according to the murine strain, but also according to the immunization protocol. For example, in C57BL/6 mice an inverse relationship exists between proliferative responses and protection. To further our understanding of cellular interactions leading to anti-sporozoite immunity, we have analyzed production of the lymphokines, IL-2, IL-3, IL-4, and IFN in cultures of sporozoite-immune total splenic lymphocytes as well as CD4+ and CD8+ enriched T cell subpopulations. Unseparated and CD4+ splenic T cell cultures demonstrate an increased production of IFN following sporozoite priming. Production of IL-2 in response to sporozoite antigen(s) parallels the proliferative response of unseparated splenic lymphocytes. While proliferative responses and IL-2 levels decrease with the onset of protection, IL-3 levels remain elevated, indicating T cell activation. In contrast, cultures of separated CD4+ T cells continue to produce IL-2 in response to sporozoite antigen(s), thus suggesting that in the unseparated splenic lymphocytes IL-2 is utilized by some cell types, such as by CD8+ T cells. Clonal expansion of this population might be required immune protection. Parallel analysis of lymphokine production are being conducted in cultures from mouse strains exhibiting unique profiles of immune responses to sporozoite antigen(s).
The pX encoding element. These differences in retroviral and cellular-induced transcriptional inducers were more effective when treatment began after virus infection, rather than before. In contrast, the disease when given therapeutically. Suboptimal concentrations of MVE-2 (single dose of 3 or 12 mg/kg, one day before infection) and AZT (3 or 10 mg/kg, qd), when combined, produced an additive antiviral effect. Another BRM, soluble glucan, did not have antiviral activity. This lack of activity might be explained by the observation that, even though glucan boosted NK activity in uninfected mice, it depressed NK activity in RLV-infected mice. If this explanation is correct, it suggests a role for NK cells in resistance to retroviruses. Although poly [I:C]-LC had therapeutic activity in the RLV model, short-term treatment of LP-BM-5-infected mice with poly [I:C]-LC did not reverse the immune suppression caused by this MAIDS virus. These results indicate that murine retrovirus models have utility for the in vivo evaluation of BRM as potential AIDS therapeutic agents.

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CD 601 CHARACTERIZATION OF AN HIV-1 INFECTED HL-60 CELL CLONE.
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In order to assess the effect of HIV-1 expression upon hematopoietic stem cell differentiation and, conversely, the effect of cellular differentiation upon HIV-1 expression, we have developed a cellular model of HIV-1 infection in progenitor cells using the promyelocytic cell line, HL 60. HIV-1 infection of HL-60 cells was achieved by co-culture with HIV-1 infected, gamma-irradiated A3.01 cells. A single Leu-1 negative, reverse transcriptase positive clone, OM, was identified and subcloned. OM subclones are biologically unstable with regard to virus production and growth characteristics. After remaining virus negative for several weeks, OM cultures spontaneously undergo a transition of rising virus titers until reaching a stable maximum. During this transition, there is a transient period of dramatically reduced cell proliferation and marked aggregation, however, there are no demonstrable changes in cell phenotype or surface myeloid marker expression. Minor fluctuations in the density of surface CD4 and glycoprotein expression and in cell size are associated with the course of viral progression.

OM cultures were tested for viral and cellular responses to differentiating and inducing agents. Retinoic acid, DMSO, and sodium butyrate all reduced the level of constitutive virus expression, as measured by reverse transcriptase and p24 antigen assays, while PMA and TNF-alpha induced HIV-1 expression. Inhibitors of protein kinase C were found to abrogate the inducible response as well as the constitutive level of HIV-1 expression. None of the agents tested were able to stimulate the expression of latent HIV-1 from the early non-virus producing OM cultures. OM cell cultures were able to differentiate normally in response to these agents, similar to the response of parental HL-60 cells. The OM cell line represents a novel reagent for the investigation of intracellular signal pathways controlling HIV-1 expression during stem cell differentiation and the factors which govern viral activation from latency.

CD 602 TRANSCRIPTION OF TNF IN HTLV-I-ASSOCIATED ATL AND HAM / TSP: RELATION TO RETROVIRAL TRANSACTIVATING GENE EXPRESSION
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The expansion of activated, antigen-reactive T-cell populations is associated with the sequential induction and coordinate expression of a battery of cytokines and corresponding receptor proteins. The capacity of HTLV-I elements to transactivate viral and cellular genes has led to speculation that this function may contribute to the subverted cellular physiologies associated with adult T-cell leukemia (ATL) and HTLV-I associated myelopathy/ tropical spinal paraparesis (HAM / TSP). Using a technique of gene amplification of splice excluded transcriptional sequences by the polymerase chain reaction (SET-PCR) the transcriptional products for tumor necrosis factor (TNF) from ATL and HAM/TSP circulating mononuclear cells were assessed and correlated with expression of the HTLV-I pX message. Cytokine mRNA from fresh ATL mononuclear cells was constitutively expressed at levels similar to that of normals and pX message was undetected. In contrast, in HAM / TSP TNF mRNA was upregulated in parallel with the consistent demonstration of the pX encoding element. These differences in retroviral and cellular-induced transcriptional products in ATL and HAM / TSP suggest alternate roles in disease pathogenesis.
A consensus peptide sequence 'QNRGLDLNLXXX' is present in 19 retroviral envelope proteins of the Swiss Protein Database (vs. 11). Others have demonstrated that a 17 amino acid immunosuppressive peptide (ISP), selected from a human consensus region of transmembrane p15E is biologically active when chemically cross-linked to albumin (Science 330:453 1985). Although no other proteins in the database display the exact p15E consensus sequence, a similar region has been identified in human alpha interferon sequences shown by others to be important for cytokine anti-viral and cytosstatic activities. We show that an ISP-containing synthetic peptide is biologically active without chemical cross-linking to a large carrier protein. This synthetic peptide, called p1SEp^ISP, is composed of the ISP sequence from feline leukemia virus attached at the NH2-terminus to a polar positive sequence also predicted to be exposed at the viral membrane surface. The activities include 40-50% maximal suppression (6pM to 19pM peptide) of [3H]TdR uptake by concanavalin A stimulated murine splenocytes, inhibition (50% at 6pM peptide) of hemolytic plaque formation and inhibition of virus release from chronically infected cell lines. Anti-viral activity was evaluated in FeLV-infected and Rauscher MuLV-infected cell lines by two methods: protein blot comparison of cellular and virus-localized p27^gag/p30^p27, and inhibition of virus release, as measured by RT activity, into conditioned growth medium. Both αβ interferon and p1SEp^ISP, but not an inactive peptide homolog (p1SEp^PSI), blocked in a nontoxic manner the cellular release of p30^p27.

Half maximal inhibition of viral RT activity in conditioned growth medium was detected at 6pM to 19pM peptide. The p1SEp^ISP, but not αβ interferon or p1SEp^PSI, also directly inhibited viral RT activity. We suggest that viral p1SE and alpha interferon may utilize a similar mechanism to effect some anti-viral and cytosstatic effects.

Both rodents and humans demonstrate decreased mitogen induced lymphoproliferation with increasing age. However, the level of decrease is not consistent among all individuals. Most elderly humans (mean age 85) demonstrate about 50% the level of response of the young, while 10-15% demonstrate <20% the proliferative response of young and 15-20% demonstrate proliferative responses comparable to young. Using Brown Norway (BN) rats, heterogeneity of lymphoproliferation was also observed. However, greater heterogeneity was observed between BN and Fisher 344 rats, raised under comparable dietary and environmental conditions, than within each strain, suggesting that genetic background may have the greatest influence upon this heterogeneity. Mean IL-2 production and IL-2 receptor expression is decreased in both humans and rats; mean IFN-g production during mitogen stimulation is decreased in humans, but increases in rats, with increasing age. Lymphokine production, however, is heterogenous among individuals in both rats and humans. Further, addition of exogenous IL-2 and/or IFN-gamma only restores the level of proliferation in 30% of elderly humans and 10% of elderly rats. Therefore, the heterogeneity observed in individuals may reflect various levels of defects in different individuals. (Supported by AG03934 and AG07719).