CYTOKINES IN GROWTH AND DEVELOPMENT

Organizers: Charles Evans and Stanley Cohen

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Keynote Address (Joint)

J 001 GENETIC ANALYSIS OF TUMOR REJECTION ANTIGENS, Thierry Boon, Ludwig Institute for Cancer Research, Brussels unit and Cellular Genetics unit, Catholic University of Louvain, Brussels, Belgium

Most mouse tumors express antigens that are potential targets for T lymphocyte-mediated rejection responses. Some of these antigens are recognized by cytolytic T cells (CTL) and do not appear to be present on normal cells. Recent progress has made it possible to understand how these new antigens can arise on tumor cells. It is now generally accepted that the antigens recognized by CTL are small peptides of about nine residues presented in a groove formed by the external domains of class I molecules of the major histocompatibility complex (MHC). A major mechanism that generates new antigenic peptides is the occurrence of point mutation in active genes. These point mutations change one amino acid in the antigenic peptides. In some instances, this confers to the peptide encoded by the mutated region the ability to bind to MHC molecules. In other instances, the point mutations generate new epitopes on peptides that were already capable of binding. All these point mutations may lie at the origin of the very diverse antigens observed on tumors induced with chemical carcinogens. A second major mechanism for the generation of new antigenic peptides on tumor cells is the activation of genes that are silent in most normal adult cells. This has recently been observed for an antigen present on a mouse mastocytoma (1). Other mastocytoma tumors have been found wherein the same gene is activated and the same antigen is present.

For human tumors, many groups have observed that lymphocytes from tumor-bearing patients ("autologous lymphocytes") can often be restimulated with the tumor cells in vitro so as to produce CTL that lyse the tumor cells and do not lyse autologous control cells. Stable clonal lines of CTL have been obtained. They recognize a limited number of antigens (3-7) on the autologous tumor cell lines. Recently a gene coding for an antigen recognized by CTL on a melanoma cell line has been isolated. This gene belongs to a new gene family that includes at least three closely related genes. The gene is expressed on several melanomas. These tumors that express both the gene and HLA-A1 present the antigen recognized by the original CTL. These results indicate that tumor rejection antigens recognized by CTL may be shared by large numbers of tumors. They also suggest that patients whose tumor expresses these antigens could be identified rapidly by HLA typing and RNA analysis of a small tumor sample. These patients could receive precisely targeted immunotherapy.

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Embryogenesis and Development

J 002 DEVELOPMENTAL ACTIVITIES OF GROWTH FACTORS, E.D. Adamson[1], J-X. Wu[1] and L.M. Wiley[2], [1]La Jolla Cancer Research Foundation, La Jolla, CA 92037; [2]Institute of Toxic and Environmental Health, University of California, Davis, CA 95616.

In a number of mammalian species, preimplantation development is affected by growth factors such as TGFα, EGF, PDGF-A, IGFs and insulin. Of these, TGFα and PDGF-A are known products of the early embryo. Other growth factors such as IL-2,TGFβ1, TGFβ2 and IGF-II are expressed by the embryo, but no precise role has yet been defined. In contrast, NGF, EGF and insulin mRNAs have not been found in embryos. *In vivo* studies have shown that CSF-1 blocks, while GM-CSF, TNFα and IL-1α assist preimplantation development in mice. CSF-1, TGFα, EGF and PDGF but not IL-2 may play roles in implantation, since they can enhance trophoblast outgrowth. Later in development, other growth factors such as FGF and TGFβ may play roles in mesoderm formation, but this has only been clearly demonstrated in Xenopus development.

Since growth factors are usually secreted diffusible molecules, embryo development can be affected by maternal factors as well as embryonic sources. Therefore, a better indicator of activity of a growth factor is the presence of cell surface receptors. Receptors for insulin, EGF/TGFα and for PDGF-A have been identified so far in preimplantation murine embryos, although their precise actions and roles in development have not yet been defined. In order to do this, we have analyzed the pattern of expression of EGF-R protein and mRNA in murine preimplantation embryos and have started to examine the effect of stimulating or blocking EGF-R activities with antibodies and antisense RNA. EGF-R protein can be visualized by immunohistochemistry at all stages of preimplantation development, but it is only strongly visible in the late 4-cell stage and later. In the one to 4-cell stages, the receptor may derive from two sources: the remains of the follicular cell membrane proteins on the oocyte surface and translation from maternal mRNA. The EGF-R mRNA is detectable by RT-PCR in varying amounts in one and 2-cell embryos, but by the 4-cell, maternal mRNA has been largely degraded. From the late 4-cell onwards, increasing levels of EGF-R transcripts can be detected, and in parallel, EGF-R protein increases to relatively high levels on trophoblast cells and to lower levels on inner cell mass cells. At the blastocyst stage, EGF-R protein synthesis can be induced by EGF application, and the receptors can respond to EGF to induce the c-*fos* protooncogene. Putting the results together with the data in the literature makes a convincing case for the involvement of EGF-Rs in preimplantation mouse development.

We have also been studying how the EGF-R gene is regulated during development. We have employed embryonal carcinoma (EC) cells as a model for these studies. In 3 out of 4 lines examined, we could find no evidence for cell surface EGF-Rs in EC stem cells, but after the induction of differentiation with various drugs, EGF-R protein and mRNA are strongly induced. PDGF-Rs are also induced. The appearance of EGF-R and PDGF-R proteins at the cell surface renders the cells responsive to EGF, TGFα and PDGFs in the medium (and to autocrine factors), and this may account for the driving force to further differentiation. Growth factors stimulate pleiotropic responses including the induction of numerous transcription factors and hence cascades and programs of gene expression leading to the production of differentiated cells.

J 003 SIGNAL TRANSDUCTION FOR LYMPHOID PROLIFERATION: ROLE OF A PUTATIVE PROTEASE (ADR). Frederick Coffman, Kerin Fresa, Iris Gonzales, Jim Sylvester and Stanley Cohen, Department of Pathology, Hahnemann University, Philadelphia PA 19102.

The initiation of DNA synthesis is a common endpoint of all cytokine-driven activation pathways. In activated T lymphocytes, the onset of DNA replication is concomitant with the appearance of a cytosolic activity which can initiate in vitro DNA replication in isolated quiescent nuclei. This activity is dependent upon a number of proteins, including a putative protease (ADR) that appears to function as an initiating factor in the replication of chromosomal DNA. Quiescent lymphocytes lack ADR activity and contain an intracellular factor which inhibits ADR-mediated initiation of DNA synthesis in isolated nuclei, indicating that lymphocyte activation requires both the induction of positive regulatory elements and the removal of negative ones. DNA replication in nuclei isolated from several transformed lymphoid cell lines is not blocked by the inhibitor derived from quiescent lymphocytes, suggesting that the failure of this antiproliferative regulator may play an important role in the continuous growth of these cells. This suggestion is supported by our recent finding of a continuously proliferating MOLT-4 T cell lymphoma subline which produces both activator and inhibitor activities, which can be separated by ammonium sulfate fractionation. Like the inhibitor derived from quiescent lymphocytes, the MOLT-4 inhibitor blocks initiation of replication in isolated normal nuclei but not MOLT-4 nuclei, is not effective if added after replication has begun, and has no effect on the replication of nicked calf thymus DNA. This indicates that the inhibitor blocks the initiation of replication from sites on intact DNA sequences and not from DNA strand breaks.

We are developing an in vitro system using plasmids containing human gene sequences as replication substrates both to facillitate the study of proteins involved in the regulation of replication initiation and to define eukaryotic DNA sequences required for these activities. To date we have seen consistant incorporation of dTTP into plasmids containing either of two regions of the human ribosomal RNA gene. Incorporation into these plasmids has many of the same characteristics as incorporation into nuclei, namely the same pH optimum and nucleotide requirements, similar kinetics, and both are inhibited by aphidicolin, n-ethylmalemide and a monoclonal antibody against DNA polymerase alpha. Further, plasmid incorporation is inhibited by aprotinin and the inhibitor from transformed lymphoid cells at the same concentrations which block incorporation into nuclei, suggesting that both ADR and the inhibitor function in a similar manner in both nuclei and plasmid replication systems. Initial DNase protection experiments show that fractions which mediate incorporation preferentially protect sequences within a 200 bp region which is AT-rich and is adjacent to an Alu element and a 40 bp Z-DNA sequence motif.

Cytokines in Inflammation and Repair

J 004 TUMOR NECROSIS FACTOR ANTAGONISM VIA SOLUBLE RECEPTORS.

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In view of a growing evidence for a key role of tumor necrosis factor (TNF) in the pathogenesis of many diseases, there are increasing attempts to find ways of inhibiting the function of this cytokine. Soluble forms of the receptors for TNF, found to be present in the serum and urine, compete for TNF with the cell-surface TNF receptors and can thus block its function[1-4]. These proteins are now being tested as inhibitors of TNF in several animal models of TNF pathogenicity. In a number of such models the soluble TNF receptors (sTNF-Rs) have already been found to provide some protection. Clues to more effective ways for applying the sTNF-Rs for therapy may perhaps be gained from the study of the physiological function of these proteins. So far there is just fragmentary information on that subject, relating to the following points:

Occurence of the sTNF-Rs: The sTNF-Rs are present constitutively in the serum, although at very low concentrations; their serum concentrations increase significantly in diseases of both an inflammatory and non-inflammatory nature and were reported to increase also after administration of TNF.

Mechanisms of formation: The sTNF-Rs are derived from the cell-surface receptors by a proteolytic cleavage mechanism which is subject to effective enhancement by certain physiological regulators. This cleavage mechanism and the structural elements in the TNF receptor taking part in it are distinct from those involved in TNF uptake and in the signalling by these receptors.

Effects: Beside their inhibitory effect on the function of TNF, which reflects their ability to bind TNF, the sTNF-Rs have also the ability to attenuate a spontaneous decay of TNF activity by stabilizing TNF structure within the complexes which they form with it. Thus, depending on the rate at which TNF is cleared from the site of its formation, the sTNF-Rs may, at different situations, affect the function of TNF in quite a different manner; in some situations they may inhibit the effects of TNF, in others, serve as carriers for TNF, and in some cases they may even augment the effects of TNF by prolonging its function.

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Viruses and Cytokines

J 005 ADENOVIRUS GENES THAT MODULATE THE SENSITIVITY OF VIRUS-INFECTED CELLS TO LYSIS BY TUMOR NECROSIS FACTOR,

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TNF-mediated cytolysis is a multistep process initiated by binding of the cytokine to a cell surface receptor. Most cultured cells express TNF receptors, but with few exceptions cell lines are resistant to TNF cytolysis. Therefore, the events which determine susceptibility or resistance to cytolysis occur intracellularly, after TNF binding. Human adenoviruses encode several proteins which alter the susceptibility of cells to TNF cytolysis. The 12S and 13S E1A proteins induce TNF susceptibility when introduced into cells either by virus infection or by transfection with appropriate expression vectors. These immediate early proteins transactivate other adenovirus genes and regulate transcription of a number of cellular genes. Mutational analysis by many laboratories has shown that specific functions tend to be associated with specific domains in these multifunctional proteins. Sensitivity to TNF maps to conserved region 1 (CR1) when E1A is introduced into cells by virus infection and to both CR1 and CR2 when transfected cells are used. These findings suggest that two different E1A functions are required for TNF sensitivity. One function is supplied by CR1 and the other requires either CR2 or a gene(s) elsewhere in adenovirus. Interestingly, the region of CR1 required for induction of TNF sensitivity is the same as the region shown by others to induce in cells TNF independent cytotoxicity and DNA degradation. Hence, one mechanism by which E1A renders cells sensitive to TNF may be by facilitating the DNA degradation so often observed in cytokine-mediated cytolysis.

It is unlikely that E1A induced cytotoxicity or TNF susceptibility is beneficial to adenovirus propagation. It is not surprising therefore that adenovirus encodes proteins that function to counteract these effects. In fact, adenovirus encodes at least four other proteins elsewhere in the genome that function to prevent E1A induced and TNF-mediated destruction of virus-infected cells. The ability of these proteins to protect from TNF lysis is cell-type dependent. The E3-14.7K protein (14,700 MW) is an abundant soluble protein found in both the cytoplasm and nucleus of infected cells. 14.7K is the most general inhibitor of TNF lysis, functioning in nearly all rodent cells tested to prevent TNF cytolysis. 14.7K protects from TNF even in spontaneously sensitive cells or in cells made sensitive to TNF by inhibition of protein synthesis.

Another set of genes, E3-14.5K/10.4K, produces a membrane-bound heterodimer that also functions to protect many, but not all mouse cell lines from TNF killing. These proteins have also been observed to cause internalization and degradation of the receptor for epidermal growth factor (EGF-R) on virus-infected cells. At present, the relationship between down-regulation of EGF-R and resistance to TNF cytolysis is unknown. The final adenovirus protein that has been implicated in protection from TNF cytolysis is E1B-19K. E1B-19K prevents TNF cytolysis of adenovirus infected human cells, but does not protect any of 15 mouse cell lines tested. E1B-19K is an interesting multi-functional protein. It co-operates with E1A in adenovirus transformation of rodent cells, it is localized to the nuclear membrane, it binds to and disrupts the nuclear lamina and vimentin containing intermediate filaments, and it transactivates certain cellular and viral genes. Significantly, E1B-19K also prevents the E1A induced cytotoxicity and DNA degradation noted above, suggesting a potential mode of action in prevention of TNF cytolysis. The finding of multiple adenovirus genes involved in modulation of cellular sensitivity to TNF has two important impications. First, it suggests that TNF, or TNF-like mechanisms, may play an important role in host resistance to virus infection or alternatively may play a significant part in pathogenesis caused during virus infection. In addition, the availability of several viral proteins, each of which probably acts at a different step in the intracellular pathway leading to TNF-mediated killing, provides a major tool for the dissection of this critical sequence of events.

J 006 ROLE OF CYTOKINES IN AUTOIMMUNE AND COXSACKIE INDUCED MYOCARDITIS,

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Infection of mice with coxsackie virus B3 (CB3) results in focal myocyte necrosis and inflammation.[1] These early pathological manifestations of viral injury gradually diminish so that by 14 days after infection the acute phase of myocarditis has resolved. In genetically predisposed mouse strains such as A/J, but not in resistant B10.A, the acute phase is followed by chronic, autoimmune myocarditis characterized by diffuse interstitial mononuclear cell and by the production of heart-specific autoantibodies.[2,3] These autoantibodies react predominantly with cardiac myosin and the late phase of myocarditis can be reproduced in genetically susceptible strains by immunization with purified cardiac myosin.[4]

LPS treatment of CB3-infected B10.A mice is capable of inducing autoimmune myocarditis in these genetically resistant animals.[5] We tested Il-1 synthetic peptide fragment 163-171 and TNF synthetic peptide 114-130 and found that both cytokines produce autoimmune myocarditis in CB3-infected resistant B10.A mice. Furthermore, elevated serum levels of both cytokines were found in the experimental groups, and immunohistochemical studies showed IL-1 and TNF producing cells among the inflammatory cells in the heart. We suggest that these cytokines play a critical role in the susceptibility of animals to post-viral autoimmune disease.

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Regulation of Normal and Neoplastic Growth

J 007 HETEROGENEITY OF PDGF RECEPTORS, Subal Bishayee, Coriell Institute For Medical Research, Camden, NJ 08103.

Platelet-derived growth factor (PDGF) is a disulfide-bonded homo-or heterodimeric protein, composed of A and B chains; B chain is the product of the c-sis proto-oncogene. The mitogenic and transforming activities of PDGF are mediated through its interaction with a high affinity cell surface receptor. PDGF receptor is a transmembrane glycoprotein (M_r 170-180 k) with a cytoplasmically facing tyrosine kinase site that is activated by ligand binding to the external domain. Two types of PDGF receptors have been cloned and sequenced. Although both the α- and β- receptors undergo ligand-induced dimerization and kinase activation, these two receptors differ in ligand-binding specificity. α-receptor binds to all three isoforms of PDGF whereas β-receptor binds with very high affinity only to PDGF BB. However, our studies revealed that in the presence of α-receptor, β-receptor is capable of binding to PDGF AB with very high affinity and such binding and subsequent activation of the β-receptor is due to the α-β-receptor heterodimer formation. In the present investigation, we have studied the structural and functional differences between α- and β-receptors. In human osteogenic sarcoma cells (MG-63), both receptors have relatively high turnover rates; $T_{1/2}$ for α-receptor is 6 h whereas that of β-receptor is 3 h. Studies conducted with antibodies directed to the peptide epitopes located at the same sites in α- and β-receptors indicated that these two receptors may differ in their conformation at least with respect to one epitope. As in the case with β-receptor, N-linked glycosylation of the α-receptor is not important in kinase activation. We have exploited the lack of an effect of N-linked oligosaccharides on the functioning of the α-receptor to develop a rapid and simple method to study the role of receptor heterodimer in kinase activation. The significance of the differences between α- and β-receptors with respect to structures, functions and glycosylation patterns in biological signal transduction and cellular transformation will be discussed.

J 008 EFFECTS OF CYTOKINES ON TUMOR CELL-ENDOTHELIAL INTERACTIONS, Marion C. Cohen, Joanna Bereta, Michal Bereta, and Stanley Cohen, Hahnemann University, Philadelphia, PA 19102.

The adherence of tumor cells to microvascular endothelium is believed to be a necessary step in their migration to sites of metastasis. It has been proposed that this process occurs when cell surface molecules on tumor cells bind to complementary sites on endothelial cells. The expression of these endothelial-derived cell adhesion molecules appears to be modulated by cytokines, a broad class of protein mediators which play important roles in immune and inflammatory reactions. It has been found by ourselves and others that exposure of endothelium to some cytokines augments the adhesion of inflammatory cells as well as some tumor cells in *in vitro* assays. Utilizing a murine model, P815 mastocytoma and microvascular endothelium, we found that pretreatment of endothelial monolayers with TNF-α, IL-1, LPS or PMA can increase the adherence of tumor cells in in a dose-dependent fashion. We found that TNF-mediated enhancement of P815 binding required both protein and RNA synthesis since the effect could be abrogated by pretreating endothelium with cycloheximide or actinomycin D before incubation with the cytokine. In addition, changes in the cell membrane protein pattern were seen following exposure of endothelium to TNF-α.

We examined the possible involvement of protein kinases in TNF-α signal transduction in endothelial cells. Quercetin, an inhibitor of tyrosine kinase, and W7, an antagonist of calmodulin, had no effect on TNF-α-mediated binding. Furthermore, PKA does not appear to play a role in the potentiation of binding by TNF-α. Although we found that H-7 and sangivamycin, inhibitors of PKC, could inhibit the TNF-α effect, our studies suggest that the "classical" PKC pathway is not completely responsible for signalling since TNF-α did not cause translocation of PKC to the cell membrane and its effect could not be completely mimicked by PMA nor by PMA in the presence of calcium-raising agents. Furthermore, staurosporine, a uniquely potent inhibitor of PKC, strongly enhanced adhesion of tumor cells to endothelium utilizing a mechanism distinct from TNF.

We also studied the effect of TGF-β, another cytokine with pleiotropic effects, on the binding of tumor cells to endothelium. Exposure of endothelium to TGF-β led to the inhibition of of both basal and TNF-α-enhanced binding of P815 cells as well as the inhibition of adherence of murine splenocytes, thymocytes, and human lymphoblastoid cells. TGF-β-mediated inhibition of P815 basal binding to endothelium is dependent on protein synthesis since cycloheximide reverses the effect of the cytokine. Inhibitors of G-proteins do not abolish TGF-β action, and PKC and PKA activators elicit an opposite effect. However, TGF-β-mediated inhibition of both basal binding and TNF-α-enhanced P815 binding to endothelium is completely abolished in the presence of the protein phosphatase inhibitor okadaic acid suggesting that TGF-β elicits its effect by stimulating protein phosphatase activity.

J 009 POTENTIATION OF ANTITUMOR IMMUNE RESPONSES USING CYTOKINE GENE-MODIFIED TUMOR VACCINES, Eli Gilboa, Bernd Gansbacher, John P. Connor, Rajat Bannerji, Warren Heston, William Fair, and Kathryn Cronin. Memorial Sloan-Kettering Cancer Center, New York, New York.

Administration of cytokines is proving to be a valuable tool in cancer immunotherapy. Short half-life in the serum dictates the use of very high doses of cytokine which often leads to side effects of varying severity, thus limiting the overall effectiveness of this treatment modality. To explore the potential advantages of localized secretion of cytokines at the site of the growing tumor, we have introduced the genes for human IL-2 and mouse IFN-gamma into several mouse-derived tumor cell lines and measured the ability of the cytokine-gene modified tumor cells to (1) grow in the mouse, and (2) elicit a protective immune response against a challenge with a tumorigenic dose of parental unmodified cells. Using three mouse tumor model systems, a chemically induced fibrosarcoma, a bladder carcinoma and a B-cell lymphoma, we found that gene transfer of IL-2 (and in one tested case, also IFN-gamma) not only prevent tumor growth in vivo but also induce a protective immune response against parental tumor cells. The cellular mechanism responsible for the induction of antitumor immune response and the implications of these findings for the development of improved tumor vaccines will be discussed.

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J 010 LYMPHOTOXIN/TNF RECEPTOR SYSTEM, Carl F. Ware[1], Matthew J. Androlewicz[1], Jeffrey L. Browning[2], Paul D. Crowe[1], Ray G. Goodwin[3], Craig A. Smith[3], Todd VanArsdale[1], [1] Division of Biomedical Sciences, Univ. of California, Riverside, CA 92521, [2]Biogen, Inc, Cambridge and [3]Immunex Corporation, Seattle.

The role of **lymphotoxin** (LT, TNF-β) in cellular immune reactions is an enigma when compared to the related potent proinflammatory cytokine, tumor necrosis factor (TNF). However, new data refute the notion that LT is a redundant form of TNF. Several cellular responses including MHC expression, leukocyte adhesion and cytotoxicity have indicated that LT functions as a partial agonist (1). The relevance of the partial agonist activity of LT is revealed in recent the characterization of membrane-anchored form of LT (2). Surface LT was initially identified on a T cell hybridoma, II-23.D7 selected for efficient lytic activity against TNF sensitive targets. Surface LT is associated with a novel 33 kDa glycoprotein (p33) in a heteromeric complex anchored to the surface by a dimer of p33. Secreted LT is not associated with p33. Signalling via IL-2R and the TCR induce expression of sLT/p33 on resting T and NK cells. Membrane TNF expression is transient on CTL in contrast to the relatively stable expression of sLT/p33. HUT-78, a T cell lymphoma, constitutively expressed sLT/p33, whereas HTLV-1 and EBV infected cell lines can be induced to express sLT/p33 suggesting that viruses may be associated with dysregulation of lymphotoxin.

Significant differences between secreted and surface LT are revealed by comparison of the receptor specificity and binding constants for the 80 and 55 kDa TNF receptors. On the II-23.D7 cell line the partial agonist activity of secreted LT is associated with coexpression of both TNFR (3), whereas LT is a full agonist on some cell types that express one receptor. That surface LT/p33 complex can function in a "juxacrine" fashion is suggested by the ability of glutaraldehyde fixed cells to lyse targets. Studies with soluble forms of the TNFR suggest a unique mode of signalling for sLT/p33 complex and thus sLT/p33 may mediate a distinct spectrum of cellular responses.

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Cytokines and The Endocrine System (Joint)

J 011 PATHOGENIC ROLE OF CYTOKINES IN AUTOIMMUNE ENDOCRINE DISEASES, Klaus Bendtzen, Laboratory of Medical Immunology TTA 7544, Rigshospitalet University Hospital, DK-2200 Copenhagen N, Denmark.

Many models have been advanced to account for the association of HLA types with autoimmune endocrine diseases. For example, aberrant expression of MHC class II molecules may facilitate a T cell-mediated attack against 'forbidden antigens' on endocrine cells. However, cytokines produced by MHC class II-bearing macrophages (MØ) may also contribute to endocrine disease (1-4). Thus, IL-1 suppresses insulin production *in vitro* through a direct cytotoxic effect on pancreatic β-cells. This effect is modulated by IRAP, a specific IL-1 receptor antagonist, suggesting the involvement of 'classical' IL-1 receptors on β-cells or other islet cells involved in β-cell damage. The toxicity afforded by IL-1 is related to disturbed glucose metabolism in β-cell mitochondria. Other cytokines such as TNFα, IL-6 and IFNγ also influence insulin production, especially in conjunction with IL-1, but these cytokines are generally not cytotoxic. Treatment of the spontaneously diabetic BB rat with antibodies to IFNγ prevents the disease (5), and drugs such as cyclosporin and fusidic acid interfere with IL-1-mediated effects on rodent β-cells and with the development of spontaneous diabetes in the BB rat. Other endocrine cells are susceptible to modulation by cytokines. For example, IL-1 suppresses, but does not kill, human thyroid cells.
A stimulatory effect on insulin and thyroglobulin production is observed at extremely low concentrations of IL-1 and of higher but *in vivo* attainable levels of IL-6. This suggests that cytokines may have important functions as **physiological** regulators of endocrine cells, for example during conditions of stress. A role of IL-6 is further supported by the fact that IL-6 is produced by glucose-stimulated rat islets and by secondary cultures of human thyrocytes, in particular after challenge with IL-1. Also, IL-6 causes similar damage to pancreatic β-cells as does IL-1.
Proposed models for a role of cytokines in insulin-dependent diabetes mellitus and, possibly, in other autoimmune endocrine diseases:
Model 1: Initial damage (virus?) affects the endocrine target cells themselves. Accumulated MØ attract T and B lymphocytes and NK cells. The MØ, and/or the MHC class II+ targets themselves, present antigen(s) to specifically reactive T cells releasing additional cytokines. IL-1 and IL-6, potentiated by TNFα and IFNγ, further damage the cells. Eventually, autoantibodies and immune complexes contribute to β-cell destruction. The specificity in this model is governed by the specifically reactive T lymphocytes and by the specificity of the infectious or toxic damage inflicted upon the target cells.
Model 2: The initial toxic, inflammatory or infectious reaction(s) take place in the vicinity of the target cells, which as 'innocent bystanders' become the victims of an immunological attack. A perivascular process and/or vasculitis in afferent blood vessels trigger the production by endothelial cells and smooth muscle cells of IL-1, TNFα and IL-6, which may accumulate at high, β-cell toxic levels in the islets. This initially nonspecific reaction eventually becomes immunologically specific resulting in accelerated β-cell destruction. This model predicts the existence of several 'etiological factors' in the course of events leading to diabetes mellitus, even in the same individual. It also explains the finding of MHC class II proteins on vascular endothelium in prediabetic BB rats and in recent-onset diabetes mellitus, even in or around islets without MØ invasion (see 2).
In both models, the magnitude of a T cell-mediated attack depends upon the MHC class II type of the individual, thus accounting for the MHC association of many autoimmune endocrine disorders. However, since the TNFα gene is polymorphic and located in the MHC region, the disease-associated HLA types may be linked to TNFα alleles coding for prolonged and/or pronounced secretion of this cytokine. Thus, activated peritoneal MØ from the spontaneously diabetic BB rat secrete more TNFα than cells from diabetes-resistant or normal rats (6). Also, MØ from HLA-DR2+ healthy individuals respond less vigorously with respect to TNFα production; this might explain the protection afforded by HLA-DR2 in diabetes mellitus (see 2).

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J 012 **NATURAL REGULATORS OF T-CELL LYMPHOKINE PRODUCTION IN VIVO,** Raymond A. Daynes, Tad A. Dowell, and Barbara A. Araneo, University of Utah School of Medicine, Salt Lake City, UT 84132.

The development of protective immunity *in vivo* requires T cells to receive, integrate, and respond appropriately to a variety of exogenous and endogenous signaling systems. Foreign antigen represents the main exogenous signal, conferring specificity to immune responses by promoting the activation of only those T cells bearing complimentary cell surface receptors. The consequences of T-cell activation *in vivo*, however, appear to be internally controlled through the activities of endogenous substances capable of regulating the functional potential of recirculating T cells. Many distinct types of naturally occurring molecules exist that can alter the functional properties of T cells. Cytokines, growth factors, other polypeptide hormones, arachidonic acid metabolites, and numerous steroid hormones, have all been thoroughly investigated for their ability to modify the biochemical behavior of T cells. Some of these agents exert their effects systemically (e.g., glucocorticoids and certain cytokines) while the actions of others (e.g., prostaglandins, growth factors, and some steroid hormones) are anatomically restricted. Such controlling mechanisms are quite important to the immune system. They provide it with an extensive degree of flexibility plus the capacity to compartmentalize specific effector responses to particular tissue microenvironments.

We have been investigating the roles played by certain species of steroid hormones that gain access to lymphoid tissues as a consequence of end-organ metabolism of inactive circulating precursors. Localized production of dehydroepiandrosterone (DHEA) from its circulating sulfated precursor (DHEAS), coupled with the enhancing influence of DHEA on activated T-cell IL-2 and γIFN production, provides a simple biochemical means to regulate the functional potential of T cells residing within tissue sites where this hormone is being effectively metabolized. Our studies also indicate that dihydrotestosterone and 1,25-dihydroxyvitamin D_3 play equally important roles in controlling the nature of T-cell responsiveness within particular lymphoid organs in the body. Therefore, alterations to the functional properties of T cells that accompany stress, trauma, or aging may, in part, be due to fluctuations in tissue localized and systemic steroid hormone levels.

The results of our very recent studies implicate platelet-derived growth factor (PDGF) as being an extremely potent modulator to the functional properties of T cells. Once again, these controlling influences only become manifest after cellular activation. T cells exposed to nanogram quantities of PDGF are able to markedly enhance their IL-2 production following activation, while simultaneously depressing the ability to secrete other lymphokines (e.g., IL-4, IL-5, γIFN). T cells residing near tissue sites of injury, where platelet activation occurs, are probably being influenced by PDGF.

We believe that a major immunobiologic role played by steroid hormones, specifically those species that undergo end-organ metabolism, is to create and maintain microenvironmental distinctions between discrete lymphoid organs. PDGF, as an example of a locally released bioactive substance, functions to create unique microenvironments anywhere in the body.

J 013 INTERACTION OF CYTOKINES WITH THE HYPOTHALAMIC-PITUITARY AXIS, James M. Krueger, University of Tennessee, Memphis, TN 38163.

Major humoral mechanisms include the endocrine and immune systems, and there is substantial literature describing interactions between these systems during infection and inflammatory processes. Within the brain, such interactions are less well known. One major brain function altered during infection and inflammation and by several endocrines is sleep. These changes in sleep provide a useful illustration of the interactions between cytokines and the hypothalamic-pituitary axis.

There is ample evidence linking IL1 to sleep regulation (reviewed 1): 1) IL1α, IL1β, and IL1$\beta_{208-240}$ enhance sleep; 2) anti-IL1β antibodies and an IL1-receptor antagonist inhibit sleep; 3) patients receiving IL1 therapy report excessive sleepiness; 4) IL1, IL1mRNA and IL1 receptors are in normal brain; and 5) IL1 levels (plasma and CSF) vary with sleep. There is also much evidence linking GHRH/GH to sleep; 1) GHRH enhances sleep; 2) a GHRH-antagonist inhibits normal sleep; 3) anti-GHRH antibodies inhibit normal sleep; 4) anti-GHRH antibodies block sleep rebound after sleep deprivation; and 5) GH release is firmly coupled to sleep cycles. These two major sleep regulatory systems are linked to each other, somnogenic doses of IL1 induce GH release via GHRH;.IL1-induced GH release is blocked with anti-GHRH antibodies.

In contrast, high doses of IL1 inhibit GH release and promote wakefulness, probably due to IL1-induced CRF release. CRF induces SOM release which inhibits GH and GHRH release. CRF itself enhances wakefulness; this effect could be mediated directly within the brain via neurons containing CRF receptors. Alternatively, CRF induces pituitary ACTH release which in turn induces release of adrenal glucocorticoids. CRF, ACTH, αMSH, and glucocorticoids inhibit sleep. CRF and αMSH block IL1-induced sleep responses. Finally glucocorticoids inhibit IL1 production but also turn on IL1-receptor production. Such complex regulatory phenomena are likely involved in physiological sleep regulation and are probably greatly amplified during pathology.

1) Krueger, J. M., *et al., Yale J. Biol. Med.* 63: 157-172, 1990.

J 014 PATHOLOGICAL EFFECTS OF CYTOKINE EXPRESSION IN VIVO Nora Sarvetnick[1], Judy Shizuru[2], Danling Gu[1], Lise Wogensen[1], Marijo Gallina[1], [1]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, [2]Stanford University, Stanford, CA

Cytokines such as IFN-g are produced during specialized circumstances such as infection. These pleiotropic molecules could potentially be responsible for some of the complex morphological changes associated with infection. We are interested in the pathological consequences of cytokine expression in vivo. We have utilized transgenic mice to target expression of several inflammatory cytokines to the pancreatic beta cells. The expression of IFN-g in the pancreas leads to lymphocytic inflammation and islet cell loss. The islet cell loss is mediated by the lymphocytes that accumulate in the pancreatic parenchyma. Additional morphological changes are observed in the IFN-g expressing pancreas including altered pancreatic vasculature as well as duct cell hyperplasia and islet cell regeneration. By analysis of SCID backcross mice some of these changes were demonstrated to be due to the infiltrating lymphocytes while others are more likely due the direct actions of the cytokine itself. More recently we have been studying the consequences of expression of other inflammatory cytokines on the morphology of the pancreas. In contrast to the studies with IFN-g, expression of the cytokine IL-10 in the pancreas leads to intense inflammation but apparently no significant islet cell loss. The discussed studies are aimed at furthering our understanding of the complex actions of these molecules in vivo.

Cytokines as Pathophysiologic Agents

J 015 Cytokine regulation of adhesive interactions between lymphocytes and fibroblasts, Joseph H. Korn and Theresa H. Piela-Smith, Newington V.A. Medical Center, Newington, CT 06111, and the Division of Rheumatic Diseases, University of Connecticut School of Medicine, Farmington, 06030.

Targeting of immune effector cells to sites of tissue injury is critical to the development and perpetuation of the immune response. This process involves both the egress of immune cells from the vasculature and the localization and persistence of cells at sites of inflammation. The latter is governed, in large part, by adhesion of lymphocytes to fibroblasts, a process that is tightly regulated and dependent on interaction of the ligands LFA-1 and ICAM-1 on lymphocytes and fibroblasts respectively. Previous studies have shown that LFA-1 function is modulated by both qualitative and quantitative changes on the cell surface following cellular activation. Similarly, ICAM-1, which functions both as a cellular adhesion ligand and a receptor for human rhinovirus (HRV) is subject to both quantitative and qualitative regulation.

Although ICAM-1 is constitutively expressed on the surface of fibroblasts and many other cells, activation of fibroblasts with γ-interferon (IFN), IL-4, and IL-1 promotes ICAM-1 function. IL-1-stimulated ICAM-1 functions as a ligand for human rhinovirus adhesion but not for resting T cell adhesion. In contrast, IL-4 and IFN-stimulated ICAM-1 functions in both systems. Furthermore, pretreatment of fibroblasts with IL-1 inhibits subsequent IFN-stimulated or IL-4-stimulated T cell adhesion but promotes the adhesion of HRV. The reasons for functional differences between IL-1 and IFN-stimulated ICAM-1 are not readily apparent. IL-1, like IL-4 and IFN treatment, increases the proportion of fibroblasts expressing high levels of ICAM-1 assessed by flow cytometry. Electron microscopic studies show no difference in clustering of IFN-induced vs. IL-1induced ICAM-1 on the fibroblast surface. Both IL-1 and IFN induce increases in ICAM-1 steady state mRNA and in ICAM-1 protein synthesis. There are no gross differences in the level of glycosylation of newly synthesized ICAM-1 following IL-1 vs. IFN treatment. However, inhibition of glycosylation or treatment of fibroblasts with neuraminidase does permit IL-1-induced ICAM-1 to function as an adhesion ligand for resting T cells. It is possible that there are conformational differences between IL-1 and IFN-induced ICAM-1 which have functional correlates.

The population of "resting" T cells which adhere to γ-IFN-treated fibroblasts is a discrete and depletable subset which includes both CD4+ and CD8+ cells. Similarly, subsets of fibroblasts appear to have greater adhesive capability. The definition of these subpopulation and their regulation should aid in understanding lymphocyte homing.

J 016 ROLE OF CYTOKINES IN BONE RESORPTION, Gregory R. Mundy, Toshiyuki Yoneda, Itsuro Kato, and Lynda F. Bonewald, University of Texas Health Science Center at San Antonio, San Antonio, Texas and OsteoSA, San Antonio, Texas.

Osteoclast function is regulated by cytokines which are generated in the microenvironment of bone remodeling surfaces. These cytokines include interleukin-1, tumor necrosis factor, gamma interferon, interleukin-6 and transforming growth factor beta. In vitro assays have allowed characterization of the effects of these cytokines on the process of osteoclast generation and activation. These cytokines may have physiologic significance for normal bone remodeling and pathologic bone destruction in disease states which cause localized bone destruction such as rheumatoid arthritis and periodontal disease, in malignancies which involve the skeleton and in osteoporosis. In one murine variant of osteopetrosis, it has been shown that the defect in osteoclast function is caused by impaired expression of the cytokine CSF-M. Recently, we have identified a novel cytokine which enhances osteoclast differentiation. This cytokine was purified from the conditioned media harvested from a human tumor which is associated with the production of colony-stimulating factors for granulocyte-macrophages, granulocytes, and macrophages. The patient from whom the tumor was derived had hypercalcemia and nude mice bearing this tumor showed increased osteoclastic bone resorption. We isolated and characterized a peptide present in the conditioned media which stimulated the formation of bone resorbing osteoclasts in human and murine marrow cultures. By the development of monoclonal and polyclonal antibodies to a synthetic peptide derived from this factor, we developed neutralizing antibodies, affinity columns and an ELISA assay which detected this cytokine in cultures of normal stromal cells which support osteoclast formation. This cytokine has different effects from that of other osteotropic cytokines, since it appears to act primarily as a differentiation agent for osteoclasts rather than as a proliferative factor for committed osteoclast progenitors. Its role in diseases associated with increased osteoclastic bone resorption is now being evaluated.

J 017 USE OF SYNTHETIC PEPTIDES TO MAP FUNCTIONAL EPITOPES OF CYTOKINES. Arnold E. Postlethwaite and Jerome M. Seyer, University of Tennessee, Memphis and Department of Veterans Affairs Medical Center, Memphis, Tennessee 38163.

Cytokines such as IL-1α, β, TNFα and IL-4 have pleotropic effects on different target cells. Mechanisms by which such cytokine pleotropism is effected are unknown but could involve interaction of specific epitopes on cytokines with different portions of the receptor(s) for each given cytokine. For example, it has been recently demonstrated by Conca et al (J. Biol. chem. 266:16265-16268, 1991) that substitution of glycine for arginine at position 127 in human IL-1β (designated IL-1 $\beta_{R\rightarrow G}$) produces a protein that will bind to the IL-1 receptor(s) with high affinity, and trigger incomplete signal transduction events characterized in fibroblasts by expression of *fos and jun* but no increased transcription of procollagenases or prostomelysin (*jun/fos*, procollagenase and prostomelysin expression are all induced by wild type IL-1β). These results suggest that a precise region of the IL-1β molecule may be essential for certain biological activities.

We have approached the question of cytokine structure-function relationships by synthesizing oligopeptides representing the complete amino acid sequences of human IL-1β, TNFα and IL-4 and have tested these peptides for some of the biological activities possessed by the parent cytokine molecule. In IL-1β, we have identified three peptides in the propeptide (1-116) portion of the molecule that possess biologic activities. The amino terminus (1-27) stimulates fibroblast proliferation and peptides 58-87 and 88-116 induce migration of monocytes and neutrophils. Peptide 58-87 induces chemokinesis while peptide 88-116 induces chemotaxis of monocytes. Peptide 1-116 is cleaved from precursor IL-1β and may function to stimulate monocyte and neutrophil accumulation and fibroblast proliferation in vivo. None of the peptides comprising 1-116 stimulated T cell proliferation nor did they displace [^{125}I] IL-1β from receptors on the murine EL4 T cell line. Oligopeptides representing human TNFα were tested for their ability to induce fibroblast chemotaxis, a property possessed by TNFα. Peptide 31-68 induced fibroblast chemotaxis, and specifically deactivated fibroblasts to respond chemotactically to TNFα, suggesting that peptide 31-68 and TNFα induce fibroblast chemotaxis through a similar mechanism (J. Exp. Med. 172:1749-1756, 1990). This chemotactic peptide also induced NREM sleep and fever in rabbits, another property of TNFα (Kapas, et al, manuscript submitted). Oligopeptides representing human IL-4 were tested for their ability to stimulate chemotaxis of fibroblasts, a property of IL-4 (J. Clin. Invest. 87:2147-2152, 1991). Two adjacent peptides induced fibroblast chemotaxis, 70-88 and 89-122. These peptide, specifically deactivated fibroblasts to chemotax to hrIL-4.

These results suggest that cytokine molecules possess distinct functional epitopes that may confer certain biological properties on the native molecule.

Cytokines and Differentiation

J 018 IDENTIFICATION OF CELLULAR PROTEINS REGULATED BY CYTOKINES.

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In an attempt to better understand the pleiotropic effects of several cytokines and to define their mechanism(s) of action at a molecular level, our laboratory has been studying the effects of pure recombinant cytokines on the proteins induced or suppressed in normal and malignant cells. First, the global pattern of proteins affected by a single cytokine or combination of cytokines in a given cell type is ascertained, using computer-based analysis of 2D gels. This permits the determination of the molecular weight and isoelectric point of such proteins and the extent of their induction or suppression in response to the dose and length of exposure of the cytokine. Attempts are then made to obtain partial amino acid sequences for several high abundance cytokine regulated proteins from Immobilon blots of preparative 2D gels or from the electroeluates of individual protein spots, using conventional N-terminal or internal microsequencing employing Mass Spectrometry. Mass Spectrometry has the advantage that all peptides are potentially sequencable including those that possess post-translational modifications. Once a given protein has been identified, its expression at the mRNA level is then evaluated. The cell types that have been examined and the cytokines that have been employed in our studies are as follows:

Cytokine(s) employed	Cell Type(s) Examined
IFN-α, IFN-γ, IL-1, TNF	Human Fibroblasts (1)
IFN-γ	A 549 Carcinoma of the Lung (2)
IFN-γ	ACHN Renal Carcinoma (2)
IFN-γ, TNF or the combination	ME 180 Cervical Carcinoma Cells (3)
IFN-γ, TNF or the combination	A375 Melanoma (4)
IL-4, IFN-γ or the combination	Human Monocytes (5)

Our studies prove that cytokines of diverse primary, secondary and tertiary structure can have overlapping patterns of proteins in a given cell type. They also demonstrate that the same two cytokines in combination (i.e. TNF and IFN-γ) can have different effects on the patterns of proteins induced in 2 different cell types, (i.e. melanoma and cervical carcinoma cells), and indicate that diverse molecular mechanisms may be involved in mediating the functional synergistic antiproliferative effects of these two agents. Furthermore, we have been able to identify several IFN-γ -induced proteins not previously associated with the action of IFN-γ, and have studied their expression at both the protein and mRNA level.

*Supported by NIH grants CA27903 and CA44446.

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(2) J. Immunol. 147, 149, 1991.
(3) Electrophoresis 11, 232, 1990.
(4) In Tumor Necrosis Factor: Structure, Mechanism of Action, Role in Disease and Therapy II, in press, 1991.
(5) Clin. Res. 39, 57A, 1991.

J 019 THE ROLE OF INTERLEUKIN-4, INTERFERON-γ AND TUMOR NECROSIS FACTOR IN THE HUMAN THYMUS: STUDIES OF THYMOCYTE PROLIFERATION AND LOCALIZATION OF CYTOKINE-PRODUCING CELLS. Marianne Murphy and Lois B. Epstein, Department of Pediatrics and the Cancer Research Institute, University of California, San Francisco, CA 94143.*

An ordered and highly regulated production and interaction of cytokines in the thymic microenvironment appears to be necessary for normal thymocyte maturation. We have examined the interaction of interleukin-4 (IL-4), interferon-γ (IFN-γ) and tumor necrosis factor α (TNF) on postnatal human thymocyte proliferation. IL-4 was found to induce proliferation of postnatal human thymocytes in the absence of exogenous mitogen. The IL-4-induced proliferation correlated with the proportion of T cell receptor γ,δ (TCRγ,δ)-bearing cells in unseparated thymocyte preparations. Using a new method for the isolation of TCRγ,δ-bearing thymocytes from the human thymus, we have directly demonstrated vigorous proliferation of these cells in response to IL-4. We also observed that both IFN-γ and TNF inhibited IL-4-induced proliferation in a dose-dependent manner. These studies define an important role for IL-4 in the proliferation of TCRγ,δ$^+$ thymocytes and suggest regulatory functions for IFN-γ and TNF in human thymocyte proliferation. We also examined the effects of these cytokines on thymocytes from children with Down syndrome (DS, trisomy 21) because children with DS have a markedly increased susceptibility to infection and abnormal patterns of thymocyte maturation (1). In addition, trisomy-21 cells are known to be more sensitive to the effects of IFN-γ making this aneuploid condition a particularly appropriate system for examining the influence of IFN-γ on thymocyte maturation. We observed that DS thymocytes also proliferated in response to IL-4, but that proliferation by these cells was inhibited to a greater extent by both IFN-γ and TNF. To confirm the presence of IFN-γ and TNF in postnatal human thymuses and to implicate their involvement in the abnormalities associated with DS thymocyte maturation, we have used the technique of *in situ* hybridization to determine the prevalence and location of IFN-γ and TNF mRNA expressing cells in frozen sections of postnatal DS and control thymuses. In control thymuses we found IFN-γ mRNA expressing cells randomly distributed throughout the thymus, predominantly in the cortex. In contrast, TNF mRNA expressing cells are localized in the connective tissue of the trabeculae, at the cortico-medullary junction and in the medulla, sometimes in close association with Hassall's corpuscles (HC). These studies indicate distinct locations for IFN-γ and TNF production in the human thymus. Current studies are in progress to confirm that these cytokines are also produced at the protein level. DS thymuses have a markedly elevated presence of TNF mRNA expression compared to age-matched control thymuses. In DS thymuses, numerous cells expressing high levels of TNF mRNA were found clustered in groups, predominantly in the connective tissue and at the cortico-medullary junctions. In addition to the known anatomical abnormalities found in DS thymuses, including cortical depletion and greatly enlarged HC, we observed that DS thymuses contain high numbers of mast cells in the same sites as the TNF mRNA expressing cells. Efforts to identify the IFN-γ and TNF producing cells in the human thymus and to determine whether mast cells could be a source of TNF in the thymus are currently underway in our laboratory. The increased presence of mast cells and their co-localization with TNF mRNA expressing cells in DS thymuses suggest that TNF produced by these cells may influence the development of morphologic and functional abnormalities in the DS thymus.

(1) Murphy M, Epstein LB. 1990. *Clin. Immunol. Immunopathol.* 55:453.
*Supported by NIH grants AG 08938 and CA 08931

J 020 ROLE OF LYMPHOKINES IN THE CONTROL OF HEMATOPOIETIC CELL DIFFERENTIATION AND ACTIVATION, Giorgio Trinchieri[1], Marek Kubin[1], Jean W. Gupta[1], and Marco Cassatella[2], [1]The Wistar Institute, Philadelphia, PA (USA) and [2]The Institute of Pathology, University of Verona, Verona, Italy.

T lymphocytes and Natural Killer (NK) cells produce a variety of lymphokines with both stimulatory and inhibitory effects on hematopoiesis, during immune response, inflammation, or when exposed to different stimuli, such as interleukin 2 (IL-2) or immune complexes. Some of these lymphokines are exclusively produced by lymphocytes, e.g. interleukin-3 (IL-3) and immune interferon (IFN-γ), whereas others are also produced by several other cell types. Among the lymphokines with stimulatory effects on hematopoiesis, T and NK cells produce several colony-stimulatory factors (CSF) including GM-CSF, M-CSF, and multi-CSF or IL-3. Other lymphokines produced by T and NK cells, such as IFN-γ and the two types of TNF (TNF-α and TNF-β or lymphotoxin) have complex effects on hematopoiesis, including radioprotection of bone marrow functions in vivo (probably mediated by secondary induction of CSF production), inhibition of proliferation of progenitor cells in vitro, induction of differentiation of myelo-monocytic precursor cells, and functional activation of terminally differentiated neutrophils and monocyte-macrophages.

T and NK cells have been shown to have an effect in vivo on hematopoiesis in both physiologic and pathologic conditions, mediated mostly through their production of lymphokines. However, the most important role of the production of humoral factors affecting hematopoiesis by lymphocytes probably resides in the recruitment and activation of effector cells of non-adaptive immunity, e.g. neutrophils and macrophages, during inflammation and immune response. Most of the lymphokines produced by T and NK cells, including the CSFs, IL-3, TNF, and IFN-γ, are potent activators of such effector cells.

TNF and IFN-γ characteristically synergize in many of their effects on hematopoiesis. For example, the two lymphokines in combination mediate a much higher inhibition of hematopoietic colony formation or induction of differentiation of leukemic myeloid cells than each lymphokine individually. In order to study the molecular mechanisms by which TNF and IFN-γ induce differentiation of myelo-monocytic cells and activation of neutrophils and monocytes, we have analyzed the effect of these lymphokines on the expression of the genes encoding the polypeptides participating in the activity of the NADPH-dependent oxidase, the enzymatic complex responsible for the formation of O_2 in phagocytic cells during the respiratory burst. *Four components of the NADPH oxidase* have been characterized in different laboratories and their genes have been molecularly cloned: gp91 and p22 are two membrane proteins corresponding to the heavy and light chains of the cytochrome B_{558}, whereas p67 and p47 are two cytoplasmic proteins necessary for NADPH oxidase activity. During differentiation of human leukemic myeloid cell lines induced by TNF and IFN-γ, the gp91, p67, and p47 genes are upregulated, at a time preceding or corresponding to the differentiation of the ability of the cells to respond to extracellular stimuli with a respiratory burst. Unlike the other three genes, the p22 gene is constitutively expressed by the myeloid cell lines and its expression is not altered by TNF and/or IFN-γ. Analysis at the single cell level by in situ hybridization indicated that during differentiation of the cell lines the accumulation of the gp91 and p47 transcripts was heterogenous. By sorting the cells on the basis of NADPH oxidase activity or expression of the monocyte differentiation antigen CD14, differentiated cells were obtained that coordinately expressed high abundance of both gp91 and p47 transcripts as well as maximal CD14 antigen expression and NADPH oxidase activity.

The molecular effects of IFN-γ on differentiated neutrophils are not identical to those observed on immature myeloid cell lines. The expression of the gp91 gene is rapidly upregulated in neutrophils treated with IFN-γ, whereas the expression of the p47 gene, unlike in the cell lines is downregulated. The increase in the gp91 gene expression is a limiting event in the enhancing effect of IFN-γ on neutrophil respiratory burst, as shown by the ability of antisense oligonucleotides complementary to the gp91 mRNA to completely prevent the enhancing effect of IFN-γ on the respiratory burst without affecting the basal respiratory burst activity of untreated neutrophils. *Because the* effect of IFN-γ on neutrophil gene expression has been demonstrated at the mRNA level and, in many cases, at the level of gene transcription, it is now clear that many of the activation effects of these lymphokines on neutrophil functions are depending on de novo transcription and translation of selected genes encoding limiting factors for these cellular functions.

Neurobiology (Joint)

J 021 CYTOKINES IN DEMYELINATION AND DYSMYELINATION, Jean E. Merrill[1], Fredricka C. Martin[1], Lisa Thomas[1], Thomas T. Lee[1], Manuel A. Orellana[1], and Irvin S.Y. Chen[2], [1]Department of Neurology, [2]Departments of Medicine and Microbiology and Immunology, U.C.L.A. School of Medicine, Los Angeles, CA 90024

The demyelinating disease multiple sclerosis (MS) is thought to be the result of autoimmune processes involving overreactive CD4+ T cells which produce Interferon gamma (IFNγ), Interleukin 2 (IL2), and other cytokines. These T cells activate peripheral blood macrophages which, as a consequence, migrate into the central nervous system (CNS) with the T cells and create inflammation. The macrophages are actively producing Interleukin 1 (IL1) and tumor necrosis factor alpha (TNFα). This inflammatory event is associated with demyelination and death of the oligodendrocyte, the glial cell producing myelin. In addition, astrocytes in the lesion or plaque respond by gliosis, an event which includes proliferation and hypertrophy. These glial cells, which do not normally express the class II major histocompatibility antigen (MHCII) or HLA-DR, become DR$^+$ in response to immune inflammation. Microglial cells are bone-marrow derived macrophages; these cells are also activated by this process. Both microglia and astrocytes have been shown to produce IL1 and TNFα in vitro. In vivo in MS lesions, these cells and the invading macrophages also appear to produce these cytokines. Both glial populations respond to IL1 and TNFα by proliferation in vitro. This response, and the in vitro gliosis seen in astrocyte cultures, is the indirect effect of other cytokines induced by IL1 and TNFα. We hypothesize that Interleukin 6 (IL6) and colony stimulating factors are the secondary cytokines induced. IL6 is evident at the lesion edge and blood brain barrier. In addition, IL1 and TNFα are implicated in the destruction of myelin and death of the oligodendrocyte. Transforming growth factor beta (TGFβ) is a natural antagonist of IL1 and TNFα. It inhibits microglia cell killing of oligodendrocytes and is seen at the edge of burnt out plaques. If IL1, IL6, and TNFα do play a role in macrophage or glial cell mediated demyelination, TGFβ might provide an autocrine feedback to downregulate the process. It might explain the limitation of plaque size or possibly be a mechanism for the relapse-remission cycles seen in some MS patients.

CNS AIDS patients experience neurological symptoms which include headache, fever, cognitive changes, abnormal reflexes, and ataxia. More severe disease is characterized by dementia and severe sensory and motor dysfunction. Histological changes include inflammation, astrogliosis, microglial nodule formation, and diffuse dysmyelination in the form of myelin pallor. In 20% of CNS AIDS, frank demyelination is seen. AIDS associated neurological diseases are correlated with greater levels of HIV-1 antigen or genome in brain tissue. In AIDS dementia, macrophages and microglial cells of the CNS are the predominant cell types infected and producing HIV-1. HIV-1 induction of IL1 and TNFα, demonstrated in cultures of peripheral blood monocyte/macrophages and glial cells, would lead to an autocrine feedback loop involving further virus replication in T cells and macrophages. IL1, IL6, and TNFα may account for many clinical and histopathological findings in AIDS nervous system disease. Since HIV-1 infected patients produce elevated cytokines in vitro and in vivo in blood, investigations of the CNS for their presence in association with activated macrophages, astrocytes, and microglia is of great importance for a formal association with the clinical and pathological conditions.

J 022 THE EMERGING NEUROPOIETIC CYTOKINE FAMILY, Paul H. Patterson, Biology Division, California Institute of Technology Pasadena, CA 91125.

As in the hematopoietic system, the enormous variety of phenotypes in the nervous system arises, in part, through the action of instructive differentiation signals. Neuronal culture assays have allowed the identification and cloning of several proteins that control the expression of phenotype-specific genes in developing neurons. The cholinergic differentiation factor (CDF; also known as leukemia inhibitory factor; LIF) induces and suppresses the expression of a number of neurotransmitter synthetic enzymes and neuropeptides in several types of postmitotic neurons. Many of these effects are reversible, and the particular genes affected depend on the type of neuron under study. Ciliary neurotrophic factor (CNTF) alters the expression of the same set of genes in sympathetic neurons as CDF/LIF. The predicted tertiary structures of these two proteins suggest they belong to a family of cytokines that includes IL-6, ONC, MGF and G-CSF. This association is strengthened by the finding that the receptors for CDF/LIF and CNTF are homologous to the transducing and ligand binding subunits, respectively, of the IL-6-R. Moreover, CDF/LIF is a polyfunctional cytokine that shares a remarkable overlap with IL-6 in its actions on non-neural tissues.

To begin to study the role of CDF/LIF *in vivo*, we have localized its mRNA using RT-PCR and RNase protection methods. CDF/LIF mRNA levels are developmentally modulated, and substantial differences are observed between tissues. Particularly high expression is found in the target tissue of cholinergic sympathetic neurons, the footpads that contain sweat glands. Sweat glands were previously shown to induce noradrenergic sympathetic neurons to become cholinergic *in vivo*. Biochemical and immunological analysis indicates that a protein resembling CNTF is also present at the appropriate time in the footpad to mediate the phenotypic conversion that sympathetic neurons undergo during normal development. In addition, CDF/LIF mRNA is selectively expressed in discrete regions of the postnatal rat brain, and in liver and spleen at particular ages. These results support the possibility that CDF/LIF plays a role in hematopoiesis and in the mature CNS.

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Cytokines and the Cardiovascular System

J 023 MODULATION OF CARDIAC MYOCYTE AND INTERSTITIUM BY IMMUNE CYTOKINES. C.A. Spilburg[1], J.Chao[1], G.F. Schreiner[2] and L.G. Lange[1], [1]Department of Medicine, Jewish Hospital of St. Louis, Washington University School of Medicine, and [2]Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.

The degradation of collagen (fibril form and gelatin) in the heart was studied in order to investigate the mechanisms of cardiac remodeling that occurs in immune-mediated cardiomyopathies, cardiac allograft rejection or after myocardial infarction. Medium from confluent rat cardiac fibroblasts showed little or no collagenase or gelatinase activity. However, when this same medium was exposed to 1 mM 4-aminophenylmercuric acetate for 2 hr at 37°C, the level of gelatinase activity increased markedly, indicating that this cell type synthesizes and secretes a progelatinase. No collagenase activity was detected. Non-reducing zymography of activated medium on gels containing 1 mg/ml gelatin showed the presence of five enzyme species -- *130 kDa, 95 kDa, 72 kDa, 67 kDa and 53 kDa*, with the 72 kDa and 67 kDa forms comprising over 80% of the zymographic activity. This proteolytic activity was enhanced by exposing fibroblasts to IL-1 (0.1 ng/ml) with most of the increase occurring in the 72 kDa species. These results indicate for the first time that gelatinases are secreted by cardiac fibroblasts and hence may play a role in heart remodeling when stimulated by immune cytokines.

Ontogeny of Aging

J 024 **AGING AND LYMPHOKINE PRODUCTION BY T CELL SUBSETS**, David N. Ernst, William O. Weigle, and Monte V. Hobbs, The Scripps Research Institute, La Jolla, CA 92037.

Recent studies on T cell clones and normal T cells from humans and experimental rodents have revealed T cell subsets which can express unique patterns of membrane differentiation antigens, activation requirements, signal transduction pathways, and cell cycle activities. These subsets can perform unique regulatory or effector functions which can reflect restricted patterns of cytokine gene expression. These findings have led to the development of T cell-subset paradigms for modeling the intercellular communications that underlie T-cell dependent immunity. We and others have begun testing whether or not the decreased immune competence of old animals more accurately reflects large shifts in the representation of functional T cell subsets rather than the accumulation of intrinsically-defective T cells. Accordingly, we have analyzed $CD8^+$ and $CD4^+$ T cells from young, middle-aged, and old mice for their expressed patterns of three membrane molecules, 3G11, CD45RB, and CD44, thought to be differentially displayed by T cell subsets. In concert, we have profiled the stimulation-dependent patterns of cytokine gene expression and cytokine release by these subsets. The results showed that increasing donor age is accompanied by increasing percentages of $3G11^{lo}$, $CD45RB^{lo}$, and $CD44^{hi}$ cells within the peripheral, $CD8^+$ and $CD4^+$ T cell populations. In comparison to young controls, stimulated $CD8^+$ T cells from old mice produced higher levels of TNFα, TNFβ, and IFNγ when cultured with plate-bound anti-CD3 antibody. Stimulated $CD4^+$ T cells from old donors produced higher levels of IL-3, IL-4, IL-5 and IFNγ, similar levels of IL-2, IL-6 and TNFβ, and lower levels of TNFα. In contrast, the DNA synthetic responses of both T cell subpopulations from old mice were diminished. Based on these findings and previous reports, it would appear that T cell populations from old donors display altered functional repertoires commensurate with changes in their subset makeup. The results from these and other studies have led to a model for the age-related decline in T cell-mediated immunity which considers the striking shifts in the subset makeup of peripheral T cells subpopulations. These subset shifts are viewed as potential sources of major deficits and/or dysregulations in T cell-dependent immunity which may contribute to immunosenescence.

J 025 REGULATION OF TGF-β1 mRNA IN ADULT RAT BRAIN: IMPLICATIONS FOR NEURODEGENERATION/REGENERATION AND AGING,

Nancy R. Nichols, Nicholas J. Laping, Todd E. Morgan, Giulio M. Pasinetti, and Caleb E. Finch, Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles CA 90089-0191.

Stress responses protect the organism against adverse conditions and mediate adaptation to stress. Impairment of stress mechanisms during aging may contribute to neurodegenerative disease. Glucocorticoids are released following stress and may facilitate adaptation by regulating response to a wound or pathology, hence their role as anti-inflammatory and immunosuppressive agents. These actions of glucocorticoids are often mediated by their interactions with cytokines. In addition, glucocorticoids can both accelerate age-related neurodegeneration in the adult rat hippocampus and prevent the loss of dentate granule neurons in developing brain. We are investigating mechanisms of neurodegeneration/regeneration at the molecular level by cloning mRNAs responsive to glucocorticoids and comparing their responses to brain lesions in young rats. We discovered that TGF-β1 mRNA expression in the brain is decreased 30% in response to glucocorticoids and subsequently showed that TGF-β1 mRNA is increased 500% in response to several different brain lesions. The mRNA is differentially expressed both at the site of injury and in the deafferented area at different times after lesion, and eventually returns to unlesioned control levels. The pattern of TGF-β1 gene expression in the deafferented area is distinctive and overlaps with that of specific antibody staining for activated microglia or reactive astrocytes depending upon the lesion. In response to a neurotoxic lesion, the pattern of TGF-β1 mRNA expression is consistent with the area of neuronal cell death. These data indicate that although various lesions increase the expression of TGF-β1 mRNA, the cellular pattern of expression may vary with the insult. In vivo TGF-β1 administration into the lateral ventricle increases three hippocampal mRNAs which are also increased after brain lesion but has no effect on other lesion-induced mRNAs. These data indicate that TGF-β1 may orchestrate select aspects of the response to brain injury. Finally, hippocampal TGF-β1 mRNA is elevated in 24 mo rats compared with 6 mo and 15 mo male Fisher 344 rats. Collectively, these data implicate TGF-β1 in neural plasticity. Therefore, TGF-β1 could play an important role in brain injury, aging and neuroendocrine adaptation, particularly in view of the similarities between tissue repair and disease processes regulated by TGF-β peptides in peripheral tissues. (Supported by PHS #AG-07909 and The Brookdale Foundation)

J 026 ACTIVATION DEFECTS IN T LYMPHOCYTES FROM OLD MICE,

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Work in many labs has now established that T cells from old mice and old people show defects both in production of and in response to IL-2. Our laboratory has focussed on two components of age-related decline in mouse T cell function: replacement of naive T cells by memory cells, and defects in activation pathways that depend on calcium signals and protein kinase activation.

Memory T cells accumulate with age, and several lines of evidence suggest that this transition is largely responsible for loss of both IL-2 production and response. (1) Purified memory T cells, from old or young mice, are less likely than naive cells to respond to Con A or to the superantigen SEB by production of IL-2 in limiting dilution assays. IL-3 producing T cells are also rare in the memory T cell subset, as are cells that can respond to Con A and IL-2 by proliferation or by differentiation into cytotoxic effectors. (2) Memory T cells, detected by expression of the CD44 marker, produce smaller changes in cytoplasmic calcium than virgin T cells when exposed either to receptor-dependent agents like Con A and aCD3, or to receptor-bypassing agents like ionomycin. (3) Subpopulations of T cells, from young or old mice, purified by their ability to produce a mitogen-triggered calcium signal are found to contain nearly all of the cells able to produce or respond to IL-2. (4) A newly developed Percoll/ionomycin gradient method that separates cells on the basis of intrinsic resistance to ionomycin-induced increases in calcium ion concentration shows that the ionophore-resistant T cells are largely $CD44^{lo}$ and function poorly in tests for IL-2 production and response. All these approaches thus show the association, in young or old mice, of three properties -- memory cell surface phenotype, resistance to calcium signal generation, and poor function in limiting dilution assays -- that distinguish T cells from young and old donors.

More recent data on protein phosphorylation, however, suggest that the accumulation of memory T cells with aging may not be the only basis for altered T cell function in old age. Using two-dimensional electrophoretic separation of "indicator" phosphoproteins we find that middle-aged and older mice exhibit a progressive and eventually quite severe decline in phosphorylation of a wide range of Con A and aCD3 responsive protein substrates. Defects in receptor-mediated signal transduction cannot by themselves account for these changes, since (a) T cells from old mice show parallel losses in phosphorylation induced by intracellular agonists like PMA and ionomycin; and since (b) Con A, aCD3, and PMA each induces the phosphorylation of three substrates in T cells from old mice that are not phosphorylated in young animals. Finally, we have used indicator phosphoproteins that distinguish virgin from memory T cells in young mice to show that the altered patterns of phosphorylation seen in old mice cannot be attributed simply to a replacement of normal naive by normal memory cells.

Cytokine Interactions in Bacterial and Parasitic Disease

J 027 CYTOKINES IN THE PATHOGENESIS OF MURINE LEISHMANIASIS.

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Infection of BALB/c mice with Leishmania major leads to progressive dissemination of the parasite and fatal disease associated with expansion of CD4+ T cells that express mRNA for IL-4 and IL-10, consistent with a Th2 phenotype (1). A number of immunologic interventions, including sublethal irradiation or administration of anti-CD4+ or anti-IL-4 monoclonal antibodies, at the time of inoculation of the parasite, results in control of Leishmania replication and establishment of immunity. In each case, such interventions are associated with expansion of CD4+ T cells that express mRNA for IL-2 and IFN-γ, consistent with a Th1 phenotype. Cell lines were established from BALB/c mice with progressive infection or with established immunity that were CD4+, expressed α/β T cell receptors, and proliferated and released cytokines in response to L. major antigens. The cell line from the animal with progressive infection released IL-4 and IL-5 and accelerated the course of infection when passively transferred into scid mouse recipients prior to challenge with parasites. The cell line from the immune mouse released IFN-γ when incubated with parasite antigens and protected recipient scid mice following passive transfer. CD4+ T cells could be recovered from the reconstituted scid mice 6 weeks after infection that maintained the cytokine profile of the parental lines. The effects of the Th2 cell line could be completely abrogated by simultaneous adminstration of anti-IL-4 antibody, whereas the effects of the Th1 cell line could be abrogated by administration of anti-IFN-γ antibody. Further analysis of the neutralizing effects of antibodies were carried out in susceptible BALB/c or resistance C57BL/6 mice. The ability of anti-IL-4 antibody to protect BALB/c mice was lost if antibody was administered later than 7 days following infection; a single dose completely cured recipient animals. Similarly, a single dose of anti-IFN-γ reversed the ability of C57BL/6 to contain infection; again, administration later than 7 days after infection had little effect. Finally, administration of large doses of recombinant murine IFN-γ to susceptible BALB/c mice from the time of infection had little effect on the course of disease, and adminstration of recombinant IL-4 had little effect on the capacity of C57BL/6 mice to heal. Co-infection of C57BL/6 mice with Nippostrongylus brasiliensis in order to elevate endogenous levels of IL-4 also had no effect on the ability of the animals to both cure L. major infection and expulse the nematode from the intestine. Although IL-4 and IFN-γ are necessary for progressive infection and establishment of Leishmania immunity, respectively, neither seems to be sufficient. The cell of origin of the early levels of IL-4 and IFN-γ that influence the later development of a mature Th2 or Th1 response remains to be identified. (Supported by NIH AI 26918)

1. Heinzel FP, MD Sadick, SS Mutha, RM Locksley. 1991. Production of interferon γ, interleukin 2, interleukin 4, and interleukin10 by CD4+ lymphocytes in vivo during healing and progressive murine leishmaniasis. Proc. Natl. Acad. Sci. USA 88:7011.

Late Abstract

ROLES FOR THE TRANSFORMING GROWTH FACTOR-beta SUPERFAMILY IN VERTEBRATE EMBRYOGENESIS.

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We are studying the function of members of the TGFß family of peptide growth factors in vertebrate embryogenesis, particularly with respect to the induction and patterning of the mesoderm in the frog *Xenopus*. We have identified the TGFß-related activins as potent mesoderm inducers in explants of blastula stage animal hemispheres, which develop a variety of dorsal/anterior mesodermal and neural tissues in response to activin treatment. These tissues include notochord, somitic muscle, neural tube, brain and eye components (including retina and lens). The induced structures are often arranged in a pattern similar to that in an intact embryo. The mechanism of this patterning is unclear but it is due in part to dorsal-ventral differences in the responsiveness of animal cap cells to activin. Mesoderm induction begins during the transcriptionally-silent early cleavage period of the blastula under the control of substances present in the egg. Two activin genes, ßA and ßB, code for homodimeric activin proteins A and B, respectively. Transcripts of the ßB gene are first detected by RNAse protection in the late blastula stage, during the latter half of the mesoderm induction period, while ßA transcripts first appear around the time of neurulation, significantly after mesoderm induction has finished. Using activin-specific primers in PCR amplification of cDNA we find no evidence for activin transcripts in fertilized eggs or early blastula stages preceding zygotic gene activation at the mid-blastula transition. Activin B therefore appears as a good candidate for a natural dorsal/anterior mesoderm inducer. Consistent with this possibility we observe that injection of synthetic activin mRNA into the ventral side of the blastula induces a partial secondary dorsal axis. The simple prediction that activin mRNA may be dorsally localized in normal embryos does not hold, however, as instead it is uniformly distributed. The temporal and spatial aspects of activin protein in the embryo remain to be determined.

Based on the activin gene expression profiles we suggest that activin B may function in the late blastula as an intermediate agent in the mesoderm induction cascade, since its mRNA appears too late in development to encode the earliest mesoderm inducing signal. Thus we have sought and identified TGFß members whose mRNAs are present maternally and which might correspond in part to the earliest acting mesoderm inducer(s). These genes are *Xenopus* homologs of mammalian bone morphogenetic proteins (BMPs) and are related to *Drosophila decepentaplegic* (*dpp*) and Vg1 (previously known to be maternal in Xenopus). They are referred to as DVR2, DVR4 and DVR7 (DVR stands for Decapentaplegic-Vg-Related). They are expressed from egg through at least neurula stages and functional tests indicate that they may be involved in ventral mesoderm formation. The latest experiments addressing spatial and temporal expression profiles and functions of TGFß genes will be presented.

Embryogenesis and Development; Inflammation and Repair

J 100 CHARACTERIZATION OF IL-1 PRODUCING CELLS IN PEYER'S PATCHES AFTER INFECTION OF MICE WITH YERSINIA ENTEROCOLITICA O8, H. Ulrich Beuscher, Ulf-Peter Rausch and Martin Röllinghoff, Institute of Clinical Microbiology, University of Erlangen-Nürnberg, Wasserturmstrasse 3, 8520 Erlangen, Germany.

In vivo production of IL-1 (i.e. IL-1α and IL-1ß) was studied in inflamed Peyer's Patches (PP) resulting from infection of mice with Y. enterocolitica O8. By using two antisera specific for IL-1α and IL-1ß, immunohistochemical analysis of frozen tissue sections revealed two types of cells producing either IL-1α or IL-1ß. In addition, immunofluorescence double stainig demonstrated a few cells coexpressing both types of IL-1. All IL-1 producing cells stained positive with anti Mac 1 antibodies and must therefore be considered as phagocytic cells. Myeloperoxidase staining of tissue sections showed that only the IL-1ß positive cells, but not the IL-1α positive cells contained the peroxidase activity. Control experiments allowed the detection of peroxidase activity in monocytes but not in mature macrophages. Finally, when comparing the kinetics of IL-1 production by infiltrating phagocytes, induction of IL-1α was delayed by at least 24 h. We conclude that cellular differentiation of mononuclear phagocytes migrating into the inflamed tissue is one mechanism involved in regulation of IL-1 expression in vivo.

J 101 **ANALYSIS OF CYTOKINE mRNA LEVELS IN INTERLEUKIN 4 TRANSGENIC MICE BY QUANTITATIVE POLYMERASE CHAIN REACTION** Thomas Blankenstein, Cornelia Platzer, Günther Richter, Klaus Überla and Tibor Diamantstein, Institute of Immunology, Klinikum Steglitz, Free University Berlin, 1000 Berlin 45, FRG

Cytokine mRNA levels were compared between spleen cells of interleukin (IL) 4 transgenic and normal mice by quantitative polymerase chain reaction (PCR). Therefore, a plasmid was constructed which contains in tandem array 5' and 3' primer specific sequences for the cytokine genes IL1-IL6, tumor necrosis factor (TNF), lymphotoxin (LT), interferon-γ (IFN-γ) and for control β-actin. A distance between 5' and 3' primers has been choosen that target and control DNA differ slightly in size. During coamplification, target and control DNA compete for the primers and the amount of PCR product is proportional to the amount of input DNA. By coamplification of constant amounts of β-actin control fragment and serially diluted cDNAs, the cDNAs were adjusted to identical concentrations. Subsequently, a fixed amount of the calibrated cDNAs was amplified in the presence of serially diluted control fragment to determine the cytokine mRNA level. The sensitivity of this approach was demonstrated by the capability to detect a two-fold difference in IL4 mRNA levels between IL4 transgenic heterozygous and homozygous animals. Upon LPS activation, the IL4 transgene which is expressed essentially in B-lymphocytes was induced approximately 50-fold. A number of cytokines (IL1, IL5, IL6, IFN-γ) and also the IL4 receptor were found to be upregulated in IL4 transgenic mice whereas IL2 and IL3 mRNA levels were not increased. These results demonstrate the usefulness of quantitative PCR to analyse the regulatory network of cytokines 'in vivo'.

J 102 POTENTIAL ROLE OF IL-1 AND METALLOPROTEINASE EXPRESSION DURING HEART DEVELOPMENT, Thomas K. Borg, Masao Nakagawa, Kristofer Rubin and Louis Terracio, University of South Carolina, Columbia, SC 29208. Recent studies have demonstrated the involvement of cytokines, especially IL-1, in embryonic development. One potential role for IL-1 in the development is the regulation of metalloproteinases secretion. These enzymes appear to be necessary for the remodeling of existing extracellular matrix (ECM) during morphogenetic events of heart development such as trabaeculation, valve and septum formation. To determine the association between IL-1 and collagenase expression, immunolocalization of these components during development was examined in the rat heart from 9.5 to 12.5 days of embryonic development. The results show a temporal and spatial localization of antisera against both IL-1 and the 72 kD collagenase which peaked at 11.5 days and correlated with the early events of valve formation in the endocardial cushions and trabaeculation of the ventricular chambers. These data document the presence of these components at the time when the ECM is undergoing remodeling to achieve the final form of the heart.

J 103 PHENOTYPIC CHARACTERIZATION OF THE CONTRA IL-2 PRODUCING HYBRIDOMA, AS-9, Elise Brownell, Lynn Fiorentino, Douglas Austen, and Takashi Maki*, Molecular Therapeutics, Inc., West Haven, CT 06516, *New England Deaconess Hospital, Boston, MA 02215

An immunosuppressive molecule named CONTRA IL2 is produced by murine spleen cells following exposure to antilymphocyte serum (ALS). This substance inhibits ConA-driven spleen cell proliferation, IL-2-dependent CTLL proliferation and CTL generation in mixed lymphocyte reactions. One of the goals of our laboratory is to characterize CONTRA IL2 at the molecular level. To this end, a CONTRA IL2-producing hybridoma line (AS-9) was developed from ALS-treated murine spleen cells. In this study, we determine the phenotype of the parental splenic cell that is the source of the immunosuppressive activity. A combination of PCR-assisted mRNA analysis, Northern analysis and cell sorting experiments suggest that parental cell type is derived from the T-cell lineage, although it fails to express any of the markers associated with the more well-known, mature T-cell compartments. One novel observation is that the AS-9 spleen cell parent is capable of expressing **spliced** T-cell receptor delta transcripts from an **unrearranged locus**. These results suggest that the CONTRA IL2 producing cell type represents a little known T-cell lineage in the adult mouse.

J 104 TGF-β OPPOSES THE STIMULATORY EFFECTS OF IL-1 AND TNF ON AMNION CELL PROSTAGLANDIN E2 PRODUCTION: IMPLICATION FOR PRETERM LABOR,

Kristina Bry, Mikko Hallman, Division of Neonatology, University of California, Irvine, CA 92717

Preterm labor is strongly associated with inflammation in the fetal membranes. Increased concentrations of cytokines, such as IL-1 and TNF, are found in the amniotic fluid in preterm labor. Prostaglandins are major inducers of uterine contractions. IL-1 and TNF promote labor by stimulating the production of prostaglandins by the fetal membranes. TGF-β influences cell growth, differentiation, immunity, and extracellular matrix formation. We studied the effect of TGF-β on the IL-1- and TNF-stimulated production of prostaglandin-E2 (PGE2) by human amnion cells in monolayer culture. Amnion cells were treated with IL-1α (6.8 ng/ml), IL-1β (5 ng/ml), TNFα (50 ng/ml), TNFβ (10 ng/ml), or vehicle (control) in the presence or absence of human TGFβ-1 (10 ng/ml). After 36 h of incubation, the media were removed and analyzed for PGE2. The table shows the results (means± SE) of seven separate experiments:

	PGE2 production (fractions of control)		decrease (%) in PGE2 production
	without TGFβ	with TGF-β	
Control	1	0.7 ± 0.1	27 ± 9
IL-1α	25.5 ± 9.6	4.1 ± 1.8	77 ± 4
IL-1β	27.3 ± 10.6	4.4 ± 2.0	75 ± 5
TNFα	48.4 ± 7.9	9.7 ± 3.1	75 ± 8
TNFβ	9.6 ± 2.6	1.4 ± 0.3	71 ± 9

TGFβ1, TGFβ2, and TGFβ1,2 were equipotent, a concentration of 0.1 ng/ml giving maximal inhibition in each case. We conclude that TGF-β strikingly suppresses the prostaglandin production induced by IL-1 or TNF. TGF-β may be an important factor in preventing the labor process provoked by these cytokines.

J 105 HYDROCOLLOID VERSUS WET-TO-DRY DRESSING TREATMENT OF CHRONIC WOUNDS.

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Chronic wounds are a common problem in bedbound geriatric patients. In this study we compare the change in surface area, and wound histology in large (15-60 cm^2) stage III-IV sacral or trochanteric chronic pressure sores treated with hydrocolloid (HCD) or normal saline wet-to- dry (WDD) dressings over an 8 week period. Individual patients (n=3) had bilateral wounds which were each randomized to HCD or WDD treatment. Change in the area of the wounds was calculated from transparency tracings obtained at entry and every two weeks using the equation: % area change= (week 0 area - week 8 area)/week 0 area. 6 mm biopsies were obtained from the center and leading edge of each wound at each time point. Biopsy specimens were divided and submitted for quantitative bacteriologic cultures (wk 0), hematoxylin and eosin, and trichrome staining. The remainder of the sample was frozen to assay for collagenase I (CSE) mRNA. The decrease in the surface area of the wounds was greater with HCD in two of three patients over the 8 week period. Quantitative cultures were positive for beta-strep in one patient. All specimens week 0 showed features of early granulation tissue with neutrophils, macrophages, histiocytes, edema, and new vessel formation. By week 4 the HCD treated wounds uniformly had increased neovascularization, fibrin deposition, increased numbers of fibroblasts and fibrillar collagen. By week 8 all the HCD treated wounds had a mature extracellular matrix with dense collagen fibrils, and a marked dimunition of inflammatory infiltrates. The WDD treated wounds were relatively unchanged demonstrating minimal fibrin and fibrillar collagen by week 8. Thus far we have been able to demonstrate improvement in the healing of chronic wounds using HCD compared to the traditional WDD in wounds with negative quantitative bacteriologic cultures. We are now developing an approach using reverse transcription and the polymerase chain reaction (RT-PCR) to quantitate collagenase expression in these wounds. A control template for collagenase I was generated using site directed mutagenesis, and cloned into a vector that contains a T3/T7 promotor for in vitro transcription. Inappropriate overproduction of collagenase may imply a role for protease inhibitors in the treatment of these wounds. A similar approach may also be used to assess the efficacy of specific growth factors in wound healing.

J 106 MOLECULAR CLONING OF THE RECEPTORS FOR HUMAN IL-8, NAP-2, AND GRO/MGSA,

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Interleukin-8, NAP-2, and GRO (also known as melanoma growth stimulatory activity) are three cytokines belonging to a growing family of peptides which are chemotactic agents for inflammatory cells such as neutrophils. Receptors have been identified for IL-8, GRO, and NAP-2 on human neutrophils and granulocytic cell lines and it has been observed that these three cytokines can compete for binding. We have recently characterized the rabbit IL-8 receptor [Beckmann *et al.*, (1991) *Biochem. Biophys. Res.*, 179, 784] and found that it binds only IL-8 at high affinity (K_a of 3 x 10^9 M^{-1}). The IL-8 receptor is a member of the rhodopsin super family of G-protein coupled receptors. Using the rabbit clone as a probe, we screened a human neutrophil library and isolated two classes of cDNAs, termed type I and type II. These cDNAs are identical to clones isolated by Holmes *et al.* [*Science* 253, 1278 (1991)] and Murphy and Tiffany [*Science* 253, 1280 (1991)], respectively. We have extended their work and found that the type I receptor binds only IL-8 while the type II receptor binds IL-8, GRO and NAP-2 at high affinity when transfected into COS-7 cells. The two cDNAs encode proteins that have an amino acid sequence identity of 77% while the type I and II receptors have an identity of 84% and 74% with the rabbit IL-8 receptor. There is also significant homology with potential coding regions of unknown function from the human cytomegalovirus genome.

J 107 EVIDENCE FOR DIFFERENCES AMONG MOUSE STRAINS AND ANATOMIC SITE IN CYTOKINE PRODUCTION BY MURINE EPIDERMAL CELLS,

Marion J.J. Gijbels, Elske Polen, Harm HogenEsch, TNO Institute of Aging and Vascular Research, Leiden, The Netherlands

There is clear evidence that the epidermis can function as a site for the initiation of an inflammatory response. Keratinocytes have the capacity to produce a variety of cytokines. In view of the fact that the inflammatory response varies in the skin of several mouse strains and in different anatomic sites, we investigated the cytokine production by epidermal cells from abdominal skin, dorsal skin and ear of C57BL/Ka, BALB/c and CBA/Rij mouse strain. Therefore, epidermal cells were cultured with and without phorbol 12-myristate 13-acetate (PMA), the supernatant was harvested after 96h and tested for interleukin (IL)-1 (D10 assay), IL-3 (FDC assay), IL-6 (B9 assay) and tumor necrosis factor alpha (TNFα) (WEHI 164 assay). After exposure to PMA, the cultured epidermis of the three mouse strains contained more IL-1 but less IL-3 and IL-6. No TNFα bioactivity was observed in supernatant of the keratinocytes. Little IL-3 was found in the supernatant of the abdominal skin and dorsal skin of the C57BL/Ka mice, whereas the IL-3 production in the epidermis of the ear was very high. However, in the BALB/c and CBA/Rij strain, the abdominal skin produced more IL-3 than the dorsal skin and in the epidermis of the ear almost no IL-3 was observed. IL-6 bioactivity in the several sites of the skin was similar for the three mouse strains. The IL-6 bioactivity was higher in the epidermis of the ear than in the epidermis of abdominal and dorsal skin.

The fact that the inflammatory response is different in the several mouse strains and in different anatomical sites seems to be related on the different cytokine production of the epidermal cells. We are now testing the contribution of Langerhans cells and dendritic epidermal T cells to the cytokine production.

J 108 THE EFFECT OF INTERLEUKIN-1 RECEPTOR ANTAGONIST ON IL-1, LPS, *STAPHYLOCOCCUS EPIDERMIDIS* AND TUMOR NECROSIS FACTOR FEVER,
Takashi Ikejima, Xixian Zhang, Heide Wen, Sheldon M. Wolff and Charles A. Dinarello, Division of Cellular Biology, Banyu-Merck Sharp & Dohme Tsukuba Research Institute, Tsukuba, Japan and Division of Geographic Medicine and Infectious Diseases, Tufts University-New England Medical Center Hospitals, Boston, MA 02111
IL-1 is one of the most potent pyrogenic cytokines and LPS is an inducer of IL-1. Using a single dose of LPS (75 µg/kg), there was no difference in the fever responses between rabbits receiving LPS plus a constant infusion of saline and rabbits receiving LPS plus an infusion of human recombinant IL-1 receptor antagonist (IL-1ra) (5 µg/kg/min). However, IL-1ra (5 µg/kg/min infusion or 1 mg/kg bolus) blocked the pyrogenicity of human IL-1β (100 ng/kg) in rabbits. Human IL-1ra is also sufficient to block 100 ng/kg rabbit IL-1β. The second peak of fever which occurs with LPS is also observed with high doses of TNF and regardless of the stimulus (LPS, TNF, *Staphylococcus epidermidis*, *E. coli*), the plasma levels of IL-1β are consistently elevated between 2 and 4 h and reach peak elavation at 3 h. The 3 h time point corresponds best with the 3 h fever peak. Since TNF induces IL-1 and IL-6 *in vitro* and *in vivo*, the second fever peak which reaches maximal levels 3 h after TNF, thought to be mediated by either IL-1 or IL-6. However, there is no diminution of the febrile response by a constant infusion of IL-1ra. We were unable to measure circulating IL-6 levels in these rabbits due to technical difficulties. TNF-induced second fever is independent of IL-1. The same dose of IL-1ra infusion did not reduce the *Staphylococcus epidermidis*-induced fever (1 x 10^9 cells/kg). These results suggest that IL-1 may not be mediating the fevers due to LPS, *Staphylococcus epidermidis* or TNF. The results are further complicated by the demonstration that blood IL-1ra levels taken at the end of IL-1ra infusion were significantly lower in rabbits receiving either LPS, *Staphylococci* or TNF than rabbits receiving saline. This would increase the requirement for IL-1ra.

J 109 TRANSCRIPTIONAL ACTIVATION OF FIBROBLAST COLLAGENASE GENE EXPRESSION BY A NOVEL LYMPHOKINE, LEUKOREGULIN
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Leukoregulin (LR) is a novel T-cell derived cytokine with unique anti-tumor properties. We have recently demonstrated that LR is also able to modulate the biosynthetic repertoire of normal human skin fibroblasts in culture (J. Cell Biol. 113:1455-1562, 1991). In this study, we have examined in detail the effects of LR on collagenase gene expression in human skin fibroblast cultures. The results indicated time- and dose-dependent induction of collagenase mRNA steady-state levels, the maximum elevation being ~35-fold. In contrast, the mRNA levels for tissue inhibitor of metalloproteases (TIMP) remained unchanged in the same RNA preparations. The enhancement of collagenase mRNA levels was shown to be dependent on protein synthesis, and it could be counter-acted by dexamethasone or all-*trans*-retinoic acid. Transient transfections of cultured fibroblast with a human collagenase promoter/reporter gene (CAT) construct indicated up-regulation of the promoter activity, which could be blocked by dexamethasone and all-*trans*-retinoic acid. The observation suggested regulation at the transcriptional level of collagenase gene expression. LR was also shown to induce the mRNA levels for *jun-B*, suggesting possible involvement of the AP-1 complex in the regulation. The ability of LR to selectively induce collagenase gene expression in skin fibroblasts suggests that this cytokine may significantly contribute to the degradation of the extracellular matrix in physiological situations, such as tissue development and repair, and in diseases characterized by excessive degradation and turnover of collagen.

J 110 HUMAN LIPOCORTIN-1 INHIBITS INTERLEUKIN-1-INDUCED NEUTROPHIL MIGRATION IN THE MOUSE AIR-POUCH MODEL,
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Lipocortin-1 (LC-1) and interleukin-1 (IL-1) are endogenous proteins with different effects on the acute inflammatory process: LC-1 has anti-inflammatory properties mediating in part the effects of glucocorticoid hormones (1,2) whereas IL-1 has a pro-inflammatory profile stimulating migration of polymorphonuclear cells (PMN) in the early stages of acute inflammation (3). Pouches were formed on the back of male mice (22-25 g) by injection of 2 ml sterile air on day 0 and 3 (4). IL-1α (20 ng) injected into the pouch on day 6 induced a fast and intensive migration of PMN at 4 h (8.9 ± 0.4 x 10^6 PMN per mouse, n=29). This migration was unaffected by pretreatment with indomethacin (0.2 and 1 mg/kg i.v.) while it was inhibited by dexamethasone in a dose-dependent manner (37.5 ± 5.8 % inhibition, n=6, p<0.05 at 0.15 mg/kg i.v. 2 h before IL-1, and 63.8 ± 4.1 %, n=6, p<0.01 at 1.5 mg/kg i.v.). A reduction of PMN influx was also evident after treatment with human recombinant LC-1. Intravenous administration of LC-1 (10 µg per mouse, 30 min before IL-1) inhibited the migration at 4 h by 89.5 ± 2.5 %, n=5, p<0.01. A significant effect was also observed with 5 µg (43.6 ± 9.2 %, n=6, p<0.05) whereas 1 µg was without effect (24.9 ± 9.0 %, n=6; N.S).
During acute inflammation, endogenous IL-1 appears to be involved with the influx of PMN (3,5): the impairment of IL-1-induced migration by LC-1 may be at least one of the mechanisms underlying the anti-inflammatory activity ascribed to this protein.

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J 111 INFLAMMATORY CYTOKINE GENE EXPRESSION AND PRODUCTION IN MONOCYTES AND ALVEOLAR MACROPHAGES: EFFECTS OF RECOMBINANT MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) THERAPY,
M.J. Thomassen, J. Antal, G.T. Budd, M. Ahmad, H. Wiedemann, D. Meeker, V. Gibson, J. Finke, R. Tubbs, and R. Bukowski, Cleveland Clinic Foundation, Cleveland, OH 44195
We compared the effect of in vivo M-CSF treatment on the inflammatory cytokine responses of monocytes and alveolar macrophages. Thirty six patients with advanced malignancy were entered into a phase I trial with recombinant M-CSF (Cetus). Treatment consisted of subcutaneous 100-12800 ug/m^2/day for days 1-5 and days 8-12. Monocytes and alveolar macrophages were isolated prestudy and day 10 of treatment. In addition, monocytes were isolated on day 3. Expression of mRNA for tumor necrosis factor-alpha (TNF) was evaluated in freshly isolated nonadherent monocytes and alveolar macrophages. Secreted TNF was measured by enzyme linked immunoassay (R & D Systems) in 24 hr culture supernatant fluids of monocytes and macrophages. No enhanced gene expression or cytokine secretion was detected at M-CSF doses below 3200 ug/m^2/day. Results for high dose M-CSF (≥3200) are shown in the Table.

	Monocytes	Alveolar Macrophages
	(number positive/number tested)	
TNF Secretion	3/5	3/4
TNF mRNA	2/3	0/3

Enhanced TNF secretion was detected in both cell populations, however, enhanced gene expression was detected only in monocytes. This discrepancy may reflect differences in sampling time or regulation between monocytes and alveolar macrophages. (N01-CM-97622).

J 112 RACIAL DIFFERENCES IN SERUM SOLUBLE INTERLEUKIN-2 RECEPTORS (sIL-2R) AMONG HEALTHY TEENAGERS. D.J.Tollerud, J.D. Harper, C.C. Kurman, L.M. Brown, E. Maloney, D.L. Nelson, W.A. Blattner. Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and the Metabolism Branch and Epidemiology and Biostatistics Program, National Cancer Institute, Bethesda, MD.

Serum sIL-2R level has proven to be a useful marker of disease progression in adults infected with the human immunodeficiency virus (HIV). HIV infection among teenagers is an increasing problem in the U.S., particularly among minority groups in certain metropolitan areas. Currently, interpretation of sIL-2R levels in this population is limited by a lack of information on potential racial differences in this important biomarker. To address this issue, we analyzed serum sIL-2R levels in 111 healthy teenagers, ages 12-19, from families enrolled in a population-based survey of adults in a large U.S. metropolitan area. Although the sera were not specifically screened for HIV, a detailed questionnaire did not reveal a history of significant illness, and all subjects had normal serum chemistry values, normal CBC and differential, and normal T- cell subset levels, including CD4 and CD8 cell counts. Geometric mean sIL-2R levels:

		AGE GROUPS (years)			
	No.	12 - 14	15 - 16	17 - 19	ALL AGES
WHITES	83	725	603	500	612
BLACKS	28	448	389	451	423

Serum sIL-2R levels were significantly lower in black teenagers compared to whites ($p<0.001$), particularly in the youngest age group. Interestingly, sIL-2R levels decreased significantly with age for white children, while levels among blacks remained relatively constant. The relationship of sIL-2R levels to other cellular and humoral immunologic parameters will be presented. This analysis provides useful normative data for clinical studies using sIL-2R levels in teenagers, and emphasizes the importance of considering ethnic differences in immunologic studies of heterogeneous populations.

J 113 EXPRESSION AND PURIFICATION OF SOLUBLE EXTRACELLULAR FORMS OF MURINE TUMOR NECROSIS FACTOR RECEPTORS: GENERATION OF TNF RECEPTOR AGONIST ANTIBODIES, Richard F. Weber*, Louis A. Tartaglia*, Irene S. Figari†, Carmen Reynolds†, Michael A. Palladino†, and David V. Goeddel*. Departments of *Molecular Biology and †Cell Biology, Genentech, Inc., South San Francisco, CA 94080

TNF-α and TNF-β are structurally and functionally homologous cytokines that mediate highly pleiotropic biological activities. These activities are transduced by two distinct cell surface receptors (TNF-R1, TNF-R2) whose cDNA's have been isolated. We have established mammalian cell lines that constitutively secrete the extracellular ligand-binding domains of the two mouse TNF receptors. Soluble murine TNF receptor proteins were purified using a combination of ligand-affinity chromatography and reverse phase HPLC and used to generate rabbit anti-murine TNF receptor polyclonal antisera. The agonist properties of these antisera were evaluated to examine the indivdual roles of the murine TNF receptors. Polyclonal antibodies directed against murine TNF-R1 induced cytotoxicity in murine LM cells and the level of manganous superoxide dismutase (MnSOD) mRNA transcripts in NIH 3T3 cells. Polyclonal antibodies directed against murine TNF-R2 had no cytotoxic activity or effect on the level of MnSOD mRNA levels. However, these antibodies, but not antibodies against murine TNF-R1 could stimulate proliferation of mouse thymoytes and the murine cytotoxic T-cell line CT-6. Our studies with agonist polyclonal antisera demonstrate that the two murine TNF receptors mediate distinct cellular responses. TNF-R1 initiates signals for cytotoxicity and the induction of MnSOD, whereas TNF-R2 initiates signals for the proliferation of thymocytes and cytotoxic T-cells.

Viruses and Cytokines; Regulation of Normal and Neoplastic Growth

J 200 TNF-α PREVENTS THE DEVELOPMENT OF RADIATION-INDUCED THYMIC LYMPHOMAS IN C57BL/KA MICE : MECHANISMS OF ACTION AND INVOLVEMENT IN THE PROTECTION BY BONE MARROW GRAFTING, Jacques Boniver, Chantal Humblet, Philippe Delvenne, Johanne Deman, Marie-Paule Defresne, Department of Pathology, University Hospital of Liège, B-4000 Liège, Belgium.

A split dose whole body irradiation induces a high incidence (>90%) of thymic lymphomas in C57BL/Ka mice. Lymphoma development is prevented if normal bone marrow cells are grafted after irradiation. The mechanism of lymphoma inhibition by such a marrow graft is the subject of the present investigation. In marrow grafted animals, radiation induced thymic preleukemia cells disappear ; meanwhile thymocyte subpopulations and thymic microenvironment, which are altered in irradiated animals in an irreversible manner, are restored as in normal mice. We have postulated that bone marrow graft induces cytokine production within the thymus, leading to elimination of preleukemia cells. Tumor necrosis factor α and Interferon γ have been investigated. Inoculation of these cytokines into split dose irradiated mice prevents lymphoma development ; indeed these treatments mimick the effects of bone marrow grafting since preleukemia cells disappear whereas thymocyte subpopulations and thymus microenvironment are restored. Thus, these cytokines might be the mediators recruited in vivo after bone marrow grafting. That a peak of intrathymic TNF-α production has been detected in marrow grafted irradiated mice and that anti-TNF-α antibodies inhibit partially the protective effects of bone marrow grafting further support this hypothesis. The results suggest that changes in the intratissular cytokine networks may be involved in carcinogenesis ; furthermore they indicate that manipulating these networks may be of interest for the prevention of cancer development, once an individual has been submitted to an oncogenic agent.

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J 201 ENHANCED EXPRESSION OF THE 75 KD TUMOR NECROSIS FACTOR RECEPTOR (TNFR) DURING HIV-1 REPLICATION IN PROMYELOCYTES.
Salvatore T. Butera and Thomas M. Folks. Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia, 30333.

It has become increasingly apparent that the control of HIV-1 replication is intertwined with normal immune regulatory pathways. We have used cellular models of acute and chronic HIV-1 infection in HL-60 promyelocytes and monoclonal reagents which distinguish the two forms of TNFR to investigate the interrelationship between HIV-1 replication and TNFR surface expression.

Using a promyelocytic model of chronic infection, OM-10.1, TNF-α treatment induced HIV-1 expression and resulted in a substantial increase of 75 kd TNFR surface levels within 24 h, as demonstrated by specific flow cytometry. A series of uninfected parental HL-60 subclones all reduced 75 kd TNFR surface expression in response to TNF-α treatment for 24 h, indicating that TNFR up-regulation by HIV-1-expressing OM-10.1 cells was not a consequence of clonal variation. Induced HIV-1 expression did not alter 55 kd TNFR levels on OM-10.1 cells, nor was there an increase of the RNA message for either TNFR over a 48 h TNF-α induction period. An increase in 75 kd TNFR expression was also evident during an acute HIV-1 infection of HL-60 promyelocytes, verifying that the response observed with OM-10.1 cells was not due merely to a selected site of viral integration. However, in acutely infected cultures this effect of HIV-1 expression was most apparent following TNF-α treatment, although this treatment did not dramatically enhance HIV-1 expression by these cultures.

In conclusion, HIV-1 replication results in a specific increase in surface expression of the 75 kd TNFR and required both active HIV-1 replication plus an exogenous TNF-α signal. Furthermore, these results demonstrate that HIV-1 expression can influence the surface expression of normal cellular receptors, which may in-turn alter immunoregulatory signal transduction.

J 202 THE ROLE OF CYTOKINES IN THE POST VIRAL FATIGUE SYNDROME. H.M.A. Cavanagh, L.J.A. Morrison and P.O. Behan, University of Glasgow, Dept. of Neurology, Southern General Hospital, Glasgow G51 4TF.

Post viral fatigue syndrome (PVFS) patients display a range of symptoms including fever, myalgia, nausea, diarrhoea and fatigue following viral infection (1). Our research has shown that >50% of PVFS patients have a persistent enterovirus infection (2). PVFS symptoms are consistent with the known side effects of an overproduction of cytokines (3). We are investigating the role of cytokines in an animal model of persistent enterovirus infection. Newborn mice were inoculated with a sub-lethal dose of Coxsackie virus B-1. The animals were then sacrificed at regular intervals. Persistent viral infection was demonstrated using the polymerase chain reaction (PCR) on purified RNA from both muscle and brain. Approximately 20% of the animals were found to be persistently infected in either brain or muscle tissue but not both. Cytokine mapping was also carried out using PCR. No differences were observed in the levels of IL-1x, IL-2, TNFx or IFN between the persistently infected mice and the control mice. Persistent infection would however appear to correlate with high levels of IL-6.

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J 203 **EFFECT OF ß-INTERFERON AND TAMOXIFEN ON THE GROWTH OF HUMAN BREAST CANCER CELL LINES** G.Di Fronzo, D Coradini, A. Biffi, V.Cappelletti.
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Antiproliferative effects of ß-interferon (ß-IFN) and tamoxifen (TAM) were studied in five breast cancer cell lines (MDAMB231, BT20, MCF7, T47D, ZR75.1) characterized by different steroid receptor status. Cultures were grown in medium containing 17 ß-estradiol. ß-IFN, at 3 different concentrations (10, 100, 500 U/ml), was tested alone and in association to TAM (10^{-7}M). ß-IFN at the two highest doses significantly inhibited (p<0.001) the growth of all cell lines, and the extent of inhibition was generally not influenced by the combined treatment with TAM. The lowest dose of ß-IFN did not inhibit the growth of ZR75.1 or MDAMB231, which were, however, inhibited by the addition of TAM (p<0.001). The inhibitory effect was superior when TAM and ß-IFN were given in association than in sequence. Anyway a modulation of the inhibitory effect following different schedules of sequencial treatment was observed in the different hormone-dependent cell lines. In fact, in the MCF-7 cell line the treatment with ß-IFN prior to TAM had a higher inhibitory effect, and the opposite was true for T47D and ZR75.1 cells. Results concerning the inhibitory effect observed with the two sequential treatments will be discussed with regard to modulation of steroid receptor content.

J 204 *IN VIVO* DELIVERY OF INTERLEUKIN 4 AND PREVENTION OF TUMOR DEVELOPMENT USING A RECOMBINANT VACCINIA VIRUS, Karen L. Elkins, David L. Ennist, Robert K. Winegar and Jerry P. Weir, Department of Cellular Immunology, Walter Reed Army Institute of Research, Rockville, MD 20850.

Our ability to modulate host responses by direct injection of cytokines is limited by their short half lives, by available quantities of purified material, and by systemic toxicities. We have investigated the use of a recombinant vaccinia virus which expresses murine IL4, VV1/IL4, as an alternate *in vivo* delivery system. Intraperitoneal injection of C3H/HeN mice with VV1/IL4 leads to the production of detectable IL4 bioactivity in peritoneal fluid within 8 hours after injection; detectable IL4 activity persisted for at least 3 days. Further, as suggested by results from other investigators, delivery of this exogenous IL4 could affect tumor transplantation. Adjacent subcutaneous inoculation of C3H/HeN mice with NCTC 2472, a syngeneic connective tissue tumor, and VV1/IL4 resulted in significantly delayed tumor development, compared to PBS-injected or control virus-injected mice (VV2/β-gal, which expresses the marker gene β-galactosidase). When VV1/IL4 was injected weekly at the site of tumor cell inoculation, tumor formation was completely ablated. Interestingly, about 40% of the mice injected weekly with VV2/β-gal also failed to develop tumors, suggesting that TK^- vaccinia virus itself has some anti-tumor properties. Since tumors developed in all athymic *nu/nu* mice, regardless of recombinant virus treatment, and since murine IL4 has no detectable anti-proliferative effect on NCTC 2472 cells *in vitro*, mature T cells contribute to the observed anti-tumor effects. These studies therefore demonstrate that murine IL4 can be readily delivered *in vivo* via a recombinant vaccinia virus in a biologically meaningful fashion. Delivery is apparently not prohibited by anti-vaccinia immunity, since repeated injections of recombinant virus are more effective than single injections alone. Such recombinant viruses may offer advantages in terms of longer delivery (on the order of several days rather than hours) and local, rather than systemic, production of cytokines, with fewer toxic side effects.

J 205 MECHANISM OF TNF RESISTANCE IN C3H MOUSE EMBRYO FIBROBLASTS. A. Fernandez and H. N. Ananthaswamy. Department of Immunology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

Our previous studies showed that transfection of nontumorigenic, TNF-resistant 10T1/2 cells with an activated Ha-ras oncogene made them tumorigenic and highly sensitive to TNF. The increased sensitivity of Ha-ras transformed cells to killing by TNF correlated with their susceptibility to killing by activated macrophages and NK cells. However, anti-TNF antibody partially blocked macrophage cytotoxicity, while it did not have any effect on NK cell cytotoxicity, suggesting that macrophage, but not NK cell cytotoxicity is mediated by TNF. To elucidate the cellular and molecular basis for TNF-resistance, we constructed somatic cell hybrids between TNF-resistant and TNF-sensitive cells and determined their sensitivity to TNF. The results indicated that the somatic cell hybrids were resistant to the cytotoxic effects of TNF. In addition, we found that treatment with cycloheximide induced sensitivity in TNF-resistant cells but had no effect on TNF-sensitive cells. Preliminary studies indicate that certain DNA-binding proteins are differentially expressed in TNF-resistant and sensitive cells. These results suggest that a dominantly acting protein(s) expressed in TNF-resistant cells may be involved in conferring protection against the cytotoxic effects of TNF. Supported by ACS grant #IM-598 to H.N.A.

J 206 EFFECTS OF CYTOKINES ON THE EXPRESSION OF CELLULAR RECEPTORS FOR VIRUSES AND VIRUS FUNCTION.

T.M. Folks. Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia 30333.

As more is understood concerning permissiveness of cells for virus expression, a greater appreciation of cytokine control over this permissiveness emerges. The work presented here describes how monokines may act together and separately in an autocrine mode to regulate virus expression. In addition, a mechanism of autoregulation between TNF and its receptor expression will be discussed in the context of viral influence over this expression. Finally, the link between ß integrins as viral receptors and cytokines will be addressed especially as they relate to two human retroviral infections.

J 207 B-CLL CELLS ARE DEFECTIVE IN THEIR RESPONSE TO IL-5, Teresa G. Hayes, Xiao-Lin Tan, Annemarie B. Moseley, and David P. Huston, Depts. of Medicine and Genetics, Baylor College of Medicine, Houston, TX 77030.

Interleukins are essential for the maturation and differentiation of B lymphocytes into Ig-secreting cells. In B cell chronic lymphocytic leukemia (B-CLL), neoplastic B lymphocytes are arrested at particular stages of development. A possible explanation for this phenomenon would be if a defect in the malignant lymphocytes renders them unable to act upon physiologic differentiation signals such as those provided by interleukins.

Purified B lymphocytes were obtained from 10 B-CLL patients and 10 healthy controls. rhIL-2 enhanced proliferation and IgM secretion in normal and B-CLL cells stimulated by *Branhamella (Moraxella) catarrhalis* (BCAT). When rhIL-5 was added to BCAT-stimulated cells, however, 8 of 10 healthy controls had a substantial increase in IgM production, compared to only 1 of 10 B-CLL patients. The difference between the increment in IgM secretion in control and B-CLL patients was statistically significant (P = 0.016).

Culture Condition	IgM ± SEM (ng/ml)	
	Normal Cells	B-CLL Cells
BCAT	6,104 ± 1,788	2,628 ± 1,340
BCAT + rhIL-5	10,248 ± 2,369	1,933 ± 782

These studies suggest that B-CLL cells may have a selective defect in their response to IL-5, an important B cell differentiation factor.

J 208 THE BIOLOGICAL ACTIVITIES OF N51/KC

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The N51/KC gene was identified by its transcriptional activation in quiescent mouse fibroblasts treated with serum or platelet-derived growth factor. It is a member of the growing family of cytokines whose predicted amino acid sequences can be aligned according to the position of their 4 cysteine residues and in which the first two cysteines are separated by a single amino acid. We used the homologies to the oncogene human *gro*/melanoma growth stimulating factor (MGSA) and the neutrophil activating factor human interleukin 8 (IL-8) as the starting point for our studies on the biological function of N51/KC.

Our source of N51/KC is from the Baculovirus expression system. The mature protein can be purified to apparent homogeneity and [^{125}I]iodinated with the retention of its biological integrity. N51/KC shows biological activities that have become synonymous for other homologs of IL-8, namely human *gro*/MGSA, human neutrophil activating peptide-2 (hNAP-2), and murine macrophage inflammatory protein-2 (muMIP-2). First, it competes for [^{125}I]IL-8 binding to human neutrophils, but it is less efficient than IL-8. This ability to compete is irreversibly abolished if N51/KC is treated with dithiothreitol and iodoacetamide. Second, it is chemotactic for human neutrophils. This activity and that of IL-8 is inhibited by pertussin toxin (PT) which suggests that like peptide N-formyl-met-leu-phe (fMLP) these cytokines are mediating their chemotactic response by a PT-sensitive GTP-binding protein. As expected [^{125}I]N51/KC specifically binds to human neutrophils, but there appears to be 10 to 30 fold fewer receptors of [^{125}I]N51/KC than for [^{125}I] IL-8. Interestingly, although N51/KC is produced in fibroblasts it does not appear to bind to a variety of different fibroblast cell lines, suggesting that it may have a paracrine mechanism of action. We are now in the process of evaluating if the N51/KC receptor is a subpopulation of the IL-8 receptor or an independent one with which IL-8 can cross react.

J 209 INTERLEUKIN 7 INDUCES CD4 T CELL DEPENDENT TUMOR REJECTION

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The potential of interleukin 7 (IL7) to induce an anti-tumor response IN VIVO was analysed. Therefore, the IL7 gene was expressed in the plasmacytoma cell line J558L. Although the growth of IL7 producing cells was not retarded IN VITRO, the IL7 producing cells were completely rejected upon injection into mice. Tumor rejection was observed only in syngeneic but not in nude mice. The tumor suppressive effect could be abolished by the parallel injection of an anti-IL7 monoclonal antibody. Immunohistochemical analysis revealed IL7-dependent infiltration of the tumor tissue by $CD4^+$ and $CD8^+$ T lymphocytes, and also type 3 complement receptor$^+$ ($CR3^+$) cells, predominantly macrophages. Depletion of T cell subsets in tumor bearing mice showed the absolute dependence of the anti-tumor response on $CD4^+$ cells whereas tumor rejection was unaffected by depletion of $CD8^+$ cells. In addition to $CD4^+$ cells, $CR3^+$ cells were also needed for tumor rejection. The anti-tumor effect of IL7 was confirmed by expression of the IL7 gene in a second tumor cell line of different cellular origin. Together, our results demonstrate that a high local IL7 concentration at the tumor site obtained by tumor cell targeted gene transfer leads to tumor rejection involving a cellular mechanism which seems to be different from the ones observed in analogous experiments with other cytokines.

J 210 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HUMAN B CELL GROWTH FACTOR-12kD (BCGF-12kD), James Jackson, Leandros Arvanitakis, Mark Sanders, Abby Maizel, and Surendra Sharma, Department of Pathology and Laboratory Medicine and Experimental Pathology, Roger Williams Medical Center-Brown University, Providence, RI 02908

Recent functional studies with recombinant lymphokines have suggested that human B cell growth and differentiation may not be efficiently regulated by the sequential action of IL-4, IL-5, and IL-6. It has become apparent that synergistic or independent actions of IL-2 and BCGF-12kD are required to potentiate B cell growth and/or differentiation. We recently identified a cDNA encoding a BCGF-12kD, which included a complete Alu repeat unit within its open reading frame. Our recent experiments indicate that a neutralizing monoclonal antibody directed against a BCGF-12kD synthetic peptide can inhibit the autocrine growth of B cells derived from Burkitt's lymphoma and chronic lymphocytic leukemia suggesting a possible pathogenetic role for BCGF-12kD in these lymphoproliferative disorders. Furthermore, characterization of the genomic organization of the BCGF-12kD gene reveals several unique features including (1) the intronless BCGF-12kD open reading frame, (2) the presence of multiple Alu repeats within and flanking the BCGF-12kD open reading frame, (3) a 5' flanking region containing putative binding sites for multiple transcription factors including OCT-1, AP-1, NFAT-1, C-EBP, and (4) the synthesis of multiple mRNA species. The results will be presented to highlight the regulation of BCGF-12kD expression in normal and malignant lymphoid cells, the expression of BCGF-12kD using the baculovirus system, and the novel regulatory role of Alu repeats.

J 211 CHANGES IN CELLULAR IMMUNITY AND CYTOKINE LEVELS IN HIV POSITIVE PATIENTS. Sergei A. Ketlinsky, Andrey A.Prokopiev, Svetlana N.Tsvetkova, Natalia M.Kalinina, Aza G.Rakhmanova, Research Institute of Pure Biopreparations, St.Petersburg, [illegible]

Peculiarities of cellular immunity and sera levels of IL-[illegible] and TNF-a in HIV positive patients [illegible] were studied. Changes in cellular immunity were typical for this infection: lowering of CD4(+) cells, augmentation of [illegible] lymphocytes and HLA-[illegible] cells. It is supposed that these changes in cellular immunity are provided by hyperproduction of IL-[illegible] and TNF-a. In [illegible] of HIV positive persons we revealed the increased level of IL-[illegible] and in [illegible] - increased level of TNF-a. In the group with PGL the increased levels of cytokines were detected rarely [illegible] and often [illegible] in persons with ARC. It should be noted that the increased levels of these two cytokines were found in the [illegible] of persons. The cytokine's concentrations in sera of HIV positive persons were: for TNFa - [illegible] ng/ml, for IL-[illegible] - [illegible] ng/ml.

J 212 PRODUCTION OF A 43kDa CELL MOTILITY FACTOR BY HUMAN LUNG ADENOCARCINOMA CELLS.

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Culture of human lung adenocarcinoma cell line WART cells on fibronectin coated substrata and in the presence of autologous serum free conditioned medium prepared at high cell density (SFCM HD) induced formation of prominent pseudopodia. The same SFCM HD in the absence of fibronectin induced cell rounding as compared to control cells incubated in serum free medium with or without bovine serum albumin. Addition of SFCM HD to modified Boyden chambers with fibronectin coated Nucleopore filters also led to chemotactic responses. No induction of cell translocation was observed on uncoated filters or filters coated with gelatin. From SFCM HD we partially purified a substance with apparent molecular weight of 43 kDa that at nM concentrations induce cell translocation on fibronectin coated substrata while it does not affect cell motility on uncoated or gelatin coated filters. Biosynthesis experiments using ^{35}S methionine incorporation and SDS-PAGE, revealed different protein synthesis patterns at high and low cell densities. We conclude that WART cells cultured at high cell density produce a substance with $M_r \approx 43$ kD that induce cell translocation on fibronectin coated substrata and that cell density is of importance for production of this molecule.

J 213 VIRUS-ENCODED HOMOLOGUES OF CELLULAR CYTOKINES AND THEIR RECEPTORS ARE SECRETED FROM POXVIRUS-INFECTED CELLS, McFadden, G., Upton, C., Smith, C.*, Graham, K., Macen, J., Opgenorth, A., and Schreiber, M., Department of Biochemistry, University of Alberta, Edmonton, Alberta, and *Immunex Corp., Seattle, Washington.

Poxviruses are a large group of complex DNA viruses that infect a variety of organisms and cause a wide range of disease pathologies. These viruses carry out their complete replicative cycles within the cytoplasm of infected cells and represent unique model systems for the study of numerous biological processes. Shope fibroma virus (SFV) is a tumorigenic leporipoxvirus which causes a localized benign fibroma in immunocompetent rabbits which regresses after two weeks due to a vigorous cell-mediated immune response. Malignant rabbit fibroma virus (MRV) and myxoma virus (MYX) are related tumorigenic leporipoxviruses which induce systemic infections characterized by severe immunosuppression and a progressive fibromatosis syndrome which is rapidly fatal. Studies to dissect the molecular mechanisms by which these viruses mediate tumorigenesis and immunosuppression has focussed on a class of viral proteins which are secreted from virus-infected cells and impinge upon exogenous secondary targets. These include virokines, such as viral analogues of cellular growth factors, and viroceptors, which are viral proteins that mimic cellular receptors and prevent host immune ligands from reaching their cognate cellular receptors. Examples of the virokine class include: (1) viral homologues of transforming growth factor-alpha/epidermal growth factor which can stimulate cells bearing the EGF receptor into mitogenesis, (2) members of the serine protease inhibitor (SERPIN) superfamily. Examples of the viroceptor class include: (1) the T2 protein, which is homologous to the tumor necrosis factor receptor and binds tumor necrosis factor and thereby short circuits host TNF-dependent immune pathways, (2) the T7 protein, which is homologous to the gamma-interferon receptor, and is the major secreted protein from myxoma virus-infected cells. Viral mutants in these and other related virulence genes will be summarized in terms of their relative contribution to viral pathogenesis. We believe these secreted proteins represent a concerted viral strategy to circumvent the cell-mediated immune response, suggesting that the highly invasive poxviruses have developed mechanisms to abrogate most, if not all, of the host immune pathways designed to clear the viral infection.

J 214 RESTORATION OF IMMUNE FUNCTIONS IN MICE INFECTED WITH CYTOMEGALOVIRUS,

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Murine cytomegalovirus (MCMV) induces a generalized immunosuppression that is characterized by depressed responses to mitogens or antigens, suppression of cytokine activity and a reduction in the secretion of Interleukin 2 (IL-2) and in the expression of IL-2 receptors. Since T lymphocytes are critical for recovery from infection with MCMV, we examined the ability of a thymic derivative, thymomodulin (TMD) to restore the impaired T cell functions in mice infected with MCMV. Female BALB/c mice 6-8 weeks old were infected by IP injection with a sublethal dose ($2X10^4$ p.f.u.) of MCMV. At 4-5 hours after infection, mice were treated with the thymic derivative TMD and treatment continued daily for 12 days. The dose of TMD was 0.05, 0.5, and 5 mg/Kg. As control, a duodenal extract (DE) prepared similarly to TMD was also given to mice infected with MCMV. Another control group included mice infected but untreated. At days 3, 6, 8, and 13 post infection mice were sacrificed, their spleens aseptically removed and processed, and the cells were examined for response to mitogens and for production of IL-2. The results showed that TMD can alleviate the immunosuppression observed in mice infected with MCMV. These studies suggest that thymic peptides are good candidates to be used in conjunction with primary antiviral therapy for the treatment of cytomegalovirus infections.

J 215 CHARACTERIZATION OF THE MGSA RECEPTOR(S): LIGAND BINDING RESULTS IN ENHANCED PHOSPHORYLATIONS.

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The human melanoma growth stimulatory activities (MGSA) are products of immediate early genes coding for cytokines which exhibit sequence similarity to platelet factor-4 and β-thromboglobulin. MGSA has been demonstrated to partially compete for binding to the 58kD neutrophil receptor for another β-thromboglobulin related chemotactic protein, IL-8. Low and high affinity IL-8 receptors have recently been cloned (Thomas et al, JBC 265:20061, 1990; Holmes et al., Science 253,1278, 1991; Murphy and Tiffany, Science 253, 1280, 1991). Murphy and Thomas demonstrated that MGSA partially competes with IL-8 for binding sites on the low affinity IL-8 receptor at concentrations greater than 10^{-8}M. We demonstrate that when ^{125}I-MGSA/GROα was crosslinked to receptors/binding proteins from human placenta, there were two major ^{125}-I-MGSA crosslinked bands of 64,000 and 84,000 M_r. Ligand binding to the receptor proteins is associated with enhanced phosphorylation of a number of substrates, including proteins in the same M_r range as the MGSA receptor/binding proteins. A number of approaches have been taken to clone the putative high affinity MGSA receptor, including expression cloning and PCR using degenerate oligonucleotides based upon the nucleotide sequence for cytokine receptors and the IL-8 receptor(s). Results from these experiments will be presented.

J 216 INFLUENCE OF ANTISENSE RNA'S OF INTERLEUKIN-1ß AND INTERLEUKIN-1 RECEPTOR ANTAGONIST ON INTERLEUKIN-1ß PRODUCTION.

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The effect of antisense RNA's to IL-1ß and IL-1ra on human monocytes was tested. IL-1ß and IL-1ra genes were cloned in correspondent orientations under T7 RNA polymerase promoter and obtained transcripts were added to cultured monocytes alone or stimulated with 10µg/ml LPS. Sense RNA's were used as a control and they did not show any detectable action on IL-1ß levels, measured by ELISA and biotest. It was shown that antisense IL-1ß RNA inhibit IL-1ß synthesis in dose-dependent manner, as was confirmed by both ELISA and biotest. IL-1ß levels in supernatants of monocytes incubated with antisense IL-1ra RNA was just the same as in control, as shown by ELISA; but the level of biologic activity in the same samples was at 70% higher, than in control. In can be proposed, that blocking of IL-1ra synthesis by antisense RNA do not leads to decreasing or increasing of IL-1ß synthesis and regulation of IL-1 biologic action by IL-1ra is carried out completely by direct competition between them for binding with IL-1 receptors.

J 217 SUPPRESSION OF INFLAMMATORY RESPONSES TO VIRAL INFECTION: VIRAL INHIBITION OF THE IL-1β CONVERTASE

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Cowpox virus is capable of inhibiting inflammatory responses mounted by the host to protect itself from viral infection. We have identified a viral gene, designated the *38K* gene, which is necessary, but not sufficient to effect this inhibition. This gene is strongly expressed immediately after viral infection. It encodes a 38 kDa protein whose amino acid sequence resembles that of plasma protein inhibitors of serine proteinases. This protein also appears to possess the stressed loop conformation typical of inhibitors of serine proteinases such as α1-antitrypsin. However, the predicted reactive center of this protein did not suggest a proteinase target, because it lacks similarity to reactive centers of known inhibitors.

One major potential mediator of inflammatory responses against virus infections is IL-1. Accordingly, we investigated whether the target of the viral 38 kDa protein might be the proteinase (IL-1β convertase) that generates mature IL-1β from its inactive precursor. Partially purified convertase was incubated with extracts of virus-infected cells prior to the addition of recombinant pro-IL-1β. Extracts of cells infected with wild-type cowpox virus completely inhibited the generation of mature IL-1β, whereas extracts from either uninfected cells, or cells infected with a cowpox virus containing an inactivated *38K* gene, had no effect. To determine whether the 38 kDa protein inhibits processing directly, the *38K* gene was placed in a viral vector for expression in mammalian cells, and the resulting protein was purified to homogeneity. This material potently inhibited the IL-1β convertase, but it did not affect representative serine proteinases of different specificities. Therefore, it appears that the 38 kDa protein of cowpox virus contributes to the suppression of host inflammatory responses by inhibiting the proteinase required to generate IL-1β.

J 218 DETECTION OF CYTOKINE mRNA EXPRESSION BY PCR IN HUMAN MONOCYTES STIMULATED WITH OKT3 MONOCLONAL ANTIBODY.

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The coordinating function in an immune response is generally believed to belong to T cells and by them produced cytokines. Although many of the soluble factors are available in recombinant form their function in vivo is still a matter of study. The complexity of the "cytokine network" is due to the fact that they are locally produced, by a small number of cells and only for a short period of time. We chose the PCR assisted mRNA amplification assay to provide the most sensitive system for this study.

In order to be able to identify a deregulated cytokine gene expression in a disease situation, we have studied the cytokine gene expression and kinetic profile in normal human peripheral mononuclear cells (MNC) activated in vitro by the polyclonal T cell activator, an anti-CD3 monoclonal antibody. Cell cultures were harvested after 2, 4, 7, 21 and 29 hours. Primer sets were designed for IL-1 , IL-2, IL-3 IL-4, IL-6, IL-10, TNF- , -IFN, GM-CSF and G -CSF. After 2 hours stimulation the mRNA for IL-1 , IL-2, IL-3 and -IFN were detectable and remained the whole time period studied. The IL-4 mRNA showed a transient expression between 4 and 7 hours of stimulation. After 4 hours of stimulation came up mRNA for IL-6 and GM-CSF and after 7 hours also mRNA for IL-10, they all remained throughout the time interval studied. No G-CSF mRNA was detected.

These results show that anti-CD3 stimulation of MNC is leading to a sequential induction of different cytokine mRNA, some with a very transient expression. The PCR assisted mRNA amplification assay is a method of choice for the study of in vivo cytokine production in tissue samples where T cell activation is anticipated: tumor biopsies, rheumatoid tissue, infectious infiltrates etc.

J 219 INTERLEUKIN 4 MEDIATED TUMOR SUPPRESSION DEPENDS ON INTERFERON GAMMA

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The molecular events during the anti-tumor response induced by tumor cell-derived interleukin (IL) 4 was investigated by semiquantitative polymerase chain reaction. The growth of IL4-producing Chinese hamster ovary cells (CHO.T1) when injected intraperitoneally into athymic nude mice was strongly suppressed and accompanied by the rapid accumulation of activated macrophages. Peritoneal cells from such mice were analysed for disappearance of IL4 mRNA, thus monitoring the kinetics of tumor cell destruction. Due to the high local IL4 concentration several mRNA were found to be upregulated during the early phase of the anti-tumor response (IL4 receptor, IL5, TNF and IFN-gamma). The functional relevance of the elevated mRNAs was analysed by injection of CHO.T1 cells together with anti-cytokine monoclonal antibodies (mAb). In contrast to anti-IL5 and anti-TNF mAbs, an anti-IFN-gamma mAb abolished almost completely the anti-tumor response demonstrating the dependence of the IL4-induced tumor suppression on IFN-gamma. This analysis allows to elucidate the mechanism of cytokine-induced tumor suppression and gets insight into the regulatory cytokine network in vivo.

J 220 IL-1 AND TNF PRODUCTION BY HUMAN TRANSFORMED MONOCYTIC CELLS.

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Among activated monocytes nearly 90% of cells were labelled with monoclonal antibodies to IL-1ß. This findings were confirmed using human transformed monocytoid U-937, THP-1 and HL-60 cell lines. Unstimulated cultured cells as well as quiescent blood monocytes did not contain IL-1ß and TNFα in their cytoplasm. After LPS stimulation nearly 20% U-937 and few THP-1 and HL-60 cells were labelled with antibodies to IL-1ß which was diffusely distributed within the cellular cytoplasm. IL-1ß synthesis was accompanied by its increased levels in the culture supernatants determined by LAF assay and ELISA. A discrepancy between IL-1ß mRNA accumulation and protein synthesis was found in THP-1 cells using RNA hybridization technique. TNFα was not found after LPS stimulation. But the addition of human recombinant TNFα to the culture fluid of U-937 cells resulted in induction of IL-1ß synthesis and secretion. After activation for 48 hours with PMA and then with LPS 60-70% cells became stained for IL-1ß and nearly one half were TNFα-positive. So the induction of TNFα production needs a differentiation of U-937 cells which may be caused by PMA activation. The data shows that the IL-1ß and TNFα synthesis is induced by different cellular activation signals. The genes of these two cytokines may be regulated separately. Using simultaneous autoradiographic study of cells pulse labelled with 3H-thymidine and immunoperoxidase staining with monoclonal AB to IL-1ß it was shown that the proliferating U-937 cells do not synthetize IL-1ß.

J 221 IN VITRO CHARACTERISTICS OF INTERLEUKIN-6 TRANSFECTED B16 MELANOMA CELLS.

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There has recently been increased awareness of the importance of Interleukin-6 (IL-6) in host defense mechanisms. Acute and chronic inflammatory responses have been shown to involve this molecule. Nevertheless, its effect upon tumor growth has yet to be fully established. In various murine and human tumor lines, IL-6 has been shown to be inhibitory, stimulatory, or have no effect. Because the molecule has an effect on the inflammatory response, we suggest that certain elements of tumor growth, such as angiogenesis and cell adhesion to the matrix (which are critical for inflammation), might even be enhanced by this molecule. It was the purpose of our studies to examine the effects of endogenous increased IL-6 production by a well characterized murine tumor line. For this, we transfected human IL-6 cDNA into murine B16 cells by standard transfection procedures. Transfected cells were shown to secrete increased levels of IL-6 by bioassay. These cells and mock controls were compared for cell growth and adhesion to matrix molecules. Transfected B16 cells (B16-IL-6) were shown to be more spindle shaped and to proliferate at a greater rate than the controls. Furthermore, B16-IL-6 cells adhered to a greater extent to fibronectin and laminin coated tissue culture wells. This adherence was blocked completely by RGDS peptides suggesting that the IL-6-mediated enhanced adherence was due to increased expression of integrin-type receptors. Flow cytometry data demonstrated that fibronectin receptor number was increased in the IL-6 transfected cells. These findings suggest that IL-6 has direct tumor cell effects that may be of importance in primary growth and in metastatic potential.

J 222 CYTOKINE REGULATION OF THE EXPRESSION OF BCRF1, THE IL-10 ANALOG IN EPSTEIN-BARR VIRUS. Chingchai Wanidworanun, Stephen P. James, and Warren Strober, Mucosal Immunity Section, NIH/NIAID, Bethesda, Maryland 20892
Interleukin-10 (IL-10) has been implicated in the cross-regulation of TH1- and TH2- types of immune responses. The Epstein-Barr virus gene, BCRF1, has extensive homology in both its DNA and protein sequences to human IL-10. This, and the fact that BCRF1 pretein also shares several biological properties with human IL-10, prompted us to investigate the role of BCRF1 in EBV-diseases in human.
We have detected mRNA of BCRF1 in B-lymphocytes of a patient with chronic EBV infection, using polymerase chain reaction. EBV-transformed B-lymphoblastoid cell lines are also found to express BCRF1 constitutively in small amounts. We are studying the effects of several cytokines on the expression of BCRF1 in the lymphoblastoid cell lines. Preliminary results indicated that interferon-gamma down-regulated the expression of BCRF1 mRNA in lymphoblastoid cells.

J 223 ENHANCED CYTOKINE PRODUCTION IN VIRUS-INDUCED MYELOPROLIFERATION. H. J. Ziltener, M. Souyri, F. Smadja-Joffe, V. Praloran, C. Jasmin, M.-C. Le Bousse-Kerdiles. The Biomedical Research Centre Vancouver BC, Canada; U 268, Villejuif and U152, Paris, France; CHRU, Nantes, France.
Myeloproliferation induced in mice by the Myeloproliferative Sarcoma Virus (MPSV) a mos-containing retrovirus results in splenic and hepatic myeloid hyperplasia, anemia, thrombocytopenia, splenic and medullary fibrosis. To characterize possible involvement of cytokines we used Northern blot analysis to compare the expression level of cytokine genes in different organs of MPSV-infected and non-infected control mice. We also measured cytokine levels in serum and in medium of cultured cells from affected mice. There were significant increases in mRNA for IL-6, and GM-CSF in liver, spleen and thymus of MPSV infected mice. In infected mice, there was also a significant increase in mRNA for CSF-1 in liver but not in the other organs. Biologically significant levels of IL-6, CSF-1 and G-CSF were found in the sera of MPSV infected but not in control mice. IL-6 and CSF-1 but not G-CSF levels were also increased in medium conditioned by spleen cells of infected mice, indicating that the enhanced cytokine production might take place in different in vivo compartments. GM-CSF activity was detected in medium conditioned by spleen cells of infected mice but could not be detected in the serum. No production of stem cell factor (steel factor, kit-ligand) or IL-3 was detected in either source. IL-1 was detected but there was no significant increase. We propose that the increased production of CSF-1, GM-CSF, G-CSF and IL-6 all of which have synergistic effects on stimulating early hemopoietic progenitor cells could be involved in the genesis of the myeloproliferation.

Cytokines and the Endocrine System; Cytokines as Pathophysiologic Agents

J 300 HEPATOTOXICITY OF TUMOR NECROSIS FACTOR α IN ISOLATED MOUSE HEPATOCYTES. Gregory M. Adamson and Ruth E. Billings, Depts of Pharmacology and Surgery, University of Nevada Medical School, Reno, NV 89557.
TNFα is known to cause liver injury when administered as a cancer drug and it may play a role in liver injury of various etiologies, such as endotoxemia, when large amounts are released from activated macrophages. Little is known about TNFα's effects on normal cells such as hepatocytes in the liver, which are the ultimate target cells in its systemic toxicity. The present study examined the effects of recombinant human TNFα (rhuTNFα) and recombinant murine TNFα (rmuTNFα) in isolated mouse hepatocytes cultured in monolayer. It was found that rmuTNFα was approximately 5-times more potent than rhuTNFα, but the effects were similar. At rhuTNFα concentrations of 1-10 μg/ml, the effects observed included the following: decreases in ATP concentrations to 10-20% of control values; decrease in cellular GSH concentrations due to accumulation and efflux of GSSG; cytotoxicity only when glutathione reductase was inhibited by pretreatment of the cells with BCNU. All of these effects could be ameliorated with anti-oxidants such as mannitol. Other protective agents were ruthenium red, which blocks futile ATP-dependent Ca^{++} cycling across the inner mitochondrial membrane, and fructose, which provides ATP via the glycolytic pathway. These studies show that TNFα has profound direct effects on hepatocytes, in the absence of other inflammatory mediators or cells. There was a correlation between effects on ATP levels, disruption to the glutathione pool, mitochondrial function, and cytotoxicity. The effects of TNFα on hepatocytes were similar to those commonly associated with an oxidative stress. It is proposed that TNFα induces the formation of free radicals in hepatocytes, an effect similar to that seen in tumor cells.

J 301 REGULATION OF Fc RECEPTOR AND MHC ANTIGEN EXPRESSION ON ISOLATED RAT MICROGLIA BY TNF, IL-1 AND LPS; EFFECTS ON IFN-γ INDUCED ACTIVATION, M. Louise Cuzner, A. Jane Loughlin, and M. Nicola Woodroofe, Multiple Sclerosis Society Laboratory, Institute of Neurology, 1, Wakefield Street, London, WC1N 1PJ.
Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (MS) characterised by the accumulation of T cells and macrophages in perivenular cuffs in the white matter. Cytokines, either produced locally by these cells or present as a result of leakage across the blood brain barrier influence local cell function. For example interferon (Ifn-γ) upregulates MHC Class II expression on microglia in MS brain, which in association with antigen could lead to local activation of T cells. Isolated rat brain microglia display enhanced expression of Fc receptors on treatment with Ifn-γ, tumour necrosis factor (TNF-α), interleukin-1 (IL-1) and lipopolysaccharide (LPS) whereas MHC antigen expression is enhanced only by Ifn-γ. Although TNF and LPS individually have no effect on MHC expression by microglia they both antagonise Ifn-γ-induced expression. The enhanced expression of Fc receptors observed in the presence of Ifn-γ, TNF or LPS is significantly inhibited by the combination of Ifn with either LPS or TNF. IL-1 has little effect on Ifn-γ-induced MHC or Fc receptor expression by microglia. Peritoneal macrophages behave in a similar way with the notable exception that IL-1 enhances Ifn-γ-induced FcR expression. These observations suggest that the functional activity of microglia during inflammation or demyelination in the CNS can be influenced by the changing profile of cytokines present during lesion development.

Woodroofe, M.N., Hayes, G.M. & Cuzner, M.L. (1989) Immunology 68, 421-426.

J 302 TUMOR NECROSIS FACTORα INDUCED OXIDATIVE STRESS IN ISOLATED MOUSE HEPATOCYTES.

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TNFα's cytotoxicity in tumor cells may be due to generation of oxygen radicals (RO) since TNFα resistance can be conferred by transfecting TNFα sensitive cells with a functioning manganese superoxide dismutase (MnSOD) gene (Wong et al, 1989 Cell, 58, 923-931). Also TNFα induces mitochondrial MnSOD in a variety of cells. TNFα impairs mitochondrial respiration in several cell types, and may increase mitochondrial generation of RO. In addition, TNFα is known to increase formation of reactive nitrogen radicals (RN) in numerous cell types, including hepatocytes. Studies in this laboratory have shown that recombinant human TNFα (rhuTNFα) may be toxic to isolated mouse hepatocytes by a free radical mechanism involving mitochondrial dysfunction and oxidative stress. The purpose of the present studies was to differentiate between RO and RN in the effects of rhuTNFα in cultured mouse hepatocytes. It was found that RN formation and cytotoxicity were potentiated by the addition of interferon τ (IFNτ) along with TNFα. However, the RN synthesis inhibitor, N-methyl-l-arginine blocked formation of RN but failed to eliminate TNFα's cytotoxicity. Conversely, RO selective antioxidants, such as mannitol, benzoate and desferrioxamine, were completely protective. Lipid peroxidation was modestly increased in TNFα-treated cells, with the effect isolated to the mitochondria. Most significantly, mitochondrial MnSOD activity was increased by 100% in hepatocytes treated with TNFα for 24 hrs. These results suggest that TNFα increases formation of RO in cultured mouse hepatocytes, possibly due to increased formation of RO in the mitochondria. RO, rather than RN, are responsible for TNF's toxic effects in these cells.

J 303 SEX STEROID REGULATION OF CYTOKINE GENE EXPRESSION

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Females animals and humans generally have a more vigorous immune system; additionally there is an increased prevalence of most autoimmune diseases in women. It is clear that the differing sex steroid profile contributes to the immunologic differences between the sexes, but the mechanisms by which these hormones alter immune responses is unknown. We have recently reported that expression of the cytokine IFN-γ can be regulation by estrogen (*J. Immunol.* 146: 4362-4367, 1991). Short-term exposure to 17β-estradiol increases IFN-γ mRNA expression and protein production in activated spleen cells, whereas no effect on IL-2 expression was found. Transient expression assays reveal that the activity of the IFN-γ promoter can be markedly increased in the presence of estrogen, an effect mediated by DNA sequences within the 5'-flanking region of the IFN-γ gene. Estrogen receptors have been identified in CD8+ T cells, thus *in vivo* estrogen may have a direct effect on these lymphocytes, altering production of cytokines such as IFN-γ and thus affecting immune system function.
Estrogen receptors have also been identified in macrophages and selected B cell lines, and we have verified their expression in a number of macrophage cell lines and a myeloma cell line. Using these cells we are currently examining whether estrogen may also directly modulate the expression of macrophage and B cell derived cytokines, including TNF-α, IL-1α, IL-1β, and IL-6. Possible indirect regulation, through specific transcription factors, is also under investigation.

J 304 ISLET CELL REGENERATION IN IFN-g TRANSGENIC MICE

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IFN-g is produced in response to infection and has immune-stimulatory and proliferative activities. To investigate the potential role of IFN-g in inflammatory autoimmune diseases, transgenic mice expressing IFN-g in pancreatic beta cells were created. These mice suffer from pancreatic inflammation leading to islet cell loss. In the course of histological examination of inflamed pancreata from ins-IFN-g transgenic mice we have noticed a dramatic cell proliferation within and around the ductal regions. Subsequent immunolabeling of histological sections with antibodies to glucagon, insulin and somatostatin reveals that hormone containing cells appear in the ductal and periductal areas. We present evidence that ductal cells from adult transgenic mice differentiate into A (glucagon) and B (insulin) and C (Somatostatin) producing cells. We have found that this process is initiated by duct cell proliferation as revealed by incorporation of thymidine analog, bromodeoxyuridine (BrdU) and with the appearance of more primitive neuroendocrine progenitor cells producing tyrosine hydroxylase (TH) along the apical regions of the ducts. The regenerative process in the ins-IFN-g transgenic mice appears similar to the events that occur during embryonic islet cell development. However, some abnormal structures and unusual cell types accumulate as these "lesions" progress. These studies underscore the lymphokine's ability to initiate a complex "transdifferentiation" pathway within a terminally differentiated structure.

J 305 TYROSINE KINASE ACTIVITY IS REQUIRED FOR THE EXPRESSION OF THE INTERLEUKIN-1β GENE IN MYELOID CELLS.

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Protein kinase C (a serine-threonine kinase) is known to be required for the expression of interleukin-1 (IL-1) genes in different cell types. To examine the role of the other type of kinases, tyrosine kinases, in the signal transduction pathway leading to the expression of the IL-1β gene, we first tested the effect of genistein (a tyrosine kinase inhibitor). In the myeloid leukemia line THP-1 genistein inhibited both the IL-1β protein production and mRNA expression which were induced either by phorbol myristate (PMA) or bacterial lipopolysaccharide (LPS). These activators also induced tyrosine-specific phosphorylation of several proteins in these cells. To localize the site of action of this tyrosine kinase activity, THP-1 cells were transfected with 5x AP-1-CAT reporter plasmid and then stimulated with PMA in the presence or absence of genistein. The same genistein concentration (30 µg/ml) which inhibited the IL-1β expression did not have any effect on the on the induced CAT activity. Thus these data suggest that the tyrosine kinase signal required acts either distal from the protein kinase C-dependent AP-1 activation or independently from it.

J 306 MODULATION BY ANTIBODIES OF THE BIOLOGICAL EFFECTS OF INTERLEUKIN 3

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Antibodies to cytokines may develop either spontaneously or as a result of therapy using these agents. These antibodies may be either neutralising or non-neutralising. We have investigated the possibility that some non-neutralising antibodies may enhance the effect of their target cytokine. Polyclonal antibodies to interleukin 3 (IL-3) were raised in rabbits using synthetic peptides which corresponded to sequences in the IL-3 molecule which were not involved in receptor binding. These antibodies did not neutralise IL-3 in bioassays on IL-3 dependent cell lines and in addition were able to bind to IL-3 when the IL-3 was complexed with its receptor in fluorescence activated cell sorts. These antibodies were demonstrated to be of high affinity by their co-elution from a Sephadex G100 column together with interleukin 3 bioactivity. *In vivo* biological studies demonstrated that the combination of antibody with IL-3 enhanced the biological activity of IL-3 between 7 and 35 fold depending on the assay used. Biological read-outs included colony forming units in the spleen, P-cell precursor frequency in the spleen and bone marrow and histology on spleen, liver, skin, jejunum and lung. No toxicity was seen. The mechanism of this enhancement appears to be an increase in the elimination half life of the interleukin 3. In the presence of antibody the α phase half life is extended from 4 minutes to 10 minutes and the β phase half life is extended from 10 minutes to 70 minutes. This demonstration of biological enhancement of cytokine activity for antibodies has a number of implications for cytokine therapy. In addition to the effects observed with exogenous cytokine therapy it may be possible to boost endogenous levels of cytokines using antibodies.

J 307 PRODUCTION OF SEVERAL CYTOKINES IN HODGKIN'S DISEASE DERIVED CELL LINES

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Hodgkin's disease derived cell lines were investigated for their capacity to produce different cytokines. The production of IL1α, IL1ß, IL2, IL3, IL4, IL5, IL6, IL8, TNFα, TNFß, G-CSF and GM-CSF was determined by enzyme linked immunosorbent tests (ELISA) and by Northern blot experiments. Our results display that Hodgkin's disease derived cell lines produce several cytokines, such as IL1α, IL4, IL5, IL6, IL8, TNFα, TNFß and GM-CSF. The cytokines IL1ß, IL2, IL3 and G-CSF could not be detected in the supernatants of the cells. In addition the receptors for IL2 and IL6 could be detected in some of the cell lines. In the cell lines with high expression of the IL2 receptor on the cell surface, we found in addition soluble IL2 receptors in the supernatants. The expression of IL6 and IL6 receptor could be confirmed in some primary tissues of Hodgkin's disease. From our data we conclude, that IL6 and additional cytokines may be involved in the biology of Hodgkin's disease.

J 308 TNF-MEDIATED DISEASE IN TRANSGENIC MICE: A GENETIC MODEL OF ARTHRITIS.

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To analyse the expression of the human tumour necrosis factor gene and to further understand its biological function, we generated several transgenic mouse lines carrying and expressing wild type and recombinant human TNF gene constructs.

A human TNF gene construct linked to the dominant control region of the human CD2 gene targets TNF expression specifically into T-cells of transgenic mice. Histopathological analysis showed hypoplastic thymuses with altered architecture and dramatically reduced cortical volume. These transgenic mice develop a severe wasting syndrome and necrotic effects are evident in liver, pancreas and lymph nodes. Thromboses are present in the lung vasculature as well as that of other organs. Detailed analysis of such transgenic mouse lines is expected to give further insight into the modulation of the immune system by cytokines.

Moreover, we are able to show that correct regulation of the expression of a wild type human TNF transgene can be established in transgenic mice. In addition, we can show that its 3′-region is necessary for macrophage-specific expression. Interestingly, transgenic mice expressing a 3′-modified huTNF gene develop major impairment in leg movement. Histological analysis of peripheral joints revealed characteristic features of inflammatory synovitis and arthritis. Our results imply direct involvement of TNF in the pathogenesis of arthritis. Transgenic mice which predictably develop arthritis constitute a novel genetic model by which the pathogenesis and treatment of this disease in humans may be further investigated.

J 309 REQUIREMENTS FOR THE INDUCTION OF AUTOIMMUNE DISEASES BY IL-2,

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Autoimmune diseases are polyetiological, insofar as several genetic defects and/or environmental triggers have to coincide to allow the disease to erupt. We have analyzed the role of cytokines and cytokine antagonists in the induction, acceleration or retardation of autoimmune processes by the lymphokine IL-2. Using a recombinant vaccinia virus (IL-2.VV) carrying the human IL-2 gene as the autonomously replicating IL-2 releasing device, we tested the *in vivo* ability of IL-2 to either block clonal deletion or to overcome anergy, i.e. the post-deletional mechanism implicated in T-cell tolerance. We have used transgenic mice for a TCR specific for male antigens, and in athymic mice, where due to the absence of intrathymic clonal deletion, autotolerance must rely entirely upon peripheral, post-deletional mechanisms. In untreated *nu/nu* mice, T cells bearing a "forbidden" potentially autoreactive T-cell repertoire - that would be clonally deleted in euthymic controls - are anergic as revealed by their inability to proliferate in response to TCR crosslinking, while T cells from IL-2.VV-treated mice do so and acquire the capacity to deliver help to B cells upon specific stimulation. Along the same line, IL-2.VV abrogated the unresponsive state of "forbidden" T cells in neonatally thymectomized mice. Since IL-2 induces autoimmune manifestations (DNA autoantibodies, rheumatoid factors, proteinuria, and interstitial nephritis) in both *nu/nu* and neonatally thymectomized mice, and not in euthymic controls, this lymphokine is likely to enhance the autoaggressive function of T cells that bear forbidden, potentially autoreactive TcR gene products. Also, IL-2.VV restored CD8 expression on $CD4^{-}CD8^{-/dull}$ T lymphocytes recovered from male mice bearing a transgenic H-Y-specific TCR, which thus fulfilled the phenotype prerequisites for class I-restricted (CD8-mediated) antigen recognition and acquired the capacity to proliferate in response to cells bearing the male-specific H-Y (auto)antigen. Finally, since IL-2 does not interfere both with thymic or peripheral clonal deletion, we conclude that IL-2 promotes autoimmune diseases by acting on the peripheral mechanisms controlling self-responses.

J 310 NITRIC OXIDE PRODUCTION IN ISLETS OF NOD MICE.

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Insulitis or the infiltration of the islet with lymphocytes begins at 4 weeks of age in the nonobese diabetic (NOD) mouse. Beta(ß) cell destruction and the development of diabetes occur between three to six months of age. Macrophages are among the first cells to infiltrate the islet and , when activated, are known to secrete a number of cytotoxic mediators among which is nitric oxide (NO). NO has been shown to be directly cytotoxic for mammalian cells. We have studied the role of macrophages in the killing of ß cells. It was observed that the numbers of cells that express both CD45 and class II increase with age in NOD islets. This population would include macrophages and B cells. CD45+ cells are essentially absent from islets obtained from (BALB/C x NOD)F1 mice, which have no evidence of insulitis. Concurrent with the increase in cell number we have observed an increased production of NO by macrophages in islets obtained from NOD mice. NO production was studied in islets obtained from 4 week and 11 week old NOD mice and age matched (NOD x BALB/C)F1 mice. In the absence of IFNγ , NO production was undetectable in all islets examined. In the presence of 100U/ml of IFNγ , 4 week old islets produced 13uM NO (per 50 islets) while 11 week old islets produced 18.5uM NO. Islets obtained from F1 animals produced undectable levels of NO when stimulated with IFNγ. The importance of NO in the specific destruction of ß cells would depend on the demonstration that ß are more sensitive to the cytotoxic action of NO than other cells resident in the islet.

J 311 1,25-DIHYDROXYVITAMIN D_3 SELECTIVELY INHIBITS FCεRII/CD23 B ISOFORM GENE EXPRESSION.

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Vitamin D_3 has been shown to effect numerous diverse immune functions in both monocytes and lymphocytes. Monocytic lineage cells can locally convert the relatively inactive 25-hydroxyvitamin D_3 (25(OH)D_3) to the more biologically active form, 1,25$(OH)_2D_3$ (calcitriol); interestingly, monocytic cells also express the low affinity receptor for IgE, FCεRII/CD23, which may be linked to the maturation process. We investigated the effect of 1,25$(OH)_2D_3$ on CD23 gene expression, specifically analyzing CD23 isoform modulation. It was observed that 1,25$(OH)_2D_3$ suppressed spontaneous and IL4-induced CD23b isoform expression in the monocytic cell line U937, at both the mRNA and protein levels. This negative modulation occurs apparently concurrent with an upregulation of CD11b, indicative of lineage maturation. However, 1,25$(OH)_2D_3$ failed to suppress CD23 expression at the protein level in EBV-transformed lymphocytes, as well as activated tonsillar B cells. This conundrum is supported by the observation that 1,25$(OH)_2D_3$ exhibits no detectable effect upon expression of spontaneous or IL4-induced CD23a mRNA in these B lineage cells. Interestingly, 1,25$(OH)_2D_3$ was found to inhibit IL4-induced CD23b mRNA expression in both EBV-transformed lymphocytes and activated tonsillar B cells, paralleling its effect on U937 cells. The distinct regulation of CD23b by 1,25$(OH)_2D_3$ in both monocytic and B cell lineages raises the possibility that the promoter region for the CD23b isoform may express regions complementary with a 1,25$(OH)_2D_3$-responsive negative regulatory element.

J 312 THE ROLE OF TNF-α AND TNF-β IN THE INDUCTION AND PATHOGENESIS OF MURINE AUTOIMMUNE TYPE 1 DIABETES: A TRANSGENIC MODEL,

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Cytokines have been shown to play a major role in several biological processes critical for the development of autoimmune type 1 diabetes. These processes include direct toxicity for β cells but not α or δ cells in the pancreas, induction of class I and class II expression on β cells, induction of expression of a variety of adhesion molecules on endothelial cells and activation of individual lymphocyte subpopulations. To better understand the role of such cytokines in the initiation or pathogenesis of type 1 diabetes, we constructed several lines of transgenic mice in which the expression of the genes encoding murine TNFα, TNFβ and human IL-1β and IL-6 is controlled by the rat insulin promoter. The following experimental questions were addressed: Is the expression of one or more of these cytokines sufficient to initiate an autoimmune reaction in the pancreas resulting in diabetes?, and secondly, do these cytokines, alone or in combination, play a role in the pathogenesis of type 1 diabetes? To address these questions, positive founder mice (CBAxB6)F2 were mated to C57Bl/6 and NOD strains. Histological examination of pancreata from F1 progeny on the B6 and NOD backgrounds indicated that expression of TNFα and TNFβ resulted in a marked perivascular infiltration without obvious insulitis by 1 month of age in both lines. By 3 months of age the infiltration had progressed to marked insulitis. Concomitant with the development of insulitis was a loss of insulin production, as determined by immnocytochemistry, in the Islets of Langerhans of transgenic mice relative to age and sex matched transgenic negative littermates. Glucagon levels were normal in both groups suggesting that the effect of the infiltration is specific for the β cells. Since a diabetic state has not been achieved in either the RIP-TNFα or RIP-TNFβ by 3 months of age, it remains unclear at this time whether the insulitis has been fully uncoupled from development of diabetes or whether the insulitis will ultimately progress to a fully diabetic state.

J 313 REGULATION OF PANCREATIC BETA-CELL GROWTH AND INSULIN SECRETION BY CYTOKINES,

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Cytokines, in particular interleukin-1 beta (IL-1), have been proposed to play a key role in the pathogenesis of type I diabetes mellitus, because they are known to be secreted by islet-infiltrating macrophages and because they inhibit beta-cell function in vitro. In this study, I have investigated the effects of various cytokines on proliferation and insulin secretion by fetal rat beta-cells or RINm5F insulinoma cells in vitro. In the fetal beta-cells it was found that IL-1 exerted a bimodal effect on beta-cell growth in that it initially suppressed DNA synthesis, an effect that was later turned into a potent stimulation. In contrast, IL-1 consistently suppressed insulin secretion and beta-cell cAMP content. Addition of the stimulatory cAMP analog Sp-cAMP[S] or pertussis toxin pretreatment (which increased cAMP content) increased beta-cell replication and insulin secretion, but failed to prevent the repressive actions of IL-1, indicating that the effects of the cytokine are not conveyed through interference with inhibitory GTP-binding proteins regulating adenylyl cyclase. Addition of the serine protease inhibitor TLCK, however, protected completely against the suppression of insulin secretion evoked by IL-1, though it also exerted a marked antiproliferative effect on its own possibly owing to its reported inhibition of tyrosine kinases. Interferon-gamma caused a moderate stimulation of beta-cell replication while suppressing insulin secretion. Interferon-alpha and TNF-alpha were without effect. IL-6 slightly enhanced long-term insulin secretion without affecting DNA synthesis; however, addition of double-stranded RNA (a known inducer of IL-6 production) failed to affect these parameters, suggesting that IL-6 cannot be formed in this system in sufficient amounts to influence beta-cell function.

In RINm5F insulinoma cells IL-1 caused a moderate inhibition of cell proliferation, but markedly suppressed insulin release and cellular insulin content. More marked inhibitory effects were exerted by IFN-gamma and IFN-alpha, the latter only at high concentrations. TNF-alpha, IL-6 or double-stranded RNA did not influence RINm5F growth or function; neither were the inhibitory actions of IL-1 or the interferons prevented by pertussis toxin pretreatment (which also itself was without effect).

J 314 PRODUCTION OF INTERLEUKIN 1, INTERLEUKIN 6 AND TUMOR NECROSIS FACTOR ALPHA IN RESPONSE TO ESTROGEN AND PROGESTERONE DURING THE PERIIMPLANTATION PERIOD OF PREGNANCY, Gary W. Wood, Mamata De, Thomas Sanford, Department of Pathology and Oncology, the University of Kansas Medical Center, Kansas City, KS 66103

The current study showed that the uterus of pregnant mice contains interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) during the periimplantation period (days 3-9). A peak of IL-1 mRNA, bioactivity and immunocytochemically detectable protein occurred on day four. IL-1 activity decreased to very low levels following implantation. Increased IL-6 activity and, later, increased TNFα activity followed the IL-1 peak. Throughout the periimplantation period, the level of cytokine bioactivity and immunocytochemically detectable protein approximately paralleled mRNA expression. The ovarian steroids, estrogen and progesterone, regulate many of the cellular and molecular changes which occur in the uterus. To explore the possibility that intrauterine production of IL-1, IL-6 and TNFα is controlled by ovarian hormones, cytokine mRNA, bioactivity and immunocytochemically detectable protein was quantitated following ovariectomy and following exposure of ovariectomized mice to estrogen and/or progesterone. Uterus from ovariectomized mice contained no detectable cytokine activity, and systemic administration of estrogen or progesterone resulted in induction of IL-1α and IL-1β mRNA expression. Significant amounts of IL-6 and TNFα mRNA appeared only following exposure of ovariectomized mice to estrogen plus progesterone. Cytokine bioactivity and immunocytochemically detectable protein also appeared following administration of either estrogen or progesterone. The highest activity levels were observed following injection of estrogen plus progesterone. Those data showing natural expression of pro-inflammatory cytokines during implantation and their induction by estrogen and progesterone raise the possibility that a cascade of cytokines and cytokine-induced mediators plays a role in estrogen and progesterone-induced cellular events during the early stages of pregnancy.

Differentiation; Neurobiology; Cardiovascular System; Ontogeny of Aging; Interactions in Bacterial and Parasitic Diseases

J 400 IL-4 DEFICIENCY IN MICE GENERATED BY GENE TARGETING, Horst Blüthmann¶, Manfred Kopf*, Rinus Lamers*, and Georges Köhler*, ¶ Department PRTB, Hoffmann-La Roche, Basel, Switzerland, and * Max-Planck-Institut für Immunbiologie, Freiburg, Germany

The cytokine IL-4 is produced by T lymphocytes and mast cells and exerts a variety of functions including the induction of growth and differentiation of B and T cells. Some of its functions have been shown to be synergized or antagonized by other cytokines. This pleiotropism as well as redundancy in the activity of cytokines makes the analysis of the action of a single one very difficult. To dissect the cytokine network we have introduced a null mutation in the *IL-4* gene by homologous recombination using the embryonic stem cell line D3M. Injection of the targeted D3M stem cells into C57BL/6 host blastocysts has yielded 21 highly chimeric male mice, 9 of which were identified as germ line transmitters of the mutation. Heterozygous *IL-4* -mutant mice were intercrossed to obtain homozygous mutant offspring, which were analyzed for the effect of *IL-4* inactivation on B and T cell development and differentiation. Thymocytes and peripheral T cells were found to be normal in homozygous mutants with respect to the total number and relative size of CD4+/CD8+ subsets. Serum IgG1 levels, however, were reduced by a factor of 20 compared to control littermates. In addition, IgE could not be detected in the serum of the mutants. These results indicate that other factors than IL-4 can induce in B cells - although much less efficiently - isotype switching to IgG1, but that the class switch to IgE is completely under the control of IL-4.

J 401 DIFFERENTIAL DEVELOPMENTAL EXPRESSION OF mRNA FOR STEM CELL FACTOR AND ITS RECEPTOR, c-KIT, IN MONONUCLEAR AND ENDOTHELIAL CELLS FROM NEWBORNS AND ADULTS, J.S. Buzby, E. Knoppel, S.D. Lyman, and M.S. Cairo. Immunex Corporation, Seattle, WA 981011, and Children's Hospital of Orange County, Orange, CA 92668.

The developmental immaturity of neonatal hematopoiesis is characterized by high incidence of cytopenia during states of increased demand, such as bacterial sepsis. We have previously demonstrated reduced levels of hematopoietic cytokine expression (GM-CSF, G-CSF, IL-3) in human umbilical cord vs. adult peripheral blood mononuclear cells (MNC) (Cairo, et al, Pediatr Res, in press). The recent discovery of stem cell (Steel) factor (SCF), which stimulates proliferation of primitive hematopoietic progenitor cells (Broxmeyer et al, Blood 77:2142) has led us to investigate its pathogenetic role in neonatal dyshematopoiesis. MNC were isolated from cord blood obtained after normal, nonstressed deliveries and from adult peripheral blood. Vascular endothelial cells (EC) were obtained from umbilical veins and adult aortas. Cells were stimulated with phorbol ester (30 ng/ml PMA), IL-1β (5U/ml), and other agonists for 4 hrs prior to RNA isolation. The RNA was then subjected to Northern blot hybridization with ^{32}P-labeled probes from pBs:hMGF and phckit/171 to detect expression of SCF and c-kit respectively. For comparison, probes from pELAM-1 and pG4H1.1ms- were used to assay expression of leukocyte adhesion molecules, ELAM-1 and ICAM-1, in EC. Expression was normalized to β-actin mRNA determined using a probe from pHFβA-1. Cell lines 3T3 and A172 were utilized as positive controls for SCF and c-kit mRNA expression respectively. The unstimulated expression of c-kit mRNA was significantly higher in cells from umbilical cords than from adults, but was equally down-modulated by PMA stimulation. In contrast, unstimulated SCF mRNA expression was significantly higher in adults, and was equally up-regulated by PMA stimulation of cord and adult EC. The response of SCF to stimulation in EC was very similar to that of ELAM-1 and ICAM-1, in that both were induced by PMA and IL-1β stimulation. This preliminary data suggests that adult MNC and EC express more mRNA for SCF but less for its receptor, c-kit, compared to newborns, and that SCF and c-kit expression may be coordinately controlled. Additionally, regulation of endothelial leukocyte adhesion proteins may parallel the regulation of SCF expression. Reduced SCF expression from newborn cellular sources (MNC and EC) may be important in the pathogenesis of the immaturity of neonatal hematopoiesis.

J 402 THE CAPACITY OF TNF, IL-1 AND LT TO INDUCE NITRIC OXIDE *IN VIVO* AS A POSSIBLE EXPLANATION FOR ALTERED MENTAL STATES IN ACUTE SYSTEMIC DISEASE, Ian A. Clark, Kirk A. Rockett, Karen M. Gray, Elizabeth J. Rockett and William B. Cowden, Deprtment of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT 2601, Australia.

Cell-mediated immunity and many of the harmul effects of parasitic infection are now thought to depend on the release of cytokines such as tumour necrosis factor (TNF), but their mechanism of action is still an open question. TNF has been shown to induce nitric oxide *in vitro* (Kilbourn, *et al.*, PNAS 82: 772; 190). Since TNF, interleukin-1 (IL-1) and lymphotoxin (LT) are all increased in the serum of malarial patients, we have compared the capacity of these and other cytokines to induce nitric oxide *in vivo*, as expressed by the measurement of reactive nitrogen intermediates (RNI) in murine plasma. Both malaria (*P. vinckei*)-primed (sub-clinically infected) and normal mice were tested.

Plasma was exposed to a copper/cadmium/zinc catalyst to convert nitrate to nitrite, then to Griess reagent, and the product measured colorimetrically. TNF, IL-1 and LT all increased the generation of products of NO, more so in mice carrying sub-clinical infections than in control animals. In contrast, IFN-γ and IL-6 induced negligible NO products in vivo. N^6-methyl-L-arginine inhibited in vivo generation of these products, demostrating their arginine origin.

NO moves freely across membranes, and has recently been recognized as a major second messenger in excitatory synaptic neurotransmission. Therefore the capacity of TNF, IL-1 and LT to induce NO inside blood vessels allows a new model of human cerebral malaria, in which cytokine-induced NO mingles with NO of neuronal origin, thus interfering with orderly cell-to-cell signalling in the brain. In particular, NO has been shown to inactivate the binding protein of glutamate receptors, thus reducing excitatory synaptic transmission. This proposal explains several key features of human cerebral malaria, such as an intact blood-brain barrier, and the rarity of residual neurological deficits, that are inconsistent with the current models for this disease.

This principle could explain how a range of acute systemic viral rickettsial and bacterial pathogens that do not enter the brain can nevertheless transiently affect brain function.

J 403 TNF RECEPTOR EXPRESSION IN T LYMPHOCYTES: DIFFERENTIAL REGULATION OF THE TYPE I TNF RECEPTOR DURING ACTIVATION OF RESTING AND EFFECTOR T CELLS. Paul D. Crowe, Carl F. Ware, Todd L. VanArsdale, Janet L. Andrews, Marcia H. Grayson, Rita Jerzy, Craig A. Smith, and Ray G. Goodwin, Division of Biomedical Sciences, University of California Riverside, CA 92521 and Immunex Corporation, 51 University Street, Seattle, WA 98101.

The expression of TNF-α receptors (TNFR) was examined on a $CD4^+$ T cell hybridoma, transformed T cell lines, CTL clones, and activated T cells from peripheral blood. Ligand crosslinking analysis and competitive binding assays using a MAb to the 80 kDa receptor (TNFR-I) demonstrated that the TNFR-I was the predominant receptor expressed on activated $CD4^+$ and $CD8^+$ T cell subsets. However, on T cell leukemic lines additional, non-TNFR-I binding was identified which was most likely due to the 55 kDa TNFR (TNFR-II). Additional subsets of T cells were readily distinguished by their expression of TNFR-I and related members of the TNFR gene family (CD40 and CD27). Signalling through the T cell receptor (TCR) for antigen or IL-2 receptor was sufficient to induce TNFR mRNA and protein expression in resting T cells. Multiple sizes of TNFR-I transcripts were detected during T cell activation. However, biosynthetic studies showed that these multiple species encode a single protein of 80 kDa. These results, and the reported ability of TNF to induce IL-2 receptor expression, indicate that TNF and IL-2 form a reciprocating receptor amplification circuit. In contrast, differentiated effector T cells triggered through the TCR or protein kinase C initiated a rapid down-regulation (transmodulation) of TNFR-I that preceded TNF or LT secretion. The mechanism of transmodulation involved proteolytic processing of the mature 80 kDa receptor, releasing a soluble 40 kDa fragment. This indicates that a TNF autocrine loop is not likely to form during activation of an effector T cell. Collectively, these results suggest that transcriptional and post-translational modification of TNFR-I are important control points regulating the expression of this receptor during T cell activation. Supported by the Haas Estate for Cancer Research and the Cancer Federation, Inc.

J 404 INACTIVATION OF INTERFERON-γ FUNCTION IN THE MOUSE, Dyana Dalton, Sharon Pitts-Meek, Bruce Hultgren and Timothy A. Stewart. Genentech Inc., South San Francisco, CA 94080

Interferon-γ, a lymphokine produced by activated T cells, has a variety of possible physiological functions. IFN-γ appears to play a major role in macrophage activation for both the killing of tumor cells *in vitro*, and for host defense against intracellular pathogens such as *Listeria*. In addition, IFN-γ has antiviral activity, induces class II antigens on some cell types, and may be involved in Ig isotype switching during an immune response.

Classical genetic analysis--analyzing the phenotype of an organism in which the function of a specific gene has been abolished--is a powerful method of determining the function of that gene. To investigate the function of IFN-γ in the mouse, we are in the process of generating mice which are deficient for production of IFN-γ. By using homologous recombination in the AB-1 embryonic stem (ES) cell line, we have replaced one normal copy of the IFN-γ gene with a defective copy of the IFN-γ gene. One of four targeted clones, when injected into C57/BL6 blastocysts, gave rise to many highly chimeric male mice (95-100% agouti coat color) indicating a high percentage contribution by the targeted ES cells to the chimeric mice. Six of these mice are transmitting only the ES cell derived genotype to their offspring. Approximately half of the F1 offspring are heterozygous for the targeted IFN-γ gene. Heterozygote matings are currently underway to generate F2 mice that are homozygous for the targeted IFN-γ gene. The viability and phenotype of the IFN-γ deficient mice will be discussed.

J 405 CYTOKINE INDUCED INFLAMMATORY/ HYPERPLASTIC RESPONSE IN THE EYE

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We are interested in assessing the potentially pathogenic effects of the lymphokine interferon gamma (IFNg) in the central nervous system (CNS). Transgenic mice in which expression of the IFNg targeted to a portion of the CNS (the retina) were used to induce morphological changes in the eye. The DNA elements required for expression in the neuroepithelium of the eye are located in a sequence of nucleic acids extending from -2174 to +70bp of the murine rhodopsin gene. We attached this rhodopsin promoter (rho) sequence to the gene encoding production of IFNg. This rho-IFNg construct was used to produce transgenic mice. The transgene was found to be present in nine lines of progeny, of which four expressed a white-eyed phenotype. These lines also displayed evidence of IFNg production by the retina as shown by *in situ* hybridization. Although expression of the transgene was found to be limited to the photoreceptors, responses to IFNg were not confined to the retina, but were far more reaching, apparently indicating the efficient secretion of the cytokine. Production of IFNg led to shortening of the rod outer segments, and eventually degenerative changes in these cells. Also within the retina, gliosis and inflammation of the ganglion layer was observed. Interestingly, we have found both degeneration and hyperplasia of the lens fibers as well as inflammation of the iris and vitreous humor of the eye. These studies demonstrate the potentially pathologic effects of induced cytokine expression in the CNS.

J 406 Regulation of IL-9 expression in susceptible and resistant mice after infection with Leishmania major

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IL-9 is a pleiotropic lymphokine, one of its activities is to stimulate growth of certain CD4+ T-lymphocytes. In murine leishmaniasis, depending on the genetic background of the host mouse strain, vigorous proliferation of either mainly Th1- in resistant C57BL/6 mice or Th2- type CD4+ T cells in susceptible BALB/c mice occurs after infection. To evaluate the possible role of IL-9 with regard to its immunregulatory function, the expression kinetics in spleen and lymph nodes of this lymphokine were compared between resistant and susceptible mice.
We show for the first time that this lymphokine is indeed produced during an immune response against an infectious agent. No principial differences in the kinetics of IL-9 expression, which had its maximum between day 5 and 7 after infection could be observed. The rate of production however appeared to be higher in susceptible BALB/c mice. After depletion of either CD4+ or CD8+ T cells the reduction of IL-9 production was more than 50 %, suggesting the functional importance of cooperation between these T cell subpopulations with regard to the production of IL-9. Studies are in progress to further evaluate the specific effects of this lymphokine on cell proliferation and differentiation in the T cell compartment.

J 407 CHANGES IN THE EXPRESSION OF TUMOR NECROSIS FACTOR RECEPTORS DURING DIFFERENTIATION OF HL-60 CELLS, Helmut Holtmann, Reinhard Winzen, Cord Brakebusch, Yaron Nophar*, Hartmut Engelmann*, Klaus Resch and David Wallach*, Institute of Molecular Pharmacology, Medical School, Hannover, FRG, and *Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot, Israel
Tumor necrosis factor (TNF), by binding to specific cell surface receptors, induces a wide variety of effects. Two distinct molecular species of these receptors have recently been identified and cloned. Expression of these two receptor species was studied in the promyelocytic leukemia cell line HL-60 before and after inducing differentiation of the cells along the granulocyte lineage (by incubation with retinoic acid) or along the macrophage lineage (by incubation with the phorbol ester PMA). The size of the complexes formed after crosslinking of TNF to its receptors on intact cells, as well as the extent of inhibition of TNF-binding by receptor-specific antisera indicated that on the surface of the undifferentiated HL-60 cells both receptor types were expressed. Differentiation into granulocyte-like cells resulted in some increase in TNF binding, apparently due to an increase in expression of the 75 kDa TNF-R, with no significant changes in the 55 kDa receptor species. In contrast, HL-60 cells induced to differentiate into macrophage-like cells lost completely the expression of the 55 kDa TNF-R. The pattern of TNF-R expression in the differentiated HL-60 cells correlated to the pattern observed in leukocytes isolated from peripheral blood: on granulocytes about equal amounts of both receptor species could be discerned, whereas on monocytes the 75 kDa receptors were predominant. The loss of 55 kDa receptors during differentiation of HL-60 cells into macrophage-like cells was accompanied by a strong decrease in the level of the mRNA for that receptor species, suggesting that at least part of the change in TNF-R expression observed is due to mechanisms controlling the amounts of the receptor mRNA. Conceivably, although there is still little knowledge on the functional differences between the two receptor species, profound changes in the pattern of their expression, as observed during differentiation of HL-60 cells, are expected to alter the kind of response of the cells to TNF and may therefore have an important role in the coordination of the effects of TNF in the organism.

J 408 IMMUNOMODULATORY EFFECTS OF TGFβ ON T LYMPHOCYTES: $CD4^-CD8^-$ THYMOCYTES BECOME CD8 SINGLE POSITIVE IN RESPONSE TO TGFβ AND IL-2.
Thomas H. Inge and Harry D. Bear, Department of Microbiology/Immunology, and Surgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298.

Although much is known about the cellular developmental pathways and molecular mechanisms of T cell differentiation, the external signals that drive differentiation of immature thymocytes are still incompletely understood. When murine $CD4^-CD8^-$ (double negative, DN) thymocytes were stimulated with phorbol dibutyrate (PDBu) + ionomycin (Io) and cultured with transforming growth factor-β (TGFβ) and interleukin-2 (IL-2), growth was inhibited by 40% compared to IL-2 alone. Concurrently, 30% of the cells exposed to TGFβ became $CD8^+$ after 2 days, while CD4 expression was virtually absent throughout 12 days of culture. DN thymocytes stimulated with PDBu + Io and cultured in IL-2 alone were never more than 10% $CD8^+$. When TCR/CD3 expression was examined, we found that 60% of the $CD8^+$ cells were $TCR/CD3^-$. Analysis of relative rates of proliferation and growth for $CD8^-$ and $CD8^+$ subsets eliminated the possibility that the TGFβ-induced increase in the proportion of $CD8^+$ cells resulted from selective survival or growth in TGFβ.

In a separate series of experiments, we obtained similar data using the CTLL-2 cell line. When CTLL-2.TH were treated with TGFβ and IL-2, growth was significantly inhibited and steady-state $CD8\alpha$ mRNA levels rose to >4-times the levels seen with IL-2 alone and peaked at 6-12 hours. De novo surface expression of $CD8\alpha/\beta$ also occurred in a dose and time dependent manner and was dependent on new RNA synthesis. Together, these data indicate that the association between TGFβ treatment and CD8 expression is generalizable and suggest that TGFβ is not only a potent inhibitor of T cell growth but also may play a role in T cell differentiation processes.

J 409 PRODUCTION AND RELEASE OF IL-1ß BY HUMAN PERIPHERAL BLOOD MONOCYTES; POSSIBLE ROLE OF "MICRODAMAGE" TO ACCOUNT FOR UNREGULATED RELEASE, John J. Jessop and Thomas Hoffman, Laboratory of Cell Biology, Division of Hematology, CBER, FDA, Bldg. 29, Rm. 222, 8800 Rockville Pike, Bethesda, MD. 20892
Three different monocyte stimuli (lipopolysaccharide (LPS), concanavalin-A (Con-A), and phorbol myristate acetate (TPA)) all induced production and release of IL-1ß from human monocytes in vitro. LPS demonstrated the greatest potency for both IL-1ß production and release and induced IL-1ß release by 3 h after activation, while Con-A was equally efficacious though less potent than LPS with release appearing at 12-24 h. TPA was a comparatively weak inducer of IL-1ß production, but a consistent inducer of release. IL-1ß release by all three activators occurred at activator concentrations greater than or equal to those required for optimal IL-1ß production, correlated best with total IL-1ß produced, and was associated with release of the cytosolic enzyme marker lactate dehydrogenase (LDH) which followed IL-1ß release temporally. LPS and Con-A had no effect on protein synthesis, a measure of overt toxicity, while TPA inhibited protein synthesis in a dose-dependent fashion. While LPS and Con-A induced expression of a 33 and a 29 kDa precursor IL-1ß, only the 17 kDa form was released. These data indicate that LPS and Con-A may stimulate release of IL-1ß from human monocytes in vitro through induction of microdamage to the cell membrane by an unregulated process. TPA induces a more profound damage to cellular integrity. The involvement of microdamage to membrane integrity in this in vitro system, as determined by LDH release, casts doubt upon its physiological relevance. However, since IL-1ß and LDH both locate in the cytosol, an IL-1ß release mechanism that coincidentally transports LDH to the outside of the cell cannot be ruled out at this time.

J 410 PLASMA LEVELS OF TNFα, IL-1ß AND IL-6 AFTER MODERATE SURGERY. M. Kristiansson*, L. Saraste*, M. Soop*, K.G. Sundqvist°. Dept of Anaesthesiology and Intensive Care* and Dept of Clinical Immunology°, Huddinge University Hospital, S-141 86 Huddinge, Sweden.
Tumor necrosis factor (TNFα) and interleukins (IL) have been reported to mediate metabolic alterations in response to trauma and infection (1): Metabolic changes are prominent 1-2 days postoperatively. A recent report indicates that IL-6 plasma levels increase after moderate surgery (2). Few published results concern cytokine levels after major surgery and in critically ill patients.
The aim of this study was to investigate plasma levels of TNFα, IL-1ß and IL-6 postoperatively in patients with and without infection. Eleven non-infected patients and nine infected patients, subjected to moderate surgery, were studied 1-2 days postoperatively. Plasma cytokine values in twelve healthy volunteers were used for comparison. Plasma IL-6 and IL-1ß levels (pg/ml) were determined by ELISA and plasma TNFα levels (pg/ml) by RIA. Values were expressed as means±SEM (medians). The Mann-Whitney U-test was used for the statistical analysis. Infected patients showed higher plasma IL-6 levels than non-infected patients, 746±201 (580) vs 179±49 (138), $p<0.05$. The plasma IL-6 concentrations in controls, 16±13 (0), were lower, $p<0.001$. Similar analyses of plasma TNFα levels revealed no significant differences. Detectable levels of plasma IL-1ß were found in only three patients (one infected and two non-infected) and in one control.
Conclusion: The results indicate that circulating levels of IL-6, but not of TNFα or of IL-1ß, are increased 1-2 days post-operatively, in particular during simultaneous infection. This suggests that IL-6 may be involved in the response to trauma and infection 24-48 hours postoperatively.
Reference:
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J 411 IL-1 AND TNF MEDIATE THE ANTI-KETOGENIC EFFECT OF ENDOTOXIN. Riaz A. Memon, Kenneth R. Feingold and Carl Grunfeld. Department of Medicine, University of California, and Metabolism Section, VA Medical Center, San Francisco, California 94121.
To determine the role of cytokines in mediating the decrease in ketones associated with infection, we studied the effect of endotoxin (LPS), IL-1 and TNF on serum and hepatic ketone body (KB) levels, serum free fatty acids (FFA) and hepatic malonyl-CoA levels. LPS (1 ug) decreased serum KB by 75% with a maximal decrease (90%) observed following 10 ug LPS in C57Bl/6 (LPS-sensitive) mice. In contrast, LPS had little effect on serum KB in C3H/HeJ (LPS-resistant) mice, whose macrophages lack the ability to produce IL-1 and TNF in response to LPS. A maximum decrease in hepatic KB was observed after 1 ug LPS in C57Bl/6 mice whereas 100 fold higher doses of LPS had little effect in C3H/HeJ mice, suggesting that IL-1 and TNF may mediate this effect. IL-1 (80 ng) and TNF (1 ug) decreased serum KB significantly in C57Bl/6 mice. IL-1 decreased hepatic KB production whereas TNF had no such effect. IL-1 and TNF increased hepatic malonyl-CoA levels, an allosteric inhibitor of carnitine palmitoyltransferase I, the rate limiting enzyme of fatty acid oxidation and ketogenesis. TNF acutely raised serum FFA (increased lipolysis), whereas IL-1 did not. By using phenylisopropyladenosine to block TNF induced lipolysis, we demonstrated that the anti-ketogenic effect of TNF is reversed by its acute effect on lipolysis, which results in an increased flux of fatty acid substrate to the liver, thereby counterbalancing the inhibitory effect of malonyl-CoA on fatty acid oxidation and ketogenesis. Taken together, these data suggest that IL-1 and TNF may mediate the anti-ketogenic effect of endotoxin and infection.

J 412 INTERLEUKIN-1 (IL-1) AND HYDROXYL RADICAL INVOLVEMENT IN THE BIOLOGICAL ACTIONS OF *BORDETELLA PERTUSSIS* TRACHEAL CYTOTOXIN, Linda J. Nixon*, Stephen A. Moser, Emil R. Unanue, and William E. Goldman, Washington University School of Medicine, St. Louis, MO 63110
Bordetella pertussis tracheal cytotoxin (TCT) is a 921 dalton muramyl peptide that is responsible for the respiratory cytopathology associated with the pertussis syndrome. The actions of TCT have been demonstrated using two in vitro models of the respiratory epithelium: destruction of ciliated cells in hamster tracheal organ culture, and dose-dependent inhibition of DNA synthesis in hamster trachea epithelial (HTE) cells. Because TCT has also been found to stimulate interleukin-1 (IL-1) production by monocytes and macrophages, we are assessing the potential role of IL-1 as the mediator of TCT toxicity for respiratory epithelial cells.

In initial studies, we used a biological assay for IL-1 activity to show that TCT induces the production of cell-associated IL-1 in both of our epithelial models. Furthermore, we tested the ability of recombinant murine IL-1β (rIL-1β) to mimic the actions of TCT. In the range of 0.5-5.0 ng/ml, rIL-1β inhibited DNA synthesis in HTE cells in a dose-dependent manner. As little as 20 ng/ml rIL-1β reproduced the pathology of TCT in hamster tracheal organ culture, as determined by light and electron microscopy.

We have begun to elucidate the mechanism by which TCT and IL-1 are toxic for the respiratory epithelium. We have tested a variety of oxygen free radical scavengers and antioxidant enzymes for their ability to alter the toxic effects of TCT and rIL-1β. Several lipid soluble hydroxyl radical scavengers, including vitamin A, dimethyl sulfoxide, dimethylthiourea, and dimethylurea, were found to attenuate the inhibition of DNA synthesis in HTE cells by at least 70%. Thus, TCT and rIL-1β appear to cause inhibitory effects on DNA synthesis via the intracellular generation of hydroxyl free radicals. Because rIL-1β mimics the biological actions of TCT through a common mechanism, there is evidence that cell-associated IL-1 may play an autocrine role in mediating the toxicity of TCT.

J 413 PKC ACTIVATION AND GROWTH FACTOR STIMULATION ACT SYNERGISTICALLY DURING NEURONAL DIFFERENTIATION OF CULTURED NEUROBLASTOMA CELLS, Sven Påhlman, Department of Pathology, University of Uppsala, S-751 85 Uppsala, Sweden
The human SH-SY5Y neuroblastoma cells differentiate into neuron-like cells when treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). This differentiation, which is induced in culture medium containing fetal calf serum, is accompanied by growth inhibition. Using a defined, serum-free, medium we have previously shown that insulin, IGF-I and IGF-II are mitogens for untreated SH-SY5Y cells, and that the corresponding receptors are expressed in these cells. After TPA treatment the cells do not replicate when stimulated with any of these three factors. However, the receptors for insulin and IGF-I are still expressed and are functional in the differentiated cells. Here we show that IGF-I strongly potentiates neurite outgrowth and neuronal differentiation (increased expression of GAP43 and neuro-peptide tyrosine) of TPA-treated SH-SY5Y cells cultured in serum-free medium. By use of blocking anti-IGF-I receptor antibodies we could show that the effect of IGF-I on differentiation, primarily was mediated via the IGF-I receptor. These data suggested a specific role for IGFs during neuronal development. However, when the study was extended to other growth factors recognizing tyrosine kinase receptors, similar results were obtained, i.e. the factors were mitogenic in non-treated cultures and potentiated, synergistically, TPA-induced neuronal differentiation. Thus, once the SH-SY5Y cells have been triggered by PKC-activating phorbolesters, a number of growth factors can induce a complementary signal resulting in the development of a neuronal phenotype.

J 414 A POSSIBLE ROLE FOR INTERLEUKIN-6 IN THE DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES IN CULTURE.
Christina Rondinone, Richard Nordan, Alan R. Kimmel, Constantine Londos, Andrew S. Greenberg. LCDB, NIDDK, Bethesda, MD 20892.
Interleukin-6 (IL-6) is produced by a diverse group of cells and can elicit a variety of responses within different cell populations. We have shown that a murine fibroblast derived preadipocyte cell line, 3T3-L1, secretes IL-6, as measured by a hybridoma growth factor (HGF) assay, during growth and at confluence. However, when confluent 3T3-L1 cells are induced to differentiate into adipocytes by addition of dexamethasone (Dex), the production of IL-6 falls to undetectable levels within 48 hr. When tumor necrosis factor (TNF) is added simultaneously with Dex, the 3T3-L1 cells did not differentiate and IL-6 production was not inhibited. To determine if IL-6 served as a negative effector of adipocyte differentiation in culture we treated confluent 3T3-L1 adipoblasts with Dex alone or with Dex plus IL-6. We found that addition of IL-6 completely blocked adipocyte differentiation (as monitored by triglyceride accumulation) in a dose dependent manner (I_{50} = ~700 HGF U/ml). Data also suggest that the inhibitory effect of IL-6 is reversible; removal of IL-6 after two days induced the rapid differentiation into adipocytes. Cytological examination of IL-6 treated cultures suggested that populations of 3T3-L1 cells were able to partially differentiate in the presence of Dex plus IL-6. We have obtained clonal isolates of 3T3-L1 adipoblasts which vary in their sensitivity to IL-6. The utility of these cell lines as well as the effects of exogenous IL-6 on the molecular aspects of adipocyte differentiation will be presented. Finally, we will discuss the possibility that cessation of IL-6 production is essential for the differentiation of adipoblasts in culture.

J 415 Ca^{2+}_i-DEPENDENT EFFECTS OF INTERLEUKIN-1 ON ELECTRICAL PROPERTIES OF CARDIAC CELLS,
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The cellular electrophysiologic effects of human recombinant interleukin-1ß (IL-1) were studied in excised tissues and isolated myocytes from guinea pig ventricles. First, transmembrane potentials were recorded from papillary muscles superfused in vitro with Tyrode's solution and paced at 1 Hz. In 44 control tissues, 1 ng/ml IL-1 shifted the plateau of the action potential to more positive potentials and significantly prolonged its duration measured at 50% of repolarization (APD_{50}) by 24.2 ± 2.2 msec ($p < 0.001$). IL-1 had no significant effect on resting membrane potential, action potential amplitude or maximum upstroke velocity. However, the same [IL-1] exposed to Ca^{2+}-loaded cells using 0.5 µM ouabain (n=8) or 25 µM Ca^{2+} ionophore (A23187; n=8), markedly suppressed the plateau and significantly shortened APD_{50} by 33.1 ± 8.7 msec (ouabain treated tissues; $p < 0.01$). As for control cells, other electrical parameters were unchanged by IL-1. To examine the transmembrane ionic mechanisms underlying the response to IL-1, ventricular myocytes were isolated from Langendorff-perfused hearts by collagenase treatment and studied using the whole-cell configuration of the patch clamp technique. Moreover, pipette solutions were formulated to dialyze the interior of cells with either a low (pCa 9) or high (pCa 7) free [Ca^{2+}]. Under voltage-clamp conditions designed to study the L-type Ca^{2+} current (I_{Ca}), IL-1 either increased or decreased I_{Ca} amplitude depending upon pipette pCa. Specifically, 1 ng/ml IL-1 increased I_{Ca} amplitude by 38.3 ± 10.4% (12.7 ± 2.2 vs 16.1 ± 2.0 pA/pF; $p < 0.01$) in 9 of 22 cells dialyzed with pipette pCa 9. The remaining cells showed no change in I_{Ca} with IL-1. In contrast, the same [IL-1] reduced I_{Ca} by 39.3 ± 6.4% (16.6 ± 2.5 vs 10.4 ± 2.1 pA/pF; $p < 0.001$) in 8 of 11 cells dialyzed with pipette pCa 7. These data suggest therefore that IL-1 modulates I_{Ca} and possibly other plateau currents in cardiac cells by intracellular mechanisms regulated by $[Ca^{2+}]_i$.

J 416 *IN VITRO* AND *IN VIVO* EFFECTS OF MONOCLONAL ANTIBODIES SPECIFIC FOR THE 55 AND 75 kDa MURINE TNF RECEPTORS,
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Two panels of monoclonal antibodies (mAb) specific for either the 55- or 75-kDa murine TNF receptor were generated by immunizing Armenian hamsters with purified extracellular domains of either protein. Antibody producing cultures were identified by immunoprecipitation assays that employed radiolabeled extracellular receptor domains. All mAb reacted only with the specific form of the receptor used for immunization. *In vitro* analyses demonstrated that three mAb specific for the 75 kDa receptor (TR75-32.4, -45.30 and -54.7) inhibited TNF-dependent killing of L929 cells. These mAb showed no agonist activity in the L cell killing assay either when used alone or following crosslinking with anti-hamster Ig. However, the same TR75 mAbs displayed agonist activity when tested in the murine thymocyte proliferation assay. In contrast, several mAb specific for the 55 kDa TNF receptor, (55R-170.1, -176.11 and -329.1) not only blocked TNF-dependent L929 cell killing but, when subsequently crosslinked with anti-hamster Ig, displayed agonist activity in the absence of exogenous TNF. In addition, one mAb (55R-593.4) induced cytocidal activity in the absence of crosslinking. The *in vivo* effects of these mAb were monitored using a model of endotoxin shock. C57BL/6 mice were pretreated with mAb or saline and then challenged 18 hr later with LPS (600 ng) and galactosamine (8 mg). The survival of the TR75-treated mice was indistinguishable from the saline controls (20%). In contrast, all of the 55R-treated animals survived. This data thus supports the concept that the two TNF receptors induce unique arrays of biologic responses and confims that this fuctional division occurs *in vivo* as well as *in vitro*.

J 417 **MODULATION BY GROWTH FACTORS OF DIFFERENTIATION TO THE MEROZOITE IN THEILERIA ANNULATA.**
Brian Shiels, Sue McKellar and Jane Kinnaird. Wellcome Unit of Molecular Parasitology, Glasgow University, U.K.
The protozoan parasite Theileria annulata is the cause of tropical theileriosis, an important disease of cattle. We are studying the differentiation of the intracellular macroschizont stage of the parasite to the merozoite. The macroschizont induces the host cell to proliferate indefinitely, and this involves cytokines functioning via an autocrine loop. Under stable conditions of culture the parasite and host divide in synchrony and the size of the macroschizont is maintained. We have developed an in vitro system for differentiation and have isolated cloned macroschizont infected cell lines which show either a diminished (<10%) or an enhanced differentiation phenotype (>60%). During the preliminary phase of differentiation we have found that parasite growth becomes asynchronous with the host cell, resulting in increased parasite size. We believe that this size increase is involved in triggering differentiation, and that the level of differentiation can be influenced by the proliferation potential of the host cell. In order to test this hypothesis the amount of foetal calf serum supplementing the culture medium was altered and the level of differentiation assessed following induction. The results showed that differentiation increased with reduced serum, and that this was associated with slower growth of the cultures. It seems likely that modulation of differentiation was due to a reduction in proliferation caused by lower levels of cytokines. It is possible, therefore, that differences in the amplitude of autocrine stimulation determine the differentiation phenotype of the cloned cell lines.

Late Abstracts

ROLE OF CYTOKINES IN THE PATHOGENESIS OF HSV-1 INDUCED CORNEAL INFLAMMATION, Robert L. Hendricks, Departments of Ophthalmology and Microbiology & Immunology, University of Illinois at Chicago, Chicago, IL 60612

We and others have demonstrated a critical role for CD4 but not CD8 T lymphocytes in the corneal inflammation resulting from infection of A/J mouse corneas with the RE strain of Herpes simplex virus type 1 (HSV-1). This study investigated the involvement of the cytokines interferon gamma (IFN-γ), and interleukins 2 and 4 (IL-2 and IL-4) in the inflammatory process. Mice were treated intraperitoneally with rat monoclonal antibodies capable of neutralizing these cytokines starting either 1 day before (day -1), or 6 days after (day +6) HSV-1 corneal infection, and continuing at weekly intervals for 30 days. Control mice were similarly treated with rat monoclonal anti-HLA-DR. The severity of inflammation (degree of stromal opacity) was evaluated by slitlamp examination and scored on a scale of 0 (no disease) to 4+ (perforated cornea). The incidence of corneal inflammation was significantly reduced by treatment with anti-IFN-γ when begun at day -1 ($p = .0002$) or day +6 ($p = 0.0063$), and by treatment with anti-IL-2 when begun at day -1 ($p = 0.0519$) or day +6 ($p = 0.0255$) as compared with control mice. The severity of corneal inflammation (including only mice with disease) was also significantly reduced when treatment with anti-IFN-γ was begun on day -1 ($p < 0.05$) or on day +6 ($p < .001$), and when treatment with anti-IL-2 was begun on day -1 ($p<0.01$) or on day +6 ($p<0.001$). Treatment with anti-IL-4 did not significantly affect the incidence ($p = 0.10$) or severity ($p>0.05$) of corneal inflammation. Inflammatory cells were extracted with collagenase from infected corneas 11, 14, and 21 days after infection. The yield of cells from inflamed corneas ranged from 10^3 to 10^5, most being neutrophils. IFN-γ-secreting cells were quantified using an ELISPOT assay. IFN-γ-secreting cells were undetectable at day 11, but were detected at comparable levels at days 14, and 21. Moreover, a significant correlation was observed between the yield of IFN-γ-secreting cells and the degree of inflammation in the cornea. Our data suggest that RE HSV-1 induced corneal inflammation is mediated by a Th1-type CD4 cell in our mouse model.

ISOLATION, EXPRESSION AND CHARACTERIZATION OF THE COWPOX VIRUS C2 GENE ENCODING A SOLUBLE FORM OF THE TYPE II TNF RECEPTOR

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T2 ORFs from the Shope Fibroma Virus (SFV) and the Myxoma virus (MV), both *Leporipoxvirus* members, encode proteins of 325 and 326 amino acids, respectively, the N terminal 160 residues of which share strong sequence similarity to the Type II TNF receptor (TNFRII, p80). Recombinant SFV T2, expressed in mammalian cells, has been shown to be secreted and to bind specifically both human TNF α and TNF β, and is therefore both structurally and functionally a soluble form of this receptor. The presumed role for such a protein is a viral antagonist of host-produced TNF, serving to protect the virus against the host inflammatory response. The Hind III C2 gene of cowpox virus (CPV), an *orthopoxvirus* capable of inhibiting the inflammatory responses to infection, appears to be the equivalent of the SFV and MV T2 genes. We have characterized this gene to assess its role in CPV anti-inflammatory activities.

C2 encodes 355 amino acids, including a 18 residue leader sequence, followed by 160 amino acids sharing strong homology with the ligand binding domain of TNFRII. Whereas the SFV and MV proteins in this region are 80% identical, the Cowpox protein is ~55% identical to either *Leporipoxvirus* member, consistent with CPV belonging to a different genus. All three proteins also contain a C-terminal domain (161 residues in C2) which shows no homology to the cysteine-rich ligand-binding domain, but clear homology to each other. All 26 cysteines, (5 of which are localized to the C-terminal element) are absolutely conserved. The recombinant C2 protein, expressed in a Vaccinia vector, is secreted from virus-infected cells and specifically binds TNF. The molecular weight of C2, by SDS gels, is ~58 kD; comparison with the protein MW of C2 (39 kD) argues glycoslytion is present. C2 is expressed early in viral infection. Taken together, these data suggest C2 likely plays an important role in dampening the host inflammatory responses to Cowpox infection.

SUPPRESSION OF HUMAN ADIPOCYTE DEVELOPMENT BY TNFα

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Recent studies demonstrate an inhibitory effect of tumor necrosis factor α (TNFα) on the expression of lipogenic enzymes in preadipocyte cell lines.

Aim of our study was to investigate the effect of TNFα on the in vitro differentiation of human adipocyte precursor cells kept in primary culture under serum-free conditions.

Exposure to TNFα of cultured preadipocytes resulted in a dose- and time-dependent decrease of the number of developing fat cells and the activity of glycerol-3-phosphate dehydrogenase (GPDH), which serves as a marker enzyme of adipogenic conversion. A 24 h incubation with TNFα at a concentration of 5 nM suppressed GPDH activity to 55% of controls. Continuous exposure of the cells to TNFα completely blocked the expression of the adipogenic phenotype and GPDH. The inhibitory effect of TNFα was not associated with changes in cell number as assessed by microscopical cell counting.

Addition of 5 nM TNFα for 24 h to newly developed fat cells also caused a reduction of GPDH activity by approximately 50%, but this effect was completely reversible. A longer exposure to TNFα of differentiated fat cells was followed by a marked delipidation of the cells and morphological changes leading to the development of long spindle-shaped cytoplasmatic extensions.

These results clearly showed that TNFα inhibits the differentiation of human adipocyte precursor cells and, in addition, promotes the delipidation of mature fat cells. It is suggested that TNFα may play a role in the physiological control of human adipose tissue cellularity.